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ABOUT JACKSON IMMUNORESEARCH LABORATORIES, INC.

Who we are

Jackson ImmunoResearch specializes in producing secondary antibodies for life science applications. Our reputation as a trusted supplier is founded on 35 years of experience in immunoglobulin purification, conjugation and lyophilization. We continue to optimize processes and develop new technologies to meet the demands of future advances in biological techniques and practices.

All of our antibodies are manufactured at our laboratories situated an hour west of Philadelphia, PA. We do not relabel (OEM) antibodies from other suppliers. In addition to secondary antibodies, we also produce complementary products such as blocking serums, control proteins and streptavidin conjugates.

We serve the United States directly from our laboratory facility in West Grove, PA, and Europe, the Middle East and North Africa from our European facility near Cambridge, UK. Our products are available globally through procurement services and local distributors.

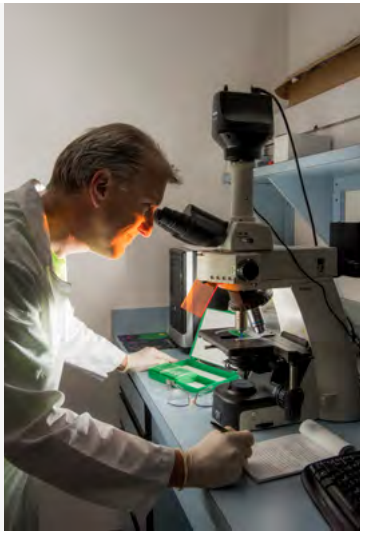
Bulk/Custom Service

We can manufacture and supply most standard inventory immunoreagents in bulk volumes upon request.

The combination of our experience, our focus on secondary antibodies, and our commitment to the highest quality standards, ensures that customers can be completely confident of product quality, consistency, and minimal lot to lot variation. These factors contribute to the efficiency of sample evaluation and approval of bulk materials, and to our goal of providing the highest quality of service.

Our Expertise

- Over 35 years of experience in a single, highly specialized field; secondary antibody manufacture, conjugation and supply
- Long-term customer relationships as an established and reliable supplier
- Fast, efficient customer services
- Maintaining the highest standards in our research, manufacturing and all operations, which contribute to ensuring guaranteed product quality, long term product consistency and reliable performance



Jackson ImmunoResearch Laboratories, Inc. is certified by BSI to ISO 9001:2015 under certificate number FM 545248.
Jackson ImmunoResearch Europe Ltd. is certified by NQA to BS EN ISO 9001:2015 and 14001:2015.

ORDERING INFORMATION

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Prices

Please check the website for pricing.
Prices are subject to change without notice.

Ordering

Please provide the following information to the Customer Service Department to expedite your order:

1. Name
2. Telephone Number and email address
3. Customer Account Number
4. Institution or Company Name
5. Shipping and Billing Address(es)
6. Payment Method
7. Product Code Number and Description
8. Quantity
9. VAT Number (if applicable - Europe only)

Discounts

We automatically apply a discount to all orders greater than $/€500. Please visit our website to see the full discount schedule.

For bulk quantities or custom orders, contact customer service for a quotation tailored to your requirements.

New Lab Start-up: The first order from a new laboratory in an educational institution is eligible for a 20% discount. If the initial order is over $/€2,000, the discount increases to 25%.

Confirming Orders

Confirming orders are required for all customs and bulk orders. Please indicate the order is a "Confirming Order" to avoid duplication.

Shipping

Packages are thoroughly checked before shipping to contain all of the items indicated on the packing list. All claims for missing items must be reported directly to Jackson ImmunoResearch within 5 days after receipt of order.

USA

Orders are shipped by FedEx or UPS 2-Day Service unless otherwise requested. Shipping charges are prepaid F.O.B., West Grove, PA, and added to the invoice.

Europe

Orders are shipped by DHL and are free of charge to educational and other non-profit institutions. If shipping charges do apply they are added to the invoice.

Returns

Please contact Customer Service for authorization to return products. All returns must be authorized in order to receive proper credit.
We reserve the right to impose handling and restocking charges on any products returned due to customer error.

Payment

Payment is due net 30 days from the invoice date.
Invoices may be paid by check, bank wire transfer, ACH, or credit card. American Express, Mastercard, and Visa credit cards are accepted for payment. Please provide the following information:
1. Credit card number
2. Expiration Date
3. Name on credit card
4. Zip code (credit card billing address)
5. CVV number


eppendorf Reference

SECONDARY ANTIBODY SELECTION

SECONDARY ANTIBODY SELECTION

Affinity-purified antibodies are isolated from antisera by immunoaffinity chromatography using antigens coupled to agarose beads. A proprietary elution process is used to dissociate antibodies from the antigen. *Unconjugated* affinity-purified antibodies are supplied sterile-filtered in phosphate buffer without stabilizers or preservatives. *Conjugated* affinity-purified antibodies are freeze-dried in phosphate buffer with stabilizers and sodium azide, with the exception of horseradish peroxidase conjugates, which do not contain a preservative. Alkaline phosphatase conjugates are freeze-dried in Tris buffer with stabilizers and sodium azide.

Selection of Affinity-Purified Secondary Antibodies

This section details how to use the product tables in this catalog and online. It may also be helpful when using the online product filter to select secondary antibodies and reagents.

Step 1. Affinity-purified secondary antibodies are offered in three different **formats**.

The secondary antibody format depends on the intended application. Select from **Whole IgG** (pages 46-71), **F(ab')$_2$ fragment** (pages 74-85), or **Fab fragment** (pages 94-103) antibodies. The whole IgG form of antibodies is suitable for the majority of immunodetection procedures. F(ab')$_2$ and Fab antibodies may be indicated for experiments with specific assay requirements.

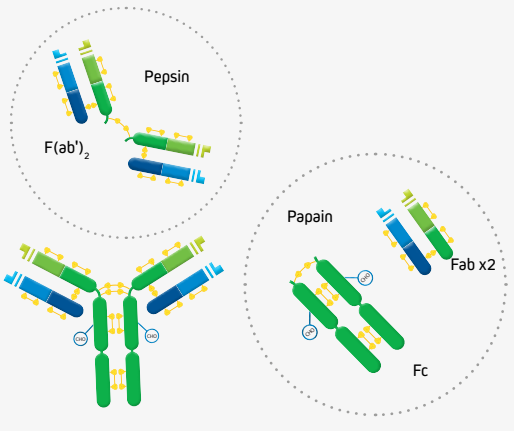


Figure 1: The digestion of IgG (approximate MW = 160 kDa) yields a number of different fragments. Papain digestion generates two monovalent Fab fragments and an Fc fragment, each about 50 kDa. Pepsin digestion degrades the Fc domain, leaving a divalent F(ab')$_2$ fragment with approximate MW of 110 kDa. For an expanded diagram with further annotation, please see page 73.

Whole IgG (pages 46-71) antibodies are isolated from antisera by immunoaffinity chromatography. They have an Fc region and two antigen binding Fab regions joined together by disulfide bonds (Figure 1), and therefore they are divalent. The average molecular weight is reported to be about 160 kDa. The whole IgG form of antibodies is suitable for the majority of immunodetection procedures and is the most cost effective.

F(ab')$_2$ fragment (pages 74-85) antibodies are generated by pepsin digestion of whole IgG antibodies to remove most of the Fc region while leaving intact some of the hinge region. F(ab')$_2$ fragments have two antigen-binding Fab regions linked together by disulfide bonds, and therefore they are divalent. The average molecular weight is about 110 kDa. They are used for specific applications, such as to avoid binding of secondary antibodies to live cells with Fc receptors or to Protein A or Protein G.

Binding of **primary** antibodies to Fc receptors also may occur if they are whole IgG antibodies, creating background regardless of the form of the **secondary** antibody. To block whole IgG primary and secondary antibodies from binding to Fc receptors, incubate cells in buffer containing 5% normal serum from the host species of the labeled secondary antibody. To prevent capping, endocytosis, and regeneration of Fc receptors on **living cells,** incubate at 4°C in buffer containing 5% normal serum with sodium azide added to inhibit metabolism. See Blocking and Controls section (pages 143-155) for more information on avoiding background.

Fab fragment (pages 86-97) antibodies are generated by papain digestion of whole IgG antibodies to remove the entire Fc portion, including the hinge region (Figure 1). These antibodies are monovalent, containing only a single antigen binding site. The molecular weight of Fab fragments is about 50 kDa. They can be used to block endogenous immunoglobulins on cells, tissues or other surfaces, and to block the exposed immunoglobulins in multiple labeling experiments using primary antibodies from the same species.

FabuLight™ (pages 98-103) antibodies are Fab fragment secondary antibodies specific to the Fc region of IgG or IgM primary antibodies. They enable labeling of primary antibodies prior to incubation with cells or tissue without compromising the active site of the primary antibody. They can also be used to label cell surface immunoglobulins without cross-linking and activating B cells.

SECONDARY ANTIBODY SELECTION

Step 2. Select the **target** species of the secondary antibody.

Antibodies are listed alphabetically according to the host species of the primary antibody. For example, if the primary antibody is made in mouse, select "Anti-Mouse".

Note : Both anti-Syrian and anti-Armenian hamster secondary antibodies are listed under "Anti-Hamster". It is important to know in which strain of hamster the primary antibody was produced since cross-reaction between the strains is not complete.

Step 3. Select the **host** species of the secondary antibody.

Selection of the host species for a secondary antibody involves many considerations, including but not limited to:

1. **Host species compatibility.** For multiple labeling, select secondary antibodies from the same host species to minimize interactions between the secondary antibodies. If different host species must be used, the secondary antibodies must each be cross-adsorbed against the host of other secondary antibodies used in the application.
2. **Cross-reacting species.** Some applications require secondary antibodies that are adsorbed against other species to minimize recognition of endogenous immunoglobulins or other primary antibodies (see step 5). Choose a secondary antibody host that provides appropriate cross-adsorptions for the intended application.
3. **Personal preference or experience.** In our experience there appears to be little difference in quality between secondary antibodies raised in different host species. Antibodies raised in rabbit are reported to have higher avidity relative to other hosts.
4. **Binding to Protein A and Protein G.** Rabbit antibodies bind well to both Protein A and Protein G, but goat and donkey antibodies bind better to Protein G.

Step 4. Select the secondary antibody **specificity.**

The following explanations of terms may assist in selecting the most appropriate antibody specificity for the experiment.

Note: Immunoglobulins from different species share similar structures, with similarities being related to closeness in phylogeny. Antibodies against immunoglobulins from one species are likely to cross-react with a number of other species, unless they have been **specifically adsorbed against the cross-reacting species.** Antibodies that have been adsorbed against other species will contain **"(min X...Sr Prot)"** in the antibody description.

Anti-IgG (H+L)

These antibodies react with both the heavy and light chains of the IgG molecule, i.e. with both the Fc and $F(ab')_2$ / Fab regions of IgG (Figure 1). Anti-IgG (H+L) antibodies also react with other immunoglobulin classes (IgM, IgA, IgD, IgE) and subclasses since they all share the same light chains (either kappa or lambda). Anti-IgG (H+L) antibodies have broader epitope recognition than anti-fragment specific antibodies. They are suggested for all general immunodetection procedures.

Anti-IgG, Fc/Fc_γ fragment specific

These antibodies react with the Fc portion of the IgG heavy chain. They have been tested by ELISA and/or adsorbed against Fab fragments. In some cases, they are additionally tested and/or adsorbed to minimize cross-reactivity to IgM and/or IgA. In such cases (anti-human, anti-mouse, and anti-rat), they are labeled "Anti-IgG, Fc_γ".

Caution: Anti-IgG, Fc_γ fragment specific antibodies do not react equally with all monoclonal primary antibodies. For an anti-mouse IgG, Fc_γ fragment specific antibody with balanced reactivity to four subclasses of IgG, select Goat Anti-Mouse IgG (subclasses 1+2a+2b+3), Fc_γ fragment specific (min X Hu, Bov, Rb Sr Prot).

SECONDARY ANTIBODY SELECTION

Anti-Mouse IgG, Fc_γ subclass specific

These antibodies react with the Fc portion of the heavy chain of individual subclasses of mouse IgG. They have been tested by ELISA and/or adsorbed to minimize cross-reactivity to other subclasses, Fab fragments and IgM; and human, bovine and rabbit serum proteins. Anti-Mouse IgG, Fc_γ subclass specific antibodies react with individual subclasses of mouse IgG. They are intended for distinguishing between different subclasses of mouse IgG primary antibodies in multiple labeling experiments, or for IgG subclass determination.

Anti-IgG, $F(ab')_2$ fragment specific

These antibodies react with the $F(ab')_2$ / Fab portion of IgG. They have been tested by ELISA and/or adsorbed against Fc fragments. They are not specific for IgG since they react with light chains, and therefore also react with other immunoglobulin classes (IgA, IgM, IgD, and IgE) and subclasses sharing the same light chains.

Anti-IgG, light chain specific

These antibodies react with the light chains shared by IgG and the other immunoglobulins. They were developed to facilitate detection of proteins around 50 kDa on Western blots after immunoprecipitation (IP), and do not react with IgG heavy chains. For details on using anti-IgG, light chain specific antibodies please see Western blotting after Immunoprecipitation (page 27).

Anti-Human Serum IgA, α Chain Specific

These antibodies react with the heavy chain of human IgA. They have been tested by ELISA and/or adsorbed to minimize cross-reactivity with human IgG and IgM.

Anti-IgM, μ chain/$Fc_{5\mu}$ fragment specific

These antibodies react with the heavy chain of IgM (in the case of anti-human, just the $Fc_{5\mu}$ portion of the heavy chain). They have been tested by ELISA and/or adsorbed against IgG. Anti-human IgM, $Fc_{5\mu}$ is additionally tested and/or adsorbed to minimize cross-reactivity to IgA.

Anti-IgG + IgM (H+L)

These antibodies react with heavy chains of IgG and IgM. They also react with the light chains that are shared among immunoglobulins, so they may also react with IgA, IgD and IgE.

Anti-Human IgA + IgG + IgM (H+L)

These antibodies react with heavy chains of human IgA, IgG and IgM. They also react with the light chains that are shared among human immunoglobulins, so they may also react with IgD and IgE.

Additional Specificities

Anti-Fluorescein, Anti-HRP, Anti-Biotin and Anti-Digoxin are available for labeling endogenous proteins or nucleic acid probes and for signal enhancement or signal conversion. For more information see page 137.

Step 5. **Cross-adsorbed** secondary antibodies (min X ... Sr Prot)

Secondary antibodies against one species are likely to cross-react with other species unless they have been specifically adsorbed against the other species. Antibodies with "(min X ... Sr Prot)" in the description have been tested and/or adsorbed against IgG and/or serum proteins of those species indicated in the parentheses. **They are recommended when the presence of immunoglobulins from other species may lead to interfering cross-reactivities.**

Note: Caution should be exercised when considering antibodies that have been adsorbed against closely related species, since they have greatly reduced epitope recognition and may recognize some monoclonals poorly. For example, choose anti-mouse IgG adsorbed against rat IgG to detect a mouse primary antibody in rat tissue which contains endogenous rat immunoglobulins, or in a multiple labeling application which includes a rat primary antibody. Use anti-mouse IgG **not** adsorbed against rat IgG to detect a mouse primary antibody in the absence of rat immunoglobulins. Two other examples of antibodies which have diminished epitope recognition after adsorption with closely related species are Anti-Rat IgG (min X ... Mouse ... Sr Prot) and Anti-Armenian Hamster IgG (min X ... Mouse, Rat ... Sr Prot).

SECONDARY ANTIBODY SELECTION

The following abbreviations are used in the parentheses:

min X = minimal cross-reaction	Ar Hms = Armenian Hamster	Rb = Rabbit
Bov = Bovine	Sy Hms = Syrian Hamster	Shp = Sheep
Ck = Chicken	Hrs = Horse	Sw = Swine
Gt = Goat	Hu = Human	Sr = Serum
GP = Guinea Pig	Ms = Mouse	Prot = Protein

ML (multiple labeling)

Some antibodies are designated (ML) to emphasize their usefulness in multiple labeling in addition to single labeling. For further information see: Applications of Secondary Antibodies - Multiple Labeling (page 35-36).

Anti-Armenian Hamster IgG vs. Anti-Syrian Hamster IgG

Most hamster monoclonal antibodies are derived from Armenian hamster spleen cell-mouse myeloma hybridomas. The IgG produced by these hybridomas is Armenian hamster IgG, while most polyclonal hamster antibodies are raised in Syrian hamsters. Antibodies raised against one hamster species are not as effective in detecting the other species, so it is important to know the origin of a hamster primary antibody.

Caution: Anti-Armenian Hamster IgG (H+L) (min X Bov, Hu, **Ms** , Rb, **Rat** Sr Prot) may not recognize all Armenian hamster monoclonal antibodies, since it has been adsorbed against closely related species (in bold). Therefore, it is **better to use an antibody adsorbed against fewer species**, such as Anti-Armenian Hamster IgG (H+L) (min X Bov Sr Prot), except in those cases where Armenian hamster monoclonals need to be detected in the presence of mouse and/or rat immunoglobulins.

Step 6. Select the desired **conjugate.**

In addition to unconjugated antibodies, JIR offers antibodies conjugated to a wide range of probes. Fluorescent dyes, fluorescent proteins, reporter enzymes, Biotin-SP™ and colloidal gold are among the conjugate choices.

For technical information about probes, see Reporter Molecules (pages 13-24).

Step 7. Complementary immunoreagents

Depending on the technique, other immunoreagents may be required to optimize the assay. Blocking reagents, experimental controls and signal enhancement molecules are available in a variety of formats. See Blocking and Controls (pages 143-155).

Streptavidin reagents are available for use with biotinylated antibodies (pages 140-142).

REPORTER MOLECULES

REPORTER MOLECULES - FLUOROPHORES

Fluorescent probes or fluorophores (fluorescent dyes or proteins) are coupled to a secondary antibody or streptavidin to allow visualization of an analyte. Each fluorophore has its own spectral characteristics, with excitation and emission spectra particular to the molecule. Fluorescence facilitates the simultaneous detection of multiple analytes for a number of techniques including flow cytometry, fluorescence microscopy (epifluorescence, confocal, multiphoton and super-resolution techniques), Western blotting and ELISA.

Fluorophore selection

The choice of fluorescent probe depends on a number of experimental variables.

Technique

Selection of a fluorophore depends on the intended application. Sample specifics influence the choice, as they may accommodate the use of particular fluorophores, and preparation may alter the way a fluorophore behaves. The following table lists some of the considerations which are pertinent to the choice of a fluorophore for a particular technique.

Technique	Considerations
Flow cytometry	Surface staining accommodates the use of larger and brighter fluorescent conjugates such as the fluorescent proteins (R-PE, APC and PerCP) or Brilliant Violet™ dyes. Smaller fluorescent conjugates can be used for both surface and intracellular staining.
IHC microscopy	• Sample penetration requirements • Sample autofluorescence • Polar (aqueous) or non-polar (plastic) mounting media • pH • Analyte abundance - choose a brighter dye for weakly expressing targets
IHC multiple labeling	• Spectral overlap of fluorophores • Filter sets available • Analyte abundance - choose a brighter dye for the least abundant analyte
Super-resolution microscopy (STED and STORM)	• High emission at the STED laser wavelength - achieving high saturation • Photostability • Brightness • Photoswitching
Western and dot blotting	• Spectral overlap • Far-red and infrared fluorescent dyes for high sensitivity

Table 1: Considerations for conjugate selection.

Instrument capabilities

Consider excitation capabilities (lamp, lasers or LEDs determine excitation wavelengths) and the number of channels and filters available. Also consider the detection system, duration of data collection and post assay analysis.

Sensitivity required

The detection level of any fluorophore-antibody conjugate depends on brightness and photostability of the dye; antibody activity, specificity and cross-reactivity; and the optimal dye:antibody ratio (moles of dye per mole of antibody). These parameters have been researched for each of JIR's dye conjugates to optimize the level of antibody detection and minimize background. The larger phycobiliproteins have high quantum yields (are very bright), but are limited to surface applications. Detection of poorly expressed analytes is enhanced by choosing a brighter fluorophore. For example, Alexa Fluor® 488 is brighter than FITC.

Experimental sample

Consider autofluorescence of the sample and possible expression of recombinant fluorescently tagged proteins. Choose fluorophores whose spectra do not overlap with endogenous fluorescence.

Degree of color separation required

For multiple labeling protocols, the dye panel choices will be constrained by instrumentation and sample specifics as described above. To achieve good color separation, choose fluorophores with minimal spectral overlap (see page 20, Figure 20). Panel developing tools are available online which can help build dye panels specific to any instrument. For more information on IHC multiple labeling see pages 35-36.

Fluorophore characteristics

Alexa Fluor® Fluorescent Dyes

Alexa Fluor® fluorescent dyes are widely recognized as superior fluorescent dyes, respected for their brightness and photostability. They are highly water soluble and remain fluorescent from pH 4 to pH 10.

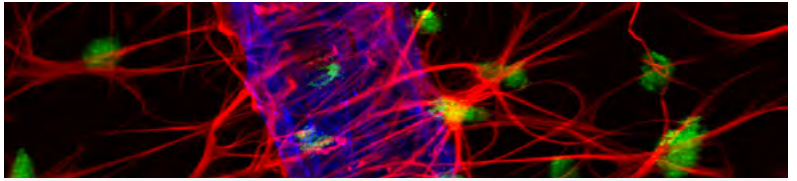


Figure 2: Rat retinal tissue. Cy™5 Streptavidin (016-170-084) and Alexa Fluor® 488-Goat Anti-Mouse IgG (H+L) (115-545-003). Mansour et al. (2008).

Brilliant Violet™ Dyes

JIR offers two BD Brilliant Violet dyes, BV421 and BV480 (these dyes are named for their emission maxima, while many fluorophores are named for the excitation maxima). BV dyes are polymer chains and can be considered as a collection of optical segments, each with the ability to absorb light and emit fluorescence signal. This results in dyes that have a bright fluorescence signal for superior resolution and sensitivity. For more information see page 118.

Cyanine dyes (Cy™2, Cy™3 and Cy™5)

Among currently available fluorescent dyes, the cyanine dyes are better able to withstand the harsh dehydration and embedding conditions required for mounting sections in non-polar plastic mounting media such as DPX and Permount™. The cyanine dyes are brighter in the non-polar environment than in aqueous media, resulting in reduced acquisition time compared with DyLight™ and Alexa Fluor® dyes, even though those dyes are brighter in aqueous mounting media. See pages 126-130 for more information about cyanine dye conjugates.

Fluorescent Proteins - Phycoerythrin, PerCP and Allophycocyanin

Jackson ImmunoResearch offers 3 fluorescent proteins, Phycoerythrin (R-PE), Allophycocyanin (APC), and Peridinin-Chlorophyll-Protein (PerCP). R-PE and APC are light-harvesting phycobiliproteins found in red, blue-green and cryptomonad algae. Jackson ImmunoResearch offers R-PE in the form found in red macrophytic algae (seaweed). APC is isolated from the blue-green alga Spirulina. PerCP is a fluorescent peridinin-chlorophyll-protein complex isolated from dinoflagellates. R-PE, PerCP and APC can be excited by light over a wide range of the visible spectrum, are highly water soluble, have relatively low isoelectric points, and lack potentially sticky carbohydrates. It should be noted that the relatively high molecular weights of these fluorescent proteins may preclude their use in procedures requiring good penetration into cells and tissues. They are predominantly intended for surface labeling of cells for flow cytometry. For more information see page 113.

The following seventeen fluorescent probes (Figures 3-20, and Table 2) are currently available from Jackson ImmunoResearch. They cover the most commonly used excitation sources and filter sets from blue to infrared emissions.

Fluorophore	Excitation Peak (nm)	Emission Peak (nm)
DyLight™ 405	400	421
Brilliant Violet 421™	407	421
Aminomethylcoumarin, AMCA	350	450
Brilliant Violet 480™	436	478
Cyanine, Cy™2	492	510
Alexa Fluor® 488	493	519
Fluorescein, FITC/DTAF	492	520
Indocarbocyanine, Cy™3	550	570
R-Phycoerythrin, R-PE	many, 488	580
Rhodamine Red™-X, RRX	570	590
Alexa Fluor® 594	591	614
Allophycocyanin, APC	many, 650	660
Alexa Fluor® 647	651	667
Indodicarbocyanine, Cy™5	650	670
Peridinin-Chlorophyll-Protein, PerCP	many, 488	675
Alexa Fluor® 680	684	702
Alexa Fluor® 790	792	803

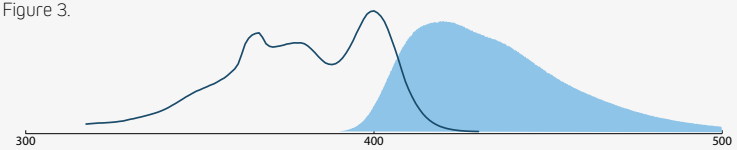
Table 2: Fluorescent probes available from Jackson ImmunoResearch.

Fluorescent conjugates

A brief description of the characteristics of fluorescent dyes and proteins in this catalog is found below (Figures 3-20). The fluorophores are listed in order of increasing wavelength.

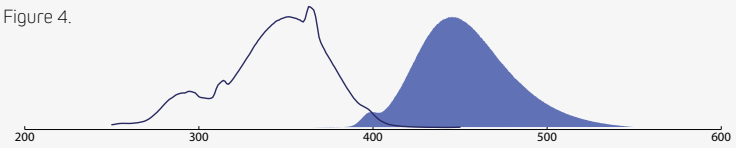
DyLight™ 405 - conjugated secondary antibodies are excited maximally at about 400 nm and fluoresce with a peak at about 421 nm. They are very bright and photostable, but their optimal use requires instruments equipped with a 405 nm laser and a 420 nm emission filter. The combination of DyLight 405, Alexa Fluor® 488, Rhodamine Red™-X, and Alexa Fluor® 647 provides for maximum color separation, good photostability and high sensitivity.

Figure 3.



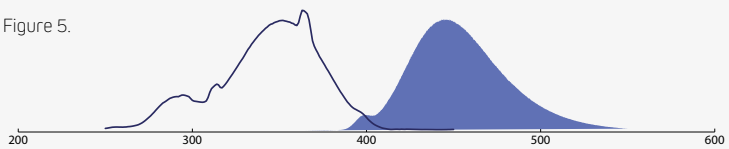
Brilliant Violet 421™ (BV421™) has an excitation peak at 407 nm with maximal emission at 421 nm. Brilliant Violet polymer chains can be considered as a collection of optical segments, each with the ability to absorb light and emit fluorescence signal. This results in conjugates that have a bright fluorescence signal for exceptional resolution and sensitivity. BV dyes are suitable for use with aqueous media (page 118).

Figure 4.



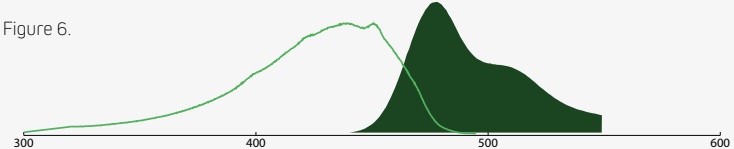
Aminomethylcoumarin Acetate (AMCA) conjugates absorb light maximally around 350 nm and fluoresce maximally around 450 nm. For fluorescence microscopy, AMCA can be excited with a mercury lamp and observed using a UV filter set. Since blue fluorescence is not well detected by the human eye, AMCA-conjugated secondary antibodies should be used with the most abundant antigens in multiple labeling experiments. Ways of improving the visibility of AMCA include dark adapting the eyes, using fluorite instead of glass objectives, avoiding mounting media that absorb UV light (such as plastic-based media), and capturing photographic images with blue-sensitive film or CCD cameras. AMCA fades rapidly in conventional epifluorescence and confocal microscopy, and therefore it should be used with mounting media containing an anti-fading agent such as n-propyl gallate. For flow cytometry, AMCA can be excited with a mercury lamp or with a water-cooled argon ion laser which emits some lines in the UV. AMCA has been used mostly for multiple labeling since there is minimal fluorescence overlap with green-fluorescing dyes and little or no overlap with longer wavelength-emitting fluorophores.

Figure 5.



Brilliant Violet 480™ (BV480™) has an excitation peak at 436 nm with maximal emission at 478 nm. Brilliant Violet polymer chains can be considered as a collection of optical segments, each with the ability to absorb light and emit fluorescence signal. This results in conjugates that have a bright fluorescence signal for exceptional resolution and sensitivity. BV dyes are suitable for use with aqueous media (page 118).

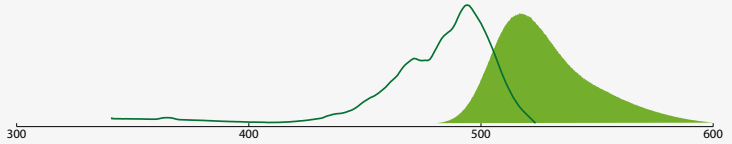
Figure 6.



REPORTER MOLECULES - FLUOROPHORES

Cy™2 conjugates have maximum absorption/excitation at 492 nm and fluoresce with a peak around 510 nm in the green region of the visible spectrum like FITC conjugates (520 nm), but they are more photostable and less sensitive to pH changes than FITC. The main advantage of Cy™2 conjugates is increased fluorescence in plastic mounting media compared with other green-fluorescing dyes. Cy™2 is available conjugated to a limited selection of antibodies and streptavidin.

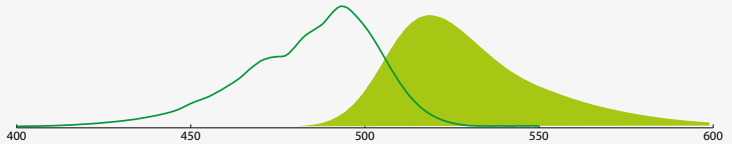
Figure 7.



Caution: Cy™2 is sensitive to p-phenylenediamine, an anti-fading agent found in some aqueous mounting media, which results in weak and diffused fluorescence after storage of stained slides.

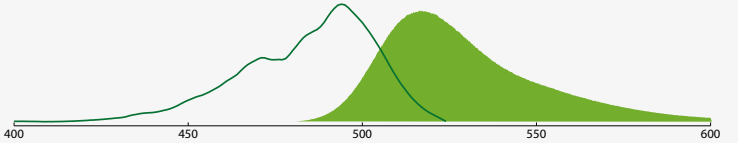
Alexa Fluor® 488 - conjugated antibodies absorb light maximally at 493 nm and fluoresce with a peak around 519 nm. In aqueous mounting media, they are brighter and more photostable than FITC, Cy™2 and DyLight™ 488. Alexa Fluor® 488 conjugates are recommended for maximum sensitivity for all immunofluorescence procedures requiring a green-fluorescing dye, except for protocols that include mounting in plastic mounting media (see Cy™2).

Figure 8.



FITC (Fluorescein isothiocyanate) is the form of fluorescein used for conjugation to all JIR antibodies and purified proteins, with the exception of streptavidin. Fluorescein conjugates absorb light maximally at 492 nm and fluoresce maximally at 520 nm. Although less bright than other green-fluorescing dyes, FITC is a widely used fluorophore for applications that don't require exquisite sensitivity. The major disadvantage of fluorescein is its rapid photobleaching (fading), which can be mitigated by the use of an anti-fading agent in the mounting medium. An alternative choice for many applications involving FITC is Alexa Fluor® 488 because it is brighter and more photostable.

Figure 9.

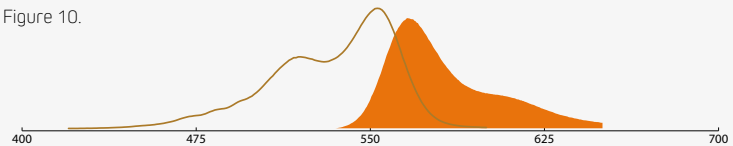


DTAF (Dichlorotriazinylamino fluorescein) is another form of fluorescein, with excitation and emission peaks identical to those of FITC. Jackson ImmunoResearch uses DTAF (instead of FITC) for conjugation with streptavidin, since fluorescence from FITC is greatly quenched after conjugation with streptavidin. This phenomenon is unique to streptavidin, and is not observed with antibodies.

REPORTER MOLECULES - FLUOROPHORES

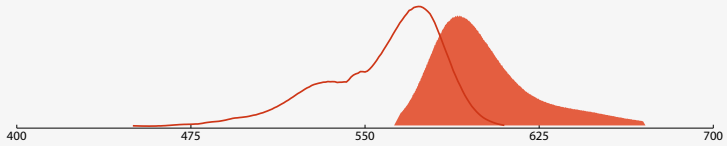
Cy™3 is brighter, more photostable and gives less background than other orange-red fluorescing dye conjugates. Cy™3 conjugates can be excited maximally at 550 nm, with peak emission at 570 nm. For fluorescence microscopy, Cy™3 can be visualized with traditional tetramethyl rhodamine (TRITC) filter sets, since the excitation and emission spectra are nearly identical to those of TRITC. Cy™3 can be excited to about 50% of maximum with an argon laser (514 nm or 528 nm lines), or to about 75% of maximum with a helium/neon laser (543 nm line) or mercury lamp (546 nm line). Cy™3 can be used with green-fluorescing dyes for double labeling. To avoid detection of Cy™3 fluorescence in the green filter set, the emission filter should have a narrow band-pass feature. Cy™3 can also be paired with Alexa Fluor® 647 for multiple labeling.

Figure 10.



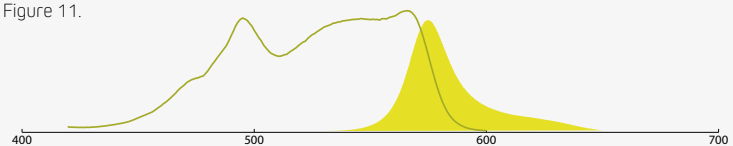
RRX (Rhodamine Red™-X) conjugates have peak excitation at 570 nm and peak emission at 590 nm. Rhodamine Red™-X is particularly useful for 3- and 4-color labeling with DyLight™ 405, Alexa Fluor® 488 and Alexa Fluor® 647 using a microscope equipped with an appropriate excitation source. In 3-color labeling, fluorescence from RRX lies about midway between that of Alexa Fluor® 488 and Alexa Fluor® 647, and it shows little overlap with either dye (Figure 20). Four-color labeling can be achieved with the addition of DyLight 405, whose emission can be separated from that of Alexa Fluor® 488.

Figure 12.



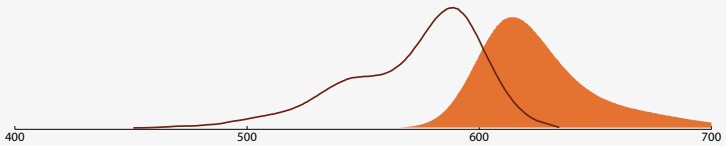
R-PE (R-Phycoerythrin) is a 240 kDa light-harvesting phycobiliprotein found in red macrophytic algae (seaweed). After phycobiliproteins are conjugated to secondary antibodies, there is little fluorescence quenching, which results in conjugates of high specific fluorescence compared with conventional fluorophore-antibody conjugates. R-PE can be excited by light over a wide range of the visible spectrum, is highly water soluble, has a relatively low isoelectric point, and lacks potentially sticky carbohydrates. The relatively high molecular weight of R-PE may preclude its use in procedures requiring good penetration into cells and tissues. It is predominantly intended for surface labeling of cells for flow cytometry.

Figure 11.



Alexa Fluor® 594 - conjugated antibodies absorb light maximally around 591 nm and fluoresce with a peak around 614 nm. They are brighter, more photostable and more hydrophilic than Texas Red conjugates. Alexa Fluor® 594 conjugates are brighter than other red-fluorescing conjugates, and they provide better color separation from green-fluorescing dyes than Cy™3 and TRITC conjugates. They are the best choice for immunofluorescence detection in the deep-red region of the visible spectrum.

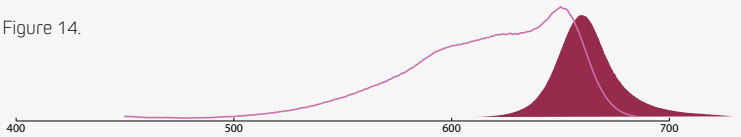
Figure 13.



REPORTER MOLECULES - FLUOROPHORES

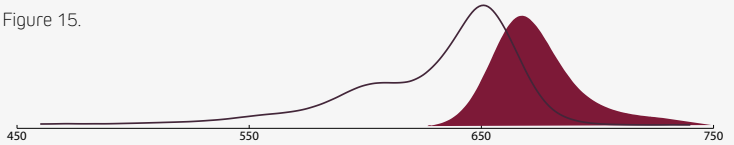
Allophycocyanin (APC) is an approximately 105 kDa phycobiliprotein containing the chromophore phycocyanobilin, isolated from the blue-green alga Spirulina. APC is chemically cross-linked to stabilize the constituent subunits making up the molecule. After phycobiliproteins are conjugated to secondary antibodies, there is little fluorescence quenching, which results in conjugates of high specific fluorescence compared with conventional fluorophore-antibody conjugates. APC can be excited by light over a wide range of the visible spectrum, is highly water soluble, has a relatively low isoelectric point, and lacks potentially sticky carbohydrates. APC conjugates are suitable for cell surface labeling techniques such as flow cytometry. See pages 39-40.

Figure 14.



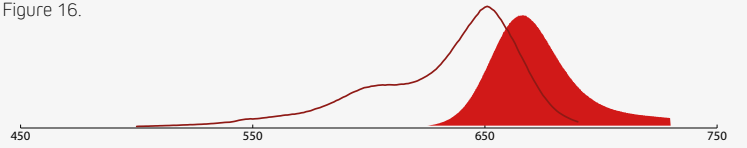
Alexa Fluor® 647 - conjugated antibodies absorb light maximally around 651 nm and fluoresce maximally around 667 nm. They are brighter than Cy™5 in aqueous mounting media. Alexa Fluor® 647 conjugates are the best choice of far-red emitting dyes for multiple labeling detection with a confocal microscope, and are also widely used in flow cytometry. A significant advantage of long wavelength dyes such as Alexa Fluor 647 is the low autofluorescence of biological specimens in this region of the spectrum. However, because of its peak emission at 667 nm, Alexa Fluor® 647 cannot be seen well by eye, and it cannot be excited optimally with a mercury lamp. Therefore, Alexa Fluor® 647 is most commonly visualized with a confocal microscope equipped with an appropriate laser for excitation and a far-red detector.

Figure 15.



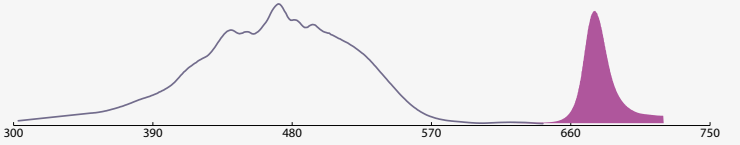
Cy™5 conjugates are excited maximally at 650 nm and fluoresce maximally at 670 nm. They can be excited to about 98% of maximum with a krypton/argon laser (647 nm line) or to about 63% of maximum with a helium/neon laser (633 nm line). Cy™5 can be used with a variety of other fluorophores for multiple labeling due to a wide separation of its emission from that of shorter wavelength-emitting fluorophores. When specimens are mounted in non-polar, plastic mounting media, Cy™5 is brighter than other far-red-fluorescing dyes, including Alexa Fluor® 647. Cy™5 is available conjugated to selected antibodies (see pages 126-130) and streptavidin (page 142).

Figure 16.



Peridinin-Chlorophyll-Protein (PerCP) is a 35.5 kDa fluorescent peridinin-chlorophyll-protein complex isolated from dinoflagellates. Jackson ImmunoResearch offers the form found in Dinophyceae sp. It has a broad spectrum of excitation with the main peak at 482 nm, and a long Stokes shift to an emission peak at 677 nm. PerCP conjugates are large complexes suitable for cell surface labeling techniques such as flow cytometry. See pages 39-40.

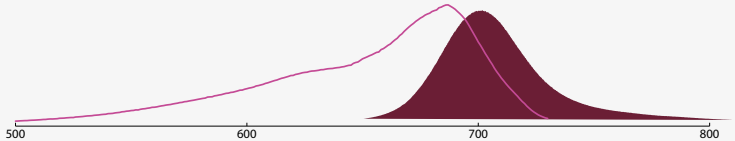
Figure 17.



REPORTER MOLECULES - FLUOROPHORES

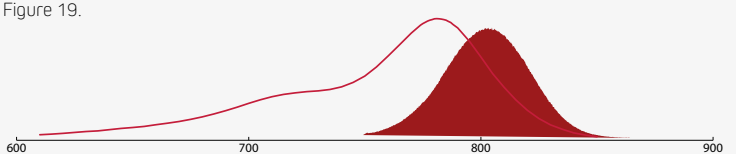
Alexa Fluor® 680 conjugates are used for very sensitive Western blots, ELISAs and multiplexing arrays. Alexa Fluor® 680 conjugates are excited with a peak around 684 nm and fluoresce with a peak around 702 nm. They can be visualized with a LI-COR Odyssey® imager and other fluorescence detection systems, and they can be used for double labeling with Alexa Fluor® 790 conjugates. (See pages 122-125 for more information).

Figure 18.



Alexa Fluor® 790 conjugates are excited with a peak around 792 nm and fluoresce at a peak around 803 nm. They are particularly suitable for highly sensitive single labeling, or double labeling in combination with Alexa Fluor® 680 with fluorescence imaged in a LI-COR Odyssey® imager and other fluorescence detection systems. (See pages 122-125 for more information).

Figure 19.



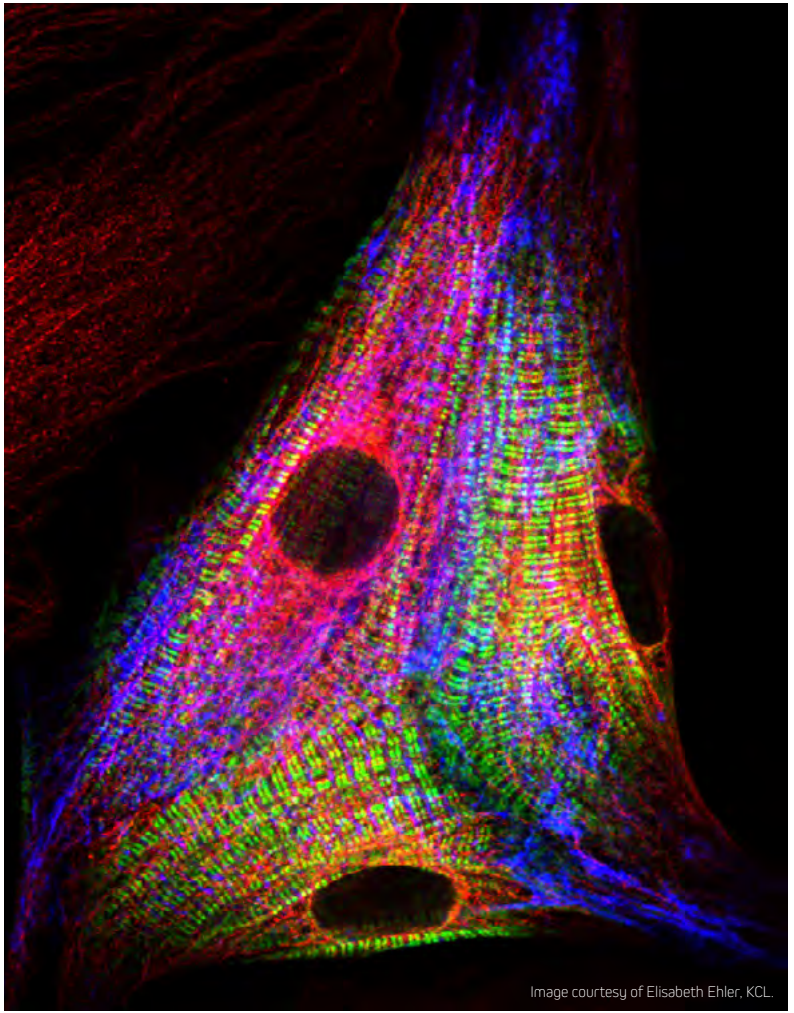
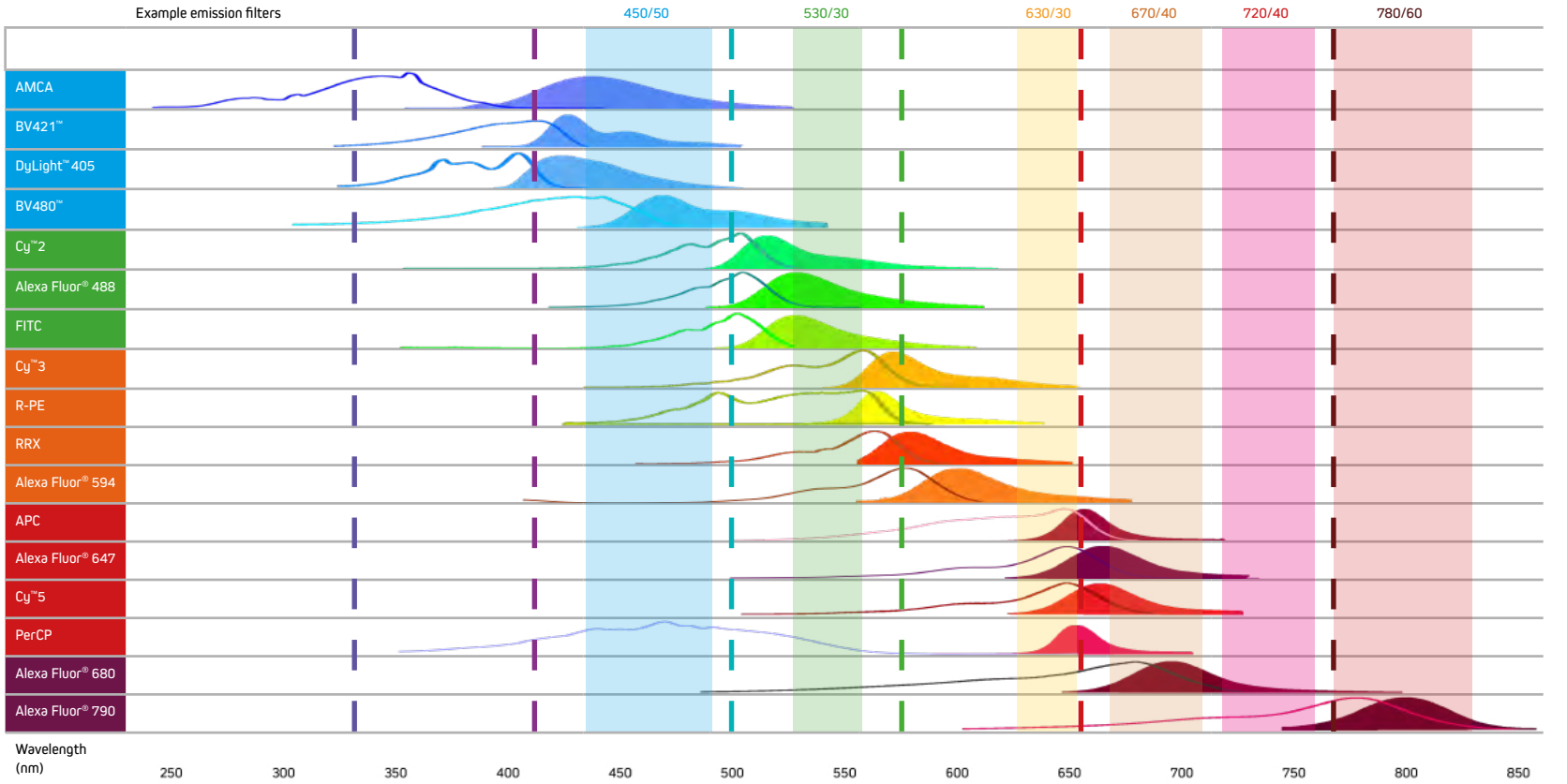


Image courtesy of Elisabeth Ehler, KCL.

REPORTER MOLECULES - FLUOROPHORES

Figure 20: Fluorescent conjugates offered by Jackson ImmunoResearch.



How to select fluorophores using this chart

Instrument

Consider excitation capabilities (lamp, lasers or LEDs determine excitation wavelengths) and the number of channels and filters available. Figure 20 shows a selection of common emission filters, but many different configurations are available. The user should review the parameters of the filter sets included with their instrument. Long-pass filters provide the best sensitivity, while narrow band-pass filters enable discrimination between signals from different fluorophores in multiple labeling applications.

Panel developing tools are available online which can help build dye panels specific to any instrument. For more information on IHC multiple labeling see pages 35-36.

Degree of color separation required

To achieve good color separation in a multiple labeling protocol, choose fluorophores with minimal spectral overlap.

Experimental sample

Choose fluorophores whose spectra do not overlap with endogenous fluorescence. Examine an unstained specimen under available filter sets to determine whether the sample exhibits autofluorescence.

Key to excitation wavelengths

Lasers
Ultra violet laser (350 nm)
Violet laser (405 nm)
Blue laser (488 nm)
Yellow laser (561 nm)
Red laser (640 nm)
Far red laser (750 nm)

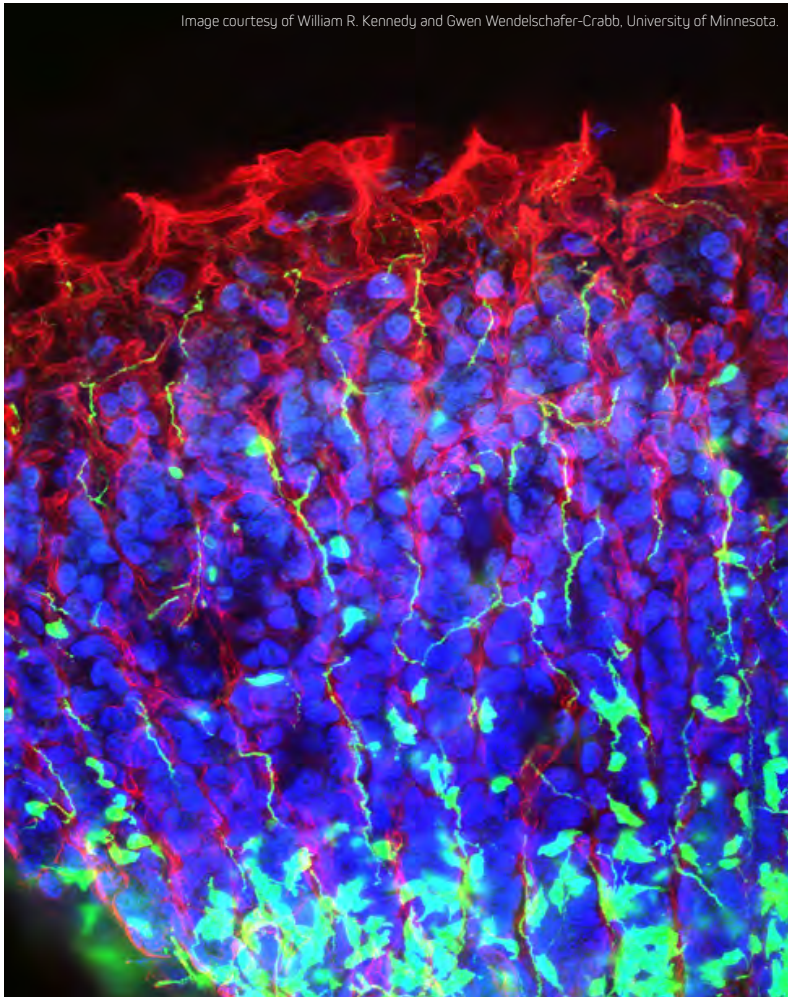


Image courtesy of William R. Kennedy and Gwen Wendelschafer-Crabb, University of Minnesota.

Reporter Enzymes

Reporter enzyme-conjugated secondary antibodies can be used for both chromogenic and chemiluminescent detection methods when combined with an appropriate substrate. Enzyme conjugates can be applied to many immunotechniques to provide robust and sensitive detection. Horseradish peroxidase and alkaline phosphatase are available conjugated to secondary antibodies, streptavidin and purified proteins.

Horseradish Peroxidase (HRP)

This commonly used reporter enzyme is derived from the root of the horseradish plant *(Armoracia rusticana)*. JIR HRP conjugates are prepared by a modified Nakane and Kawaoi procedure (1974).

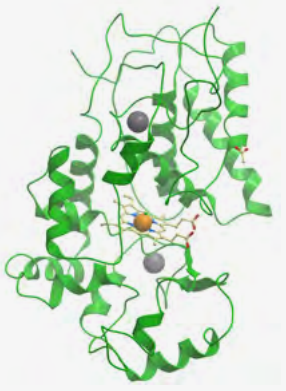


Figure 21: Horseradish peroxidase crystal structure at 1.57A from PDB 1HCH. Images generated using Molsoft ICM browser. Image shows two calcium molecules (grey spheres) and the catalytic iron (orange sphere) within the protoporphyrin IX container.

HRP conjugates are suitable for all immunotechniques employing colorimetric and chemiluminescent detection methods, including Western blotting, immunohistochemistry and ELISA.

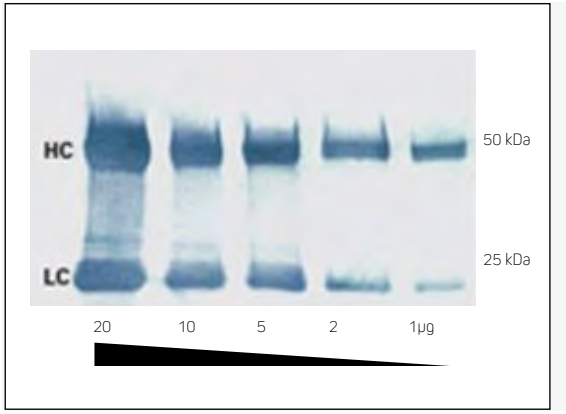


Figure 22: A colorimetric Western blot. Heavy (HC 50 kDa) and light (LC 25 kDa) chains of reduced and SDS-denatured mouse IgG separated by SDS-PAGE and detected by Western blot using HRP-Goat Anti-Mouse IgG (H+L) (115-035-003) and visualized with TMB chromogenic substrate.

Note about endogenous peroxidase. Some tissues contain endogenous peroxidase-like enzymes which can react with peroxidase substrates, resulting in background staining. Pre-treatment of sample with hydrogen peroxidase will exhaust the endogenous enzyme activity, allowing clear detection of specific signal.

Cautions regarding reagent compatibility

1. Do not add sodium azide to solutions containing HRP. It will inactivate the enzyme. See online guides **for details on how to check enzyme and substrate reactivity.**
2. If glycerol is added to extend shelf life of reconstituted product, confirm that glycerol is ACS grade or better. Lower grades of glycerol may seriously inhibit peroxidase enzyme activity.

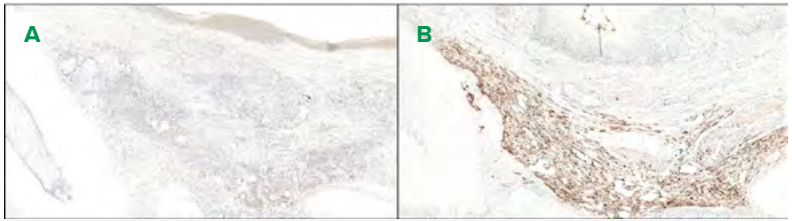


Figure 23: Anti-GFP immunohistochemical staining using Biotin-SP-conjugated Donkey Anti-Rabbit IgG (H+L) (711-065-152) secondary antibody followed by HRP-conjugated Streptavidin (016-030-084): (A) At 4 weeks post-transplantation, no GFP signal could be detected in the *in vivo* specimens. (B) GFP-producing cells were visualized by brown staining in the positive control; signal was visualized using the chromogenic substrate 3,3' diaminobenzidine (DAB). Nuclei were counter-stained with hematoxylin.

Alkaline Phosphatase

Alkaline phosphatase (from calf intestine) conjugates are prepared by a method modified from Avremeas et al (1978). The resulting conjugates contain heterogeneous, high molecular weight complexes. They are sensitive reagents suitable for solid-phase immunoassays such as ELISA and Western blotting. Although alkaline phosphatase conjugates are sometimes used for immunohistochemistry, penetration into tissues may be limited by their large sizes.

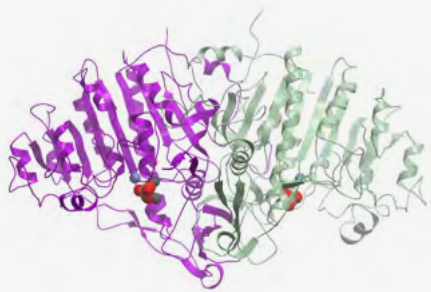


Figure 24: The alkaline phosphatase homodimer from the 2Å crystal structure PDB 1ALK showing phosphate, zinc and magnesium ions. The image was generated using Molsoft ICM browser.

Biotin-SP (long spacer)

Biotin is a small molecule which non-covalently binds to avidin and streptavidin with very high affinity. The affinity of the interaction makes biotin an excellent conjugate for detection when used in immunohistochemistry techniques. Biotin conjugates can be employed in signal amplification techniques (see pages 30 and 141).

Biotin-SP is our trade name for biotin with a 6-atom spacer positioned between biotin and the protein to which it is conjugated. When Biotin-SP-conjugated antibodies are used in enzyme immunoassays, there is an increase in sensitivity compared to biotin-conjugated antibodies without the spacer. This is especially notable when Biotin-SP-conjugated antibodies are used with alkaline phosphatase-conjugated streptavidin. Apparently, the long spacer extends the biotin moiety away from the antibody surface, making it more accessible to binding sites on streptavidin.

Biotinylated antibodies require additional reagents for visualization. We offer streptavidin (page 141) and Mouse Anti-Biotin (page 137) conjugated to fluorophores and enzymes.

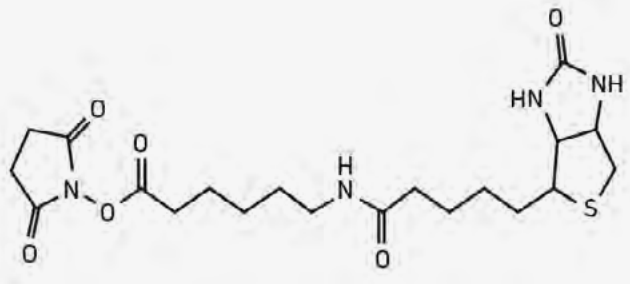


Figure 25: Biotin-SP molecule. Molecular weight 454.54 Da. Linker 22.4 Å.

REPORTER MOLECULES - ENZYMES AND OTHER PROBE MOLECULES

ImmunoGold reagents

ImmunoGold reagents offer excellent tissue penetration due to their small particle size (Dixon et al., 2015). ImmunoGold colloidal gold reagents are available either for transmission (TEM) and scanning electron microscopy (SEM) (EM Grade 6, 12 and 18 nm) or for brightfield microscopy or immunoblotting (LM Grade 4 nm). For more information about EM see page 43.

ImmunoGold reagents for electron microscopy

The EM Grade is distinguished from other commercial preparations by careful separation of monomeric particles from small aggregates using ultracentrifugation in density gradients. The resulting monomeric colloidal gold-protein complexes are recommended for multiple labeling applications, as different antigenic sites can be distinguished by particle size. The complexes are suspended in sterile-filtered buffer containing stabilizers and a preservative.

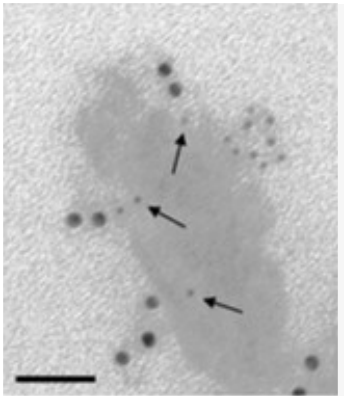


Figure 26: Transmission scanning microscopy of fixed human cornea sections showing colocalization of MUC16 (large gold particles (12 nm Colloidal Gold Donkey Anti-Mouse IgG (H+L) (715-205-150)) and ezrin (small gold particles, arrowheads (6 nm Colloidal Gold Donkey Anti–Goat IgG (705-195-147)) on the microplicae. Scale bar, 0.1 µm. (Blalock, et al, 2007).

The 4 nm size may be used for electron microscopy in studies that require smaller particles since they are relatively uniform in size (coefficient of variation less than or equal to 15%), though small aggregates are not removed from this grade. The 4 nm particles are not suitable for multiple labeling with EM Grade reagents, since size uniformity is paramount and aggregated material may be mistaken for a larger particle.

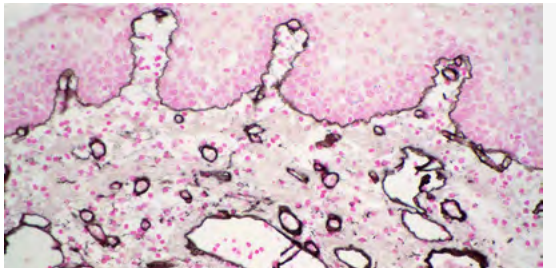


Figure 27: Immunolabeling for collagen type IV in normal human skin using ImmunoGold reagents 4 nm with silver enhancement. Prof. Jurgen Roth, Dept. Path. University of Zurich.

Silver enhancement for light microscopy with ImmunoGold reagents

Silver enhancement allows the excellent penetration properties of ImmunoGold reagents to be used with light microscopy. The gold particles act as a nucleation site for the silver ions, which accumulate around the particle until enough contrast is generated to be visualized (Dixon et al., 2015). A detailed protocol for silver enhancement, using reagents that are easily prepared in the laboratory, is provided with all orders for LM Grade products and is also available online. Alternatively, silver enhancement kits are commercially available.

Signal intensity is relatively independent of particle size when silver enhancement is used, so all particle sizes may be used for light microscopy or immunoblotting. For light microscopy, 4 nm particles (LM Grade) may penetrate tissues better than larger particles.

All LM Grade colloidal gold-protein complexes are freeze-dried in buffer with stabilizers and a preservative. After reconstitution, they may be frozen in aliquots for extended storage.

References:

Avremeas, S., Ternynck, T. And Guesdon, J.-l. (1978), Coupling Of Enzymes To Antibodies And Antigens. Scandinavian Journal Of Immunology, 8: 7–23. Doi:10.1111/J.1365-3083.1978.Tb03880.X

Paul K. Nakane, Akira Kawaoi (1974). Peroxidase-labeled Antibody A New Method Of Conjugation Journal of Histochemistry & Cytochemistry Vol 22, Issue 12, pp. 1084 - 1091. 10.1177/22.12.1084

Dixon, A. R., Bathany, C., Tsuei, M., White, J., Barald, K. F., & Takayama, S. (2015). Recent developments in multiplexing techniques for immunohistochemistry. Expert Review of Molecular Diagnostics, 15 (9), 1171–1186. http://doi.org/10.1586/14737159.2015.1069182.

Timothy D. Blalock, et al; Functions of MUC16 in Corneal Epithelial Cells. Invest. Ophthalmol. Vis. Sci. 2007;48(10):4509-4518.

APPLICATIONS OF SECONDARY ANTIBODIES

Western blotting with JIR secondary antibodies

Western blotting is an analytical technique used to detect specific proteins or peptides in biological samples or solutions containing complex mixtures. Initially, linearized proteins are separated by gel electrophoresis to resolve them by size. They are then transferred onto a membrane such as nitrocellulose or polyvinylidene difluoride (PVDF) which immobilizes the protein. Subsequently, the membrane is blocked and then probed with a primary antibody directed against the specific protein of interest. A secondary antibody conjugated to a reporter molecule is then used to identify the analyte using colorimetric, chemiluminescent or fluorescent detection.

Colorimetric detection

Reporter enzyme conjugates alkaline phosphatase (AP) and horseradish peroxidase (HRP) can be used for colorimetric detection. The conjugated reporter enzyme catalyzes the conversion of the chromogenic substrate to a colored precipitate, which is visualized directly on the blotting membrane. Colorimetric detection can offer quick and easily obtained results without the need for expensive detectors or extensive optimization.

Chemiluminescent detection

Enzyme-linked conjugates can also be used for chemiluminescent signal detection. HRP conjugates produce signal by oxidizing a chemiluminescent substrate (luminol) to a form which emits light. AP conjugates produce signal when the enzyme dephosphorylates a specific substrate (e.g. 1,2-dioxetane) to a light emitting product. The signal can then be captured by exposing photographic film to the membrane or using a cooled charge-coupled device (CCD) camera. Chemiluminescent detection offers excellent sensitivity, however quantification and probing for multiple targets can be limited, and development may require refinement to optimize signal capture.

Fluorescent detection

Fluorescent detection uses a fluorescent dye conjugate to visualize antigen on the membrane. Light at a wavelength specific to the dye's spectral characteristic is absorbed, exciting the dye's electrons to a higher electronic state, and as they return to their ground state they emit photons at the emission wavelength characteristic to the fluorophore. The light emitted is detected by a digital imager fitted with appropriate filters. Fluorescent Western blotting allows for quantitative analysis and multiplex probing without the need for stripping and reblotting. Jackson ImmunoResearch offers a range of fluorescent dyes covering the spectrum (see pages 15-19).

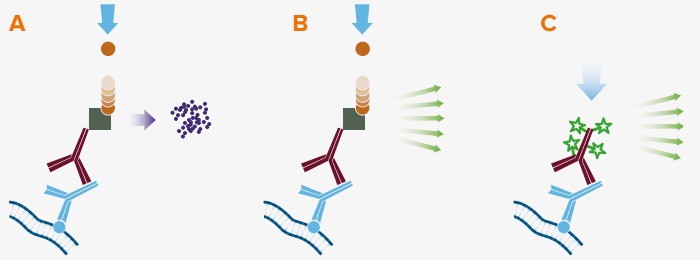


Figure 28: The 3 detection methods for Western blot: (A) Colorimetric, (B) Chemiluminescent, and (C) Fluorescent; A. The reporter enzyme conjugate catalyzes the conversion of a chromogenic substrate to a colored insoluble precipitate, visible by eye on the blotting membrane. B. The reporter enzyme conjugate catalyzes a reaction which converts the chemiluminescent substrate to a light emitting form, and the emitted light is detected by X-ray film or CCD camera. C. The reporter fluorescent dye is excited by its characteristic wavelength light, and resulting emitted light is captured by a digital imager .

Jackson ImmunoResearch produces the largest diversity of species specific secondary antibody conjugates for use in Western blotting. JIR offers antibodies and streptavidin conjugated with horseradish peroxidase (HRP), alkaline phosphatase and Biotin-SP for use in traditional blots developed with chromogenic or chemiluminescent (ECL) substrates. In addition, secondary antibodies conjugated with fluorescent dyes, including far-red and infrared-emitting dyes, are available for multicolor imaging in modern readers.

Jackson ImmunoResearch also offers affinity-purified anti-horseradish peroxidase which may be used to detect HRP or to enhance signal by binding to HRP-conjugated molecules. Conjugated anti-HRP may be used to convert an HRP conjugate into a different signal.

Recommended dilution ranges for our conjugated secondary antibodies can be found in the Appendix, pages 163-164.

For Western blotting troubleshooting see www.jacksonimmuno.com/secondary-antibody-resource.

Membrane blocking buffer

Normal serum from the host species of the labeled antibody (5% v/v) is an excellent block, although 5% non-fat milk and 3% BSA are commonly used and may also be effective. Avoid milk or BSA when using a primary antibody derived from goat or sheep.

Diluting antibodies for Western blotting

PBS/Tween 20 (0.05% v/v) or TBS/Tween, without carrier proteins, is recommended as the secondary antibody diluent. Especially when using anti-goat or anti-sheep secondary antibodies, avoid using milk or BSA in the diluent buffer. Bovine IgG in the milk or BSA may interact with the antibody due to homologous epitopes of the related species.

See page 147 for a list of normal serums available from Jackson ImmunoResearch.

Western blotting after Immunoprecipitation

For researchers who perform Western blotting following immunoprecipitation, antibodies specific for light chains or Fc fragments allow unobstructed detection of antigens in the 50 kDa or 25 kDa ranges, respectively.

Anti-light chain specific antibodies

Anti-IgG (H+L) antibodies react with native primary antibodies used for detecting specific protein bands on Western blots (Figure 29 A). If diluted properly, anti-light chain specific antibodies do not bind to the reduced and denatured IgG heavy chain band (50 kDa) on blots (Figure 29 B). Therefore, detection of antigens with molecular weights near 50 kDa is not obscured by reduced and denatured IgG heavy chains from primary antibodies used for immunoprecipitation (IP). Although the antibodies react strongly with native IgG light chains, some do not react as strongly with reduced and denatured light chains on blots. Therefore, they are not recommended for sensitive and quantitative detection of reduced and denatured light chains. The antibodies have been adsorbed to minimize cross-reactivity with immunoglobulins from many other species, which also may be present on blots.

Light chain specific antibodies are available directed against goat, mouse, rabbit, rat and sheep, see pages 108-111.

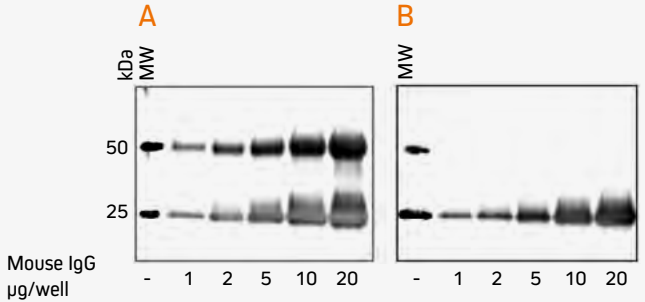


Figure 29: Western blotting after IP. Use Anti-light chain specific antibodies to avoid obscuring analytes in the 50 kDa range.

Gels were loaded with reduced and denatured Mouse IgG, whole molecule.

After SDS-PAGE and transfer to nitrocellulose, blots were blocked with BSA (10% w/v). After incubation with secondary antibody, blots were developed with ECL substrate. Blots were imaged simultaneously, with auto exposure time based on bright bands.

A: The gel was probed using HRP-conjugated Goat Anti-Mouse IgG (H+L) (115-035-003), revealing bands corresponding to both heavy chains (50 kDa) and light chains (25 kDa).

B: The gel was probed using HRP-conjugated Goat Anti-Mouse IgG, light chain specific (115-035-174), revealing only the 25 kDa band corresponding to Ig light chains. The IP antibody heavy chain is not detected, allowing visualization of the protein of interest near 50 kDa.

APPLICATIONS OF SECONDARY ANTIBODIES - WESTERN BLOTTING

Anti-Fc specific antibodies

Anti-IgG, Fc fragment specific antibodies may be used to detect native IgG primary antibodies without binding to the 25 kDa band of reduced and denatured IgG light chains on Western blots. Using these antibodies allows clear detection of a 25 kDa analyte, without interference from the light chains of an IP antibody. However, this detection is complicated by the appearance of degraded heavy chain antibody at 25 kDa, see Figure 30 panel A.

To avoid signal from degraded heavy chain at 25 kDa, block with monovalent Fab fragment anti-Fc (FabuLight™), see Figure 30, panels B and C. The extreme sensitivity of Western blotting requires high concentrations of the blocking reagent.

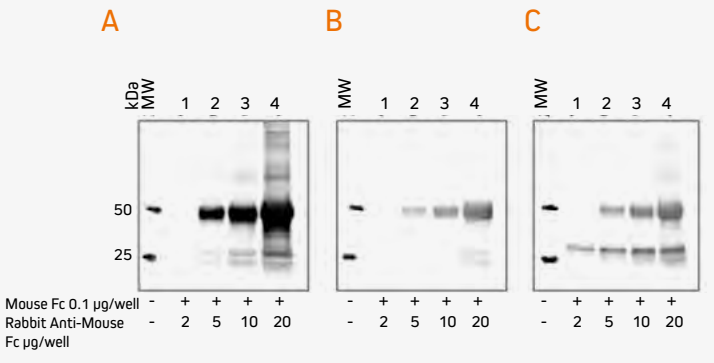


Figure 30: Western blotting after IP - FabuLight blocking to avoid obscuring analytes in the 25 kDa range.

Figure 30 Protocol.

Rabbit Anti-Mouse IgG, Fc_γ fragment specific (315-005-008) was mixed with ChromPure™ Mouse Fc (015-000-008) to simulate immunoprecipitation (IP).

Three identical gels were run, lanes loaded with denatured and reduced:

Lane 1: 2 µg Rabbit Anti-Mouse Fc + 0.1 µg Mouse Fc
Lane 2: 5 µg Rabbit Anti-Mouse Fc + 0.1 µg Mouse Fc
Lane 3: 10 µg Rabbit Anti-Mouse Fc + 0.1 µg Mouse Fc
Lane 4: 20 µg Rabbit Anti-Mouse Fc + 0.1 µg Mouse Fc

After SDS-PAGE and transfer to nitrocellulose, blots were blocked with BSA (10% w/v). Subsequent incubations are shown below. After incubation with secondary antibody, blots were developed with ECL substrate.

Blots were imaged simultaneously, with auto exposure time based on bright bands.

A: No FabuLight block
No primary antibody
Secondary antibody: HRP Goat Anti-Rabbit IgG, Fc (111-035-008), 1:200K
Secondary antibody detects IP antibody heavy chain (HC) at 50 kDa, and degraded HC at 25 kDa.

B: FabuLight block: Fab Goat Anti-Rabbit IgG, Fc (111-007-008), 200 µg/ml
No primary antibody
Secondary antibody: HRP Goat Anti-Rabbit IgG, Fc (111-035-008), 1:200K
Signal from IP antibody HC is greatly reduced. At lower loading amounts, the degraded HC at 25 kDa is not detectable.

C: FabuLight block : Fab Goat Anti-Rabbit IgG, Fc (111-007-008), 200 µg/ml
Primary antibody: Rabbit Anti-Mouse IgG, Fc_γ (min X Hu Sr Prot)(315-035-046), 1 µg/ml
Secondary antibody: HRP Goat Anti-Rabbit IgG, Fc (111-035-008), 1:200K
Secondary antibody detects the primary antibody, revealing protein of interest at 25 kDa. Note that the protein of interest shows the sharpest band when amount of IP antibody is low.

Fab fragment Anti-Fc specific antibodies are found in the FabuLight tables (see pages 100-103). To block signal from a mouse IP antibody, use the Fab fragment corresponding to the subclass of the IP antibody.

Fluorescent Western blotting for quantitative and multiplex detection

Fluorescent Western blotting allows multiplex detection without stripping and reprobing for analytes and can be used for quantitative detection. Various fluorescent conjugates can be employed depending on the instrument setup. For a list of fluorescent conjugates offered by Jackson ImmunoResearch please see pages 15-19.

For optimal detection, Alexa Fluor® 680 and Alexa Fluor® 790 conjugates can be used to achieve high sensitivity Western blots. See pages 122-125 for details.

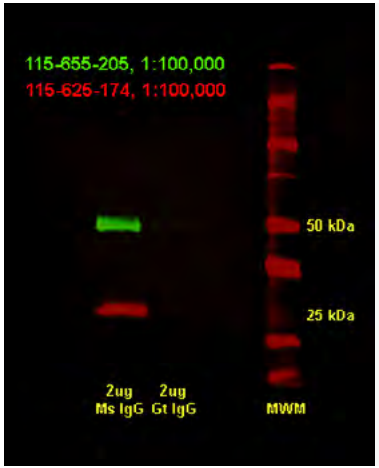
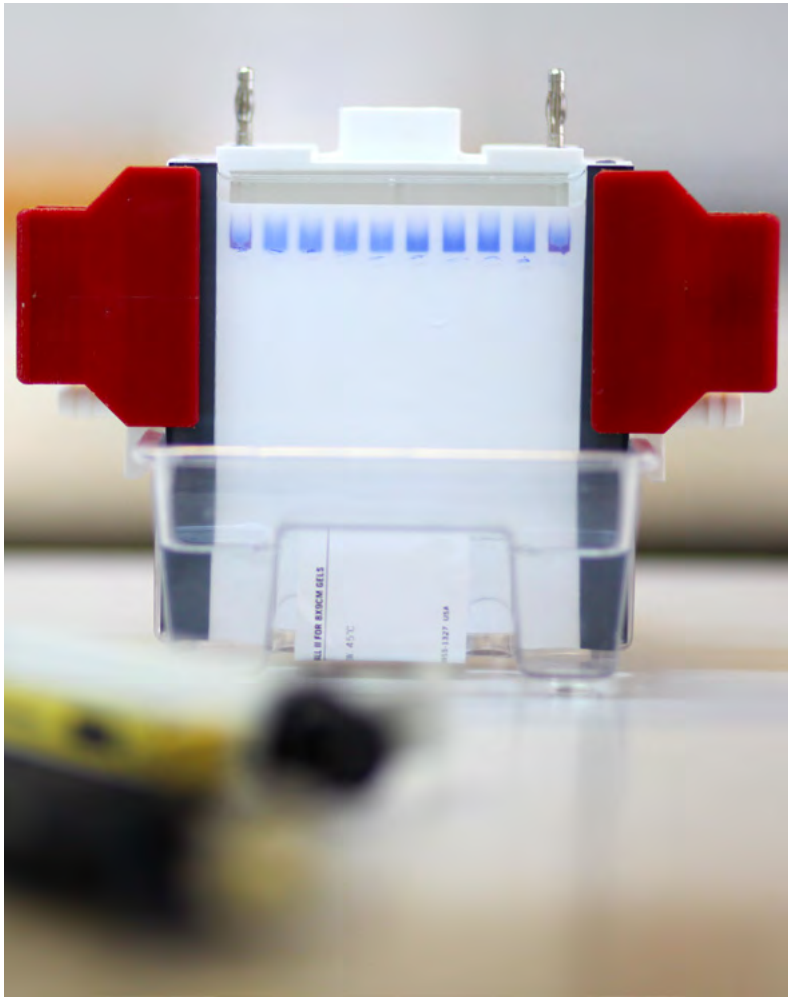


Figure 31: Multiplex detection of multiple protein targets without stripping and reprobing. Double immunofluorescence staining on a Western blot using Alexa Fluor® 680 far-red dye and Alexa Fluor® 790 infrared dye. Mouse IgG was reduced and denatured with β-mercaptoethanol and SDS. The heavy and light chains were separated by SDS-PAGE, transferred to nitrocellulose, and double labeled with a 1:100,000 dilution of Alexa Fluor® 790-Goat Anti-Mouse IgG, Fc_γ Subclass 1 specific (min X Hu, Bov, Rb Sr Prot, 115-655-205) (green) detecting heavy chains at 50 kDa and a 1:100,000 dilution of Alexa Fluor® 680-Goat Anti-Mouse IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Rb, Rat, Shp Ig, 115-625-174) (red) detecting light chains at 25 kDa). Fluorescence was imaged in a LI-COR Odyssey® imager.



IHC with JIR Secondary Antibodies

Immunohistochemistry (IHC) is a powerful technique, indispensable in research and in clinical diagnostics. It is a staple of the pathology lab for disease diagnostics and classification. In research, IHC is used to interrogate many biological processes, including visualization of protein expression patterns, characterization of protein interactions, and identification of tissue boundaries. The researcher can thereby observe the distribution and localization of specific structures within the context of the cellular architecture.

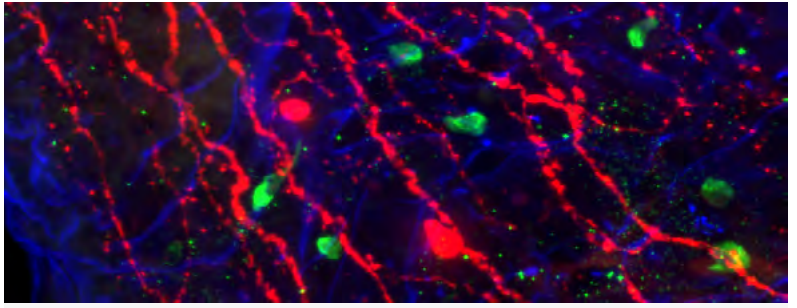


Figure 32: Triple immunofluorescence. Jejunum villus probed for Ulex - Biotin using Cy™5 Streptavidin (blue 016-170-084), GIP by Cy™2 AffiniPure Donkey Anti-Goat IgG (H+L) (green 705-225-147), tubulin by Cy™3 AffiniPure Donkey Anti-Mouse IgG (H+L) (red 715-165-150). Image courtesy of Brian McAdams and William Kennedy, University of Minnesota.

Principles of Immunohistochemistry

A tissue analyte is specifically recognized by a primary antibody, which may be directly conjugated (direct IHC); or the primary may itself be detected by a conjugated secondary antibody (indirect IHC), allowing visualization of the analyte. The conjugated antibody can be labeled with any of several reporter molecules, including enzymes, fluorophores and colloidal gold. The expression of multiple analytes can be observed and characterized by individual primary/secondary antibody pairs in multiple labeling protocols.

Direct or Indirect Immunohistochemistry

Analytes of interest may be detected on the surface of the tissue or interrogated internally through permeabilization of the sample. The analyte (antigen) can be detected directly (Figure 33 A), or indirectly (Figure 33 B) using a fluorescent (immunofluorescence) or reporter enzyme (colorimetric) probe conjugated to a secondary antibody.

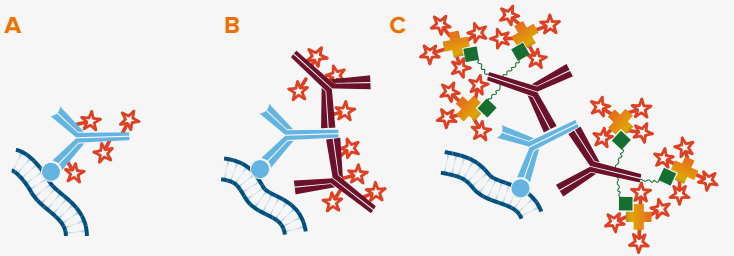


Figure 33: A: Direct IHC, B: Indirect IHC, C: Signal enhancement with biotinylated secondary and labeled streptavidin

The indirect method using a secondary antibody offers many advantages. It preserves the antigen binding site on the primary antibody by conjugating to the secondary antibody. Since secondary antibodies are available conjugated to a wide variety of fluorophores, the indirect method enhances the possibilities for multiple labeling. In addition, it provides inherent signal enhancement, whereby multiple secondary antibodies bind to one antigen-bound primary antibody. Signal can be further enhanced by using a biotinylated secondary antibody followed by conjugated streptavidin (Figure 33C), or by using the peroxidase-anti-peroxidase (PAP) method (Figure 61, Page 136).

Designing the indirect IHC protocol

Factors that contribute to the success of IHC experiments are the tissue of interest, primary antibody choice, secondary antibody specificity, and options for signal detection. Proper blocking assures optimal signal to noise ratio, and control proteins aid in the interpretation of results.

Tissue or sample specifics

Tissue species

The source (species) of experimental sample will influence the choice of antibodies. Certain tissue types contain endogenous immunoglobulin (Ig), and others exhibit vasculature in which remnant blood may provide endogenous Ig. For indirect IHC, the secondary antibody must recognize the specific primary antibody but not endogenous Ig. It is most convenient to use a primary antibody that is derived from a different host animal than the tissue of interest: in this case select a secondary antibody that has been cross-adsorbed (min X) to minimize recognition of endogenous Ig.
If the experimental strategy includes using a primary antibody from the same host species as the tissue of interest, endogenous Ig can be masked by blocking with monovalent Fab fragments of secondary antibodies. It is also possible to label primary antibodies with Fab fragments prior to IHC incubation. For more information on Fab fragment protocols see pages 86-98.

Fixation

Tissue fixation is the process of stabilizing and preserving a specimen in a configuration that approximates its natural state. As the innate aqueous environment is altered by chemical fixation, an antigenic site may be modified so that it cannot be recognized by a primary antibody that would detect its native state. Many primary antibodies are marketed as "validated" to perform under specified fixation conditions, though this information may not be available. In some cases, antigen retrieval protocols can return antigenic sites to their native state.

Note that the concept of antibodies validated to perform in IHC is unique to primary antibodies. If a primary antibody binds its target site, a labeled secondary antibody will bind to it regardless of tissue fixation.

Intrinsic signal

Tissue samples may reveal several types of intrinsic signal. Autofluorescence (signal in the absence of fluorescent probe molecules) may be evident in some regions of the spectrum. When planning an immunofluorescent protocol, observe an unlabeled tissue sample using available filter sets. Choose fluorophores that are compatible with spectral regions that have limited autofluorescence. It is possible to suppress autofluorescence with various reagents, though the efficiency of these treatments varies depending on both tissue type and fixation method.

Intrinsic signal may also derive from endogenous enzymes and/or biotin. To test for and control these types of background signal, see suggestions under Blocking and Controls pages 144-146.

Primary antibody choice for IHC

Target

The primary antibody should recognize the protein of interest under the intended experimental conditions. Some primary antibodies have been validated for use under specific conditions, e.g. fixation methods. An antibody that has been validated only for Western blotting may not be appropriate for IHC.

Monoclonal or polyclonal

Polyclonal antibodies recognize multiple epitopes, maximizing the opportunity for the antibody to detect the antigen, but may result in detection of homologous proteins and lead to background signal. Monoclonal antibodies consist of identical immunoglobulin molecules produced from a single cell line, and are appropriate when the detection needs are epitope specific. However, they may give a weaker signal than polyclonals. Controls can be used to help determine the suitability of antibodies for each assay system.

Host species of the primary antibody

Ideally, the host species of the primary antibody should be a different species from the sample species to avoid cross-reactivity with endogenous proteins. If the primary antibody and the tissue of interest are the same species, blocking protocols can be used to prevent off-target signal. See pages 86-93 for Fab blocking protocols.

Detecting multiple targets

Multiple labeling is used for the detection of more than one analyte in the same assay. Primary antibodies derived from different species, or mouse monoclonal antibodies of different subclasses, are optimal choices for multiple labeling. If these choices are unavailable, multiple labeling can be achieved through special strategies.

See the Multiple Labeling section (pages 35-36) for further guidelines on setting up a multiple labeling experiment.

Antibody optimization - internal/in-house validation

Primary antibodies may detect the target protein satisfactorily for one experimental protocol, but not work as well under different conditions. It is good practice to optimize and therefore validate the antibody each time the experimental conditions are altered, for example, when the tissue type is changed or a different secondary antibody detection system is used.

Selecting the secondary antibody for IHC and IF

When selecting secondary antibodies for IHC and multiple labeling experiments, consider the criteria in the following table.

The affinity-purified antibodies marked **"ML"** (multiple labeling) have been specifically prepared to meet these criteria.

Caution: Avoid the possible formation of immune complexes by diluting antibodies in PBS/Tween only (without carrier proteins such as normal serum). To limit unwanted interactions incubate reagents sequentially rather than mixing multiple antibodies.

Requirement	Antibody	Example
The secondary antibody must not recognize endogenous tissue immunoglobulins.	Choose a secondary antibody cross-adsorbed against the species of interest.	In Rat tissue, choose Goat Anti-Mouse IgG (H+L)(min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot)
	For visualizing a primary antibody on tissue from the same species (e.g. mouse on mouse labeling) block endogenous Ig with Fab fragments.	See pages 86-93 for Fab fragment blocking protocols.
The secondary antibody must not bind to endogenous Fc receptors and proteins.	Block with normal serum from the same species as the secondary antibody.	See page 147 for normal serums.
Multiple secondary antibodies must not recognize one another.	Use secondary antibodies that are derived from the same host species if possible.	Donkey Anti-Mouse Donkey Anti-Rabbit Donkey Anti-Guinea Pig
Each secondary antibody must only recognize its target primary antibody.	For multiple labeling protocols, choose secondary antibodies which have been cross-adsorbed against the other species employed in the experiment.	Antibodies raised in donkeys have been highly cross-adsorbed against other species.
	For labeling different mouse subclasses, use subclass specific antibodies.	Goat Anti-Mouse IgG1, IgG2a, etc. See pages 105-107 for anti-Mouse IgG subclass specific antibodies.
	If appropriate cross-adsorbed antibodies are unavailable use a Fab protocol for multiple labeling.	See pages 86-93 for Fab fragment blocking protocols.

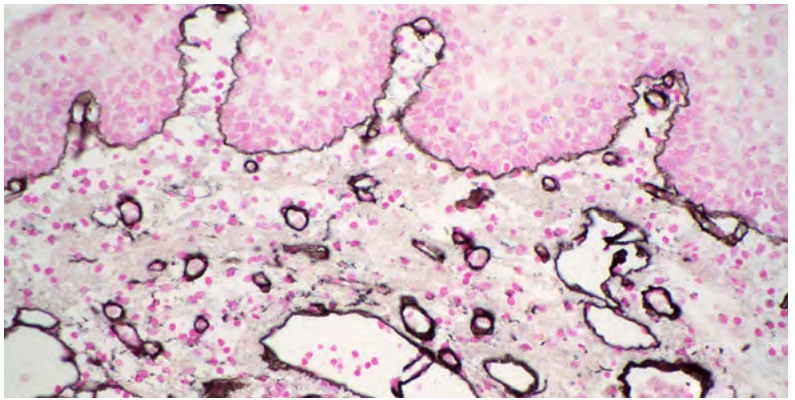
Table 3: Secondary antibody selection criteria for IHC.

APPLICATIONS OF SECONDARY ANTIBODIES - IMMUNOHISTOCHEMISTRY

Detection

Analytes can be visualized under a fluorescence microscope by using a fluorescent conjugate, or under a light microscope by using an enzyme conjugate followed by a chromogenic substrate (colorimetric detection). Detection by light microscopy is also possible using silver enhancement of a colloidal gold antibody.

Each detection method can offer advantages, in terms of sensitivity, quantification, cost-per-assay or capacity for detection of multiple analytes.



Colorimetric detection

Alkaline Phosphatase (AP) and Horseradish Peroxidase (HRP) conjugates can be used for colorimetric detection. For more information on reporter enzymes see pages 22-24. Colorimetric detection can offer quick and easily obtained results, with excellent detection sensitivity. Multiple analyte detection can be achieved by using different chromogens in sequential incubations.

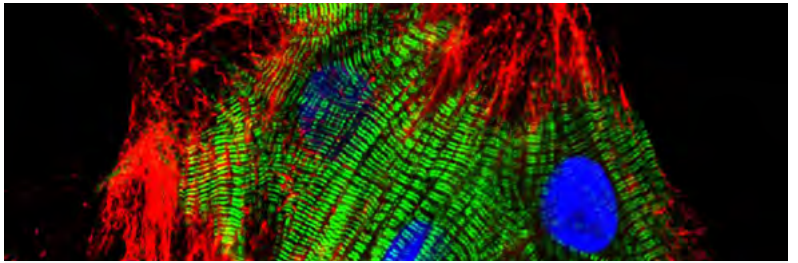


Figure 34: Primary culture of neonatal rat cardiomyocytes stained with Cy™3-AffiniPure Goat Anti-Mouse IgG (H+L) (115-165-146) and Cy™2-AffiniPure Goat Anti-Rabbit IgG (H+L) (111-225-144) secondary antibodies together with DAPI counterstain. Image courtesy of Elisabeth Ehler, KCL.

Fluorescent detection

Immunofluorescence uses a fluorescent dye conjugate to visualize antigens. For more information on fluorescent probes see pages 15-21. Fluorescent IHC allows for detection of multiple analytes, and up to 5 color immunofluorescence is possible using the appropriate fluorescent dyes and filter sets. Stripping and reincubating with additional antibodies has resulted in 20 analytes being probed on a single tissue sample.

APPLICATIONS OF SECONDARY ANTIBODIES - IMMUNOHISTOCHEMISTRY

The table below compares the two detection methods.

	Colorimetric	Fluorescent
Sensitivity	HRP conjugates are more sensitive than fluorescent conjugates.	Fluorophores such as Alexa Fluors® and Cyanine dyes are brighter and more photostable than FITC, increasing their sensitivity.
	ImmunoGold with silver enhancement is also very sensitive.	
Expense and ease	Simple light microscopes are commonly available.	Epifluorescent microscopes are readily available and versatile. Expensive multichannel microscopes may require use of core facilities, increasing cost and difficulty of experiment.
Resolution	Detail and depth of interrogation are limited by the quality and thickness of the samples.	Maximum detail can be detected using confocal microscopy, including compilation of 3D images using scanning laser techniques to visualize within much thicker sample slices.
	Electron microscopy using ImmunoGold reagents offers excellent resolution.	Super-resolution microscopy allows images to be resolved down to 50 nm (see pages 37-38 for more information).
Multiplexing (simultaneous detection of multiple antigens)	Multiple analytes can be labeled by using different chromogens for detection in sequential incubations.	Up to 5 color immunofluorescence can be performed with appropriate microscope laser and filter set up. Additional multiplexing has been reported using rounds of stripping and reincubating.
Colocalization		Analytes can be identified by channel, and their signals merged to identify overlap by color.
Color stability	Stains are stable and do not fade during examination under the microscope. Slides can be stored for years.	Photostability can be variable across fluorescent conjugates, making data collection time sensitive. Permanent mounting media can increase longevity of archived slides.
Advanced techniques		Fluorescent dyes can be used for a number of techniques to characterize biological interactions. FRET (Förster Resonance Energy Transfer) can be used to examine structural characteristics, binding stoichiometries, and affinities. Super-resolution microscopy allows images to be resolved down to 50 nm. Fluorescent reagents make it possible to observe live cells in real time.

Table 4: IHC can be performed with either colorimetric or fluorescent detection. Comparative advantages to the user range from simplicity of use to simultaneous detection of multiple antigens.

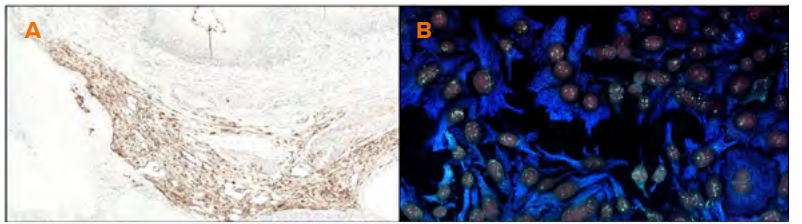


Figure 35: IHC can be performed using colorimetric and fluorescent detection. A: Colorimetric detection using DAB. B: Double immunofluorescence in HEP2 cells. Staining using Brilliant Violet™ 421 and 480, nuclear staining with DRAQ5™.

Blocking reagents

Blocking steps can dramatically improve results and simplify analysis of IHC experiments. To avoid unexpected signal from non-specific, conserved sequence and/or Fc-receptor binding, we recommend blocking with normal serum from the host species of the labeled antibody (5% v/v). Other blocking reagents, such as BSA or commercially available blockers, may also be effective at minimizing background.

Controls

The addition of experimental controls will improve analysis of results and aid troubleshooting. For more information see Blocking and Controls (pages 143-155).

Multiple labeling for simultaneous detection of several targets

Multiple labeling is the process of sequential immunolabeling to detect multiple antigens by either immunofluorescent or colorimetric IHC/ICC. The successful detection of more than one antigen requires rational experimental design, taking into account a large number of variables accumulated by each of the experimental steps. Here we detail how to set up the multiple labeling experiment and some considerations essential to executing a successful assay.

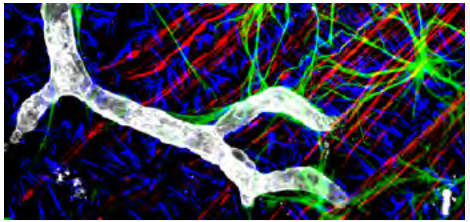


Figure 36 . Mouse GFAP (green) NF (red) Collagen IV (grey) Vimentin (blue) z1. Image courtesy of Gabe Luna, Neuroscience Research Institute, UC Santa Barbara.

Designing the multiple labeling experiment

Selection of antibodies for simultaneous detection of more than one antigen depends on at least two important criteria:

1. **Availability of secondary antibodies that do not recognize**
   (a) **one another** (are derived from the same host species),
   (b) other **primary antibodies** used in the assay system,
   (c) endogenous immunoglobulins present in the **tissues or cells under investigation.**
2. **Use of probes (enzyme-reaction products, fluorophores, or electron-dense particles) that are well resolved.**

The affinity-purified antibodies marked "ML" (multiple labeling) have been specifically prepared to meet these criteria. They include mouse IgG subclass specific antibodies (see pages 105-107) and antibodies cross-adsorbed (min X) against other species.

Multiple labeling example:

Tissue	Mouse	Mouse	Mouse
Antigen	Antigen A	Antigen B	Antigen C
Blocking Step	**Step 1**	**Step 4**	**Step 7**
	5% Normal Donkey serum to block	5% Normal Donkey serum to block (if needed)	5% Normal Donkey serum to block (if needed)
	Wash	Wash	Wash
Primary antibody step	**Step 2**	**Step 5**	**Step 8**
	Goat Anti-Antigen A	Rabbit Anti-Antigen B	Rat Anti-Antigen C
	Wash	Wash	Wash
Secondary antibody step	**Step 3**	**Step 6**	**Step 9**
	Probe 1 Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, **Ms, Rb, Rat** Sr Prot)	**Probe 2** Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, **Gt,** GP, Sy Hms, Hrs, Hu, **Ms, Rat,** Shp Sr Prot)	**Probe 3** Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, **Gt,** GP, Sy Hms, Hrs, Hu, **Ms, Rb,** Shp Sr Prot)

Note: In this example, the secondary antibodies used do not recognize each other since they are all made in **donkey.** They have been solid-phase adsorbed so that they do not recognize the other primary antibodies used (min X) in steps, 2, 5, and 8. Also, they do not react with endogenous mouse Ig, which may be present in the mouse tissue.

Caution: Do not dilute any antibody with normal serum or mix antibodies together to save time, which may result in immune complex formation which could increase background.

Further considerations

Blocking

For general blocking purposes, normal serum (5% v/v) from the same species as the secondary antibody host provides efficient background reduction for non-specific, conserved-sequence, and/or Fc-receptor binding.

Specific unwanted reactions with antibodies can be blocked with monovalent Fab fragments of secondary antibodies. This type of blocking is indicated for situations in which the specimen and primary antibodies are of the same species (e.g.mouse on mouse labeling), or when multiple primary antibodies are raised in the same host animal. See pages 86-93 for Fab fragment blocking protocols.

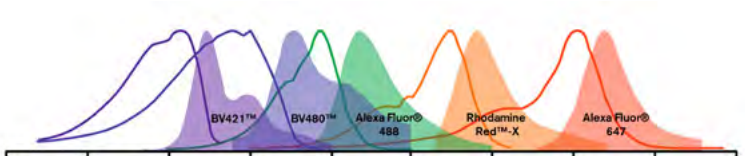
Visualization

Successful multiple labeling depends on the use of probes whose signals can be distinguished by available equipment.

Fluorescence microscopy is a common platform for multiple labeling, since filter sets have been designed to discriminate among the many fluorophores available. Narrow band-pass emission filters are critical for separating signals from multiple fluorophores, suppressing detection of fluorescence from overlapping spectra. When planning a multiple labeling protocol, formulate a dye panel from fluorophores with well separated emission spectra that are compatible with available instrumentation. See pages 15-21.

Multiple labeling can also be achieved with enzyme-linked antibodies. An antigenic site is labeled with a primary and secondary antibody, followed by color development with a chromogenic substrate such as DAB, TMB or AEC. Additional antigenic sites are labeled sequentially, with different chromogens used for each antigenic site.

For multiple labeling in electron microscopy, different sizes of colloidal gold particles complexed with secondary antibodies allow clear visualization of separate antigenic sites. See Electron Microscopy section for more information (page 43).



Channel	Blue	Cyan	Green	Orange	Red
Option 1	DAPI	BV480	Alexa Fluor® 488	RR-X	Alexa Fluor® 647
Option 2	BV421	BV480	Alexa Fluor® 488	RR-X	DRAQ5™
Option 3	BV421	BV480	Alexa Fluor® 488	RR-X	Alexa Fluor® 647

Figure 37: Three examples of dye panels. Options 1 and 2 combine nuclear stains with immunofluorescence. Option 3 shows 5 color immunofluorescence.

Controls

Prior to performing a multiple labeling protocol, optimize conditions for each primary/secondary antibody pair. Titrating both the primary and secondary antibody will identify conditions with low background and best positive signal. To demonstrate the specificity of each secondary antibody for its intended primary, attempt to label primaries with the "wrong" secondary antibodies (negative controls).

When using primary antibodies raised in the same host as the specimen species (e.g. mouse on mouse), controls can be used to determine the level of non-specific signal. See pages 144-146 for more information on controls.

For a review of multi-color immunofluorescence labeling with confocal microscopy see Brelje, Wessendorf, and Sorenson, "Multi-color laser scanning confocal immunofluorescence microscopy: Practical application and limitations." In Cell Biological Applications of Confocal Microscopy (Methods in Cell Biology. vol. 38). Ed. B. Matsumoto. Orlando, FL: Academic Press, Inc. 1993, pp. 98-181.

SRM with JIR Secondary Antibodies

Super-resolution microscopy (SRM) encompasses any optical technique which circumvents the resolution limits of light diffraction found in conventional light microscopy. SRM techniques allow cellular structures to be resolved to the sub-organelle level, enabling information about the 3D structure of cellular components to be determined, and single molecule colocalization to be observed.

Each SRM technique has its own requirements for probe selection. Jackson ImmunoResearch offers a wide selection of labeled secondary antibodies with dyes known to be robust in SRM methods. It should be noted that the field of SRM changes rapidly and guidance for each of the techniques is beyond the scope of this catalog. It is therefore recommended that information from specialized technical literature is sought to aid fluorophore selection.

The following sections briefly outline the principles of some of the most popular SRM techniques.

Stimulated Emission Depletion (STED) microscopy

Diffraction of light by a microscope lens causes light from a single point to appear over a larger area, known as a point spread function or PSF (see Figure 38).

Stimulated emission depletion (STED) microscopy produces super-resolution images by confining the fluorescing region (PSF) of a sample. STED microscopes use two overlapping lasers, the first of which excites the fluorophores as per conventional microscopy (Figure 39A). The second laser, called a depletion laser (STED laser), excites a "donut" of light, with a very small (~30 nm) zero intensity (unexcited) point at its center. This second laser essentially "switches off" the fluorescence generated by the first laser except at the center of the donut, thereby reducing the excited fluorescent molecules to those at the zero point. This effectively reduces the PSF to produce a very small focused region of single molecule fluorescence. Without overlapping interference patterns, high-resolution images can be obtained (Figure 39D). Images with a resolution up to 30 nm in the axial (x-y) plane have been reported.

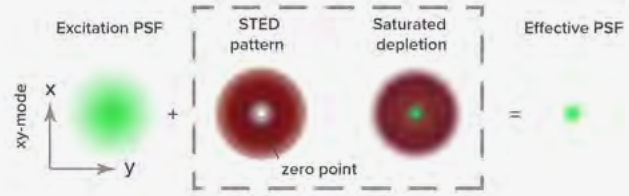


Figure 38. In optical microscopy, imaging is achieved by light rays from a point source converging to a single point at the image plane. Beyond the limits of light diffraction exact convergence of the rays is prevented, leading to the image of the object blurring. The resolution of a microscope is determined by the size of the point spread function (PSF), or three-dimensional intensity distribution of the object at a point. In STED a donut-shaped depletion laser is applied with the zero point overlapping the maximum of the excitation laser focus. The STED laser causes "saturated depletion" of fluorescence, whereby "fluorescence from regions near the zero point are suppressed, leading to a decreased size of the effective PSF". Reproduced from Huang et al. (2009).

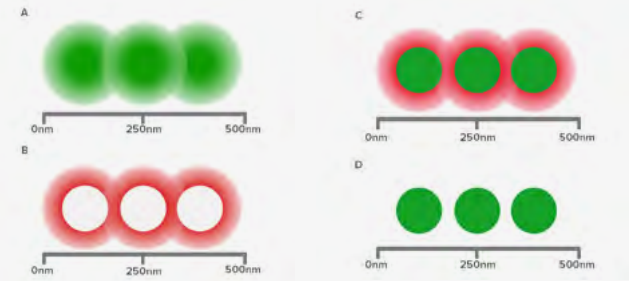


Figure 39:
A. Individual proteins closer than 250 nm cannot be resolved by standard confocal imaging, resulting in a blurred image.
B. The STED depletion laser creates a "donut" of quenched fluorescence.
C. The saturated depletion functionally reduces the excitation PSF.
D. The individual proteins are now resolved.

APPLICATIONS OF SECONDARY ANTIBODIES - SUPER-RESOLUTION MICROSCOPY

Fluorophore-conjugated antibodies for STED

To achieve super-resolution, dyes must have a high emission cross section with the STED laser wavelength and efficiently achieve a high saturation. This intense illumination ensures that all molecules to be "turned off" by the STED laser are dominated by stimulated emission. Suitable dyes should have a low propensity for photo-bleaching, with high quantum yields and contrast, and contain sufficient density of labeling in close proximity to the target.

Jackson ImmunoResearch offers secondary antibodies conjugated to dyes over a broad spectral range that have been successfully employed in STED: Alexa Fluor® 488, FITC, Alexa Fluor® 594 and Alexa Fluor® 647.

Stochastic Optical Reconstruction Microscopy (STORM)

Single molecule localization can be achieved using Stochastic Optical Reconstruction Microscopy (STORM) using photoswitchable fluorophores to generate images with resolution superior to those collected using conventional methods. There are a number of SRM techniques which exploit the principle of reversible saturable optically linear fluorescence (RESOLFT), using photoswitching or photoactivation of fluorescent dyes. Examples include direct stochastic optical reconstruction microscopy (dSTORM), photoactivated localization microscopy (PALM), fluorescence photoactivation localization microscopy (fPALM), ground state depletion followed by individual molecule return (GSDIM), and super-resolution optical fluctuation imaging (SOFI).

These techniques can be performed using either a pair of dyes to function as an effector and an activator dye to achieve photoswitching, or using dyes which "self-switch" in the case of direct STORM (dSTORM) (Heilemann et al., 2008). Jackson ImmunoResearch provides secondary antibody conjugates suitable for dSTORM applications. Briefly, a relatively low-intensity excitation laser excites the sample, randomly activating a small number of dye molecules. The individually fluorescing dye molecules are spread apart enough so the center of their point spread function can be calculated, which infers the exact location of the dye. A second laser is then used to switch all the molecules off and the process is repeated. High resolution images are then mathematically generated by overlapping all of the mapped point spread functions of each dye.

Fluorophore-conjugated antibodies for single molecule localization experiments

The best dyes for single molecule localization are typically very bright and result in enough photons to reliably produce tight Gaussian distributions. Jackson ImmunoResearch offers several proven dyes in a broad spectral range such as Alexa Fluor® 488, Alexa Fluor® 647 and Cy™5 for use in these types of experiments.

Suggested fluorescent dye conjugates for super-resolution microscopy

STED	Excitation (nm)	Emission (nm)
Alexa Fluor® 488	493	519
Fluorescein/FITC	492	520
Alexa Fluor® 594	591	614
Alexa Fluor® 647	651	667

Table 5: Fluors reported for use in STED by Farahani, J.N. et al. (2010).

dSTORM	Excitation (nm)	Emission (nm)
Alexa Fluor® 488	493	519
Alexa Fluor® 647	651	667
Cy™5	650	670

Table 6: Fluors reported for use in dSTORM by Dempsey et al. (2011).

References:

Huang, B., Bates, M., & Zhuang, X. (2009). Super-resolution fluorescence microscopy. *Annual Review of Biochemistry*, 78 , 993–1016.

Farahani, J.N. et al. (2010). Stimulated Emission Depletion (STED) Microscopy: from Theory to Practice. *Microscopy: Science, Technology, Applications and Education*. 1539-1547.

Heilemann M, et al. (2008) Subdiffraction-resolution fluorescence imaging with conventional fluorescent probes. Angew Chem Int Ed Engl. 47:6172–6176

Dempsey, et al. (2011). Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. *Nature Methods*, 8, 1027-1036.

APPLICATIONS OF SECONDARY ANTIBODIES - FLOW CYTOMETRY

Flow cytometry is a powerful technique for measuring and analyzing the physical characteristics of single particles in solution as they travel past a beam of light. Properties such as relative size and fluorescence can be measured, and fluorescently tagged antibodies enable cells to be interrogated for multiple proteins and molecular dynamics. Isotype controls are used for experiment validation and analysis of results.

Indirect flow cytometry

Flow cytometry can be performed directly, using conjugated primary antibodies, or indirectly, using a conjugated secondary antibody to bind an unconjugated primary. Indirect flow cytometry allows the choice of a wide range of probe molecules, enabling the user to match the desired probe with any primary antibody. Secondary antibody conjugates can improve a flow cytometry experiment by preserving the active site of the primary antibody, and by signal amplification (see Figure 40 B).

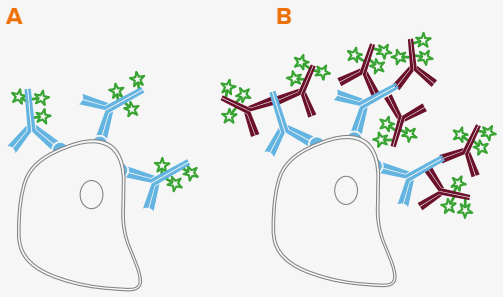


Figure 40: Direct (A) and indirect (B) flow cytometry.

Secondary antibody format for flow cytometry

The format of secondary antibody can impact the success of an experiment. In addition to **whole molecule IgG**, Jackson ImmunoResearch offers fragments of secondary antibodies.

F(ab')$_2$ fragments are generated by proteolysis of the whole IgG to yield a divalent fragment containing two Fab arms and no Fc domain (see page 8). When used to stain tissue or cells, the F(ab')$_2$ secondary antibodies can help to avoid background caused by off-target binding. The absence of an Fc region prevents F(ab')$_2$ antibodies from being captured by Fc receptors expressed on cell surfaces. Please note that if **a primary** antibody is trapped by an Fc receptor, the F(ab')$_2$ secondary antibody will detect the off-target binding, so blocking is critical. For a list of F(ab')$_2$ antibodies please see pages 74-85.

FabuLight™ secondary antibodies are created by papain digestion of IgG, followed by removal of Fc fragments. These monovalent Fab fragments are specific for the Fc region of primary antibodies, so they don't interact with the primary's antigen-binding region. Conjugated FabuLights are convenient for labeling primary antibodies prior to incubation with an experimental sample, saving incubation and wash steps. Like F(ab')$_2$ fragments, these Fab fragments can minimize background staining due to Fc receptor binding. For more information on FabuLight secondary antibodies see pages 98-103.

Fluorescent conjugates for flow cytometry

The choice of fluorescent dye conjugate depends on a number of experimental variables.

- Instrument capabilities. Consider excitation capabilities (excitation wavelengths or laser colors), and which filters are available.
- Experimental sample. It may be necessary to consider autofluorescence or the expression of recombinant fluorescently tagged proteins, which may preclude using fluorophores with spectral overlap.
- Sensitivity required. Several fluorophores may have similar excitation and emission spectra, but differences in inherent brightness can result in one fluorophore showing a larger population shift. For example, Alexa Fluor® 488 is brighter than FITC.
- Degree of color separation required. For multiple labeling, the dye panel choices will be constrained by the equipment available. To achieve good color separation it is important to look at the emission overlap of the fluorophores in the dye panel. Panel developing tools are available online which can help build dye panels specific to any instrument.

Flow cytometry with JIR Secondary Antibodies

Fluorescent dyes from UV to far red can be used for flow cytometry, depending on instrument capabilities. Jackson ImmunoResearch offers a range of fluorescent dye conjugates spanning the spectrum, making it possible to design a flow cytometry dye panel that accommodates instrument capabilities and recombinant proteins incorporated in the experiment. These secondary antibody conjugates can be found listed in the tables of Whole IgG (pages 46-71) and F(ab')$_2$ Fragments (pages 74-85).

Fluorescent protein conjugates for flow cytometry

Jackson ImmunoResearch offers three large, bright fluorescent proteins (R-PE, APC, and PerCP) conjugated to a selection of highly adsorbed secondary antibodies, streptavidin, and purified immunoglobulin controls. The conjugates are excellent choices for surface labeling, but their size may preclude their use as intracellular probes. A table of fluorescent proteins conjugated to F(ab')$_2$ and whole IgG secondary antibodies can be found on pages 113-117.

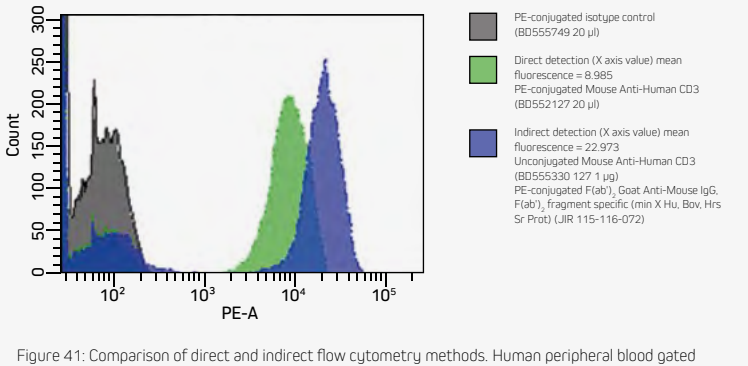


Figure 41: Comparison of direct and indirect flow cytometry methods. Human peripheral blood gated lymphocytes after ammonium chloride lysis of erythrocytes were analyzed for CD3 expression using direct and indirect methods. Comparison of mean fluorescence showed that the indirect method produced a brighter signal (22,973) compared to the direct method (8,985). (Experiment performed on BD FACSCelesta).

Biotin-SP conjugates for flow cytometry

Jackson ImmunoResearch offers Biotin-SP-conjugated secondary antibodies in both whole IgG and F(ab')$_2$ format. Biotin-SP conjugates require the use of fluorescently labeled streptavidin for visualization. A table of Streptavidin conjugates can be found on page 142.

Controls for flow cytometry

Controls are essential to validate an experiment and interpret results. An isotype control is a negative control which estimates the non-specific binding of an antibody. Isotype controls are antibodies which match the host species and class of antibodies used in the experiment but are not directed against the antigen of interest. ChromPure™ proteins are purified from the serum of non-immunized animals and are appropriate experimental controls. An isotype control should be conjugated with the same reporter molecule as the specific antibody.

Isotype controls for flow cytometry

An isotype control for **direct immunofluorescence** will be conjugated to the same fluorophore as the primary antibody. For polyclonal primary antibodies (e.g. rabbit or goat primaries), conjugated ChromPure purified proteins are good experimental controls. Conjugated ChromPure proteins can also be used as controls for monoclonal primaries, though it may be preferable to use a control that is the same subclass as the monoclonal.

Indirect immunofluorescence may require isotype controls for both the primary and secondary antibodies.

a. Unconjugated ChromPure purified proteins can be used as experimental controls for unlabeled polyclonal primary antibodies. Some users choose a control that is the same subclass as their monoclonal primary antibody, but the mixed subclass ChromPure proteins may also be acceptable controls.

b. ChromPure purified proteins conjugated with the same reporter molecule as the labeled secondary antibody are good isotype controls. The isotype control should have the same format (whole molecule or antibody fragment) as the secondary antibody.

See Blocking and Controls pages 143-155 for more information.

ELISA with JIR Secondary Antibodies

An enzyme-linked immunosorbent assay (ELISA) is a robust and sensitive technique used to detect and quantify specific proteins in samples which may contain complex mixtures of proteins. Antibodies are used to detect the specific proteins immobilized on the surface of microplate wells. The technique facilitates high volume and fast throughput analysis, ideal for analyzing large numbers of samples.

ELISA formats

ELISAs are performed in a number of ways, some of which are illustrated in Figure 42.

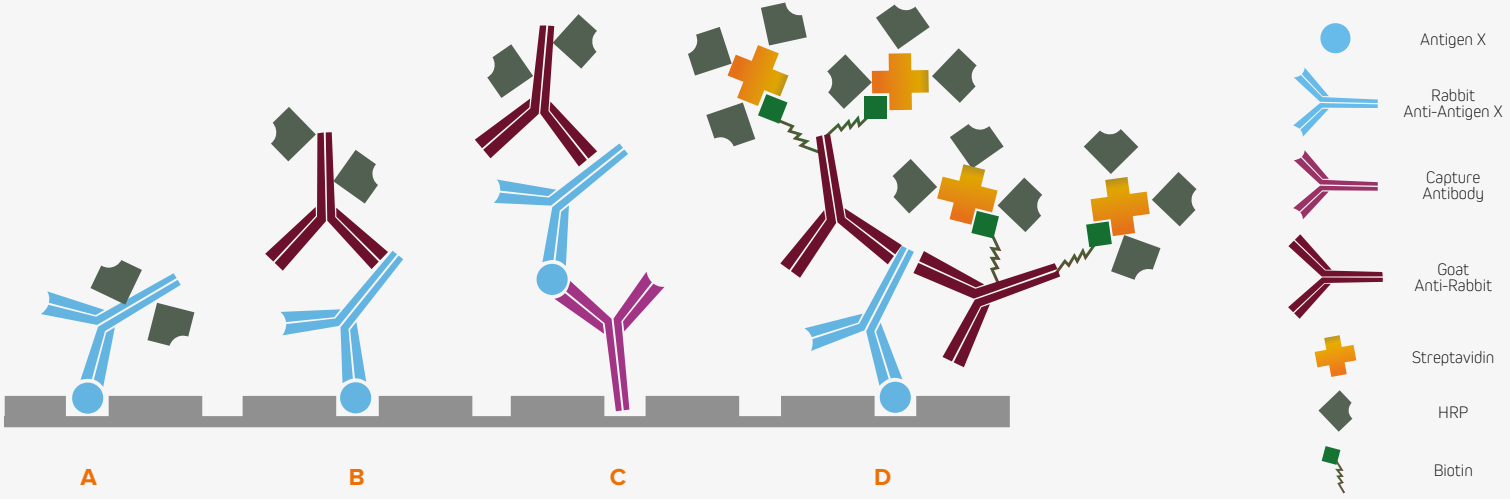


Figure 42: A. In direct ELISA a conjugated primary antibody detects plate-bound antigen. B. In the indirect ELISA method multiple conjugated secondary antibodies are able to bind the primary antibody, leading to signal amplification. C. Sandwich ELISA uses a capture antibody bound to the plate, which binds antigen from the sample, which is then visualised using a conjugated secondary antibody. D. Biotin/streptavidin signal amplification. Biotinylated secondary antibodies bind the primary antibody which has reacted with plate-bound antigen. Conjugated streptavidin then binds to multiple biotin molecules on the secondary antibody, leading to maximal signal amplification.

APPLICATIONS OF SECONDARY ANTIBODIES - ELISA

Blocking

Blocking reagents are especially important in ELISA. To block all unsaturated binding sites on the microplate, use normal serum (5% v/v) derived from the host species of the labeled antibody.

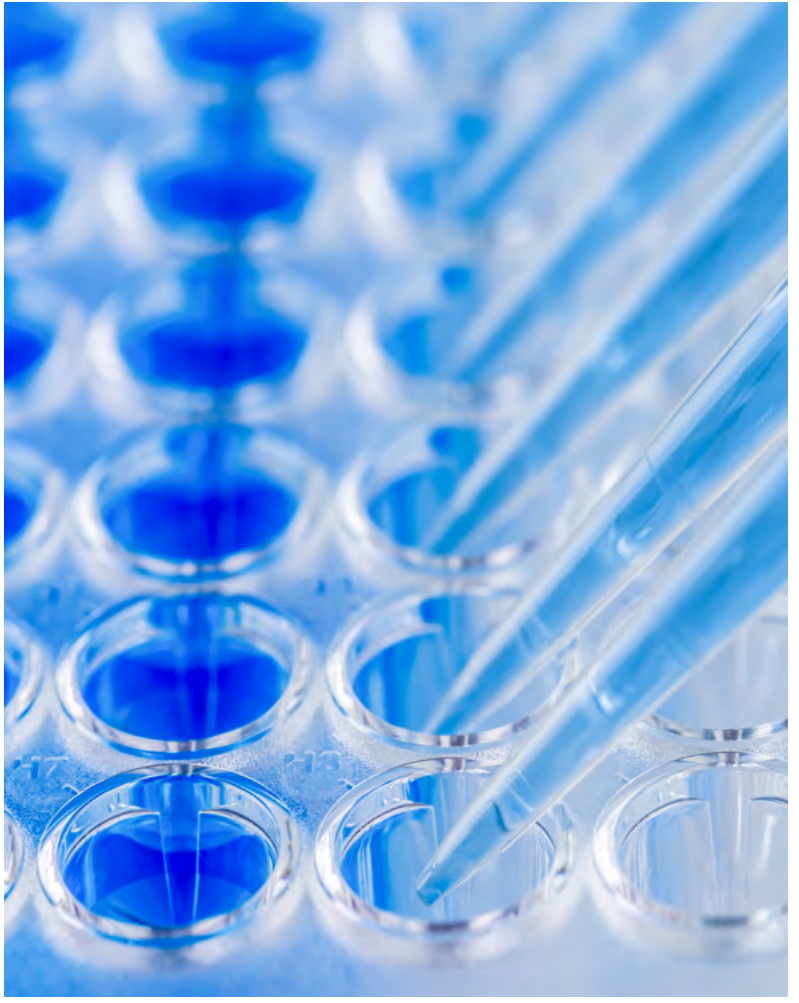
Secondary antibody conjugates for ELISA

Jackson ImmunoResearch alkaline phosphatase (AP) and horseradish peroxidase (HRP) conjugates can be used for colorimetric assays using a chromogenic substrate. For chemiluminescent detection, a luminol based substrate is commonly used with peroxidase conjugates for highly sensitive detection. For more information on reporter enzyme conjugates see pages 22-23.

ELISAs can also be performed using fluorescent conjugates to allow simultaneous detection of multiple primary antibodies derived from different species. By using labeled secondary antibodies each antigen can be distinguished specifically by the individual fluorescent signal. The detection limit for fluorescent ELISA is typically lower than colorimetric or chemiluminescent detection using a reporter enzyme.

Labeled streptavidin with biotinylated antibodies for enhanced sensitivity

Signal enhancement can be achieved using labeled streptavidin to detect a biotinylated antibody (primary or secondary antibody). Each antibody can present multiple biotin molecules, which are then able to bind to multiple streptavidin molecules. These combined factors mean that multiple probe molecules are available to either catalyze the detection substrate to its end product or generate fluorescent emission, achieving a brighter signal and greater sensitivity. Recommended dilution ranges for ELISA can be found in the Appendix, pages 163-164.



EM with JIR Secondary Antibodies

Electron microscopy (EM) allows the collection of high-resolution images using electron beams in the place of light. Staining and immunolabeling with electron dense material enables the visualization of the structures of the sample by adding contrast to the image. Transmission electron microscopy (TEM) requires electrons to pass through thinly sliced specimen sections, allowing 3D images to be compiled and subcellular organelles to be observed. Scanning electron microscopy (SEM) detects the scattered electrons or those emitted from the sample surface. The angle of collection gives the images a 3D quality.

Jackson ImmunoResearch ImmunoGold secondary antibodies for EM

Jackson ImmunoResearch ImmunoGold reagents are colloidal gold particles complexed to secondary antibodies. The electron-dense gold increases electron scatter to reveal high contrast "spots" which allow analytes to be visualized. Labeling of multiple analytes can be achieved by using secondary antibodies complexed to differently sized particles (Figure 45). In addition to TEM and SEM applications, ImmunoGold reagents can be used for brightfield microscopy.

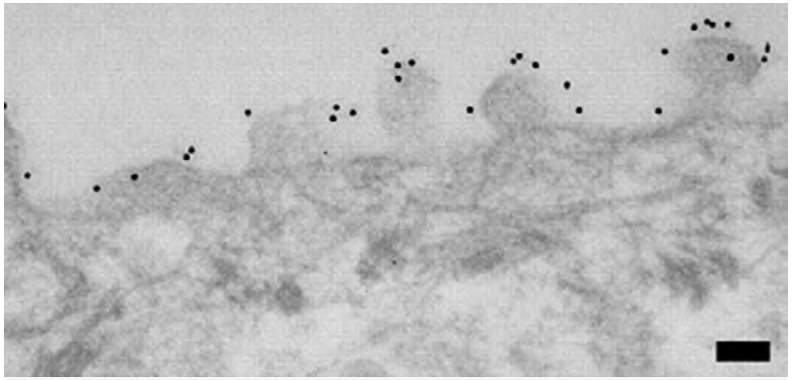


Figure 43: Localization of MUC16 protein on human corneal epithelial cell cultures. Localization using monoclonal Mouse Anti–Human MUC16 antibody and 12 nm Colloidal Gold Donkey Anti-Mouse IgG (H+L) (715-205-150), in stratified HCLE cell cultures. Scale bar: 0.4 µm. (Blalock, et al, 2007).

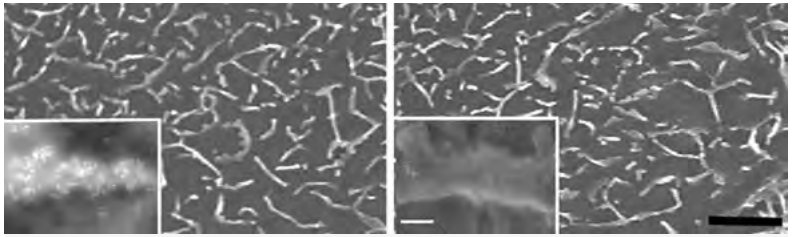


Figure 44: Scanning electron microscopy of the surface of human corneal epithelial cell cultures labeled with 6 nm Colloidal Gold Donkey Anti–Mouse IgG (715-195-150). (left) Non-transfected and (right) MUC16 siRNA sequence 2-transfected HCLE cells. (left, right) Field emission scanning electron microscopy showing localization of MUC16 on microplicae (insets). Scale bar: (left, right) 1 µm; (left, right insets) 0.1 µm. Images from Timothy D. Blalock, et al; Functions of MUC16 in Corneal Epithelial Cells. Invest. Ophthalmol. Vis. Sci. 2007;48(10):4509-4518.

For information on Jackson ImmunoResearch ImmunoGold reagents for electron microscopy and light microscopy see Technical - selecting conjugates - ImmunoGold page 24.

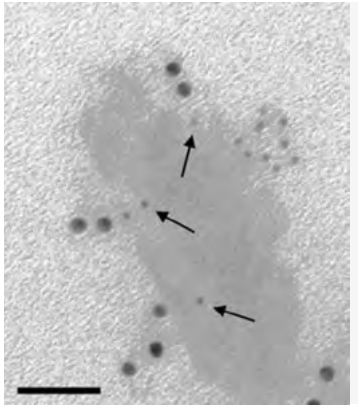
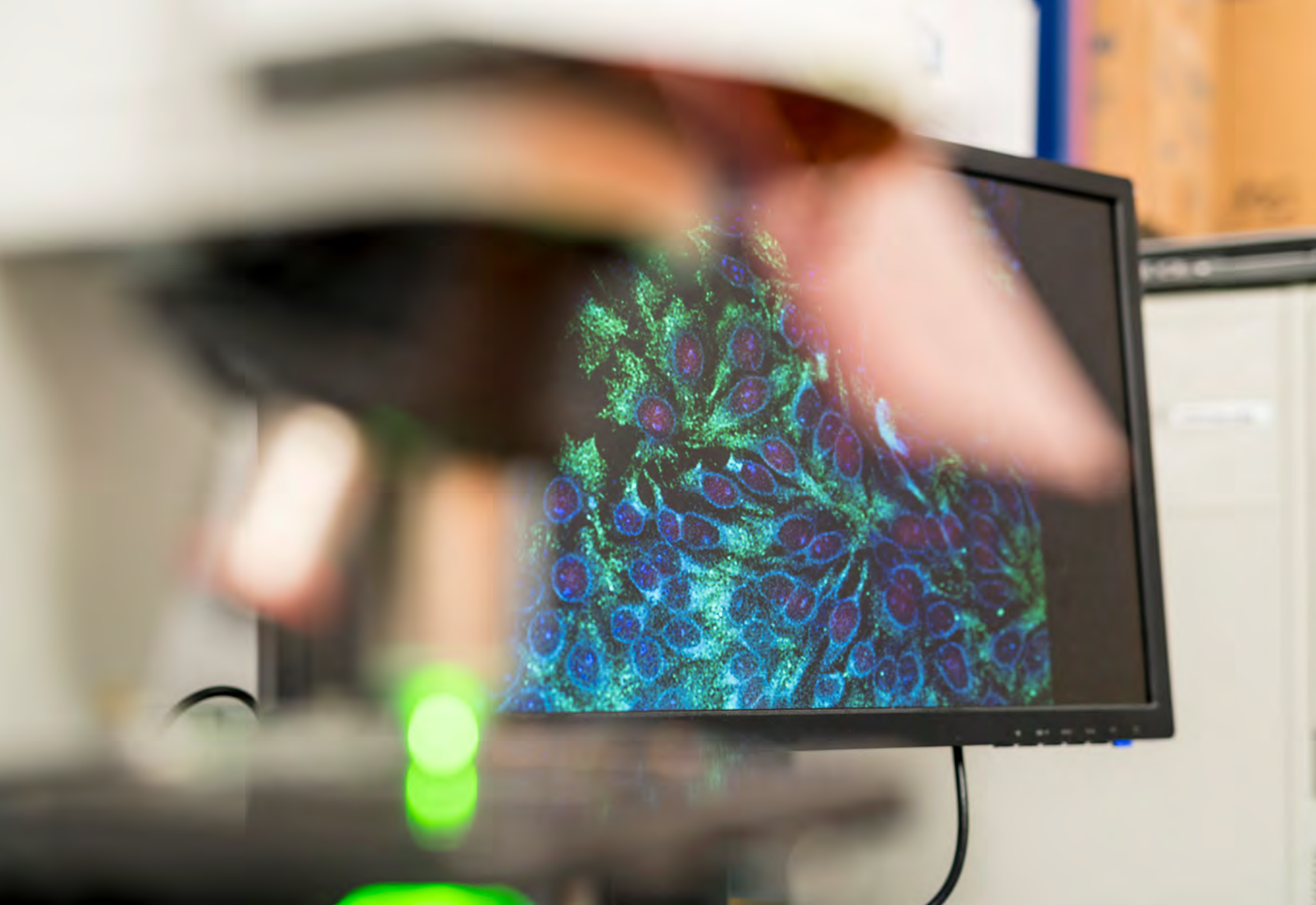


Figure 45: Transmission scanning microscopy of fixed human cornea sections showing colocalization of MUC16 (large gold particles (12 nm Colloidal Gold Donkey Anti-Mouse IgG (H+L) (715-205-150)) and ezrin (small gold particles, arrowheads (6 nm Colloidal Gold Donkey Anti–Goat IgG (705-195-147)) on the microplicae. Scale bar, 0.1 µm. (Blalock, et al, 2007).



AFFINITY-PURIFIED SECONDARY ANTIBODIES

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-BOVINE				
GOAT	Goat Anti-Bovine IgG (H+L) (!)	101-005-003 2.0 mg	101-035-003 2.0 ml	101-055-003 1.0 ml	101-065-003 2.0 ml
GOAT	Goat Anti-Bovine IgG (H+L) (min X Ar Hms, Hu, Ms, Rat Sr Prot) (ML) (!)	101-005-165 1.5 mg	101-035-165 1.5 ml	101-055-165 1.0 ml	101-065-165 1.5 ml
GOAT	Goat Anti-Bovine IgG, Fc fragment specific (!)		101-035-008 2.0 ml		
RABBIT	Rabbit Anti-Bovine IgG (H+L) (!)	301-005-003 2.0 mg	301-035-003 1.5 ml	301-055-003 1.0 ml	301-065-003 1.5 ml
	ANTI-CAT				
GOAT	Goat Anti-Cat IgG (H+L)	102-005-003 2.0 mg	102-035-003 2.0 ml	102-055-003 1.0 ml	102-065-003 2.0 ml
GOAT	Goat Anti-Cat IgG, Fc fragment specific	102-005-008 2.0 mg	102-035-008 2.0 ml	102-055-008 1.0 ml	102-065-008 2.0 ml
GOAT	Goat Anti-Cat IgG, $F(ab')_2$ fragment specific	102-005-006 2.0 mg	102-035-006 2.0 ml	102-055-006 1.0 ml	102-065-006 2.0 ml
	ANTI-CHICKEN				
DONKEY	Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	703-005-155 1.0 mg	703-035-155 0.5 ml	703-055-155 0.5 ml	703-065-155 0.5 ml
GOAT	Goat Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	103-005-155 1.0 mg	103-035-155 0.5 ml	103-055-155 0.5 ml	103-065-155 0.5 ml
RABBIT	Rabbit Anti-Chicken IgY (IgG) (H+L)	303-005-003 2.0 mg	303-035-003 1.5 ml	303-055-003 1.0 ml	303-065-003 1.5 ml
RABBIT	Rabbit Anti-Chicken IgY (IgG), Fc fragment specific	303-005-008 2.0 mg	303-035-008 1.5 ml	303-055-008 1.0 ml	303-065-008 1.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
101-475-003 1.5 mg	101-155-003 2.0 mg	101-545-003 1.5 mg	101-095-003 2.0 mg	101-165-003 2.0 mg	101-295-003 2.0 mg	101-585-003 1.5 mg	101-605-003 1.5 mg
101-475-165 1.0 mg	101-155-165 1.5 mg	101-545-165 1.0 mg	101-095-165 1.5 mg	101-165-165 1.5 mg	101-295-165 1.5 mg	101-585-165 1.0 mg	101-605-165 1.0 mg
301-475-003 1.0 mg	301-155-003 1.5 mg	301-545-003 1.0 mg	301-095-003 1.5 mg	301-165-003 1.5 mg	301-295-003 1.5 mg	301-585-003 1.0 mg	301-605-003 1.0 mg
102-475-003 1.5 mg	102-155-003 2.0 mg	102-545-003 1.5 mg	102-095-003 2.0 mg	102-165-003 2.0 mg	102-295-003 2.0 mg	102-585-003 1.5 mg	102-605-003 1.5 mg
102-475-008 1.5 mg	102-155-008 2.0 mg	102-545-008 1.5 mg	102-095-008 2.0 mg	102-165-008 2.0 mg	102-295-008 2.0 mg	102-585-008 1.5 mg	102-605-008 1.5 mg
102-475-006 1.5 mg	102-155-006 2.0 mg	102-545-006 1.5 mg	102-095-006 2.0 mg	102-165-006 2.0 mg	102-295-006 2.0 mg	102-585-006 1.5 mg	102-605-006 1.5 mg
703-475-155 0.5 mg	703-155-155 0.5 mg	703-545-155 0.5 mg	703-095-155 0.5 mg	703-165-155 0.5 mg	703-295-155 0.5 mg	703-585-155 0.5 mg	703-605-155 0.5 mg
103-475-155 0.5 mg	103-155-155 0.5 mg	103-545-155 0.5 mg	103-095-155 0.5 mg	103-165-155 0.5 mg	103-295-155 0.5 mg	103-585-155 0.5 mg	103-605-155 0.5 mg
303-475-003 1.0 mg	303-155-003 1.5 mg	303-545-003 1.0 mg	303-095-003 1.5 mg	303-165-003 1.5 mg	303-295-003 1.5 mg	303-585-003 1.0 mg	303-605-003 1.0 mg
303-475-008 1.0 mg	303-155-008 1.5 mg	303-545-008 1.0 mg	303-095-008 1.5 mg	303-165-008 1.5 mg	303-295-008 1.5 mg	303-585-008 1.0 mg	303-605-008 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-CHICKEN				
RABBIT	Rabbit Anti-Chicken IgY (IgG), $F(ab')_2$ fragment specific	303-005-006 2.0 mg	303-035-006 1.5 ml	303-055-006 1.0 ml	303-065-006 1.5 ml
	ANTI-DOG				
RABBIT	Rabbit Anti-Dog IgG (H+L)	304-005-003 2.0 mg	304-035-003 1.5 ml	304-055-003 1.0 ml	304-065-003 1.5 ml
RABBIT	Rabbit Anti-Dog IgG, Fc fragment specific	304-005-008 2.0 mg	304-035-008 1.5 ml	304-055-008 1.0 ml	304-065-008 1.5 ml
	ANTI-GOAT				
BOVINE	Bovine Anti-Goat IgG (H+L) (min X Bov, Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (ML)	805-005-180 1.0 mg	805-035-180 0.5 ml	805-055-180 0.5 ml	805-065-180 0.5 ml
DONKEY	Donkey Anti-Goat IgG (H+L) (!)	705-005-003 1.0 mg	705-035-003 1.0 ml	705-055-003 1.0 ml	705-065-003 1.0 ml
DONKEY	Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (ML) (!)	705-005-147 1.0 mg	705-035-147 0.5 ml	705-055-147 0.5 ml	705-065-147 0.5 ml
MOUSE	Mouse Anti-Goat IgG (H+L) (min X Hu, Ms, Rb Sr Prot) (ML) (!)	205-005-108 1.5 mg	205-035-108 1.0 ml	205-055-108 0.5 ml	205-065-108 1.0 ml
MOUSE	IgG Fraction Monoclonal Mouse Anti-Goat IgG, light chain specific (min X Hrs, Hu, Ms, Rb, Rat Ig) (!)	205-002-176 1.0 mg	205-032-176 0.5 ml	205-052-176 0.5 ml	205-062-176 0.5 ml
RABBIT	Rabbit Anti-Goat IgG (H+L) (!)	305-005-003 2.0 mg	305-035-003 1.5 ml	305-055-003 1.0 ml	305-065-003 1.5 ml
RABBIT	Rabbit Anti-Goat IgG (H+L) (min X Hu Sr Prot) (!)	305-005-045 1.5 mg	305-035-045 1.0 ml	305-055-045 0.5 ml	305-065-045 1.0 ml
RABBIT	Rabbit Anti-Goat IgG, Fc fragment specific (!)	305-005-008 2.0 mg	305-035-008 1.5 ml	305-055-008 1.0 ml	305-065-008 1.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
303-475-006 1.0 mg	303-155-006 1.5 mg	303-545-006 1.0 mg	303-095-006 1.5 mg	303-165-006 1.5 mg	303-295-006 1.5 mg	303-585-006 1.0 mg	303-605-006 1.0 mg
304-475-003 1.0 mg	304-155-003 1.5 mg	304-545-003 1.0 mg	304-095-003 1.5 mg	304-165-003 1.5 mg	304-295-003 1.5 mg	304-585-003 1.0 mg	304-605-003 1.0 mg
304-475-008 1.0 mg	304-155-008 1.5 mg	304-545-008 1.0 mg	304-095-008 1.5 mg	304-165-008 1.5 mg	304-295-008 1.5 mg	304-585-008 1.0 mg	304-605-008 1.0 mg
805-475-180 0.5 mg	805-155-180 0.5 mg	805-545-180 0.5 mg	805-095-180 0.5 mg	805-165-180 0.5 mg	805-295-180 0.5 mg	805-585-180 0.5 mg	805-605-180 0.5 mg
705-475-003 1.0 mg	705-155-003 1.0 mg	705-545-003 1.0 mg	705-095-003 1.0 mg	705-165-003 1.0 mg	705-295-003 1.0 mg	705-585-003 1.0 mg	705-605-003 1.0 mg
705-475-147 0.5 mg	705-155-147 0.5 mg	705-545-147 0.5 mg	705-095-147 0.5 mg	705-165-147 0.5 mg	705-295-147 0.5 mg	705-585-147 0.5 mg	705-605-147 0.5 mg
205-475-108 1.0 mg	205-155-108 1.0 mg	205-545-108 1.0 mg	205-095-108 1.0 mg	205-165-108 1.0 mg	205-295-108 1.0 mg	205-585-108 1.0 mg	205-605-108 1.0 mg
		205-542-176 0.5 mg		205-162-176 0.5 mg		205-582-176 0.5 mg	205-602-176 0.5 mg
305-475-003 1.0 mg	305-155-003 1.5 mg	305-545-003 1.0 mg	305-095-003 1.5 mg	305-165-003 1.5 mg	305-295-003 1.5 mg	305-585-003 1.0 mg	305-605-003 1.0 mg
305-475-045 1.0 mg	305-155-045 1.0 mg	305-545-045 1.0 mg	305-095-045 1.0 mg	305-165-045 1.0 mg	305-295-045 1.0 mg	305-585-045 1.0 mg	305-605-045 1.0 mg
305-475-008 1.0 mg	305-155-008 1.5 mg	305-545-008 1.0 mg	305-095-008 1.5 mg	305-165-008 1.5 mg	305-295-008 1.5 mg	305-585-008 1.0 mg	305-605-008 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description		Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-GOAT					
RABBIT	Rabbit Anti-Goat IgG, Fc fragment specific (min X Hu Sr Prot)	(!)	305-005-046 1.5 mg	305-035-046 1.0 ml	305-055-046 0.5 ml	305-065-046 1.0 ml
RABBIT	Rabbit Anti-Goat IgG, F(ab')$_2$ fragment specific	(!)	305-005-006 2.0 mg	305-035-006 1.5 ml	305-055-006 1.0 ml	305-065-006 1.5 ml
RABBIT	Rabbit Anti-Goat IgG, F(ab')$_2$ fragment specific (min X Hu Sr Prot)	(!)	305-005-047 1.5 mg	305-035-047 1.0 ml	305-055-047 0.5 ml	305-065-047 1.0 ml
	ANTI-GUINEA PIG					
DONKEY	Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	(ML)	706-005-148 1.0 mg	706-035-148 0.5 ml	706-055-148 0.5 ml	706-065-148 0.5 ml
GOAT	Goat Anti-Guinea Pig IgG (H+L)		106-005-003 2.0 mg	106-035-003 2.0 ml	106-055-003 1.0 ml	106-065-003 2.0 ml
GOAT	Goat Anti-Guinea Pig IgG, Fc fragment specific		106-005-008 2.0 mg	106-035-008 2.0 ml	106-055-008 1.0 ml	106-065-008 2.0 ml
GOAT	Goat Anti-Guinea Pig IgG, F(ab')$_2$ fragment specific		106-005-006 2.0 mg	106-035-006 2.0 ml	106-055-006 1.0 ml	106-065-006 2.0 ml
	ANTI-ARMENIAN HAMSTER					
GOAT	Goat Anti-Armenian Hamster IgG (H+L) (min X Bov Sr Prot)		127-005-099 1.5 mg	127-035-099 1.5 ml	127-055-099 1.0 ml	127-065-099 1.5 ml
GOAT	Goat Anti-Armenian Hamster IgG (H+L) (min X Bov, Hu, **Ms**, Rb, **Rat** Sr Prot)	(SP)	127-005-160 1.0 mg	127-035-160 0.5 ml	127-055-160 0.5 ml	127-065-160 0.5 ml
	ANTI-SYRIAN HAMSTER					
GOAT	Goat Anti-Syrian Hamster IgG (H+L) (min X Bov, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(ML)	107-005-142 1.0 mg	107-035-142 1.0 ml	107-055-142 0.5 ml	107-065-142 1.0 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
305-475-046 1.0 mg	305-155-046 1.0 mg	305-545-046 1.0 mg	305-095-046 1.0 mg	305-165-046 1.0 mg	305-295-046 1.0 mg	305-585-046 1.0 mg	305-605-046 1.0 mg
305-475-006 1.0 mg	305-155-006 1.5 mg	305-545-006 1.0 mg	305-095-006 1.5 mg	305-165-006 1.5 mg	305-295-006 1.5 mg	305-585-006 1.0 mg	305-605-006 1.0 mg
305-475-047 1.0 mg	305-155-047 1.0 mg	305-545-047 1.0 mg	305-095-047 1.0 mg	305-165-047 1.0 mg	305-295-047 1.0 mg	305-585-047 1.0 mg	305-605-047 1.0 mg
706-475-148 0.5 mg	706-155-148 0.5 mg	706-545-148 0.5 mg	706-095-148 0.5 mg	706-165-148 0.5 mg	706-295-148 0.5 mg	706-585-148 0.5 mg	706-605-148 0.5 mg
106-475-003 1.5 mg	106-155-003 2.0 mg	106-545-003 1.5 mg	106-095-003 2.0 mg	106-165-003 2.0 mg	106-295-003 2.0 mg	106-585-003 1.5 mg	106-605-003 1.5 mg
106-475-008 1.5 mg	106-155-008 2.0 mg	106-545-008 1.5 mg	106-095-008 2.0 mg	106-165-008 2.0 mg	106-295-008 2.0 mg	106-585-008 1.5 mg	106-605-008 1.5 mg
106-475-006 1.5 mg	106-155-006 2.0 mg	106-545-006 1.5 mg	106-095-006 2.0 mg	106-165-006 2.0 mg	106-295-006 2.0 mg	106-585-006 1.5 mg	106-605-006 1.5 mg
127-475-099 1.0 mg	127-155-099 1.5 mg	127-545-099 1.0 mg	127-095-099 1.5 mg	127-165-099 1.5 mg	127-295-099 1.5 mg	127-585-099 1.0 mg	127-605-099 1.0 mg
127-475-160 0.5 mg	127-155-160 0.5 mg	127-545-160 0.5 mg	127-095-160 0.5 mg	127-165-160 0.5 mg	127-295-160 0.5 mg	127-585-160 0.5 mg	127-605-160 0.5 mg
107-475-142 1.0 mg	107-155-142 1.0 mg	107-545-142 1.0 mg	107-095-142 1.0 mg	107-165-142 1.0 mg	107-295-142 1.0 mg	107-585-142 1.0 mg	107-605-142 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-SYRIAN HAMSTER				
RABBIT	Rabbit Anti-Syrian Hamster IgG (H+L)	307-005-003 2.0 mg	307-035-003 1.5 ml	307-055-003 1.0 ml	307-065-003 1.5 ml
	ANTI-HORSE				
GOAT	Goat Anti-Horse IgG (H+L)	108-005-003 2.0 mg	108-035-003 2.0 ml	108-055-003 1.0 ml	108-065-003 2.0 ml
GOAT	Goat Anti-Horse IgG, Fc fragment specific	108-005-008 2.0 mg	108-035-008 2.0 ml	108-055-008 1.0 ml	108-065-008 2.0 ml
RABBIT	Rabbit Anti-Horse IgG (H+L) (!)	308-005-003 2.0 mg	308-035-003 1.5 ml	308-055-003 1.0 ml	308-065-003 1.5 ml
	ANTI-HUMAN				
ALPACA	Alpaca Anti-Human IgG (H+L) (min X Bov, Ms, Rb Sr Prot) (ML)	609-005-213 1.0 mg	609-035-213 1.0 ml	609-055-213 1.0 ml	609-065-213 1.0 ml
DONKEY	Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot) (ML)	709-005-149 1.0 mg	709-035-149 0.5 ml	709-055-149 0.5 ml	709-065-149 0.5 ml
DONKEY	Donkey Anti-Human IgG, Fc_{γ} fragment specific (min X Bov, Hrs, Ms Sr Prot)	709-005-098 1.0 mg	709-035-098 1.0 ml	709-055-098 1.0 ml	709-065-098 1.0 ml
DONKEY	Donkey Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov, Hrs Sr Prot)	709-005-073 1.5 mg	709-035-073 1.5 ml	709-055-073 1.0 ml	709-065-073 1.5 ml
GOAT	Goat Anti-Human IgG (H+L)	109-005-003 2.0 mg	109-035-003 2.0 ml	109-055-003 1.0 ml	109-065-003 2.0 ml
GOAT	Goat Anti-Human IgG (H+L) (min X Bov, Hrs, Ms Sr Prot)	109-005-088 1.5 mg	109-035-088 1.5 ml	109-055-088 1.0 ml	109-065-088 1.5 ml
GOAT	Goat Anti-Human IgG, Fc_{γ} fragment specific	109-005-008 2.0 mg	109-035-008 2.0 ml	109-055-008 1.0 ml	109-065-008 2.0 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
307-475-003 1.0 mg	307-155-003 1.5 mg	307-545-003 1.0 mg	307-095-003 1.5 mg	307-165-003 1.5 mg	307-295-003 1.5 mg	307-585-003 1.0 mg	307-605-003 1.0 mg
108-475-003 1.5 mg	108-155-003 2.0 mg	108-545-003 1.5 mg	108-095-003 2.0 mg	108-165-003 2.0 mg	108-295-003 2.0 mg	108-585-003 1.5 mg	108-605-003 1.5 mg
108-475-008 1.5 mg	108-155-008 2.0 mg	108-545-008 1.5 mg	108-095-008 2.0 mg	108-165-008 2.0 mg	108-295-008 2.0 mg	108-585-008 1.5 mg	108-605-008 1.5 mg
308-475-003 1.0 mg	308-155-003 1.5 mg	308-545-003 1.0 mg	308-095-003 1.5 mg	308-165-003 1.5 mg	308-295-003 1.5 mg	308-585-003 1.0 mg	308-605-003 1.0 mg
609-475-213 1.0 mg	609-155-213 1.0 mg	609-545-213 1.0 mg	609-095-213 1.0 mg	609-165-213 1.0 mg	609-295-213 1.0 mg	609-585-213 1.0 mg	609-605-213 1.0 mg
709-475-149 0.5 mg	709-155-149 0.5 mg	709-545-149 0.5 mg	709-095-149 0.5 mg	709-165-149 0.5 mg	709-295-149 0.5 mg	709-585-149 0.5 mg	709-605-149 0.5 mg
709-475-098 1.0 mg	709-155-098 1.0 mg	709-545-098 1.0 mg	709-095-098 1.0 mg	709-165-098 1.0 mg	709-295-098 1.0 mg	709-585-098 1.0 mg	709-605-098 1.0 mg
709-475-073 1.0 mg	709-155-073 1.5 mg	709-545-073 1.0 mg	709-095-073 1.5 mg	709-165-073 1.5 mg	709-295-073 1.5 mg	709-585-073 1.0 mg	709-605-073 1.0 mg
109-475-003 1.5 mg	109-155-003 2.0 mg	109-545-003 1.5 mg	109-095-003 2.0 mg	109-165-003 2.0 mg	109-295-003 2.0 mg	109-585-003 1.5 mg	109-605-003 1.5 mg
109-475-088 1.0 mg	109-155-088 1.5 mg	109-545-088 1.0 mg	109-095-088 1.5 mg	109-165-088 1.5 mg	109-295-088 1.5 mg	109-585-088 1.0 mg	109-605-088 1.0 mg
109-475-008 1.5 mg	109-155-008 2.0 mg	109-545-008 1.5 mg	109-095-008 2.0 mg	109-165-008 2.0 mg	109-295-008 2.0 mg	109-585-008 1.5 mg	109-605-008 1.5 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-HUMAN				
GOAT	Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot) (ML)	109-005-098 1.0 mg	109-035-098 1.0 ml	109-055-098 1.0 ml	109-065-098 1.0 ml
GOAT	Goat Anti-Human IgG, $F(ab')_2$ fragment specific	109-005-006 2.0 mg	109-035-006 2.0 ml	109-055-006 1.0 ml	109-065-006 2.0 ml
GOAT	Goat Anti-Human IgG, $F(ab')_2$ fragment specific (min X Bov, Hrs, Ms Sr Prot)	109-005-097 1.5 mg	109-035-097 1.5 ml	109-055-097 1.0 ml	109-065-097 1.5 ml
GOAT	Goat Anti-Human IgG + IgM (H+L)	109-005-044 2.0 mg	109-035-044 2.0 ml	109-055-044 1.0 ml	109-065-044 2.0 ml
GOAT	Goat Anti-Human IgG + IgM (H+L) (min X Bov Sr Prot)	109-005-127 1.5 mg	109-035-127 1.5 ml	109-055-127 1.0 ml	109-065-127 1.5 ml
GOAT	Goat Anti-Human IgA + IgG + IgM (H+L)	109-005-064 2.0 mg	109-035-064 2.0 ml	109-055-064 1.0 ml	109-065-064 2.0 ml
GOAT	Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific	109-005-043 2.0 mg	109-035-043 2.0 ml	109-055-043 1.0 ml	109-065-043 2.0 ml
GOAT	Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov Sr Prot) (ML)	109-005-129 1.5 mg	109-035-129 1.5 ml	109-055-129 1.0 ml	109-065-129 1.5 ml
GOAT	Goat Anti-Human Serum IgA, α chain specific (ML)	109-005-011 2.0 mg	109-035-011 2.0 ml	109-055-011 1.0 ml	109-065-011 2.0 ml
MOUSE	Mouse Anti-Human IgG (H+L) (min X Ms Sr Prot)	209-005-082 2.0 mg	209-035-082 1.5 ml	209-055-082 1.0 ml	209-065-082 1.5 ml
MOUSE	Mouse Anti-Human IgG (H+L) (min X Bov, Hrs, Ms Sr Prot)	209-005-088 1.5 mg	209-035-088 1.0 ml	209-055-088 0.5 ml	209-065-088 1.0 ml
MOUSE	Mouse Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	209-005-098 1.5 mg	209-035-098 1.0 ml	209-055-098 0.5 ml	209-065-098 1.0 ml
MOUSE	Mouse Anti-Human IgG, $F(ab')_2$ fragment specific (min X Bov, Hrs, Ms Sr Prot)	209-005-097 1.5 mg	209-035-097 1.0 ml	209-055-097 0.5 ml	209-065-097 1.0 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
109-475-098 1.0 mg	109-155-098 1.0 mg	109-545-098 1.0 mg	109-095-098 1.0 mg	109-165-098 1.0 mg	109-295-098 1.0 mg	109-585-098 1.0 mg	109-605-098 1.0 mg
109-475-006 1.5 mg	109-155-006 2.0 mg	109-545-006 1.5 mg	109-095-006 2.0 mg	109-165-006 2.0 mg	109-295-006 2.0 mg	109-585-006 1.5 mg	109-605-006 1.5 mg
109-475-097 1.0 mg	109-155-097 1.5 mg	109-545-097 1.0 mg	109-095-097 1.5 mg	109-165-097 1.5 mg	109-295-097 1.5 mg	109-585-097 1.0 mg	109-605-097 1.0 mg
109-475-044 1.5 mg	109-155-044 2.0 mg	109-545-044 1.5 mg	109-095-044 2.0 mg	109-165-044 2.0 mg	109-295-044 2.0 mg	109-585-044 1.5 mg	109-605-044 1.5 mg
109-475-127 1.0 mg	109-155-127 1.5 mg	109-545-127 1.0 mg	109-095-127 1.5 mg	109-165-127 1.5 mg	109-295-127 1.5 mg	109-585-127 1.0 mg	109-605-127 1.0 mg
109-475-064 1.5 mg	109-155-064 2.0 mg	109-545-064 1.5 mg	109-095-064 2.0 mg	109-165-064 2.0 mg	109-295-064 2.0 mg	109-585-064 1.5 mg	109-605-064 1.5 mg
109-475-043 1.5 mg	109-155-043 2.0 mg	109-545-043 1.5 mg	109-095-043 2.0 mg	109-165-043 2.0 mg	109-295-043 2.0 mg	109-585-043 1.5 mg	109-605-043 1.5 mg
109-475-129 1.0 mg	109-155-129 1.5 mg	109-545-129 1.0 mg	109-095-129 1.5 mg	109-165-129 1.5 mg	109-295-129 1.5 mg	109-585-129 1.0 mg	109-605-129 1.0 mg
109-475-011 1.5 mg	109-155-011 2.0 mg	109-545-011 1.5 mg	109-095-011 2.0 mg	109-165-011 2.0 mg	109-295-011 2.0 mg	109-585-011 1.5 mg	109-605-011 1.5 mg
209-475-082 1.0 mg	209-155-082 1.5 mg	209-545-082 1.0 mg	209-095-082 1.5 mg	209-165-082 1.5 mg	209-295-082 1.5 mg	209-585-082 1.0 mg	209-605-082 1.0 mg
209-475-088 1.0 mg	209-155-088 1.0 mg	209-545-088 1.0 mg	209-095-088 1.0 mg	209-165-088 1.0 mg	209-295-088 1.0 mg	209-585-088 1.0 mg	209-605-088 1.0 mg
209-475-098 1.0 mg	209-155-098 1.0 mg	209-545-098 1.0 mg	209-095-098 1.0 mg	209-165-098 1.0 mg	209-295-098 1.0 mg	209-585-098 1.0 mg	209-605-098 1.0 mg
209-475-097 1.0 mg	209-155-097 1.0 mg	209-545-097 1.0 mg	209-095-097 1.0 mg	209-165-097 1.0 mg	209-295-097 1.0 mg	209-585-097 1.0 mg	209-605-097 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-HUMAN				
RABBIT	Rabbit Anti-Human IgG (H+L)	309-005-003 2.0 mg	309-035-003 1.5 ml	309-055-003 1.0 ml	309-065-003 1.5 ml
RABBIT	Rabbit Anti-Human IgG (H+L) (min X Ms Sr Prot)	309-005-082 1.5 mg	309-035-082 1.0 ml	309-055-082 0.5 ml	309-065-082 1.0 ml
RABBIT	Rabbit Anti-Human IgG, Fc_{γ} fragment specific	309-005-008 2.0 mg	309-035-008 1.5 ml	309-055-008 1.0 ml	309-065-008 1.5 ml
RABBIT	Rabbit Anti-Human IgG, $F(ab')_2$ fragment specific	309-005-006 2.0 mg	309-035-006 1.5 ml	309-055-006 1.0 ml	309-065-006 1.5 ml
RABBIT	Rabbit Anti-Human IgG + IgM (H+L) (min X Ms Sr Prot)	309-005-107 1.5 mg	309-035-107 1.0 ml	309-055-107 0.5 ml	309-065-107 1.0 ml
RABBIT	Rabbit Anti-Human IgA + IgG + IgM (H+L)	309-005-064 2.0 mg	309-035-064 1.5 ml	309-055-064 1.0 ml	309-065-064 1.5 ml
RABBIT	Rabbit Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Ms Sr Prot)	309-005-095 1.5 mg	309-035-095 1.0 ml	309-055-095 0.5 ml	309-065-095 1.0 ml
RABBIT	Rabbit Anti-Human Serum IgA, α chain specific	309-005-011 2.0 mg	309-035-011 1.5 ml	309-055-011 1.0 ml	309-065-011 1.5 ml
RABBIT	Rabbit Anti-Human Lactoferrin	309-005-015 2.0 mg	309-035-015 1.5 ml	309-055-015 1.0 ml	309-065-015 1.5 ml
	ANTI-MOUSE				
ALPACA	Alpaca Anti-Mouse IgG (H+L) (min X Bov, Hu, Rb Sr Prot) (ML)	615-005-214 1.0 mg	615-035-214 1.0 ml	615-055-214 1.0 ml	615-065-214 1.0 ml
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot) (ML)	715-005-150 1.0 mg	715-035-150 0.5 ml	715-055-150 0.5 ml	715-065-150 0.5 ml
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot) (SP) (ML)	715-005-151 1.0 mg	715-035-151 0.5 ml	715-055-151 0.5 ml	715-065-151 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
309-475-003 1.0 mg	309-155-003 1.5 mg	309-545-003 1.0 mg	309-095-003 1.5 mg	309-165-003 1.5 mg	309-295-003 1.5 mg	309-585-003 1.0 mg	309-605-003 1.0 mg
309-475-082 1.0 mg	309-155-082 1.0 mg	309-545-082 1.0 mg	309-095-082 1.0 mg	309-165-082 1.0 mg	309-295-082 1.0 mg	309-585-082 1.0 mg	309-605-082 1.0 mg
309-475-008 1.0 mg	309-155-008 1.5 mg	309-545-008 1.0 mg	309-095-008 1.5 mg	309-165-008 1.5 mg	309-295-008 1.5 mg	309-585-008 1.0 mg	309-605-008 1.0 mg
309-475-006 1.0 mg	309-155-006 1.5 mg	309-545-006 1.0 mg	309-095-006 1.5 mg	309-165-006 1.5 mg	309-295-006 1.5 mg	309-585-006 1.0 mg	309-605-006 1.0 mg
309-475-107 1.0 mg	309-155-107 1.0 mg	309-545-107 1.0 mg	309-095-107 1.0 mg	309-165-107 1.0 mg	309-295-107 1.0 mg	309-585-107 1.0 mg	309-605-107 1.0 mg
309-475-064 1.0 mg	309-155-064 1.5 mg	309-545-064 1.0 mg	309-095-064 1.5 mg	309-165-064 1.5 mg	309-295-064 1.5 mg	309-585-064 1.0 mg	309-605-064 1.0 mg
309-475-095 1.0 mg	309-155-095 1.0 mg	309-545-095 1.0 mg	309-095-095 1.0 mg	309-165-095 1.0 mg	309-295-095 1.0 mg	309-585-095 1.0 mg	309-605-095 1.0 mg
309-475-011 1.0 mg	309-155-011 1.5 mg	309-545-011 1.0 mg	309-095-011 1.5 mg	309-165-011 1.5 mg	309-295-011 1.5 mg	309-585-011 1.0 mg	309-605-011 1.0 mg
309-475-015 1.0 mg	309-155-015 1.5 mg	309-545-015 1.0 mg	309-095-015 1.5 mg	309-165-015 1.5 mg	309-295-015 1.5 mg	309-585-015 1.0 mg	309-605-015 1.0 mg
615-475-214 1.0 mg	615-155-214 1.0 mg	615-545-214 1.0 mg	615-095-214 1.0 mg	615-165-214 1.0 mg	615-295-214 1.0 mg	615-585-214 1.0 mg	615-605-214 1.0 mg
715-475-150 0.5 mg	715-155-150 0.5 mg	715-545-150 0.5 mg	715-095-150 0.5 mg	715-165-150 0.5 mg	715-295-150 0.5 mg	715-585-150 0.5 mg	715-605-150 0.5 mg
715-475-151 0.5 mg	715-155-151 0.5 mg	715-545-151 0.5 mg	715-095-151 0.5 mg	715-165-151 0.5 mg	715-295-151 0.5 mg	715-585-151 0.5 mg	715-605-151 0.5 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE				
DONKEY	Donkey Anti-Mouse IgM, μ chain specific	715-005-020 2.0 mg	715-035-020 2.0 ml	715-055-020 1.0 ml	715-065-020 2.0 ml
DONKEY	Donkey Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs, **Rat** Sr Prot) (SP)	715-005-140 1.0 mg	715-035-140 0.5 ml	715-055-140 0.5 ml	715-065-140 0.5 ml
GOAT	Goat Anti-Mouse IgG (H+L)	115-005-003 2.0 mg	115-035-003 2.0 ml	115-055-003 1.0 ml	115-065-003 2.0 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs Sr Prot)	115-005-062 1.5 mg	115-035-062 1.5 ml	115-055-062 1.0 ml	115-065-062 1.5 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot) (ML)	115-005-146 1.5 mg	115-035-146 1.5 ml	115-055-146 1.0 ml	115-065-146 1.5 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot) (SP) (ML)	115-005-166 1.0 mg	115-035-166 0.5 ml	115-055-166 0.5 ml	115-065-166 0.5 ml
GOAT	Goat Anti-Mouse IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Rb, Rat, Shp Ig) (kLC)	115-005-174 1.0 mg	115-035-174 0.5 ml	115-055-174 0.5 ml	115-065-174 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (ML)	115-005-008 2.0 mg	115-035-008 2.0 ml	115-055-008 1.0 ml	115-065-008 2.0 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-005-071 1.5 mg	115-035-071 1.5 ml	115-055-071 1.0 ml	115-065-071 1.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-205 1.0 mg	115-035-205 0.5 ml	115-055-205 0.5 ml	115-065-205 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-206 1.0 mg	115-035-206 0.5 ml	115-055-206 0.5 ml	115-065-206 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-207 1.0 mg	115-035-207 0.5 ml	115-055-207 0.5 ml	115-065-207 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2c specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-208 1.0 mg	115-035-208 0.5 ml	115-055-208 0.5 ml	115-065-208 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (kLC) This antibody reacts primarily with kappa light chains. It is not suitable for detection of primary antibodies with lambda light chains.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
715-475-020 1.5 mg	715-155-020 2.0 mg	715-545-020 1.5 mg	715-095-020 2.0 mg	715-165-020 2.0 mg	715-295-020 2.0 mg	715-585-020 1.5 mg	715-605-020 1.5 mg
715-475-140 0.5 mg	715-155-140 0.5 mg	715-545-140 0.5 mg	715-095-140 0.5 mg	715-165-140 0.5 mg	715-295-140 0.5 mg	715-585-140 0.5 mg	715-605-140 0.5 mg
115-475-003 1.5 mg	115-155-003 2.0 mg	115-545-003 1.5 mg	115-095-003 2.0 mg	115-165-003 2.0 mg	115-295-003 2.0 mg	115-585-003 1.5 mg	115-605-003 1.5 mg
115-475-062 1.0 mg	115-155-062 1.5 mg	115-545-062 1.0 mg	115-095-062 1.5 mg	115-165-062 1.5 mg	115-295-062 1.5 mg	115-585-062 1.0 mg	115-605-062 1.0 mg
115-475-146 1.0 mg	115-155-146 1.5 mg	115-545-146 1.0 mg	115-095-146 1.5 mg	115-165-146 1.5 mg	115-295-146 1.5 mg	115-585-146 1.0 mg	115-605-146 1.0 mg
115-475-166 0.5 mg	115-155-166 0.5 mg	115-545-166 0.5 mg	115-095-166 0.5 mg	115-165-166 0.5 mg	115-295-166 0.5 mg	115-585-166 0.5 mg	115-605-166 0.5 mg
		115-545-174 0.5 mg		115-165-174 0.5 mg		115-585-174 0.5 mg	115-605-174 0.5 mg
115-475-008 1.5 mg	115-155-008 2.0 mg	115-545-008 1.5 mg	115-095-008 2.0 mg	115-165-008 2.0 mg	115-295-008 2.0 mg	115-585-008 1.5 mg	115-605-008 1.5 mg
115-475-071 1.0 mg	115-155-071 1.5 mg	115-545-071 1.0 mg	115-095-071 1.5 mg	115-165-071 1.5 mg	115-295-071 1.5 mg	115-585-071 1.0 mg	115-605-071 1.0 mg
115-475-205 0.5 mg	115-155-205 0.5 mg	115-545-205 0.5 mg	115-095-205 0.5 mg	115-165-205 0.5 mg	115-295-205 0.5 mg	115-585-205 0.5 mg	115-605-205 0.5 mg
115-475-206 0.5 mg	115-155-206 0.5 mg	115-545-206 0.5 mg	115-095-206 0.5 mg	115-165-206 0.5 mg	115-295-206 0.5 mg	115-585-206 0.5 mg	115-605-206 0.5 mg
115-475-207 0.5 mg	115-155-207 0.5 mg	115-545-207 0.5 mg	115-095-207 0.5 mg	115-165-207 0.5 mg	115-295-207 0.5 mg	115-585-207 0.5 mg	115-605-207 0.5 mg
115-475-208 0.5 mg	115-155-208 0.5 mg	115-545-208 0.5 mg	115-095-208 0.5 mg	115-165-208 0.5 mg	115-295-208 0.5 mg	115-585-208 0.5 mg	115-605-208 0.5 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE				
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-209 1.0 mg	115-035-209 0.5 ml	115-055-209 0.5 ml	115-065-209 0.5 ml
GOAT	Goat Anti-Mouse IgG, (subclasses 1+2a+2b+3), Fc_γ fragment specific (min X Hu, Bov, Rb Sr Prot) (ML)	115-005-164 1.0 mg	115-035-164 1.0 ml	115-055-164 0.5 ml	115-065-164 1.0 ml
GOAT	Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific	115-005-006 2.0 mg	115-035-006 2.0 ml	115-055-006 1.0 ml	115-065-006 2.0 ml
GOAT	Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)	115-005-072 1.5 mg	115-035-072 1.5 ml	115-055-072 1.0 ml	115-065-072 1.5 ml
GOAT	Goat Anti-Mouse IgG + IgM (H+L)	115-005-044 2.0 mg	115-035-044 2.0 ml	115-055-044 1.0 ml	115-065-044 2.0 ml
GOAT	Goat Anti-Mouse IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)	115-005-068 1.5 mg	115-035-068 1.5 ml	115-055-068 1.0 ml	115-065-068 1.5 ml
GOAT	Goat Anti-Mouse IgM, μ chain specific (ML)	115-005-020 2.0 mg	115-035-020 2.0 ml	115-055-020 1.0 ml	115-065-020 2.0 ml
GOAT	Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-005-075 1.0 mg	115-035-075 1.0 ml	115-055-075 1.0 ml	115-065-075 1.0 ml
RABBIT	Rabbit Anti-Mouse IgG (H+L)	315-005-003 2.0 mg	315-035-003 1.5 ml	315-055-003 1.0 ml	315-065-003 1.5 ml
RABBIT	Rabbit Anti-Mouse IgG (H+L) (min X Hu Sr Prot)	315-005-045 1.5 mg	315-035-045 1.0 ml	315-055-045 0.5 ml	315-065-045 1.0 ml
RABBIT	Rabbit Anti-Mouse IgG, Fc_γ fragment specific	315-005-008 2.0 mg	315-035-008 1.5 ml	315-055-008 1.0 ml	315-065-008 1.5 ml
RABBIT	Rabbit Anti-Mouse IgG, Fc_γ fragment specific (min X Hu Sr Prot)	315-005-046 1.5 mg	315-035-046 1.0 ml	315-055-046 0.5 ml	315-065-046 1.0 ml
RABBIT	Rabbit Anti-Mouse IgG, $F(ab')_2$ fragment specific	315-005-006 2.0 mg	315-035-006 1.5 ml	315-055-006 1.0 ml	315-065-006 1.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation)

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
115-475-209 0.5 mg	115-155-209 0.5 mg	115-545-209 0.5 mg	115-095-209 0.5 mg	115-165-209 0.5 mg	115-295-209 0.5 mg	115-585-209 0.5 mg	115-605-209 0.5 mg
115-475-164 1.0 mg	115-155-164 1.0 mg	115-545-164 1.0 mg	115-095-164 1.0 mg	115-165-164 1.0 mg	115-295-164 1.0 mg	115-585-164 1.0 mg	115-605-164 1.0 mg
115-475-006 1.5 mg	115-155-006 2.0 mg	115-545-006 1.5 mg	115-095-006 2.0 mg	115-165-006 2.0 mg	115-295-006 2.0 mg	115-585-006 1.5 mg	115-605-006 1.5 mg
115-475-072 1.0 mg	115-155-072 1.5 mg	115-545-072 1.0 mg	115-095-072 1.5 mg	115-165-072 1.5 mg	115-295-072 1.5 mg	115-585-072 1.0 mg	115-605-072 1.0 mg
115-475-044 1.5 mg	115-155-044 2.0 mg	115-545-044 1.5 mg	115-095-044 2.0 mg	115-165-044 2.0 mg	115-295-044 2.0 mg	115-585-044 1.5 mg	115-605-044 1.5 mg
115-475-068 1.0 mg	115-155-068 1.5 mg	115-545-068 1.0 mg	115-095-068 1.5 mg	115-165-068 1.5 mg	115-295-068 1.5 mg	115-585-068 1.0 mg	115-605-068 1.0 mg
115-475-020 1.5 mg	115-155-020 2.0 mg	115-545-020 1.5 mg	115-095-020 2.0 mg	115-165-020 2.0 mg	115-295-020 2.0 mg	115-585-020 1.5 mg	115-605-020 1.5 mg
115-475-075 1.0 mg	115-155-075 1.0 mg	115-545-075 1.0 mg	115-095-075 1.0 mg	115-165-075 1.0 mg	115-295-075 1.0 mg	115-585-075 1.0 mg	115-605-075 1.0 mg
315-475-003 1.0 mg	315-155-003 1.5 mg	315-545-003 1.0 mg	315-095-003 1.5 mg	315-165-003 1.5 mg	315-295-003 1.5 mg	315-585-003 1.0 mg	315-605-003 1.0 mg
315-475-045 1.0 mg	315-155-045 1.0 mg	315-545-045 1.0 mg	315-095-045 1.0 mg	315-165-045 1.0 mg	315-295-045 1.0 mg	315-585-045 1.0 mg	315-605-045 1.0 mg
315-475-008 1.0 mg	315-155-008 1.5 mg	315-545-008 1.0 mg	315-095-008 1.5 mg	315-165-008 1.5 mg	315-295-008 1.5 mg	315-585-008 1.0 mg	315-605-008 1.0 mg
315-475-046 1.0 mg	315-155-046 1.0 mg	315-545-046 1.0 mg	315-095-046 1.0 mg	315-165-046 1.0 mg	315-295-046 1.0 mg	315-585-046 1.0 mg	315-605-046 1.0 mg
315-475-006 1.0 mg	315-155-006 1.5 mg	315-545-006 1.0 mg	315-095-006 1.5 mg	315-165-006 1.5 mg	315-295-006 1.5 mg	315-585-006 1.0 mg	315-605-006 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description		Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE					
RABBIT	Rabbit Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot)		315-005-047 1.5 mg	315-035-047 1.0 ml	315-055-047 0.5 ml	315-065-047 1.0 ml
RABBIT	Rabbit Anti-Mouse IgG + IgM (H+L)		315-005-044 2.0 mg	315-035-044 1.5 ml	315-055-044 1.0 ml	315-065-044 1.5 ml
RABBIT	Rabbit Anti-Mouse IgG + IgM (H+L) (min X Hu Sr Prot)		315-005-048 1.5 mg	315-035-048 1.0 ml	315-055-048 0.5 ml	315-065-048 1.0 ml
RABBIT	Rabbit Anti-Mouse IgM, μ chain specific		315-005-020 2.0 mg	315-035-020 1.5 ml	315-055-020 1.0 ml	315-065-020 1.5 ml
RABBIT	Rabbit Anti-Mouse IgM, μ chain specific (min X Hu Sr Prot)		315-005-049 1.5 mg	315-035-049 1.0 ml	315-055-049 0.5 ml	315-065-049 1.0 ml
RAT	Rat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot)	(ML)	415-005-166 1.0 mg	415-035-166 1.0 ml	415-055-166 0.5 ml	415-065-166 1.0 ml
SHEEP	Sheep Anti-Mouse IgG (H+L)		515-005-003 2.0 mg	515-035-003 2.0 ml	515-055-003 1.0 ml	515-065-003 2.0 ml
SHEEP	Sheep Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs Sr Prot)		515-005-062 1.5 mg	515-035-062 1.5 ml	515-055-062 1.0 ml	515-065-062 1.5 ml
SHEEP	Sheep Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)		515-005-071 1.5 mg	515-035-071 1.5 ml	515-055-071 1.0 ml	515-065-071 1.5 ml
SHEEP	Sheep Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)		515-005-072 1.5 mg	515-035-072 1.5 ml	515-055-072 1.0 ml	515-065-072 1.5 ml
	ANTI-RABBIT					
ALPACA	Alpaca Anti-Rabbit IgG (H+L) (min X Bov, Hu, Ms Sr Prot)	(ML)	611-005-215 1.0 mg	611-035-215 1.0 ml	611-055-215 1.0 ml	611-065-215 1.0 ml
DONKEY	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	(ML)	711-005-152 1.0 mg	711-035-152 0.5 ml	711-055-152 0.5 ml	711-065-152 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation)

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
315-475-047 1.0 mg	315-155-047 1.0 mg	315-545-047 1.0 mg	315-095-047 1.0 mg	315-165-047 1.0 mg	315-295-047 1.0 mg	315-585-047 1.0 mg	315-605-047 1.0 mg
315-475-044 1.0 mg	315-155-044 1.5 mg	315-545-044 1.0 mg	315-095-044 1.5 mg	315-165-044 1.5 mg	315-295-044 1.5 mg	315-585-044 1.0 mg	315-605-044 1.0 mg
315-475-048 1.0 mg	315-155-048 1.0 mg	315-545-048 1.0 mg	315-095-048 1.0 mg	315-165-048 1.0 mg	315-295-048 1.0 mg	315-585-048 1.0 mg	315-605-048 1.0 mg
315-475-020 1.0 mg	315-155-020 1.5 mg	315-545-020 1.0 mg	315-095-020 1.5 mg	315-165-020 1.5 mg	315-295-020 1.5 mg	315-585-020 1.0 mg	315-605-020 1.0 mg
315-475-049 1.0 mg	315-155-049 1.0 mg	315-545-049 1.0 mg	315-095-049 1.0 mg	315-165-049 1.0 mg	315-295-049 1.0 mg	315-585-049 1.0 mg	315-605-049 1.0 mg
415-475-166 1.0 mg	415-155-166 1.0 mg	415-545-166 1.0 mg	415-095-166 1.0 mg	415-165-166 1.0 mg	415-295-166 1.0 mg	415-585-166 1.0 mg	415-605-166 1.0 mg
515-475-003 1.5 mg	515-155-003 2.0 mg	515-545-003 1.5 mg	515-095-003 2.0 mg	515-165-003 2.0 mg	515-295-003 2.0 mg	515-585-003 1.5 mg	515-605-003 1.5 mg
515-475-062 1.0 mg	515-155-062 1.5 mg	515-545-062 1.0 mg	515-095-062 1.5 mg	515-165-062 1.5 mg	515-295-062 1.5 mg	515-585-062 1.0 mg	515-605-062 1.0 mg
515-475-071 1.0 mg	515-155-071 1.5 mg	515-545-071 1.0 mg	515-095-071 1.5 mg	515-165-071 1.5 mg	515-295-071 1.5 mg	515-585-071 1.0 mg	515-605-071 1.0 mg
515-475-072 1.0 mg	515-155-072 1.5 mg	515-545-072 1.0 mg	515-095-072 1.5 mg	515-165-072 1.5 mg	515-295-072 1.5 mg	515-585-072 1.0 mg	515-605-072 1.0 mg
611-475-215 1.0 mg	611-155-215 1.0 mg	611-545-215 1.0 mg	611-095-215 1.0 mg	611-165-215 1.0 mg	611-295-215 1.0 mg	611-585-215 1.0 mg	611-605-215 1.0 mg
711-475-152 0.5 mg	711-155-152 0.5 mg	711-545-152 0.5 mg	711-095-152 0.5 mg	711-165-152 0.5 mg	711-295-152 0.5 mg	711-585-152 0.5 mg	711-605-152 0.5 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-RABBIT				
GOAT	Goat Anti-Rabbit IgG (H+L)	111-005-003 2.0 mg	111-035-003 2.0 ml	111-055-003 1.0 ml	111-065-003 2.0 ml
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu Sr Prot)	111-005-045 1.5 mg	111-035-045 1.5 ml	111-055-045 1.0 ml	111-065-045 1.5 ml
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot) (ML)	111-005-144 1.5 mg	111-035-144 1.5 ml	111-055-144 1.0 ml	111-065-144 1.5 ml
GOAT	Goat Anti-Rabbit IgG, Fc fragment specific	111-005-008 2.0 mg	111-035-008 2.0 ml	111-055-008 1.0 ml	111-065-008 2.0 ml
GOAT	Goat Anti-Rabbit IgG, Fc fragment specific (min X Hu Sr Prot)	111-005-046 1.5 mg	111-035-046 1.5 ml	111-055-046 1.0 ml	111-065-046 1.5 ml
GOAT	Goat Anti-Rabbit IgG, $F(ab')_2$ fragment specific	111-005-006 2.0 mg	111-035-006 2.0 ml	111-055-006 1.0 ml	111-065-006 2.0 ml
GOAT	Goat Anti-Rabbit IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot)	111-005-047 1.5 mg	111-035-047 1.5 ml	111-055-047 1.0 ml	111-065-047 1.5 ml
MOUSE	Mouse Anti-Rabbit IgG (H+L) (min X Hu, Gt, Ms, Shp Sr Prot) (ML)	211-005-109 1.5 mg	211-035-109 1.0 ml	211-055-109 0.5 ml	211-065-109 1.0 ml
MOUSE	IgG Fraction Monoclonal Mouse Anti-Rabbit IgG, light chain specific (min X Bov, Gt, Ar Hms, Hrs, Hu, Ms, Rat, Shp Ig)	211-002-171 1.0 mg	211-032-171 0.5 ml	211-052-171 0.5 ml	211-062-171 0.5 ml
	ANTI-RAT				
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot) (ML)	712-005-150 1.0 mg	712-035-150 0.5 ml	712-055-150 0.5 ml	712-065-150 0.5 ml
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms**, Rb, Shp Sr Prot) (SP) (ML)	712-005-153 1.0 mg	712-035-153 0.5 ml	712-055-153 0.5 ml	712-065-153 0.5 ml
GOAT	Goat Anti-Rat IgG (H+L)	112-005-003 2.0 mg	112-035-003 2.0 ml	112-055-003 1.0 ml	112-065-003 2.0 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
111-475-003 1.5 mg	111-155-003 2.0 mg	111-545-003 1.5 mg	111-095-003 2.0 mg	111-165-003 2.0 mg	111-295-003 2.0 mg	111-585-003 1.5 mg	111-605-003 1.5 mg
111-475-045 1.0 mg	111-155-045 1.5 mg	111-545-045 1.0 mg	111-095-045 1.5 mg	111-165-045 1.5 mg	111-295-045 1.5 mg	111-585-045 1.0 mg	111-605-045 1.0 mg
111-475-144 1.0 mg	111-155-144 1.5 mg	111-545-144 1.0 mg	111-095-144 1.5 mg	111-165-144 1.5 mg	111-295-144 1.5 mg	111-585-144 1.0 mg	111-605-144 1.0 mg
111-475-008 1.5 mg	111-155-008 2.0 mg	111-545-008 1.5 mg	111-095-008 2.0 mg	111-165-008 2.0 mg	111-295-008 2.0 mg	111-585-008 1.5 mg	111-605-008 1.5 mg
111-475-046 1.0 mg	111-155-046 1.5 mg	111-545-046 1.0 mg	111-095-046 1.5 mg	111-165-046 1.5 mg	111-295-046 1.5 mg	111-585-046 1.0 mg	111-605-046 1.0 mg
111-475-006 1.5 mg	111-155-006 2.0 mg	111-545-006 1.5 mg	111-095-006 2.0 mg	111-165-006 2.0 mg	111-295-006 2.0 mg	111-585-006 1.5 mg	111-605-006 1.5 mg
111-475-047 1.0 mg	111-155-047 1.5 mg	111-545-047 1.0 mg	111-095-047 1.5 mg	111-165-047 1.5 mg	111-295-047 1.5 mg	111-585-047 1.0 mg	111-605-047 1.0 mg
211-475-109 1.0 mg	211-155-109 1.0 mg	211-545-109 1.0 mg	211-095-109 1.0 mg	211-165-109 1.0 mg	211-295-109 1.0 mg	211-585-109 1.0 mg	211-605-109 1.0 mg
		211-542-171 0.5 mg		211-162-171 0.5 mg		211-582-171 0.5 mg	211-602-171 0.5 mg
712-475-150 0.5 mg	712-155-150 0.5 mg	712-545-150 0.5 mg	712-095-150 0.5 mg	712-165-150 0.5 mg	712-295-150 0.5 mg	712-585-150 0.5 mg	712-605-150 0.5 mg
712-475-153 0.5 mg	712-155-153 0.5 mg	712-545-153 0.5 mg	712-095-153 0.5 mg	712-165-153 0.5 mg	712-295-153 0.5 mg	712-585-153 0.5 mg	712-605-153 0.5 mg
112-475-003 1.5 mg	112-155-003 2.0 mg	112-545-003 1.5 mg	112-095-003 2.0 mg	112-165-003 2.0 mg	112-295-003 2.0 mg	112-585-003 1.5 mg	112-605-003 1.5 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-RAT				
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs Sr Prot)	112-005-062 1.5 mg	112-035-062 1.5 ml	112-055-062 1.0 ml	112-065-062 1.5 ml
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot) (ML)	112-005-143 1.5 mg	112-035-143 1.5 ml	112-055-143 1.0 ml	112-065-143 1.5 ml
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Rb Sr Prot) (SP) (ML)	112-005-167 1.0 mg	112-035-167 0.5 ml	112-055-167 0.5 ml	112-065-167 0.5 ml
GOAT	Goat Anti-Rat IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Ms, Rb, Shp Ig) (kLC)	112-005-175 1.0 mg	112-035-175 0.5 ml	112-055-175 0.5 ml	112-065-175 0.5 ml
GOAT	Goat Anti-Rat IgG, Fc_γ fragment specific (ML)	112-005-008 2.0 mg	112-035-008 2.0 ml	112-055-008 1.0 ml	112-065-008 2.0 ml
GOAT	Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-005-071 1.5 mg	112-035-071 1.5 ml	112-055-071 1.0 ml	112-065-071 1.5 ml
GOAT	Goat Anti-Rat IgG, $F(ab')_2$ fragment specific	112-005-006 2.0 mg	112-035-006 2.0 ml	112-055-006 1.0 ml	112-065-006 2.0 ml
GOAT	Goat Anti-Rat IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)	112-005-072 1.5 mg	112-035-072 1.5 ml	112-055-072 1.0 ml	112-065-072 1.5 ml
GOAT	Goat Anti-Rat IgG + IgM (H+L)	112-005-044 2.0 mg	112-035-044 2.0 ml	112-055-044 1.0 ml	112-065-044 2.0 ml
GOAT	Goat Anti-Rat IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)	112-005-068 1.5 mg	112-035-068 1.5 ml	112-055-068 1.0 ml	112-065-068 1.5 ml
GOAT	Goat Anti-Rat IgM, μ chain specific (ML)	112-005-020 2.0 mg	112-035-020 2.0 ml	112-055-020 1.0 ml	112-065-020 2.0 ml
GOAT	Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-005-075 1.0 mg	112-035-075 1.0 ml	112-055-075 1.0 ml	112-065-075 1.0 ml
MOUSE	Mouse Anti-Rat IgG (H+L) (min X Ms Sr Prot)	212-005-082 2.0 mg	212-035-082 1.5 ml	212-055-082 1.0 ml	212-065-082 1.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (kLC) This antibody reacts primarily with kappa light chains. It is not suitable for detection of primary antibodies with lambda light chains.

(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
112-475-062 1.0 mg	112-155-062 1.5 mg	112-545-062 1.0 mg	112-095-062 1.5 mg	112-165-062 1.5 mg	112-295-062 1.5 mg	112-585-062 1.0 mg	112-605-062 1.0 mg
112-475-143 1.0 mg	112-155-143 1.5 mg	112-545-143 1.0 mg	112-095-143 1.5 mg	112-165-143 1.5 mg	112-295-143 1.5 mg	112-585-143 1.0 mg	112-605-143 1.0 mg
112-475-167 0.5 mg	112-155-167 0.5 mg	112-545-167 0.5 mg	112-095-167 0.5 mg	112-165-167 0.5 mg	112-295-167 0.5 mg	112-585-167 0.5 mg	112-605-167 0.5 mg
		112-545-175 0.5 mg		112-165-175 0.5 mg		112-585-175 0.5 mg	112-605-175 0.5 mg
112-475-008 1.5 mg	112-155-008 2.0 mg	112-545-008 1.5 mg	112-095-008 2.0 mg	112-165-008 2.0 mg	112-295-008 2.0 mg	112-585-008 1.5 mg	112-605-008 1.5 mg
112-475-071 1.0 mg	112-155-071 1.5 mg	112-545-071 1.0 mg	112-095-071 1.5 mg	112-165-071 1.5 mg	112-295-071 1.5 mg	112-585-071 1.0 mg	112-605-071 1.0 mg
112-475-006 1.5 mg	112-155-006 2.0 mg	112-545-006 1.5 mg	112-095-006 2.0 mg	112-165-006 2.0 mg	112-295-006 2.0 mg	112-585-006 1.5 mg	112-605-006 1.5 mg
112-475-072 1.0 mg	112-155-072 1.5 mg	112-545-072 1.0 mg	112-095-072 1.5 mg	112-165-072 1.5 mg	112-295-072 1.5 mg	112-585-072 1.0 mg	112-605-072 1.0 mg
112-475-044 1.5 mg	112-155-044 2.0 mg	112-545-044 1.5 mg	112-095-044 2.0 mg	112-165-044 2.0 mg	112-295-044 2.0 mg	112-585-044 1.5 mg	112-605-044 1.5 mg
112-475-068 1.0 mg	112-155-068 1.5 mg	112-545-068 1.0 mg	112-095-068 1.5 mg	112-165-068 1.5 mg	112-295-068 1.5 mg	112-585-068 1.0 mg	112-605-068 1.0 mg
112-475-020 1.5 mg	112-155-020 2.0 mg	112-545-020 1.5 mg	112-095-020 2.0 mg	112-165-020 2.0 mg	112-295-020 2.0 mg	112-585-020 1.5 mg	112-605-020 1.5 mg
112-475-075 1.0 mg	112-155-075 1.0 mg	112-545-075 1.0 mg	112-095-075 1.0 mg	112-165-075 1.0 mg	112-295-075 1.0 mg	112-585-075 1.0 mg	112-605-075 1.0 mg
212-475-082 1.0 mg	212-155-082 1.5 mg	212-545-082 1.0 mg	212-095-082 1.5 mg	212-165-082 1.5 mg	212-295-082 1.5 mg	212-585-082 1.0 mg	212-605-082 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-RAT				
MOUSE	Mouse Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Ms, Gt, Rb Sr Prot) (ML)	212-005-168 1.5 mg	212-035-168 1.0 ml	212-055-168 0.5 ml	212-065-168 1.0 ml
MOUSE	Mouse Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs, Ms Sr Prot)	212-005-104 1.5 mg	212-035-104 1.0 ml	212-055-104 0.5 ml	212-065-104 1.0 ml
MOUSE	Mouse Anti-Rat IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs, Ms Sr Prot)	212-005-106 1.5 mg	212-035-106 1.0 ml	212-055-106 0.5 ml	212-065-106 1.0 ml
RABBIT	Rabbit Anti-Rat IgG (H+L)	312-005-003 2.0 mg	312-035-003 1.5 ml	312-055-003 1.0 ml	312-065-003 1.5 ml
RABBIT	Rabbit Anti-Rat IgG (H+L) (min X Hu Sr Prot)	312-005-045 1.5 mg	312-035-045 1.0 ml	312-055-045 0.5 ml	312-065-045 1.0 ml
RABBIT	Rabbit Anti-Rat IgG, Fc_γ fragment specific (min X Hu Sr Prot)	312-005-046 1.5 mg	312-035-046 1.0 ml	312-055-046 0.5 ml	312-065-046 1.0 ml
RABBIT	Rabbit Anti-Rat IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot)	312-005-047 1.5 mg	312-035-047 1.0 ml	312-055-047 0.5 ml	312-065-047 1.0 ml
RABBIT	Rabbit Anti-Rat IgG + IgM (H+L)	312-005-044 2.0 mg	312-035-044 1.5 ml	312-055-044 1.0 ml	312-065-044 1.5 ml
RABBIT	Rabbit Anti-Rat IgG + IgM (H+L) (min X Hu Sr Prot)	312-005-048 1.5 mg	312-035-048 1.0 ml	312-055-048 0.5 ml	312-065-048 1.0 ml
RABBIT	Rabbit Anti-Rat IgM, μ chain specific	312-005-020 2.0 mg	312-035-020 1.5 ml	312-055-020 1.0 ml	312-065-020 1.5 ml
RABBIT	Rabbit Anti-Rat IgM, μ chain specific (min X Hu Sr Prot)	312-005-049 1.5 mg	312-035-049 1.0 ml	312-055-049 0.5 ml	312-065-049 1.0 ml
	ANTI-SHEEP				
DONKEY	Donkey Anti-Sheep IgG (H+L) (!)	713-005-003 1.0 mg	713-035-003 1.0 ml	713-055-003 1.0 ml	713-065-003 1.0 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
212-475-168 1.0 mg	212-155-168 1.0 mg	212-545-168 1.0 mg	212-095-168 1.0 mg	212-165-168 1.0 mg	212-295-168 1.0 mg	212-585-168 1.0 mg	212-605-168 1.0 mg
212-475-104 1.0 mg	212-155-104 1.0 mg	212-545-104 1.0 mg	212-095-104 1.0 mg	212-165-104 1.0 mg	212-295-104 1.0 mg	212-585-104 1.0 mg	212-605-104 1.0 mg
212-475-106 1.0 mg	212-155-106 1.0 mg	212-545-106 1.0 mg	212-095-106 1.0 mg	212-165-106 1.0 mg	212-295-106 1.0 mg	212-585-106 1.0 mg	212-605-106 1.0 mg
312-475-003 1.0 mg	312-155-003 1.5 mg	312-545-003 1.0 mg	312-095-003 1.5 mg	312-165-003 1.5 mg	312-295-003 1.5 mg	312-585-003 1.0 mg	312-605-003 1.0 mg
312-475-045 1.0 mg	312-155-045 1.0 mg	312-545-045 1.0 mg	312-095-045 1.0 mg	312-165-045 1.0 mg	312-295-045 1.0 mg	312-585-045 1.0 mg	312-605-045 1.0 mg
312-475-046 1.0 mg	312-155-046 1.0 mg	312-545-046 1.0 mg	312-095-046 1.0 mg	312-165-046 1.0 mg	312-295-046 1.0 mg	312-585-046 1.0 mg	312-605-046 1.0 mg
312-475-047 1.0 mg	312-155-047 1.0 mg	312-545-047 1.0 mg	312-095-047 1.0 mg	312-165-047 1.0 mg	312-295-047 1.0 mg	312-585-047 1.0 mg	312-605-047 1.0 mg
312-475-044 1.0 mg	312-155-044 1.5 mg	312-545-044 1.0 mg	312-095-044 1.5 mg	312-165-044 1.5 mg	312-295-044 1.5 mg	312-585-044 1.0 mg	312-605-044 1.0 mg
312-475-048 1.0 mg	312-155-048 1.0 mg	312-545-048 1.0 mg	312-095-048 1.0 mg	312-165-048 1.0 mg	312-295-048 1.0 mg	312-585-048 1.0 mg	312-605-048 1.0 mg
312-475-020 1.0 mg	312-155-020 1.5 mg	312-545-020 1.0 mg	312-095-020 1.5 mg	312-165-020 1.5 mg	312-295-020 1.5 mg	312-585-020 1.0 mg	312-605-020 1.0 mg
312-475-049 1.0 mg	312-155-049 1.0 mg	312-545-049 1.0 mg	312-095-049 1.0 mg	312-165-049 1.0 mg	312-295-049 1.0 mg	312-585-049 1.0 mg	312-605-049 1.0 mg
713-475-003 1.0 mg	713-155-003 1.0 mg	713-545-003 1.0 mg	713-095-003 1.0 mg	713-165-003 1.0 mg	713-295-003 1.0 mg	713-585-003 1.0 mg	713-605-003 1.0 mg

WHOLE IgG SECONDARY ANTIBODIES

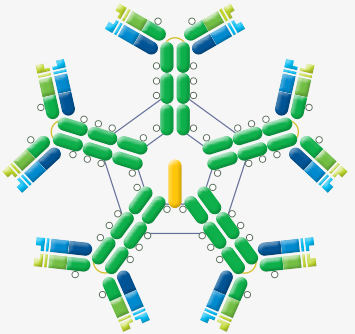
	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-SHEEP				
DONKEY	Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (ML) (!)	713-005-147 1.0 mg	713-035-147 0.5 ml	713-055-147 0.5 ml	713-065-147 0.5 ml
MOUSE	IgG Fraction Monoclonal Mouse Anti-Sheep IgG, light chain specific (min X Bov, Hrs, Hu, Ms, Rb, Rat Ig)	213-002-177 1.0 mg	213-032-177 0.5 ml	213-052-177 0.5 ml	213-062-177 0.5 ml
RABBIT	Rabbit Anti-Sheep IgG (H+L) (!)	313-005-003 2.0 mg	313-035-003 1.5 ml	313-055-003 1.0 ml	313-065-003 1.5 ml
	Rabbit Anti-Sheep IgG (H+L) (min X Hu Sr Prot) (!)	313-005-045 1.5 mg	313-035-045 1.0 ml	313-055-045 0.5 ml	313-065-045 1.0 ml
	Rabbit Anti-Sheep IgG, Fc fragment specific (min X Hu Sr Prot) (!)	313-005-046 1.5 mg	313-035-046 1.0 ml	313-055-046 0.5 ml	313-065-046 1.0 ml
	Rabbit Anti-Sheep IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot) (!)	313-005-047 1.5 mg	313-035-047 1.0 ml	313-055-047 0.5 ml	313-065-047 1.0 ml
	ANTI-SWINE				
GOAT	Goat Anti-Swine IgG (H+L)	114-005-003 2.0 mg	114-035-003 2.0 ml	114-055-003 1.0 ml	114-065-003 2.0 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

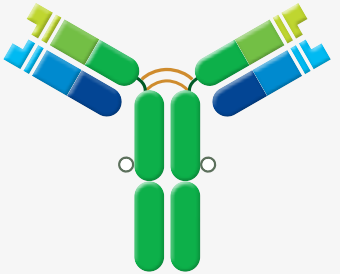
(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
713-475-147 0.5 mg	713-155-147 0.5 mg	713-545-147 0.5 mg	713-095-147 0.5 mg	713-165-147 0.5 mg	713-295-147 0.5 mg	713-585-147 0.5 mg	713-605-147 0.5 mg
		213-542-177 0.5 mg		213-162-177 0.5 mg		213-582-177 0.5 mg	213-602-177 0.5 mg
313-475-003 1.0 mg	313-155-003 1.5 mg	313-545-003 1.0 mg	313-095-003 1.5 mg	313-165-003 1.5 mg	313-295-003 1.5 mg	313-585-003 1.0 mg	313-605-003 1.0 mg
313-475-045 1.0 mg	313-155-045 1.0 mg	313-545-045 1.0 mg	313-095-045 1.0 mg	313-165-045 1.0 mg	313-295-045 1.0 mg	313-585-045 1.0 mg	313-605-045 1.0 mg
313-475-046 1.0 mg	313-155-046 1.0 mg	313-545-046 1.0 mg	313-095-046 1.0 mg	313-165-046 1.0 mg	313-295-046 1.0 mg	313-585-046 1.0 mg	313-605-046 1.0 mg
313-475-047 1.0 mg	313-155-047 1.0 mg	313-545-047 1.0 mg	313-095-047 1.0 mg	313-165-047 1.0 mg	313-295-047 1.0 mg	313-585-047 1.0 mg	313-605-047 1.0 mg
114-475-003 1.5 mg	114-155-003 2.0 mg	114-545-003 1.5 mg	114-095-003 2.0 mg	114-165-003 2.0 mg	114-295-003 2.0 mg	114-585-003 1.5 mg	114-605-003 1.5 mg

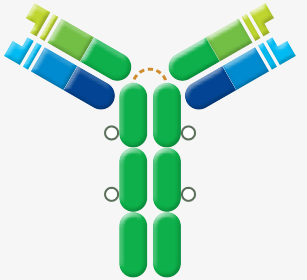
IgM



IgG



IgY



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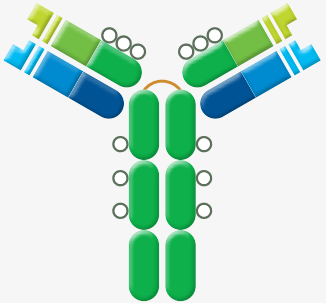

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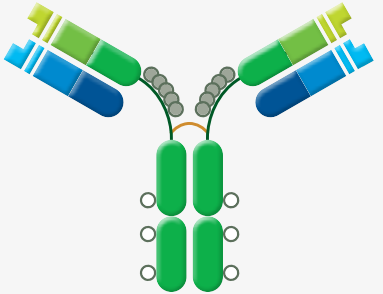
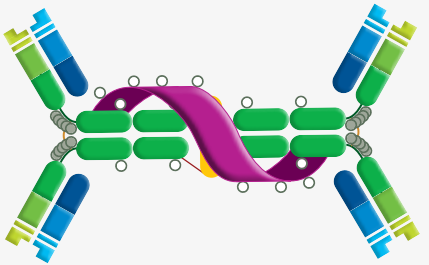
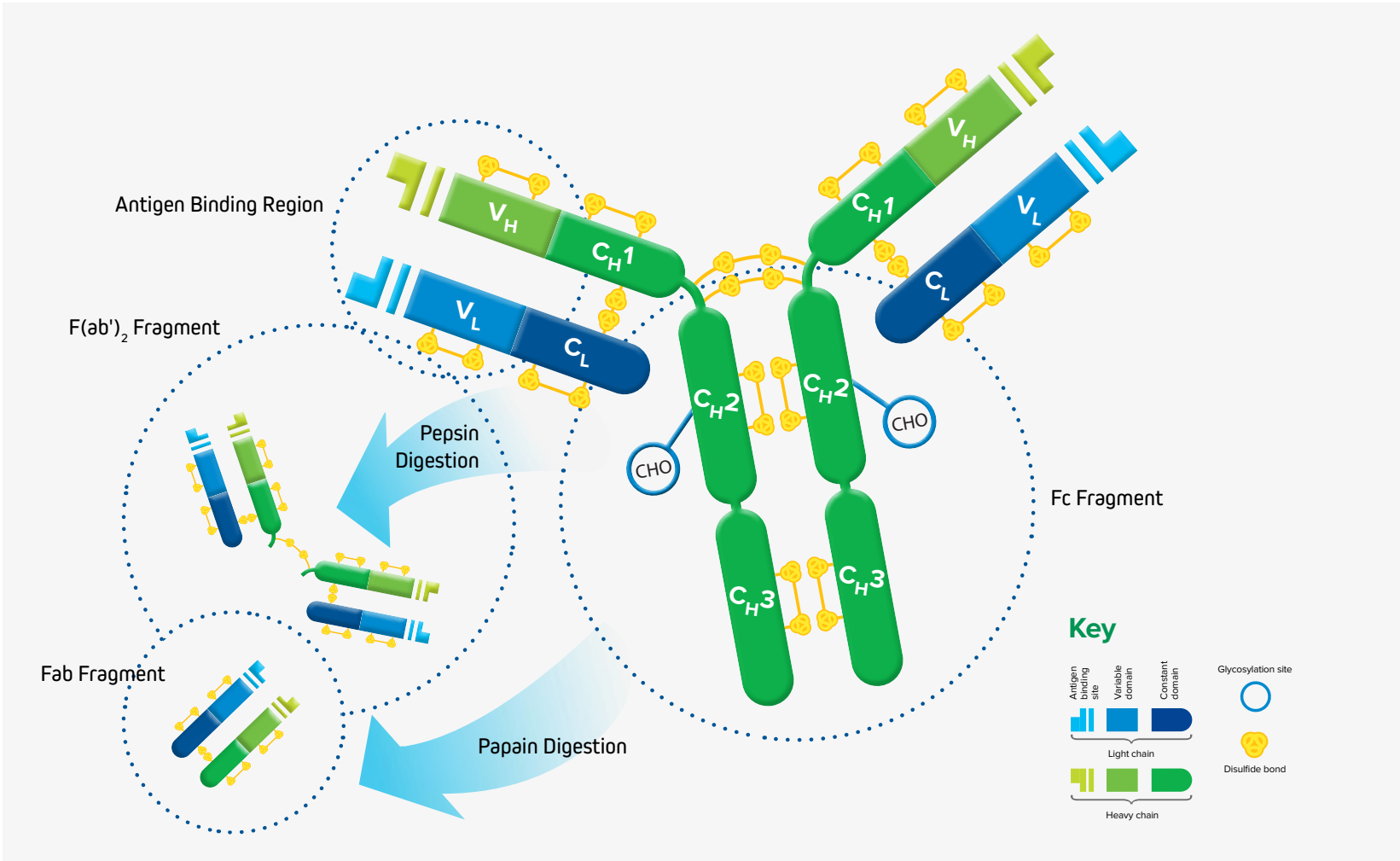


IgA




Antigen Binding Region
F(ab')2 Fragment
Fab Fragment
Pepsin Digestion
Papain Digestion
Fc Fragment
VH
CH1
VL
CL
CH2
CH3
CHO
Key
Antigen binding site
Variable domain
Constant domain
Light chain
Heavy chain
Glycosylation site
Disulfide bond

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-BOVINE				
GOAT	$F(ab')_2$ Fragment Goat Anti-Bovine IgG (H+L) (!)	101-006-003 1.0 mg	101-036-003 0.5 ml	101-056-003 0.5 ml	101-066-003 0.5 ml
	ANTI-CHICKEN				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	703-006-155 0.5 mg	703-036-155 0.3 ml	703-056-155 0.3 ml	703-066-155 0.3 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Chicken IgY (IgG) (H+L)	303-006-003 1.0 mg	303-036-003 0.5 ml	303-056-003 0.5 ml	303-066-003 0.5 ml
	ANTI-GOAT				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (ML) (!)	705-006-147 0.5 mg	705-036-147 0.3 ml	705-056-147 0.3 ml	705-066-147 0.3 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG (H+L) (!)	305-006-003 1.0 mg	305-036-003 0.5 ml	305-056-003 0.5 ml	305-066-003 0.5 ml
	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG (H+L) (min X Hu Sr Prot) (!)	305-006-045 0.5 mg	305-036-045 0.5 ml	305-056-045 0.5 ml	305-066-045 0.5 ml
	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG, Fc fragment specific (!)	305-006-008 1.0 mg	305-036-008 0.5 ml	305-056-008 0.5 ml	305-066-008 0.5 ml
	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG, Fc fragment specific (min X Hu Sr Prot) (!)	305-006-046 0.5 mg	305-036-046 0.5 ml	305-056-046 0.5 ml	305-066-046 0.5 ml
	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG, $F(ab')_2$ fragment specific (!)	305-006-006 1.0 mg	305-036-006 0.5 ml	305-056-006 0.5 ml	305-066-006 0.5 ml
	$F(ab')_2$ Fragment Rabbit Anti-Goat IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot) (!)	305-006-047 0.5 mg	305-036-047 0.5 ml	305-056-047 0.5 ml	305-066-047 0.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
	101-156-003 1.0 mg	101-546-003 0.75 mg	101-096-003 1.0 mg	101-166-003 1.0 mg	101-296-003 1.0 mg	101-586-003 0.75 mg	101-606-003 0.75 mg
703-476-155 0.3 mg	703-156-155 0.3 mg	703-546-155 0.3 mg	703-096-155 0.3 mg	703-166-155 0.3 mg	703-296-155 0.3 mg	703-586-155 0.3 mg	703-606-155 0.3 mg
	303-156-003 0.5 mg	303-546-003 0.5 mg	303-096-003 0.5 mg	303-166-003 0.5 mg	303-296-003 0.5 mg	303-586-003 0.5 mg	303-606-003 0.5 mg
705-476-147 0.3 mg	705-156-147 0.3 mg	705-546-147 0.3 mg	705-096-147 0.3 mg	705-166-147 0.3 mg	705-296-147 0.3 mg	705-586-147 0.3 mg	705-606-147 0.3 mg
	305-156-003 0.5 mg	305-546-003 0.5 mg	305-096-003 0.5 mg	305-166-003 0.5 mg	305-296-003 0.5 mg	305-586-003 0.5 mg	305-606-003 0.5 mg
	305-156-045 0.5 mg	305-546-045 0.5 mg	305-096-045 0.5 mg	305-166-045 0.5 mg	305-296-045 0.5 mg	305-586-045 0.5 mg	305-606-045 0.5 mg
	305-156-008 0.5 mg	305-546-008 0.5 mg	305-096-008 0.5 mg	305-166-008 0.5 mg	305-296-008 0.5 mg	305-586-008 0.5 mg	305-606-008 0.5 mg
	305-156-046 0.5 mg	305-546-046 0.5 mg	305-096-046 0.5 mg	305-166-046 0.5 mg	305-296-046 0.5 mg	305-586-046 0.5 mg	305-606-046 0.5 mg
	305-156-006 0.5 mg	305-546-006 0.5 mg	305-096-006 0.5 mg	305-166-006 0.5 mg	305-296-006 0.5 mg	305-586-006 0.5 mg	305-606-006 0.5 mg
	305-156-047 0.5 mg	305-546-047 0.5 mg	305-096-047 0.5 mg	305-166-047 0.5 mg	305-296-047 0.5 mg	305-586-047 0.5 mg	305-606-047 0.5 mg

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-GUINEA PIG				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	706-006-148 0.5 mg	706-036-148 0.3 ml	706-056-148 0.3 ml	706-066-148 0.3 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Guinea Pig IgG (H+L)	106-006-003 1.0 mg	106-036-003 0.5 ml	106-056-003 0.5 ml	106-066-003 0.5 ml
	ANTI-SYRIAN HAMSTER				
GOAT	$F(ab')_2$ Fragment Goat Anti-Syrian Hamster IgG (H+L) (min X Bov, Hrs, Hu, Ms, Rb, Rat Sr Prot) (ML)	107-006-142 0.5 mg	107-036-142 0.5 ml	107-056-142 0.5 ml	107-066-142 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Syrian Hamster IgG (H+L)	307-006-003 1.0 mg	307-036-003 0.5 ml	307-056-003 0.5 ml	307-066-003 0.5 ml
	ANTI-HORSE				
GOAT	$F(ab')_2$ Fragment Goat Anti-Horse IgG (H+L)	108-006-003 1.0 mg	108-036-003 0.5 ml	108-056-003 0.5 ml	108-066-003 0.5 ml
	ANTI-HUMAN				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot) (ML)	709-006-149 0.5 mg	709-036-149 0.3 ml	709-056-149 0.3 ml	709-066-149 0.3 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	709-006-098 1.0 mg	709-036-098 0.5 ml	709-056-098 0.5 ml	709-066-098 0.5 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov, Hrs Sr Prot)	709-006-073 1.0 mg	709-036-073 0.5 ml	709-056-073 0.5 ml	709-066-073 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG (H+L)	109-006-003 1.0 mg	109-036-003 0.5 ml	109-056-003 0.5 ml	109-066-003 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG (H+L) (min X Bov, Hrs, Ms Sr Prot)	109-006-088 1.0 mg	109-036-088 0.5 ml	109-056-088 0.5 ml	109-066-088 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
706-476-148 0.3 mg	706-156-148 0.3 mg	706-546-148 0.3 mg	706-096-148 0.3 mg	706-166-148 0.3 mg	706-296-148 0.3 mg	706-586-148 0.3 mg	706-606-148 0.3 mg
	106-156-003 1.0 mg	106-546-003 0.75 mg	106-096-003 1.0 mg	106-166-003 1.0 mg	106-296-003 1.0 mg	106-586-003 0.75 mg	106-606-003 0.75 mg
	107-156-142 0.5 mg	107-546-142 0.5 mg	107-096-142 0.5 mg	107-166-142 0.5 mg	107-296-142 0.5 mg	107-586-142 0.5 mg	107-606-142 0.5 mg
	307-156-003 0.5 mg	307-546-003 0.5 mg	307-096-003 0.5 mg	307-166-003 0.5 mg	307-296-003 0.5 mg	307-586-003 0.5 mg	307-606-003 0.5 mg
	108-156-003 1.0 mg	108-546-003 0.75 mg	108-096-003 1.0 mg	108-166-003 1.0 mg	108-296-003 1.0 mg	108-586-003 0.75 mg	108-606-003 0.75 mg
709-476-149 0.3 mg	709-156-149 0.3 mg	709-546-149 0.3 mg	709-096-149 0.3 mg	709-166-149 0.3 mg	709-296-149 0.3 mg	709-586-149 0.3 mg	709-606-149 0.3 mg
	709-156-098 1.0 mg	709-546-098 0.75 mg	709-096-098 1.0 mg	709-166-098 1.0 mg	709-296-098 1.0 mg	709-586-098 0.75 mg	709-606-098 0.75 mg
	709-156-073 1.0 mg	709-546-073 0.75 mg	709-096-073 1.0 mg	709-166-073 1.0 mg	709-296-073 1.0 mg	709-586-073 0.75 mg	709-606-073 0.75 mg
	109-156-003 1.0 mg	109-546-003 0.75 mg	109-096-003 1.0 mg	109-166-003 1.0 mg	109-296-003 1.0 mg	109-586-003 0.75 mg	109-606-003 0.75 mg
109-476-088 0.75 mg	109-156-088 1.0 mg	109-546-088 0.75 mg	109-096-088 1.0 mg	109-166-088 1.0 mg	109-296-088 1.0 mg	109-586-088 0.75 mg	109-606-088 0.75 mg

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description		Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-HUMAN					
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, Fc_γ fragment specific		109-006-008 1.0 mg	109-036-008 0.5 ml	109-056-008 0.5 ml	109-066-008 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	(ML)	109-006-098 1.0 mg	109-036-098 0.5 ml	109-056-098 0.5 ml	109-066-098 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Ms, Rb Sr Prot)	(ML)	109-006-170 1.0 mg	109-036-170 0.5 ml	109-056-170 0.5 ml	109-066-170 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, $F(ab')_2$ fragment specific		109-006-006 1.0 mg	109-036-006 0.5 ml	109-056-006 0.5 ml	109-066-006 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, $F(ab')_2$ fragment specific (min X Bov, Hrs, Ms Sr Prot)		109-006-097 1.0 mg	109-036-097 0.5 ml	109-056-097 0.5 ml	109-066-097 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG + IgM (H+L) (min X Bov Sr Prot)		109-006-127 1.0 mg	109-036-127 0.5 ml	109-056-127 0.5 ml	109-066-127 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgA + IgG + IgM (H+L)		109-006-064 1.0 mg	109-036-064 0.5 ml	109-056-064 0.5 ml	109-066-064 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov Sr Prot)	(ML)	109-006-129 1.0 mg	109-036-129 0.5 ml	109-056-129 0.5 ml	109-066-129 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human Serum IgA, α chain specific	(ML)	109-006-011 1.0 mg	109-036-011 0.5 ml	109-056-011 0.5 ml	109-066-011 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Human IgG (H+L)		309-006-003 1.0 mg	309-036-003 0.5 ml	309-056-003 0.5 ml	309-066-003 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Human IgG, Fc_γ fragment specific		309-006-008 1.0 mg	309-036-008 0.5 ml	309-056-008 0.5 ml	309-066-008 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Human IgM, $Fc_{5\mu}$ fragment specific		309-006-043 1.0 mg	309-036-043 0.5 ml	309-056-043 0.5 ml	309-066-043 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
	109-156-008 1.0 mg	109-546-008 0.75 mg	109-096-008 1.0 mg	109-166-008 1.0 mg	109-296-008 1.0 mg	109-586-008 0.75 mg	109-606-008 0.75 mg
109-476-098 0.75 mg	109-156-098 1.0 mg	109-546-098 0.75 mg	109-096-098 1.0 mg	109-166-098 1.0 mg	109-296-098 1.0 mg	109-586-098 0.75 mg	109-606-098 0.75 mg
109-476-170 0.75 mg	109-156-170 1.0 mg	109-546-170 0.75 mg	109-096-170 1.0 mg	109-166-170 1.0 mg	109-296-170 1.0 mg	109-586-170 0.75 mg	109-606-170 0.75 mg
	109-156-006 1.0 mg	109-546-006 0.75 mg	109-096-006 1.0 mg	109-166-006 1.0 mg	109-296-006 1.0 mg	109-586-006 0.75 mg	109-606-006 0.75 mg
109-476-097 0.75 mg	109-156-097 1.0 mg	109-546-097 0.75 mg	109-096-097 1.0 mg	109-166-097 1.0 mg	109-296-097 1.0 mg	109-586-097 0.75 mg	109-606-097 0.75 mg
	109-156-127 1.0 mg	109-546-127 0.75 mg	109-096-127 1.0 mg	109-166-127 1.0 mg	109-296-127 1.0 mg	109-586-127 0.75 mg	109-606-127 0.75 mg
	109-156-064 1.0 mg	109-546-064 0.75 mg	109-096-064 1.0 mg	109-166-064 1.0 mg	109-296-064 1.0 mg	109-586-064 0.75 mg	109-606-064 0.75 mg
109-476-129 0.75 mg	109-156-129 1.0 mg	109-546-129 0.75 mg	109-096-129 1.0 mg	109-166-129 1.0 mg	109-296-129 1.0 mg	109-586-129 0.75 mg	109-606-129 0.75 mg
	109-156-011 1.0 mg	109-546-011 0.75 mg	109-096-011 1.0 mg	109-166-011 1.0 mg	109-296-011 1.0 mg	109-586-011 0.75 mg	109-606-011 0.75 mg
	309-156-003 0.5 mg	309-546-003 0.5 mg	309-096-003 0.5 mg	309-166-003 0.5 mg	309-296-003 0.5 mg	309-586-003 0.5 mg	309-606-003 0.5 mg
	309-156-008 0.5 mg	309-546-008 0.5 mg	309-096-008 0.5 mg	309-166-008 0.5 mg	309-296-008 0.5 mg	309-586-008 0.5 mg	309-606-008 0.5 mg
	309-156-043 0.5 mg	309-546-043 0.5 mg	309-096-043 0.5 mg	309-166-043 0.5 mg	309-296-043 0.5 mg	309-586-043 0.5 mg	309-606-043 0.5 mg

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot) (ML)	715-006-150 0.5 mg	715-036-150 0.3 ml	715-056-150 0.3 ml	715-066-150 0.3 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot) (SP) (ML)	715-006-151 0.5 mg	715-036-151 0.3 ml	715-056-151 0.3 ml	715-066-151 0.3 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Mouse IgM, μ chain specific	715-006-020 1.0 mg	715-036-020 0.5 ml	715-056-020 0.5 ml	715-066-020 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L)	115-006-003 1.0 mg	115-036-003 0.5 ml	115-056-003 0.5 ml	115-066-003 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs Sr Prot)	115-006-062 1.0 mg	115-036-062 0.5 ml	115-056-062 0.5 ml	115-066-062 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot) (ML)	115-006-146 1.0 mg	115-036-146 0.5 ml	115-056-146 0.5 ml	115-066-146 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, Fc_γ fragment specific (ML)	115-006-008 1.0 mg	115-036-008 0.5 ml	115-056-008 0.5 ml	115-066-008 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-006-071 1.0 mg	115-036-071 0.5 ml	115-056-071 0.5 ml	115-066-071 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific	115-006-006 1.0 mg	115-036-006 0.5 ml	115-056-006 0.5 ml	115-066-006 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)	115-006-072 1.0 mg	115-036-072 0.5 ml	115-056-072 0.5 ml	115-066-072 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)	115-006-068 1.0 mg	115-036-068 0.5 ml	115-056-068 0.5 ml	115-066-068 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgM, μ chain specific (ML)	115-006-020 1.0 mg	115-036-020 0.5 ml	115-056-020 0.5 ml	115-066-020 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-006-075 1.0 mg	115-036-075 0.5 ml	115-056-075 0.5 ml	115-066-075 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
715-476-150 0.3 mg	715-156-150 0.3 mg	715-546-150 0.3 mg	715-096-150 0.3 mg	715-166-150 0.3 mg	715-296-150 0.3 mg	715-586-150 0.3 mg	715-606-150 0.3 mg
715-476-151 0.3 mg	715-156-151 0.3 mg	715-546-151 0.3 mg	715-096-151 0.3 mg	715-166-151 0.3 mg	715-296-151 0.3 mg	715-586-151 0.3 mg	715-606-151 0.3 mg
715-476-020 0.75 mg	715-156-020 1.0 mg	715-546-020 0.75 mg	715-096-020 1.0 mg	715-166-020 1.0 mg	715-296-020 1.0 mg	715-586-020 0.75 mg	715-606-020 0.75 mg
	115-156-003 1.0 mg	115-546-003 0.75 mg	115-096-003 1.0 mg	115-166-003 1.0 mg	115-296-003 1.0 mg	115-586-003 0.75 mg	115-606-003 0.75 mg
	115-156-062 1.0 mg	115-546-062 0.75 mg	115-096-062 1.0 mg	115-166-062 1.0 mg	115-296-062 1.0 mg	115-586-062 0.75 mg	115-606-062 0.75 mg
115-476-146 0.75 mg	115-156-146 1.0 mg	115-546-146 0.75 mg	115-096-146 1.0 mg	115-166-146 1.0 mg	115-296-146 1.0 mg	115-586-146 0.75 mg	115-606-146 0.75 mg
	115-156-008 1.0 mg	115-546-008 0.75 mg	115-096-008 1.0 mg	115-166-008 1.0 mg	115-296-008 1.0 mg	115-586-008 0.75 mg	115-606-008 0.75 mg
115-476-071 0.75 mg	115-156-071 1.0 mg	115-546-071 0.75 mg	115-096-071 1.0 mg	115-166-071 1.0 mg	115-296-071 1.0 mg	115-586-071 0.75 mg	115-606-071 0.75 mg
	115-156-006 1.0 mg	115-546-006 0.75 mg	115-096-006 1.0 mg	115-166-006 1.0 mg	115-296-006 1.0 mg	115-586-006 0.75 mg	115-606-006 0.75 mg
115-476-072 0.75 mg	115-156-072 1.0 mg	115-546-072 0.75 mg	115-096-072 1.0 mg	115-166-072 1.0 mg	115-296-072 1.0 mg	115-586-072 0.75 mg	115-606-072 0.75 mg
	115-156-068 1.0 mg	115-546-068 0.75 mg	115-096-068 1.0 mg	115-166-068 1.0 mg	115-296-068 1.0 mg	115-586-068 0.75 mg	115-606-068 0.75 mg
	115-156-020 1.0 mg	115-546-020 0.75 mg	115-096-020 1.0 mg	115-166-020 1.0 mg	115-296-020 1.0 mg	115-586-020 0.75 mg	115-606-020 0.75 mg
115-476-075 0.75 mg	115-156-075 1.0 mg	115-546-075 0.75 mg	115-096-075 1.0 mg	115-166-075 1.0 mg	115-296-075 1.0 mg	115-586-075 0.75 mg	115-606-075 0.75 mg

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description		Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE					
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Mouse IgG (H+L)		315-006-003 1.0 mg	315-036-003 0.5 ml	315-056-003 0.5 ml	315-066-003 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Mouse IgG (H+L) (min X Hu Sr Prot)		315-006-045 0.5 mg	315-036-045 0.5 ml	315-056-045 0.5 ml	315-066-045 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Mouse IgG, Fc_γ fragment specific (min X Hu Sr Prot)		315-006-046 0.5 mg	315-036-046 0.5 ml	315-056-046 0.5 ml	315-066-046 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot)		315-006-047 0.5 mg	315-036-047 0.5 ml	315-056-047 0.5 ml	315-066-047 0.5 ml
	ANTI-RABBIT					
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	(ML)	711-006-152 0.5 mg	711-036-152 0.3 ml	711-056-152 0.3 ml	711-066-152 0.3 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG (H+L)		111-006-003 1.0 mg	111-036-003 0.5 ml	111-056-003 0.5 ml	111-066-003 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG (H+L) (min X Hu Sr Prot)		111-006-045 1.0 mg	111-036-045 0.5 ml	111-056-045 0.5 ml	111-066-045 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot)	(ML)	111-006-144 1.0 mg	111-036-144 0.5 ml	111-056-144 0.5 ml	111-066-144 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG, Fc fragment specific (min X Hu Sr Prot)		111-006-046 1.0 mg	111-036-046 0.5 ml	111-056-046 0.5 ml	111-066-046 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG, $F(ab')_2$ fragment specific (min X Hu Sr Prot)		111-006-047 1.0 mg	111-036-047 0.5 ml	111-056-047 0.5 ml	111-066-047 0.5 ml
	ANTI-RAT					
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	(ML)	712-006-150 0.5 mg	712-036-150 0.3 ml	712-056-150 0.3 ml	712-066-150 0.3 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
	315-156-003 0.5 mg	315-546-003 0.5 mg	315-096-003 0.5 mg	315-166-003 0.5 mg	315-296-003 0.5 mg	315-586-003 0.5 mg	315-606-003 0.5 mg
	315-156-045 0.5 mg	315-546-045 0.5 mg	315-096-045 0.5 mg	315-166-045 0.5 mg	315-296-045 0.5 mg	315-586-045 0.5 mg	315-606-045 0.5 mg
	315-156-046 0.5 mg	315-546-046 0.5 mg	315-096-046 0.5 mg	315-166-046 0.5 mg	315-296-046 0.5 mg	315-586-046 0.5 mg	315-606-046 0.5 mg
	315-156-047 0.5 mg	315-546-047 0.5 mg	315-096-047 0.5 mg	315-166-047 0.5 mg	315-296-047 0.5 mg	315-586-047 0.5 mg	315-606-047 0.5 mg
711-476-152 0.3 mg	711-156-152 0.3 mg	711-546-152 0.3 mg	711-096-152 0.3 mg	711-166-152 0.3 mg	711-296-152 0.3 mg	711-586-152 0.3 mg	711-606-152 0.3 mg
	111-156-003 1.0 mg	111-546-003 0.75 mg	111-096-003 1.0 mg	111-166-003 1.0 mg	111-296-003 1.0 mg	111-586-003 0.75 mg	111-606-003 0.75 mg
	111-156-045 1.0 mg	111-546-045 0.75 mg	111-096-045 1.0 mg	111-166-045 1.0 mg	111-296-045 1.0 mg	111-586-045 0.75 mg	111-606-045 0.75 mg
111-476-144 0.75 mg	111-156-144 1.0 mg	111-546-144 0.75 mg	111-096-144 1.0 mg	111-166-144 1.0 mg	111-296-144 1.0 mg	111-586-144 0.75 mg	111-606-144 0.75 mg
111-476-046 0.75 mg	111-156-046 1.0 mg	111-546-046 0.75 mg	111-096-046 1.0 mg	111-166-046 1.0 mg	111-296-046 1.0 mg	111-586-046 0.75 mg	111-606-046 0.75 mg
111-476-047 0.75 mg	111-156-047 1.0 mg	111-546-047 0.75 mg	111-096-047 1.0 mg	111-166-047 1.0 mg	111-296-047 1.0 mg	111-586-047 0.75 mg	111-606-047 0.75 mg
712-476-150 0.3 mg	712-156-150 0.3 mg	712-546-150 0.3 mg	712-096-150 0.3 mg	712-166-150 0.3 mg	712-296-150 0.3 mg	712-586-150 0.3 mg	712-606-150 0.3 mg

$F(ab')_2$ FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-RAT				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms,** Rb, Shp Sr Prot) (SP) (ML)	712-006-153 0.5 mg	712-036-153 0.3 ml	712-056-153 0.3 ml	712-066-153 0.3 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG (H+L)	112-006-003 1.0 mg	112-036-003 0.5 ml	112-056-003 0.5 ml	112-066-003 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs Sr Prot)	112-006-062 1.0 mg	112-036-062 0.5 ml	112-056-062 0.5 ml	112-066-062 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot) (ML)	112-006-143 1.0 mg	112-036-143 0.5 ml	112-056-143 0.5 ml	112-066-143 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-006-071 1.0 mg	112-036-071 0.5 ml	112-056-071 0.5 ml	112-066-071 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)	112-006-072 1.0 mg	112-036-072 0.5 ml	112-056-072 0.5 ml	112-066-072 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)	112-006-068 1.0 mg	112-036-068 0.5 ml	112-056-068 0.5 ml	112-066-068 0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-006-075 1.0 mg	112-036-075 0.5 ml	112-056-075 0.5 ml	112-066-075 0.5 ml
MOUSE	$F(ab')_2$ Fragment Mouse Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Gt, Rb Sr Prot) (SP) (ML)	212-006-168 0.5 mg	212-036-168 0.5 ml	212-056-168 0.5 ml	212-066-168 0.5 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Rat IgG (H+L) (min X Hu Sr Prot)	312-006-045 0.5 mg	312-036-045 0.5 ml	312-056-045 0.5 ml	312-066-045 0.5 ml
	ANTI-SHEEP				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	713-006-147 0.5 mg	713-036-147 0.3 ml	713-056-147 0.3 ml	713-066-147 0.3 ml
RABBIT	$F(ab')_2$ Fragment Rabbit Anti-Sheep IgG (H+L) (!)	313-006-003 1.0 mg	313-036-003 0.5 ml	313-056-003 0.5 ml	313-066-003 0.5 ml

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450	Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667
712-476-153 0.3 mg	712-156-153 0.3 mg	712-546-153 0.3 mg	712-096-153 0.3 mg	712-166-153 0.3 mg	712-296-153 0.3 mg	712-586-153 0.3 mg	712-606-153 0.3 mg
	112-156-003 1.0 mg	112-546-003 0.75 mg	112-096-003 1.0 mg	112-166-003 1.0 mg	112-296-003 1.0 mg	112-586-003 0.75 mg	112-606-003 0.75 mg
	112-156-062 1.0 mg	112-546-062 0.75 mg	112-096-062 1.0 mg	112-166-062 1.0 mg	112-296-062 1.0 mg	112-586-062 0.75 mg	112-606-062 0.75 mg
112-476-143 0.75 mg	112-156-143 1.0 mg	112-546-143 0.75 mg	112-096-143 1.0 mg	112-166-143 1.0 mg	112-296-143 1.0 mg	112-586-143 0.75 mg	112-606-143 0.75 mg
112-476-071 0.75 mg	112-156-071 1.0 mg	112-546-071 0.75 mg	112-096-071 1.0 mg	112-166-071 1.0 mg	112-296-071 1.0 mg	112-586-071 0.75 mg	112-606-071 0.75 mg
112-476-072 0.75 mg	112-156-072 1.0 mg	112-546-072 0.75 mg	112-096-072 1.0 mg	112-166-072 1.0 mg	112-296-072 1.0 mg	112-586-072 0.75 mg	112-606-072 0.75 mg
	112-156-068 1.0 mg	112-546-068 0.75 mg	112-096-068 1.0 mg	112-166-068 1.0 mg	112-296-068 1.0 mg	112-586-068 0.75 mg	112-606-068 0.75 mg
	112-156-075 1.0 mg	112-546-075 0.75 mg	112-096-075 1.0 mg	112-166-075 1.0 mg	112-296-075 1.0 mg	112-586-075 0.75 mg	112-606-075 0.75 mg
	212-156-168 0.5 mg	212-546-168 0.5 mg	212-096-168 0.5 mg	212-166-168 0.5 mg	212-296-168 0.5 mg	212-586-168 0.5 mg	212-606-168 0.5 mg
	312-156-045 0.5 mg	312-546-045 0.5 mg	312-096-045 0.5 mg	312-166-045 0.5 mg	312-296-045 0.5 mg	312-586-045 0.5 mg	312-606-045 0.5 mg
713-476-147 0.3 mg	713-156-147 0.3 mg	713-546-147 0.3 mg	713-096-147 0.3 mg	713-166-147 0.3 mg	713-296-147 0.3 mg	713-586-147 0.3 mg	713-606-147 0.3 mg
	313-156-003 0.5 mg	313-546-003 0.5 mg	313-096-003 0.5 mg	313-166-003 0.5 mg	313-296-003 0.5 mg	313-586-003 0.5 mg	313-606-003 0.5 mg

Monovalent Fab Fragment Affinity-Purified Antibodies for Blocking and Double Labeling Primary Antibodies from the Same Host Species

Monovalent Fab fragments of affinity-purified secondary antibodies are offered to cover (block) the surface of immunoglobulins for double labeling primary antibodies from the same host species, or to block endogenous immunoglobulins in tissue sections or on cell surfaces. They can be used for these purposes because Fab fragments have only a single antigen binding site (i.e. they are monovalent).

In contrast, divalent antibodies (whole IgG and $F(ab')_2$ fragments) have two antigen binding sites. After labeling the first primary antibody, some antigen binding sites on the first secondary antibody may remain open which could capture the second primary antibody introduced in a subsequent step. Consequently, it will appear as overlapping labeling, even though there may not be overlapping antigens. Therefore, divalent antibodies should not be used for blocking or for double labeling two primary antibodies from the same species.

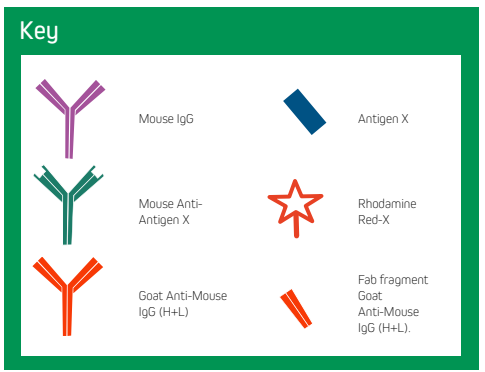
Monovalent Fab secondary antibodies are not necessary when primary antibodies from the same host species are different classes of immunoglobulins, such as IgG and IgM, or different subclasses of IgG, such as Mouse IgG1 and Mouse IgG2a. In these cases, it is much easier and more effective to use class specific or subclass specific antibodies, respectively, to distinguish between the two primary antibodies.

Caution: Whole IgG and $F(ab')_2$ fragments are divalent antibodies, with two antigen binding sites, therefore they cannot be used in the following protocols which specifically require Fab fragments.

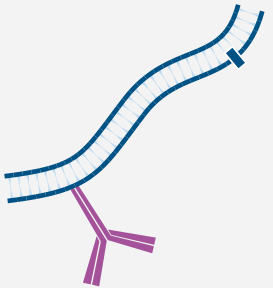
Blocking Endogenous Immunoglobulins With Fab Fragments
Background staining may be observed if a labeled secondary antibody is not adsorbed to minimize recognition of endogenous tissue Ig. When a primary antibody is the same species as the tissue under study (e.g. mouse primary used on mouse tissue), blocking endogenous Ig suppresses the off-target signal.

To block endogenous immunoglobulins on cells or tissue sections, incubate with an excess (20-40 µg/ml) of unconjugated Fab antibody just after blocking with 5% normal serum. Blocking efficiency can be confirmed by eliminating the primary antibody from the protocol and incubating with labeled secondary antibody. It may be necessary to increase the concentration of Fab antibody up to 100 µg/ml to suppress signal from high levels of endogenous IgG.

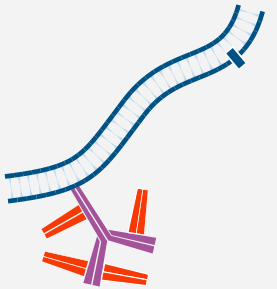
To avoid displacement of the Fab antibody by the labeled secondary antibody, a light post-fixation with glutaraldehyde may be necessary, provided that it does not affect antigenicity of the target proteins. Fab antibodies are not as effective for blocking immunoglobulins in Western blotting or ELISA applications. For more information see the blocking and controls section (pages 143-155).



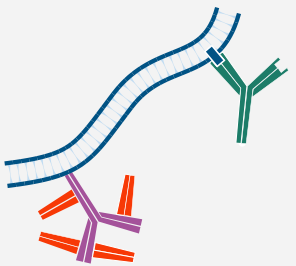
Blocking: **Use of unconjugated Fab fragments** to **block endogenous immunoglobulins** and avoid off target signal (Figure 46).



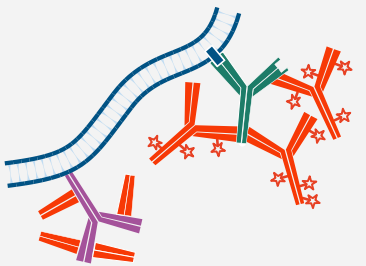
1. Samples may express endogenous immunoglobulins, in this example mouse IgG.



2. After blocking with normal serum, incubate with an excess of unconjugated Fab antibody, in this example Fab fragment Goat Anti-Mouse IgG (H+L). Wash.



3. Incubate with primary antibody, in this example Mouse Anti-Antigen X. Wash.



4. Incubate with conjugated secondary antibody, in this example Rhodamine Red™-X-Goat Anti-Mouse IgG (H+L). Wash.

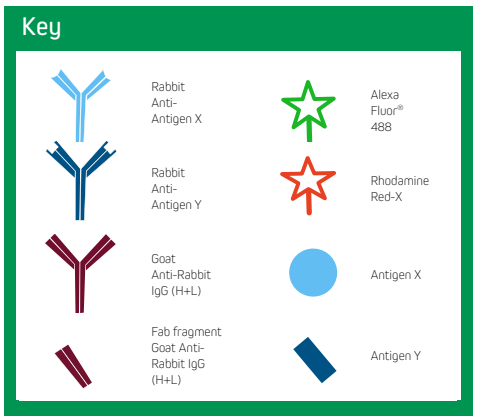
Detection of two unlabeled primary antibodies from the same host species

The following examples show some of the possible protocols used for double labeling two unconjugated primary antibodies from the same host species.

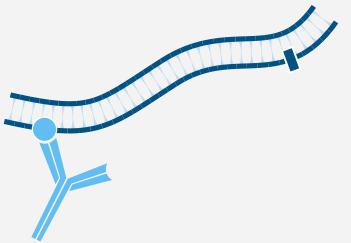
The success of these experimental designs will require some empirical manipulations. Optimizing reagent concentrations in each step or switching the labeling sequence of the two antigens may influence the outcome.

- Labeling the less abundant primary antibody first increases blocking efficiency.
- Blocking with an appropriate normal serum helps to reduce background.
- To avoid displacement of the Fab antibody by the labeled secondary antibody, a light post-fixation with glutaraldehyde may be used, provided that it does not affect antigenicity of the target proteins.

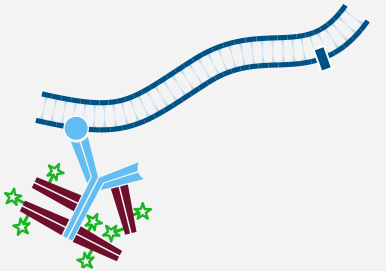
Important note: The monovalent Fab fragments have not been adsorbed to remove cross-reactivities to other species. If the experimental sample contains endogenous immunoglobulins Example C should be used. Example A or B could introduce background.



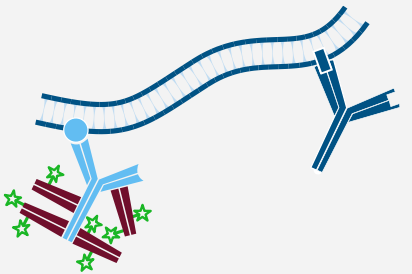
Example A: Use of conjugated Fab fragments for labeling and blocking (Figure 47).



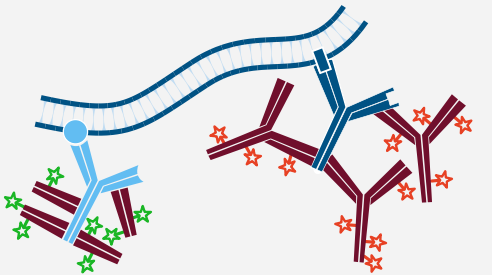
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.



2. Incubate with excess conjugated secondary antibody, in this example Alexa Fluor® 488-Fab fragment Goat Anti-Rabbit IgG (H+L). Wash.



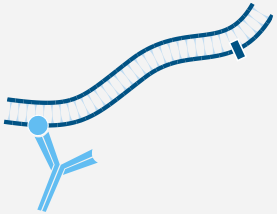
3. Incubate with the second primary antibody, Rabbit Anti-Antigen Y.



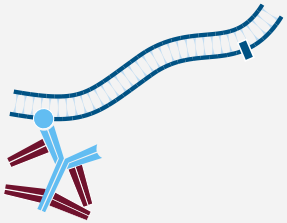
4. Incubate with a second conjugated secondary antibody, in this example Rhodamine Red™-X-Goat Anti-Rabbit IgG (H+L). Wash.

Application notes (1) Monovalent Fab fragments have not been adsorbed against other species, so they may cross-react with endogenous Ig. Use Example C to avoid detection of endogenous Ig. (2) Example A may require a high concentration of conjugated Fab to saturate the first primary antibody. If this results in unacceptable background, try a lower concentration of the conjugated Fab, followed by further blocking with unconjugated Fab.

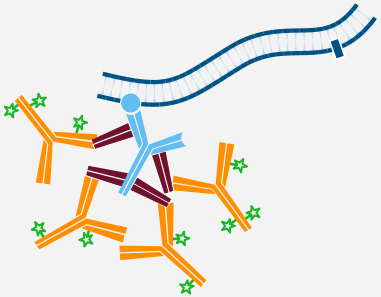
Example B: Use of unconjugated Fab fragments to cover the first primary antibody, presenting it as a different species (Figure 48).



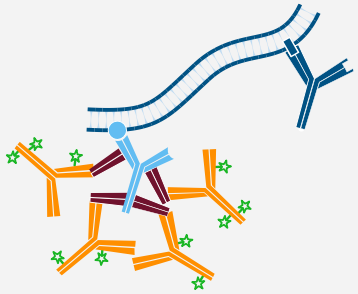
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.



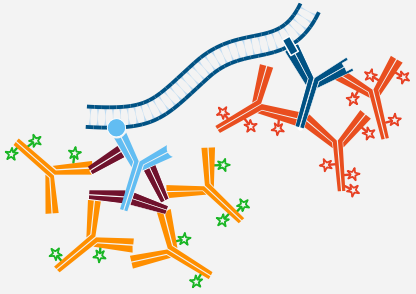
2. Incubate with an excess of unconjugated Fab antibody against the host species of the primary antibody, in this example unconjugated Fab fragment Goat Anti-Rabbit IgG (H+L). This presents the rabbit IgG as goat Fab. Wash.



3. Incubate with conjugated tertiary antibody directed against the host species of the Fab antibody. The tertiary antibody must not recognize the host species of either the primary antibodies or the second secondary antibody. This example used Alexa Fluor® 488-Mouse Anti-Goat IgG (H+L) (min X **Ms**, Hu, **Rb** Sr Prot). Wash.



4. Incubate with the second primary antibody, in this example Rabbit Anti-Antigen Y. Wash.

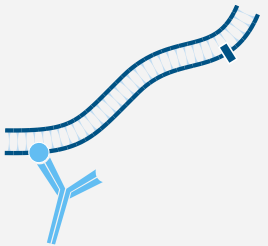


5. Incubate with second conjugated secondary antibody, that does not recognize the host species of either the Fab antibody used in step 2 or the tertiary antibody used in step 3. In this example, Rhodamine Red™-X-Mouse Anti-Rabbit IgG (H+L) (min X Hu, **Gt**, **Ms**, Shp Sr Prot) was used. Wash.

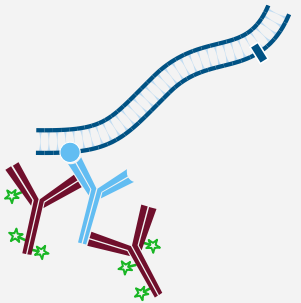
Application note: Monovalent Fab fragments have not been adsorbed against other species, so they may cross-react with endogenous Ig. Use Example C to avoid detection of endogenous Ig.

Key

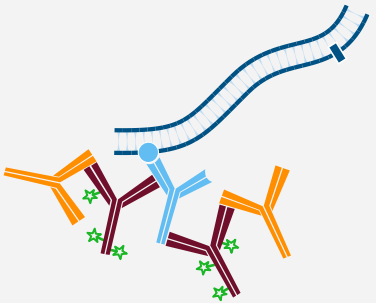
Example C: Use of unconjugated Fab fragments for blocking after the first secondary antibody step. This example is suggested to avoid detection of endogenous Ig (Figure 49).



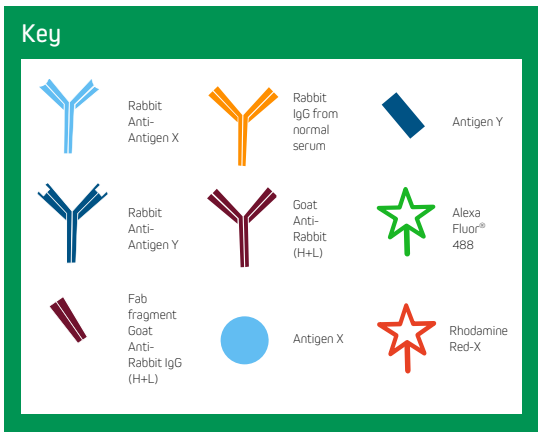
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.

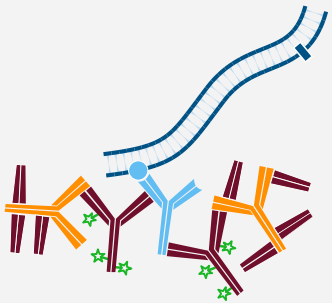


2. Incubate with conjugated secondary antibody, in this example Alexa Fluor® 488-Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot). Wash.

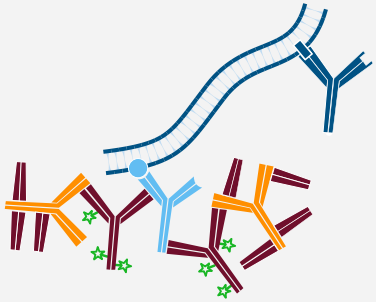


3. Incubate with normal serum from the same host species as the primary antibodies, in this example normal rabbit serum. The purpose of this step is to saturate open binding sites on the first secondary antibody with IgG so that they cannot capture the second primary antibody. Wash.

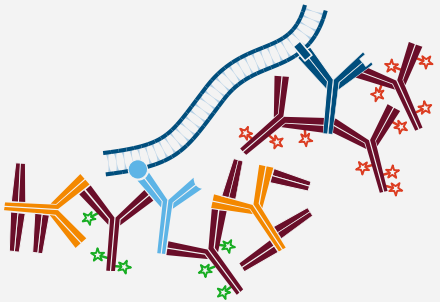




4. Incubate with an excess of unconjugated Fab antibody against the host species of the primary antibodies, in this example Fab Goat Anti-Rabbit IgG (H+L). The host species of the Fab antibody should be the same as the host species of the conjugated secondary antibody. This step covers the rabbit IgG so that the second secondary antibody will not bind to it. Wash.



5. Incubate with the second primary antibody, in this example Rabbit Anti-Antigen Y. Wash.

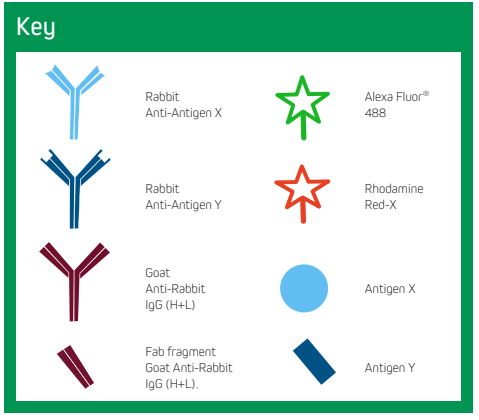


6. Incubate with the same secondary antibody as used in step 2, conjugated to a different probe, in this example Rhodamine Red™-X-Goat Anti-Rabbit IgG (H+L)(min X Hu, Ms, Rat Sr Prot). Wash.

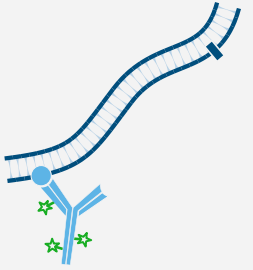
Detection of one unlabeled and one or more labeled primary antibodies from the same host species

Examples D and E illustrate multiple labeling protocols that include a directly labeled and an unlabeled primary antibody. It is advisable to incubate the less abundant primary first. In Example D, the directly labeled primary antibody is incubated first, then blocked with Fab fragments prior to applying the unlabeled primary antibody.

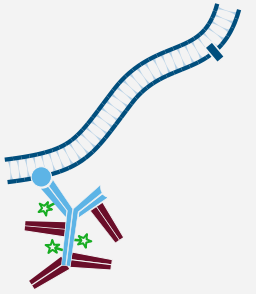
If the unlabeled primary antibody is incubated first (Example E), double labeling can be achieved without using Fab fragments. Following incubation with the labeled secondary antibody, normal serum is used to block open binding arms of the secondary, preventing capture of the labeled primary.



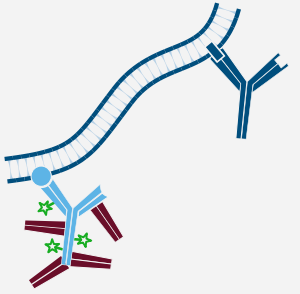
Example D: Use of unconjugated Fab fragments for detection of one unlabeled and one or more labeled primary antibodies (Figure 50).



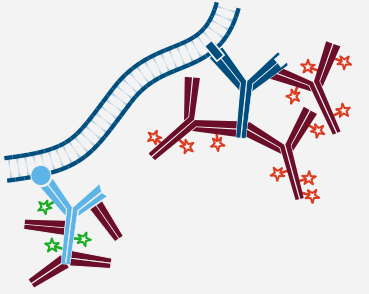
1. After blocking with normal serum, incubate with conjugated primary antibody, in this example Alexa Fluor® 488-Rabbit Anti-Antigen X. Wash.



2. Incubate with an excess of unconjugated Fab Goat Anti-Rabbit IgG (H+L). Wash.

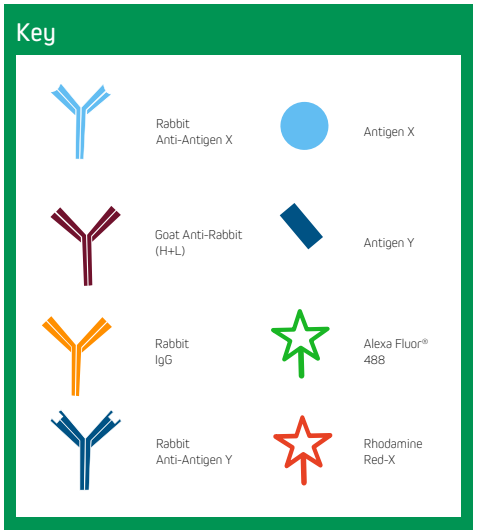


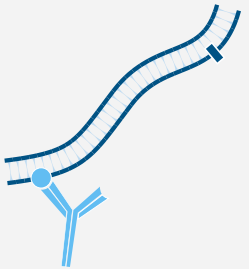
3. Incubate with the unconjugated primary antibody, in this example Rabbit Anti-Antigen Y. Wash.



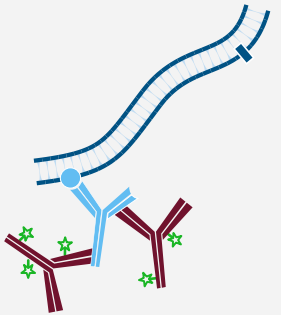
4. Incubate with conjugated secondary antibody, in this example Rhodamine Red™-X-Goat Anti-Rabbit IgG (H+L). Wash.

Example E: Detection of one unlabeled and one or more labeled primary antibodies without the use of Fab fragments (Figure 51).

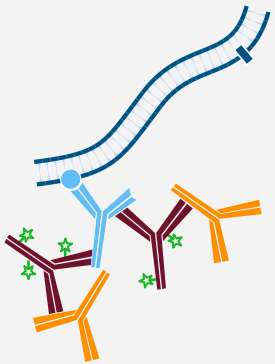




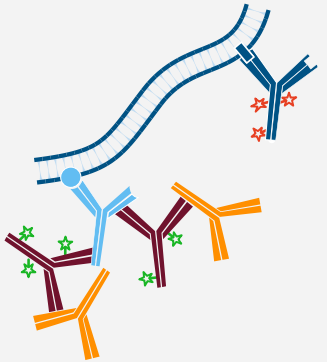
1. After blocking with normal serum, incubate with the unlabeled primary antibody, in this example Rabbit Anti-Antigen X. Wash.



2. Incubate with conjugated secondary antibody, in this example Alexa Fluor® 488-Goat Anti-Rabbit IgG (H+L). Wash.



3. Incubate with normal serum from the host species of the primary antibody, in this example normal rabbit serum. Wash.



4. Incubate with conjugated primary antibody, in this example Rhodamine Red™-X-Rabbit Anti-Antigen Y. Wash.

Fab FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated		Biotin-SP (long spacer)		Alexa Fluor® 488 A=493, E=519	
	ANTI-GOAT						
DONKEY	Fab Fragment Donkey Anti-Goat IgG (H+L) (!)	705-007-003	1.0 mg	705-067-003	0.5 ml	705-547-003	0.75 mg
RABBIT	Fab Fragment Rabbit Anti-Goat IgG (H+L) (!)	305-007-003	1.0 mg	305-067-003	0.5 ml	305-547-003	0.5 mg
	ANTI-HUMAN						
GOAT	Fab Fragment Goat Anti-Human IgG (H+L)	109-007-003	1.0 mg	109-067-003	0.5 ml	109-547-003	0.75 mg
GOAT	Fab Fragment Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific	109-007-043	1.0 mg	109-067-043	0.5 ml	109-547-043	0.5 mg
	ANTI-MOUSE						
DONKEY	Fab Fragment Donkey Anti-Mouse IgG (H+L)	715-007-003	1.0 mg	715-067-003	0.5 ml	715-547-003	0.75 mg
GOAT	Fab Fragment Goat Anti-Mouse IgG (H+L)	115-007-003	1.0 mg	115-067-003	0.5 ml	115-547-003	0.75 mg
GOAT	Fab Fragment Goat Anti-Mouse IgM, μ chain specific	115-007-020	1.0 mg	115-067-020	0.5 ml	115-547-020	0.5 mg
RABBIT	Fab Fragment Rabbit Anti-Mouse IgG (H+L)	315-007-003	1.0 mg	315-067-003	0.5 ml	315-547-003	0.5 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titre.

Fluorescein FITC A=492, E=520		Cyanine Cy™3 A=550, E= 570		Rhodamine Red™-X A=570, E=590		Alexa Fluor® 594 A=591, E=614		Alexa Fluor® 647 A=651, E=667	
705-097-003	1.0 mg	705-167-003	1.0 mg	705-297-003	1.0 mg	705-587-003	0.75 mg	705-607-003	0.75 mg
305-097-003	0.5 mg	305-167-003	0.5 mg	305-297-003	0.5 mg	305-587-003	0.5 mg	305-607-003	0.5 mg
109-097-003	1.0 mg	109-167-003	1.0 mg	109-297-003	1.0 mg	109-587-003	0.75 mg	109-607-003	0.75 mg
109-097-043	0.5 mg	109-167-043	0.5 mg	109-297-043	0.5 mg	109-587-043	0.5 mg	109-607-043	0.5 mg
715-097-003	1.0 mg	715-167-003	1.0 mg	715-297-003	1.0 mg	715-587-003	0.75 mg	715-607-003	0.75 mg
115-097-003	1.0 mg	115-167-003	1.0 mg	115-297-003	1.0 mg	115-587-003	0.75 mg	115-607-003	0.75 mg
115-097-020	0.5 mg	115-167-020	0.5 mg	115-297-020	0.5 mg	115-587-020	0.5 mg	115-607-020	0.5 mg
315-097-003	0.5 mg	315-167-003	0.5 mg	315-297-003	0.5 mg	315-587-003	0.5 mg	315-607-003	0.5 mg

Fab FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated		Biotin-SP (long spacer)		Alexa Fluor® 488 A=493, E=519	
	ANTI-RABBIT						
DONKEY	Fab Fragment Donkey Anti-Rabbit IgG (H+L)	711-007-003	1.0 mg	711-067-003	0.5 ml	711-547-003	0.75 mg
GOAT	Fab Fragment Goat Anti-Rabbit IgG (H+L)	111-007-003	1.0 mg	111-067-003	0.5 ml	111-547-003	0.75 mg
	ANTI-RAT						
DONKEY	Fab Fragment Donkey Anti-Rat IgG (H+L)	712-007-003	1.0 mg	712-067-003	0.5 ml	712-547-003	0.75 mg
GOAT	Fab Fragment Goat Anti-Rat IgG (H+L)	112-007-003	1.0 mg	112-067-003	0.5 ml	112-547-003	0.75 mg
	ANTI-SHEEP						
RABBIT	Fab Fragment Rabbit Anti-Sheep IgG (H+L) (!)	313-007-003	1.0 mg	313-067-003	0.5 ml	313-547-003	0.5 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titre.

Fluorescein FITC A=492, E=520		Cyanine Cy™3 A=550, E= 570		Rhodamine Red™-X A=570, E=590		Alexa Fluor® 594 A=591, E=614		Alexa Fluor® 647 A=651, E=667	
711-097-003	1.0 mg	711-167-003	1.0 mg	711-297-003	1.0 mg	711-587-003	0.75 mg	711-607-003	0.75 mg
111-097-003	1.0 mg	111-167-003	1.0 mg	111-297-003	1.0 mg	111-587-003	0.75 mg	111-607-003	0.75 mg
712-097-003	1.0 mg	712-167-003	1.0 mg	712-297-003	1.0 mg	712-587-003	0.75 mg	712-607-003	0.75 mg
112-097-003	1.0 mg	112-167-003	1.0 mg	112-297-003	1.0 mg	112-587-003	0.75 mg	112-607-003	0.75 mg
313-097-003	0.5 mg	313-167-003	0.5 mg	313-297-003	0.5 mg	313-587-003	0.5 mg	313-607-003	0.5 mg

FABULIGHT™ - Fc SPECIFIC Fab FRAGMENTS

FabuLight antibodies are Fab fragment secondary antibodies specific to the Fc region of IgG or IgM primary antibodies. They are available conjugated with 9 different fluorophores and biotin, and enable labeling of primary antibodies in solution, prior to incubation with cells or tissue. They can be used as a time-saving alternative to sequential incubation for flow cytometry and immunohistochemistry procedures.

Possible uses of FabuLights also include labeling cell surface immunoglobulins without cross-linking and activating B cells, and labeling Fc chimeras (fusion proteins). See page 28 for the use of FabuLight antibodies in a protocol for avoiding detection of IP (immunoprecipitating) light chains in Western blotting.

Label primary antibodies in solution with FabuLights

FabuLights can be used to label primary antibodies in solution. The FabuLight binds to the Fc portion of the primary antibody, leaving the antigen-binding region active. FabuLight-primary antibody complexes do not precipitate or aggregate because the dye-conjugated Fab fragments are monovalent. The complexes offer good tissue penetration.

Incubation with FabuLight-labeled primary antibodies requires fewer washes than sequential incubation with primary antibodies and labeled secondary antibodies, thereby reducing damage to cells in flow cytometry protocols. Incubation steps in protocols requiring multiple primary antibodies from the same host animal are also reduced.

Note: FabuLights are not provided cross-adsorbed against other species, so blocking steps may be required to avoid labeling endogenous immunoglobulins. Find our FabuLight white paper at www.jacksonimmuno.com for further advice on protocol development.

Using FabuLights

Optimal protocols for each application must be established empirically. Complexing at a 3:1 molar ratio of FabuLight:primary antibody (equal weight ratios) provides a good degree of labeling of the primary antibody without excessive amounts of unbound Fab. Titrating Fab-labeled complexes vs. their target antigens will minimize the amount of free FabuLight, thereby minimizing potential cross-talk in a multiple labeling application. Label the least abundant target antigen first for optimal results.

- FabuLight antibodies have not been adsorbed to remove cross-reactivities to other species, so it may be necessary to block endogenous IgG prior to incubation with an unlabeled Fab anti-IgG (H+L) prior to application of a FabuLight-labeled complex (See Figure 46, page 87).
- While the use of a FabuLight-labeled primary antibody is convenient, the protocol is not as sensitive as sequential incubations with a primary antibody and labeled secondary antibody.
- To avoid displacement of the FabuLight-primary antibody by a subsequently applied secondary antibody, a light cross-linking with glutaraldehyde may be used, provided that it does not affect epitope recognition of subsequent target proteins.
- For multiple labeling applications, we recommend incubating FabuLight-primary antibody complexes sequentially to minimize cross-reactivity.

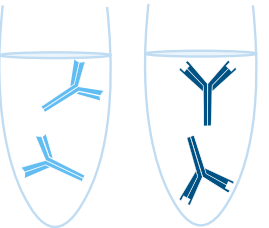
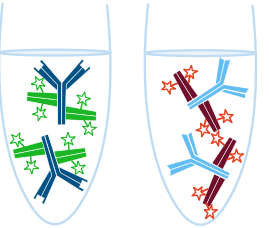
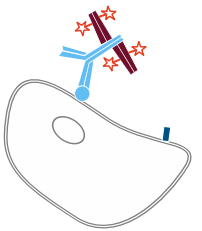


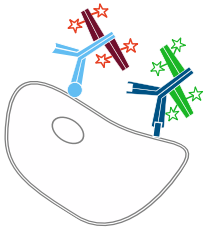
Figure 52: Pre-label primary antibodies with FabuLight antibodies prior to incubation with experimental sample. Step 1: Place primary antibodies in separate tubes.



Step 2: Mix primary antibody and FabuLight to create complexes.



Step 3: Incubate sample with the FabuLight-primary antibody complex for the least abundant antigen first.



Step 4: Incubate the sample with subsequent FabuLight-primary antibody complexes.

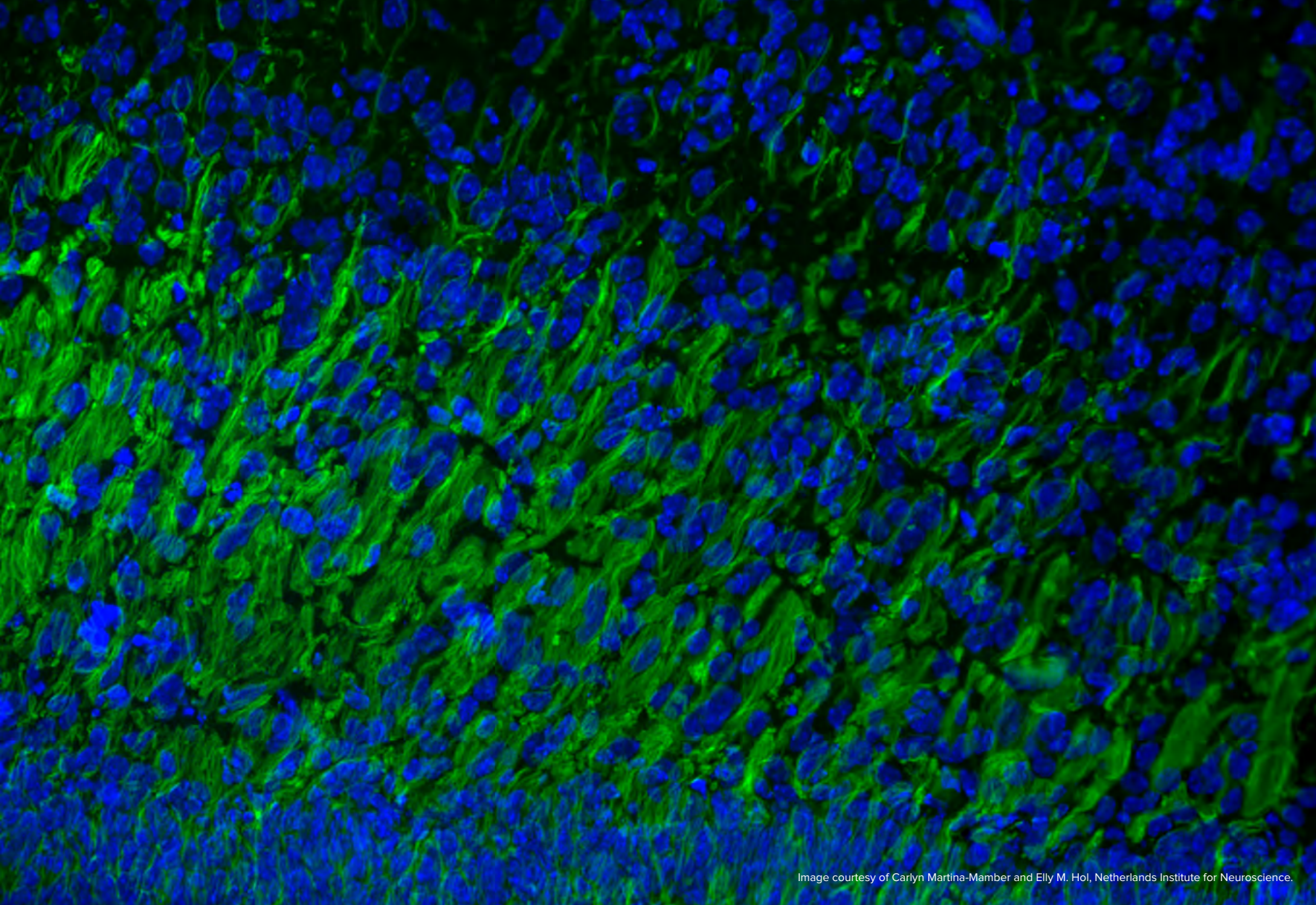


Image courtesy of Carlyn Martina-Mamber and Elly M. Hol, Netherlands Institute for Neuroscience.

FABULIGHT™ - Fc SPECIFIC Fab FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Biotin-SP (long spacer)	DyLight™ 405 A=400, E=421
	ANTI-CHICKEN			
GOAT	Fab Fragment Goat Anti-Chicken IgY (IgG), Fc fragment specific	103-007-008 1.0 mg	103-067-008 0.5 ml	103-477-008 0.5 mg
	ANTI-GOAT			
BOVINE	Fab Fragment Bovine Anti-Goat IgG, Fc fragment specific	805-007-008 1.0 mg	805-067-008 0.5 ml	805-477-008 0.5 mg
	ANTI-GUINEA PIG			
GOAT	Fab Fragment Goat Anti-Guinea Pig IgG, Fc fragment specific	106-007-008 1.0 mg	106-067-008 0.5 ml	106-477-008 0.5 mg
	ANTI-HUMAN			
GOAT	Fab Fragment Goat Anti-Human IgG, Fc_{γ} fragment specific	109-007-008 1.0 mg	109-067-008 0.5 ml	109-477-008 0.5 mg
	Fab Fragment Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific	109-007-043 1.0 mg	109-067-043 0.5 ml	109-477-043 0.5 mg
	ANTI-MOUSE			
GOAT	Fab Fragment Goat Anti-Mouse IgG1, Fc_{γ} fragment specific	115-007-185 1.0 mg	115-067-185 0.5 ml	115-477-185 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2a, Fc_{γ} fragment specific	115-007-186 1.0 mg	115-067-186 0.5 ml	115-477-186 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2b, Fc_{γ} fragment specific	115-007-187 1.0 mg	115-067-187 0.5 ml	115-477-187 0.5 mg
	Fab Fragment Goat Anti-Mouse IgG2c, Fc_{γ} fragment specific	115-007-188 1.0 mg	115-067-188 0.5 ml	115-477-188 0.5 mg

Alexa Fluor® 488 A=493, E=519	Cyanine Cy™3 A=550, E= 570	Phycoerythrin R-PE A=488, E=580	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
103-547-008 0.5 mg	103-167-008 0.5 mg	103-117-008 0.5 ml	103-297-008 0.5 mg	103-587-008 0.5 mg	103-607-008 0.5 mg	103-627-008 0.5 mg	103-657-008 0.5 mg
805-547-008 0.5 mg	805-167-008 0.5 mg	805-117-008 0.5 ml	805-297-008 0.5 mg	805-587-008 0.5 mg	805-607-008 0.5 mg	805-627-008 0.5 mg	805-657-008 0.5 mg
106-547-008 0.5 mg	106-167-008 0.5 mg	106-117-008 0.5 ml	106-297-008 0.5 mg	106-587-008 0.5 mg	106-607-008 0.5 mg	106-627-008 0.5 mg	106-657-008 0.5 mg
109-547-008 0.5 mg	109-167-008 0.5 mg	109-117-008 0.5 ml	109-297-008 0.5 mg	109-587-008 0.5 mg	109-607-008 0.5 mg	109-627-008 0.5 mg	109-657-008 0.5 mg
109-547-043 0.5 mg	109-167-043 0.5 mg	109-117-043 0.5 ml	109-297-043 0.5 mg	109-587-043 0.5 mg	109-607-043 0.5 mg	109-627-043 0.5 mg	109-657-043 0.5 mg
115-547-185 0.5 mg	115-167-185 0.5 mg	115-117-185 0.5 ml	115-297-185 0.5 mg	115-587-185 0.5 mg	115-607-185 0.5 mg	115-627-185 0.5 mg	115-657-185 0.5 mg
115-547-186 0.5 mg	115-167-186 0.5 mg	115-117-186 0.5 ml	115-297-186 0.5 mg	115-587-186 0.5 mg	115-607-186 0.5 mg	115-627-186 0.5 mg	115-657-186 0.5 mg
115-547-187 0.5 mg	115-167-187 0.5 mg	115-117-187 0.5 ml	115-297-187 0.5 mg	115-587-187 0.5 mg	115-607-187 0.5 mg	115-627-187 0.5 mg	115-657-187 0.5 mg
115-547-188 0.5 mg	115-167-188 0.5 mg	115-117-188 0.5 ml	115-297-188 0.5 mg	115-587-188 0.5 mg	115-607-188 0.5 mg	115-627-188 0.5 mg	115-657-188 0.5 mg

FABULIGHT™ - Fc SPECIFIC Fab FRAGMENT SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Biotin-SP (long spacer)	DyLight™ 405 A=400, E=421
	ANTI-MOUSE			
GOAT	Fab Fragment Goat Anti-Mouse IgG3, Fc_γ fragment specific	115-007-189 1.0 mg	115-067-189 0.5 ml	115-477-189 0.5 mg
GOAT	Fab Fragment Goat Anti-Mouse IgM, μ Chain Specific	115-007-020 1.0 mg	115-067-020 0.5 ml	115-477-020 0.5 mg
	ANTI-RABBIT			
GOAT	Fab Fragment Goat Anti-Rabbit IgG, Fc fragment specific	111-007-008 1.0 mg	111-067-008 0.5 ml	111-477-008 0.5 mg
	ANTI-RAT			
GOAT	Fab fragment Goat Anti-Rat IgG, Fc_γ fragment specific	112-007-008 1.0 mg	112-067-008 0.5 ml	112-477-008 0.5 mg
GOAT	Fab fragment Goat Anti-Rat IgM, μ chain specific	112-007-020 1.0 mg	112-067-020 0.5 ml	112-477-020 0.5 mg

Alexa Fluor® 488 A=493, E=519	Cyanine Cy™3 A=550, E= 570	Phycoerythrin R-PE A=488, E=580	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
115-547-189 0.5 mg	115-167-189 0.5 mg	115-117-189 0.5 ml	115-297-189 0.5 mg	115-587-189 0.5 mg	115-607-189 0.5 mg	115-627-189 0.5 mg	115-657-189 0.5 mg
115-547-020 0.5 mg	115-167-020 0.5 mg	115-117-020 0.5 ml	115-297-020 0.5 mg	115-587-020 0.5 mg	115-607-020 0.5 mg	115-627-020 0.5 mg	115-657-020 0.5 mg
111-547-008 0.5 mg	111-167-008 0.5 mg	111-117-008 0.5 ml	111-297-008 0.5 mg	111-587-008 0.5 mg	111-607-008 0.5 mg	111-627-008 0.5 mg	111-657-008 0.5 mg
112-547-008 0.5 mg	112-167-008 0.5 mg	112-117-008 0.5 ml	112-297-008 0.5 mg	112-587-008 0.5 mg	112-607-008 0.5 mg	112-627-008 0.5 mg	112-657-008 0.5 mg
112-547-020 0.5 mg	112-167-020 0.5 mg	112-117-020 0.5 ml	112-297-020 0.5 mg	112-587-020 0.5 mg	112-607-020 0.5 mg	112-627-020 0.5 mg	112-657-020 0.5 mg

SPECIALIZED SECONDARY ANTIBODIES

Mouse IgG subclasses

Mice express 4 of the 5 available IgG subclasses making up their IgG isotype. They typically produce IgG1, IgG2b and IgG3, and depending on their strain will also express either IgG2a or IgG2c (Collins, 2016). IgG2a and IgG2c subclasses are seen as functionally comparable, being the most active of the subclasses to bind complement (Collins, 2016).

Inbred mouse strains, such as C57Bl/6, C57Bl/10, SJL and NOD mice, possess an IgH-1b allele which results in expression of IgG2c instead of the IgG2a subclass expressed by BALB/c and Swiss Webster mice. The IgH-1a haplotype of these mice strains include the IGHG2C gene alongside the other isotypes, but not the IGHG2A present on the IgH-1b haplotype of the BALB/c mice (Martin et al. 1998).

A number of monoclonal antibodies originate from these inbred stains, and some IgG2c clones have been incorrectly isotyped as IgG2a by reagents that cannot distinguish between these two subclasses. Subclass specific antibodies from Jackson ImmunoResearch provide exquisite discrimination among the subclasses.

The following example illustrates the performance of the Goat Anti-Mouse IgG subclass specific antibodies in a dot blot experiment. In each case, the subclass specific antibody only recognizes its specific target in the presence of additionally blotted subclasses.

Distinguishing between different mouse IgG subclasses

Jackson ImmunoResearch Anti-Mouse IgG subclass specific antibodies offer specificity to the 5 individual mouse IgG subclasses. These highly specific antibodies are designed to distinguish between two or more different subclasses of mouse IgG in multiple labeling experiments, or for mouse IgG subclass determination.

They have been adsorbed against human, bovine and rabbit serum proteins to minimize cross-reactivity with tissue immunoglobulins, adherent bovine IgG on cultured cells and rabbit primary antibodies.

Anti-Mouse IgG, subclass specific antibodies are available conjugated to our complete range of fluorescent conjugates, reporter enzymes and biotin.

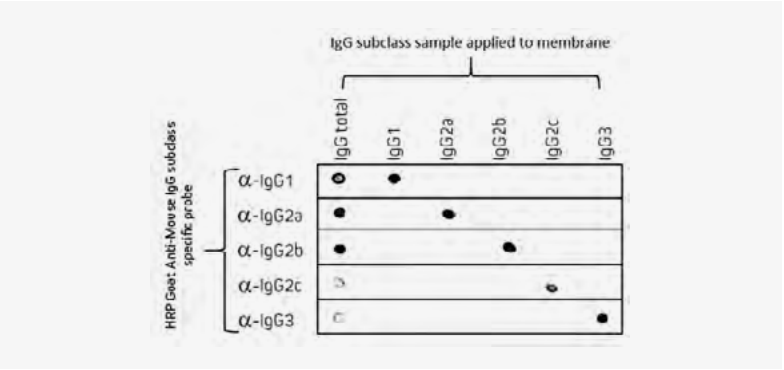


Figure 53: Dot blot showing the specificity of Goat Anti-Mouse IgG, Fc_γ subclass specific antibodies. Separate nitrocellulose strips (rows) received 100 ng "dots" of mouse IgG and each subclass, and then were blocked with 5% (w/v) BSA in PBST. After probing with HRP-conjugated Goat Anti-Mouse subclass specific antibodies, the strips were developed with TMBM substrate. The grid of positive signals shows the specificity of each subclass directed antibody. Some subclasses are poorly represented in a total IgG pool and thus give weak signal (IgG total vs. Anti-IgG2c and -IgG3). HRP-conjugates used for probing were 115-035-205 (Anti-Mouse IgG1), 115-035-206 (Anti-Mouse IgG2a), 115-035-207 (Anti-Mouse IgG2b), 115-035-208 (Anti-Mouse IgG2c), and 115-035-209 (Anti-Mouse IgG3).

Subclass specific antibodies are not necessary for general detection of mouse monoclonal antibodies in single-labeling experiments or in multiple labeling experiments involving one mouse monoclonal and primary antibodies from other species.

References

Collins, A. (2016). IgG subclass co-expression brings harmony to the quartet model of murine IgG function; Immunol Cell Biology, 94, 10, 949- 954. http://dx.doi.org/10.1038/icb.2016.65

Martin, R., Brady, J., Lew, A. (1998) The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice; Journal of Immunological Methods, Volume 212, Issue 2, 1998, Pages 187-192, ISSN 0022-1759. http://dx.doi.org/10.1016/S0022-1759(98)00015-5

ANTI-MOUSE IgG SUBCLASS SPECIFIC SECONDARY ANTIBODIES

	Antibody Description		Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-MOUSE					
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-005-205 1.0 mg	115-035-205 0.5 ml	115-055-205 0.5 ml	115-065-205 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-005-206 1.0 mg	115-035-206 0.5 ml	115-055-206 0.5 ml	115-065-206 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-005-207 1.0 mg	115-035-207 0.5 ml	115-055-207 0.5 ml	115-065-207 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2c specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-005-208 1.0 mg	115-035-208 0.5 ml	115-055-208 0.5 ml	115-065-208 0.5 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-005-209 1.0 mg	115-035-209 0.5 ml	115-055-209 0.5 ml	115-065-209 0.5 ml

	Antibody Description		Cyanine Cy™3 A=550, E= 570	Phycoerythrin R-PE A=488, E=580	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614
	ANTI-MOUSE					
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-165-205 0.5 mg	115-115-205 0.5 ml	115-295-205 0.5 mg	115-585-205 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-165-206 0.5 mg	115-115-206 0.5 ml	115-295-206 0.5 mg	115-585-206 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-165-207 0.5 mg	115-115-207 0.5 ml	115-295-207 0.5 mg	115-585-207 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2c specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-165-208 0.5 mg	115-115-208 0.5 ml	115-295-208 0.5 mg	115-585-208 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-165-209 0.5 mg	115-115-209 0.5 ml	115-295-209 0.5 mg	115-585-209 0.5 mg

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

DyLight™ 405 A=400, E=421		Brilliant Violet 421™ A=407, E=421		Coumarin AMCA A=350, E=450		Brilliant Violet 480™ A=436, E=478		Cyanine Cy™2 A=492, E=510		Alexa Fluor® 488 A=493, E=519		Fluorescein FITC A=492, E=520	
115-475-205	0.5 mg	115-675-205	0.25 ml	115-155-205	0.5 mg	115-685-205	0.25 ml	115-225-205	0.5 mg	115-545-205	0.5 mg	115-095-205	0.5 mg
115-475-206	0.5 mg	115-675-206	0.25 ml	115-155-206	0.5 mg	115-685-206	0.25 ml	115-225-206	0.5 mg	115-545-206	0.5 mg	115-095-206	0.5 mg
115-475-207	0.5 mg	115-675-207	0.25 ml	115-155-207	0.5 mg	115-685-207	0.25 ml	115-225-207	0.5 mg	115-545-207	0.5 mg	115-095-207	0.5 mg
115-475-208	0.5 mg			115-155-208	0.5 mg					115-545-208	0.5 mg	115-095-208	0.5 mg
115-475-209	0.5 mg	115-675-209	0.25 ml	115-155-209	0.5 mg	115-685-209	0.25 ml	115-225-209	0.5 mg	115-545-209	0.5 mg	115-095-209	0.5 mg

Allophycocyanin APC A=650, E=660		Alexa Fluor® 647 A=651, E=667		Cyanine Cy™5 A=650, E=670		PerCP A=488, E=675		Alexa Fluor® 680 A=684, E=702		Alexa Fluor® 790 A=792, E=803	
115-135-205	0.3 ml	115-605-205	0.5 mg	115-175-205	0.5 mg	115-125-205	0.3 ml	115-625-205	0.3 mg	115-655-205	0.3 mg
115-135-206	0.3 ml	115-605-206	0.5 mg	115-175-206	0.5 mg	115-125-206	0.3 ml	115-625-206	0.3 mg	115-655-206	0.3 mg
115-135-207	0.3 ml	115-605-207	0.5 mg	115-175-207	0.5 mg	115-125-207	0.3 ml	115-625-207	0.3 mg	115-655-207	0.3 mg
115-135-208	0.3 ml	115-605-208	0.5 mg			115-125-208	0.3 ml				
115-135-209	0.3 ml	115-605-209	0.5 mg	115-175-209	0.5 mg	115-125-209	0.3 ml	115-625-209	0.3 mg	115-655-209	0.3 mg

ANTI-IgG LIGHT CHAIN SPECIFIC SECONDARY ANTIBODIES

Anti-IgG, light chain specific antibodies react with native primary antibodies used for detecting specific protein bands on Western blots after immunoprecipitation

Probing in the 50 kDa range after IP

If diluted properly, anti-light chain specific antibodies do not bind to the reduced and denatured IgG heavy chain band (50 kDa) on blots. Therefore, by using anti-light chain specific antibodies, detection of antigens with molecular weights near 50 kDa is not obscured by large amounts of reduced and denatured IgG heavy chains from primary antibodies used for immunoprecipitation (IP). For more information see Western blotting section pages 26-29.

Caution: Although the antibodies react strongly with native IgG light chains, some do not react as strongly with reduced and denatured light chains on blots. Therefore, they are not recommended for sensitive and quantitative detection of reduced and denatured light chains.

Specificity

The antibodies have been thoroughly adsorbed to minimize cross-reactivity with immunoglobulins from many other species, which also may be present on blots.

25 kDa Protein of interest

If the protein of interest has a reduced and denatured molecular weight near 25 kDa, anti-IgG, **Fc fragment specific** antibodies may be used to detect IgG primary antibodies, without binding to the 25 kDa band of reduced and denatured IgG light chains on Western blots.
See page 28 for more information.

ANTI-IgG LIGHT CHAIN SPECIFIC SECONDARY ANTIBODIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)
	ANTI-GOAT				
MOUSE	IgG Fraction Monoclonal Mouse Anti-Goat IgG, light chain specific (min X Hrs, Hu, Ms, Rb, Rat Ig) (!)	205-002-176 1.0 mg	205-032-176 0.5 ml	205-052-176 0.5 ml	205-062-176 0.5 ml
	ANTI-MOUSE				
GOAT	Goat Anti-Mouse IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Rb, Rat, Shp Ig) (kLC)	115-005-174 1.0 mg	115-035-174 0.5 ml	115-055-174 0.5 ml	115-065-174 0.5 ml
	ANTI-RABBIT				
MOUSE	IgG Fraction Monoclonal Mouse Anti-Rabbit IgG, light chain specific (min X Bov, Gt, Ar Hms, Hrs, Hu, Ms, Rat, Shp Ig)	211-002-171 1.0 mg	211-032-171 0.5 ml	211-052-171 0.5 ml	211-062-171 0.5 ml
	ANTI-RAT				
GOAT	Goat Anti-Rat IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Ms, Rb, Shp Ig) (kLC)	112-005-175 1.0 mg	112-035-175 0.5 ml	112-055-175 0.5 ml	112-065-175 0.5 ml
	ANTI-SHEEP				
MOUSE	IgG Fraction Monoclonal Mouse Anti-Sheep IgG, light chain specific (min X Bov, Hrs, Hu, Ms, Rb, Rat Ig)	213-002-177 1.0 mg	213-032-177 0.5 ml	213-052-177 0.5 ml	213-062-177 0.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

(kLC) This antibody reacts primarily with kappa light chains. It is not suitable for detection of primary antibodies with lambda light chains.

Alexa Fluor® 488 A=493, E=519	Cyanine Cy™3 A=550, E= 570	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
205-542-176 0.5 mg	205-162-176 0.5 mg	205-582-176 0.5 mg	205-602-176 0.5 mg	205-622-176 0.3 mg	205-652-176 0.3 mg
115-545-174 0.5 mg	115-165-174 0.5 mg	115-585-174 0.5 mg	115-605-174 0.5 mg	115-625-174 0.3 mg	115-655-174 0.3 mg
211-542-171 0.5 mg	211-162-171 0.5 mg	211-582-171 0.5 mg	211-602-171 0.5 mg	211-622-171 0.3 mg	211-652-171 0.3 mg
112-545-175 0.5 mg	112-165-175 0.5 mg	112-585-175 0.5 mg	112-605-175 0.5 mg	112-625-175 0.3 mg	112-655-175 0.3 mg
213-542-177 0.5 mg	213-162-177 0.5 mg	213-582-177 0.5 mg	213-602-177 0.5 mg	213-622-177 0.3 mg	213-652-177 0.3 mg

ADDITIONAL SECONDARY ANTIBODY CONJUGATES

FLUORESCENT PROTEIN CONJUGATES

Introduction

Jackson ImmunoResearch offers secondary antibodies, streptavidin and immunoglobulin controls conjugated to 3 fluorescent proteins: Phycoerythrin (R-PE), Allophycocyanin (APC), and Peridinin-Chlorophyll-Protein (PerCP). R-PE and APC are light-harvesting phycobiliproteins found in red, blue-green and cryptomonad algae.

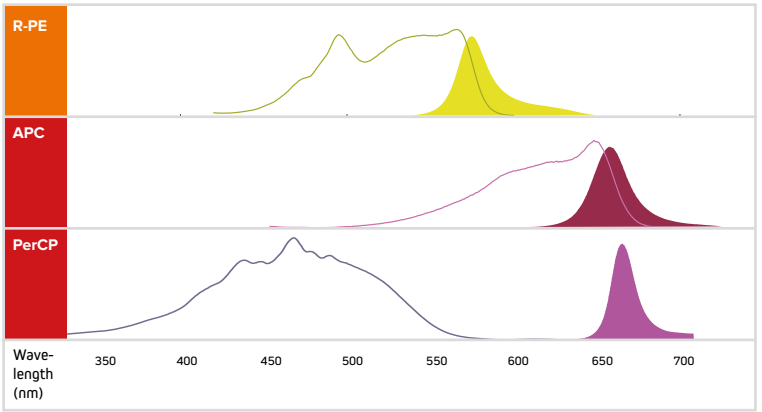


Figure 54: Excitation and emission characteristics of R-PE, APC and PerCP.

	Excitation range (nm)	Excitation laser (nm)	Emission peak (nm)
R-PE	450 to 570	488	580
APC	500 to 670	650	660
PerCP	370 to 570	488	675

Application

R-PE, PerCP, and APC can be excited by light over a wide range of the visible spectrum, are highly water soluble, have relatively low isoelectric points, and lack potentially sticky carbohydrates.

The fluorescent protein conjugates are predominantly used for surface labeling of cells for flow cytometry. Their relatively high molecular weights may preclude their use in procedures requiring penetration into cells and tissues.

For more information on fluorescent protein conjugates see pages 14-18.

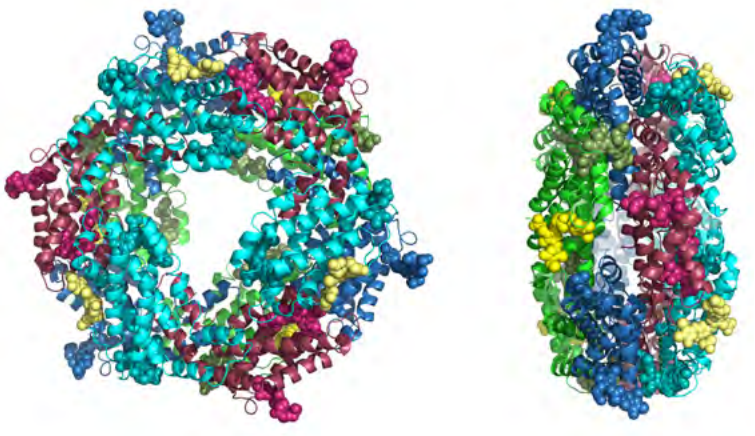


Figure 55: Crystal structure of a representative R-PE (from Polysiphonia urceolata). Illustrated using PDB 1LIA (Chang et al. (1996)) with the PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

FLUORESCENT PROTEIN CONJUGATES

	Antibody Description		Phycoerythrin R-PE A=488, E=580		Allophycocyanin APC A=650, E=660		PerCP A=488, E=675	
	ANTI-CHICKEN							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	(ML)	703-116-155	1.0 ml	703-136-155	0.5 ml	703-126-155	0.5 ml
	ANTI-GOAT							
DONKEY	Whole IgG Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(!)	705-115-147	1.0 ml				
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(!) (ML)	705-116-147	1.0 ml	705-136-147	0.5 ml	705-126-147	0.5 ml
	ANTI-GUINEA PIG							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	(ML)	706-116-148	1.0 ml	706-136-148	0.5 ml	706-126-148	0.5 ml
	ANTI-ARMENIAN HAMSTER							
GOAT	Whole IgG Goat Anti-Armenian Hamster IgG (H+L) (min X Bov, Hu, **Ms**, Rb, **Rat** Sr Prot)	(SP)	127-115-160	1.0 ml	127-135-160	0.5 ml	127-125-160	0.5 ml
	ANTI-HUMAN							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot)	(ML)	709-116-149	1.0 ml	709-136-149	0.5 ml	709-126-149	0.5 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)		709-116-098	1.0 ml	709-136-098	0.5 ml	709-126-098	0.5 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov, Hrs Sr Prot)		709-116-073	1.0 ml	709-136-073	0.5 ml	709-126-073	0.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

	Antibody Description		Phycoerythrin R-PE A=488, E=580		Allophycocyanin APC A=650, E=660		PerCP A=488, E=675	
	ANTI-HUMAN							
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG (H+L) (min X Bov, Hrs, Ms Sr Prot)		109-116-088	1.0 ml	109-136-088	0.5 ml	109-126-088	0.5 ml
GOAT	Whole IgG Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	(ML)	109-115-098	1.0 ml	109-135-098	0.5 ml	109-125-098	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	(ML)	109-116-098	1.0 ml	109-136-098	0.5 ml	109-126-098	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Ms, Rb Sr Prot)	(ML)	109-116-170	1.0 ml	109-136-170	0.5 ml	109-126-170	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG, $F(ab')_2$ fragment specific (min X Bov, Hrs, Ms Sr Prot)		109-116-097	1.0 ml	109-136-097	0.5 ml	109-126-097	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgG + IgM (H+L) (min X Bov Sr Prot)		109-116-127	1.0 ml	109-136-127	0.5 ml	109-126-127	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov Sr Prot)	(ML)	109-116-129	1.0 ml				
GOAT	Whole IgG Goat Anti-Human Serum IgA, α chain specific	(ML)	109-115-011	1.0 ml	109-135-011	0.5 ml	109-125-011	0.5 ml
	ANTI-MOUSE							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	(ML)	715-116-150	1.0 ml	715-136-150	0.5 ml	715-126-150	0.5 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat,** Shp Sr Prot)	(SP) (ML)	715-116-151	1.0 ml	715-136-151	0.5 ml	715-126-151	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot)	(ML)	115-116-146	1.0 ml	115-136-146	0.5 ml	115-126-146	0.5 ml

FLUORESCENT PROTEIN CONJUGATES

	Antibody Description		Phycoerythrin R-PE A=488, E=580		Allophycocyanin APC A=650, E=660		PerCP A=488, E=675	
	ANTI-MOUSE							
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-116-071	1.0 ml	115-136-071	0.5 ml	115-126-071	0.5 ml
GOAT	Whole IgG Goat Anti-Mouse IgG (subclasses 1+2a+2b+3), Fc_γ fragment specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-164	1.0 ml	115-135-164	0.5 ml	115-125-164	0.5 ml
GOAT	Whole IgG Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-205	0.5 ml	115-135-205	0.3 ml	115-125-205	0.3 ml
GOAT	Whole IgG Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-206	0.5 ml	115-135-206	0.3 ml	115-125-206	0.3 ml
GOAT	Whole IgG Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-207	0.5 ml	115-135-207	0.3 ml	115-125-207	0.3 ml
GOAT	Whole IgG Goat Anti-Mouse IgG, Fc_γ subclass 2c specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-208	0.5 ml	115-135-208	0.3 ml	115-125-208	0.3 ml
GOAT	Whole IgG Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-115-209	0.5 ml	115-135-209	0.3 ml	115-125-209	0.3 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)		115-116-072	1.0 ml	115-136-072	0.5 ml	115-126-072	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)		115-116-068	1.0 ml	115-136-068	0.5 ml	115-126-068	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-116-075	1.0 ml	115-136-075	0.5 ml	115-126-075	0.5 ml
	ANTI-RABBIT							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	ML	711-116-152	1.0 ml	711-136-152	0.5 ml	711-126-152	0.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

	Antibody Description		Phycoerythrin R-PE A=488, E=580		Allophycocyanin APC A=650, E=660		PerCP A=488, E=675	
	ANTI-RABBIT							
GOAT	$F(ab')_2$ Fragment Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot)	(ML)	111-116-144	1.0 ml	111-136-144	0.5 ml	111-126-144	0.5 ml
	ANTI-RAT							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	(ML)	712-116-150	1.0 ml	712-136-150	0.5 ml	712-126-150	0.5 ml
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms,** Rb, Shp Sr Prot)	(SP) (ML)	712-116-153	1.0 ml	712-136-153	0.5 ml	712-126-153	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot)	(ML)	112-116-143	1.0 ml	112-136-143	0.5 ml	112-126-143	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	112-116-071	1.0 ml	112-136-071	0.5 ml	112-126-071	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgG, $F(ab')_2$ fragment specific (min X Hu, Bov, Hrs Sr Prot)		112-116-072	1.0 ml	112-136-072	0.5 ml	112-126-072	0.5 ml
GOAT	$F(ab')_2$ Fragment Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	112-116-075	1.0 ml	112-136-075	0.5 ml	112-126-075	0.5 ml
	ANTI-SHEEP							
DONKEY	$F(ab')_2$ Fragment Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(!) (ML)	713-116-147	1.0 ml	713-136-147	0.5 ml	713-126-147	0.5 ml
	Streptavidin		016-110-084	1.0 ml	016-130-084	0.5 ml	016-120-084	0.5 ml

BRILLIANT VIOLET™ CONJUGATES

Brilliant Violet™ polymer chains can be considered as a collection of optical segments, each with the ability to absorb light and emit fluorescence signal. This results in materials that have a bright fluorescence signal for better resolution and sensitivity.

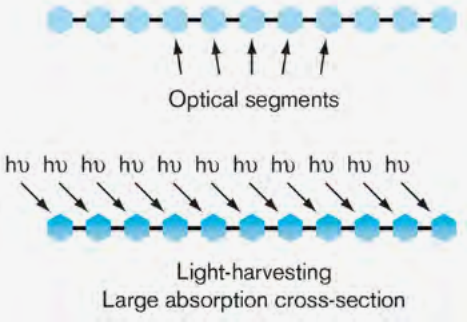


Figure 56: Image from BD Biosciences. Brilliant Violet dyes are provided under an intellectual property license from Sirigen Inc., a Becton, Dickinson and Company affiliate.

Excitation and Emission characteristics

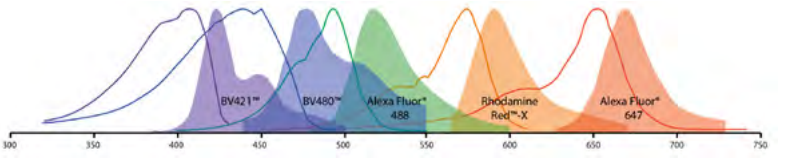
	Excitation max (nm)	Emission max (nm)
Brilliant Violet 421™	407	421
Brilliant Violet 480™	436	478

Applications

Add more colors to multiple labeling assays with Brilliant Violet 421™ (BV421™) and Brilliant Violet 480™ (BV480™) conjugated secondary antibodies. When combined with Alexa Fluor® 488, Rhodamine Red™-X, and Alexa Fluor® 647 conjugates, effective 5-color fluorescent labeling is possible.

Four-color antibody staining is possible using either DAPI or DRAQ5™ as a nuclear counterstain. See Figure 57 for examples of 4- and 5-color fluorescent staining using BV dye conjugates.

Figure 57: Options for 5 color immunofluorescence with BV dyes.



Channel	Blue	Cyan	Green	Orange	Red
Option 1	DAPI	BV480	Alexa Fluor® 488	RR-X	Alexa Fluor® 647
Option 2	BV421	BV480	Alexa Fluor® 488	RR-X	DRAQ5
Option 3	BV421	BV480	Alexa Fluor® 488	RR-X	Alexa Fluor® 647

Jackson ImmunoResearch offers a range of BV dye-conjugated secondary antibodies that are recommended for multiple labeling, See multiple labeling guidelines, page 35.

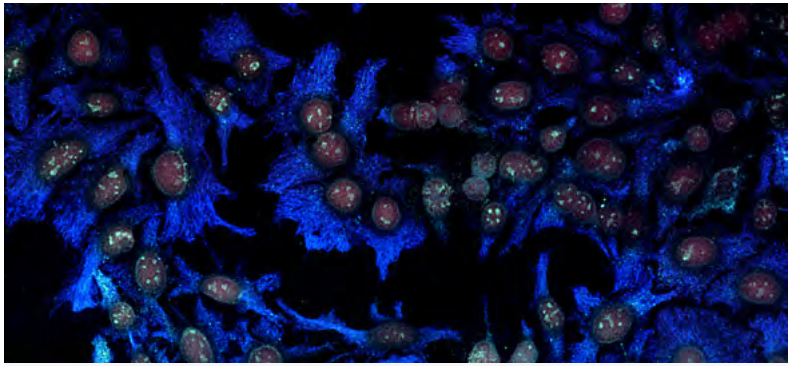


Figure 58: Double immunofluorescence of Hep-2 cells using Brilliant Violet 421 and 480 conjugated secondary antibodies (Rabbit Anti-Ki67 visualized with BV 480 Goat Anti-Rabbit 111-685-144; Mouse Anti-Tubulin visualized with BV 421 Goat Anti-Mouse 115-675-166).

BRILLIANT VIOLET™ CONJUGATES

	Antibody Description		Brilliant Violet 421™ A=407, E=421	Brilliant Violet 480™ A=436, E=478
	ANTI-CHICKEN			
DONKEY	Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	(ML)	703-675-155 0.25 ml	703-685-155 0.25 ml
	ANTI-GOAT			
DONKEY	Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(!) (ML)	705-675-147 0.25 ml	705-685-147 0.25 ml
	ANTI-GUINEA PIG			
DONKEY	Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	(ML)	706-675-148 0.25 ml	706-685-148 0.25 ml
	ANTI-HUMAN			
DONKEY	Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot)	(ML)	709-675-149 0.25 ml	709-685-149 0.25 ml
GOAT	Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	(ML)	109-675-098 0.25 ml	109-685-098 0.25 ml
	ANTI-MOUSE			
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	(ML)	715-675-150 0.25 ml	715-685-150 0.25 ml
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot)	(SP) (ML)	715-675-151 0.25 ml	715-685-151 0.25 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot)	(ML)	115-675-146 0.25 ml	115-685-146 0.25 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

BRILLIANT VIOLET™ CONJUGATES

	Antibody Description		Brilliant Violet 421™ A=407, E=421	Brilliant Violet 480™ A=436, E=478
	ANTI-MOUSE			
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot)	SP ML	115-675-166 0.25 ml	115-685-166 0.25 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-675-071 0.25 ml	115-685-071 0.25 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-675-205 0.25 ml	115-685-205 0.25 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	ML	115-675-206 0.25 ml	115-685-206 0.25 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	ML	115-675-207 0.25 ml	115-685-207 0.25 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-675-209 0.25 ml	115-685-209 0.25 ml
GOAT	Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-675-075 0.25 ml	115-685-075 0.25 ml
	ANTI-RABBIT			
DONKEY	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	ML	711-675-152 0.25 ml	711-685-152 0.25 ml
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot)	ML	111-675-144 0.25 ml	111-685-144 0.25 ml
	ANTI-RAT			
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	ML	712-675-150 0.25 ml	712-685-150 0.25 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

	Antibody Description		Brilliant Violet 421™ A=407, E=421	Brilliant Violet 480™ A=436, E=478
	ANTI-RAT			
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms**, Rb, Shp Sr Prot)	SP ML	712-675-153 0.25 ml	712-685-153 0.25 ml
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot)	ML	112-675-143 0.25 ml	112-685-143 0.25 ml
	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Rb Sr Prot)	SP ML	112-675-167 0.25 ml	112-685-167 0.25 ml
	Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	ML	112-675-071 0.25 ml	112-685-071 0.25 ml
	Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	ML	112-675-075 0.25 ml	112-685-075 0.25 ml
	ANTI-SHEEP			
DONKEY	Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	! ML	713-675-147 0.25 ml	713-685-147 0.25 ml

NEAR-INFRARED (NIR) FLUORESCENT CONJUGATES

Antibodies conjugated with far-red- and infrared-emitting dyes are suitable for a variety of techniques requiring the highest sensitivity. Alexa Fluor® 680 and 790 dyes are more sensitive than visible light-emitting dyes due to lower fluorescence quenching of the conjugates and higher extinction coefficients of the dyes. Low sample autofluorescence in this region of the spectrum results in lower background signal compared with other fluorophores.

Applications

The increased brightness of far-red and infrared dye conjugates (Alexa Fluor® 680 and 790) allows for a wide range of immunofluorescence detection and imaging modalities.

Western Blotting

Alexa Fluor® 680 and 790 conjugates can be used for high sensitivity Western blots, quantitative Western blots, in-gel Western blots, microWestern arrays, in-cell Western assays, on-cell Western assays, and other techniques that require the brightest dyes.

Fluorescence microscopy

Alexa Fluor® 680 and 790 secondary antibodies are adsorbed to minimize cross-reactions with others species and/or with other immunoglobulin classes, facilitating multiple labeling.

Flow cytometry

With modern flow cytometers now able to accommodate longer wavelengths, Alexa Fluor® 680 and 790 provide expanded choice for flow cytometry dye panels. See pages 39-40 for more information on JIR secondary antibodies for flow cytometry.

Format

Jackson ImmunoResearch offers a comprehensive selection of Alexa Fluor® 680 and Alexa Fluor® 790 dyes conjugated to secondary antibodies, streptavidin, signal enhancing antibodies (see page 139) and purified Donkey, Goat and Mouse IgG controls (see pages 150 – 153).

	Antibody Description	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
	ANTI-CHICKEN		
DONKEY	Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	703-625-155 0.5 mg	703-655-155 0.5 mg
	ANTI-GOAT		
DONKEY	Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	705-625-147 0.5 mg	705-655-147 0.5 mg
MOUSE	IgG Fraction Monoclonal Mouse Anti-Goat IgG, light chain specific (min X Hrs, Hu, Ms, Rb, Rat Ig) (!)	205-622-176 0.3 mg	205-652-176 0.3 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

	Antibody Description		Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
	ANTI-GUINEA PIG			
DONKEY	Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot)	ML	706-625-148 0.5 mg	706-655-148 0.5 mg
	ANTI-ARMENIAN HAMSTER			
GOAT	Goat Anti-Armenian Hamster IgG (H+L) (min X Bov, Hu, **Ms**, Rb, **Rat** Sr Prot)	SP	127-625-160 0.3 mg	127-655-160 0.3 mg
	ANTI-HUMAN			
DONKEY	Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot)	ML	709-625-149 0.5 mg	709-655-149 0.5 mg
GOAT	Goat Anti-Human IgG, Fc_{γ} fragment specific (min X Bov, Hrs, Ms Sr Prot)	ML	109-625-098 0.5 mg	109-655-098 0.5 mg
	Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific (min X Bov Sr Prot)	ML	109-625-129 0.5 mg	109-655-129 0.5 mg
	ANTI-MOUSE			
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	ML	715-625-150 0.5 mg	715-655-150 0.5 mg
	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot)	SP ML	715-625-151 0.3 mg	715-655-151 0.3 mg
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot)	ML	115-625-146 0.5 mg	115-655-146 0.5 mg
	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot)	SP ML	115-625-166 0.3 mg	115-655-166 0.3 mg

NEAR-INFRARED (NIR) FLUORESCENT CONJUGATES

	Antibody Description		Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
	ANTI-MOUSE			
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	115-625-071 0.5 mg	115-655-071 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-625-205 0.3 mg	115-655-205 0.3 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-625-206 0.3 mg	115-655-206 0.3 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-625-207 0.3 mg	115-655-207 0.3 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	(ML)	115-625-209 0.3 mg	115-655-209 0.3 mg
GOAT	Goat Anti-Mouse IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Rb, Rat, Shp Ig)	(kLC)	115-625-174 0.3 mg	115-655-174 0.3 mg
GOAT	Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	115-625-075 0.5 mg	115-655-075 0.5 mg
	ANTI-RABBIT			
DONKEY	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	(ML)	711-625-152 0.5 mg	711-655-152 0.5 mg
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot)	(ML)	111-625-144 0.5 mg	111-655-144 0.5 mg
MOUSE	IgG Fraction Monoclonal Mouse Anti-Rabbit IgG, light chain specific (min X Bov, Gt, Ar Hms, Hrs, Hu, Ms, Rat, Shp Ig)		211-622-171 0.3 mg	211-652-171 0.3 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).
(kLC) This antibody reacts primarily with kappa light chains. It is not suitable for detection of primary antibodies with lambda light chains.

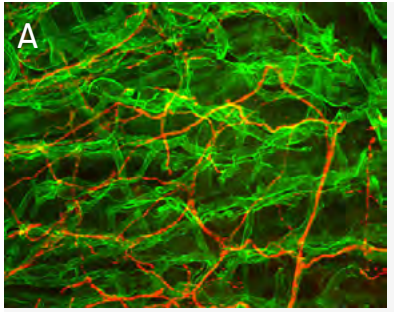
	Antibody Description		Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
	ANTI-RAT			
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	(ML)	712-625-150 0.5 mg	712-655-150 0.5 mg
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms**, Rb, Shp Sr Prot)	(SP) (ML)	712-625-153 0.3 mg	712-655-153 0.3 mg
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot)	(ML)	112-625-143 0.5 mg	112-655-143 0.5 mg
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Rb Sr Prot)	(SP) (ML)	112-625-167 0.3 mg	112-655-167 0.3 mg
GOAT	Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	112-625-071 0.5 mg	112-655-071 0.5 mg
GOAT	Goat Anti-Rat IgG, light chain specific (min X Bov, Gt, Hrs, Hu, Ms, Rb, Shp Ig)	(kLC)	112-625-175 0.3 mg	112-655-175 0.3 mg
GOAT	Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	(ML)	112-625-075 0.5 mg	112-655-075 0.5 mg
	ANTI-SHEEP			
DONKEY	Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	(!) (ML)	713-625-147 0.5 mg	713-655-147 0.5 mg
MOUSE	IgG Fraction Monoclonal Mouse Anti-Sheep IgG, light chain specific (min X Bov, Hrs, Hu, Ms, Rb, Rat Ig)		213-622-177 0.3 mg	213-652-177 0.3 mg
	Streptavidin		016-620-084 0.5 mg	016-650-084 0.5 mg

CYANINE CONJUGATES FOR PERMANENT MOUNTING

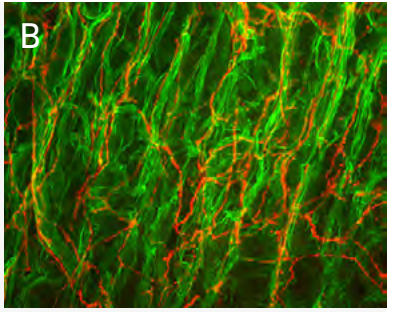
Among currently available fluorescent dyes, the cyanine dyes offer the best choice for permanent mounting. The dyes are better able to withstand the harsh dehydration and embedding conditions required for mounting sections in non-polar plastic media, such as DPX and Permount. The major advantages of plastic over aqueous mounting media are brightness, contrast, longevity of fluorescence and sample storage lifetime.

The cyanine dyes are brighter in the non-polar environment than in aqueous media, resulting in less acquisition time in the confocal microscope than that required for Alexa Fluor® dyes, even though Alexa Fluors are brighter in aqueous mounting media.

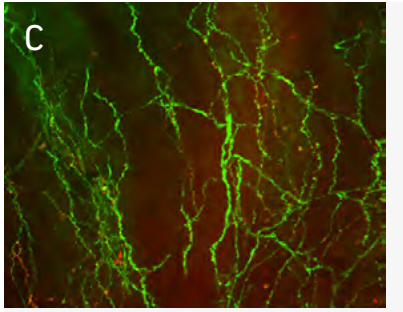
Figure 59 shows comparable images generated using shorter exposure times with Cy™2 dyes compared to other fluorophores with similar spectral characteristics.



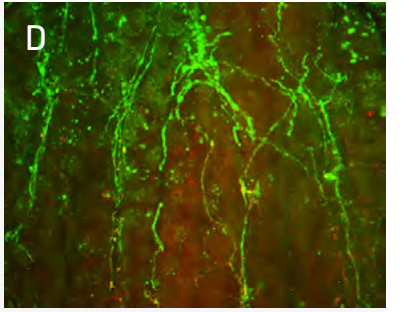
Cy2-Goat Anti-Mouse IgG (H+L)
Exposure time **36.7ms**



Alexa Fluor® 488-Goat Anti-Mouse IgG (H+L)
Exposure time **222.4ms**



Cy2-Goat Anti-Mouse IgG (H+L)
Exposure time **222.4ms**



Alexa Fluor® 488-Goat Anti-Mouse IgG (H+L)
Exposure time **889.6ms**

Figure 59. Sections of gastric mucosa were stained and mounted in DPX. Sections A and B were stained with primary antibodies for abundant antigens (mouse anti-Type IV collagen [green] and rabbit anti-PGP 9.5 [red]). Sections C and D were stained with primary antibodies for less abundant antigens (mouse anti-PGP 9.5 [green] and rabbit anti-substance P [red]).

All sections were stained with fluorophore-conjugated secondary antibodies from Jackson ImmunoResearch. Sections were exposed to light long enough to achieve approximately the same brightness. Exposure times, expressed in milliseconds (ms), are therefore an indication of the relative brightness of each fluorophore.

Note that Cy2 conjugates required significantly less exposure time in DPX than Alexa Fluor® 488, both with abundant and less abundant antigens. Other antigens that are less visible in Cy2-stained sections were reportedly not visible with Alexa Fluor® 488. Similar differences were observed when the other cyanines, Cy™3 and Cy™5, were compared with corresponding Alexa Fluor® dyes.

Images and results are courtesy of Dr. Gwen Wendelschafer-Crabb, Kennedy Lab, University of Minnesota. Similar results were reported to us by Dr. Barbara Jones, Department of Neurology and Neurosurgery, McGill University.

CYANINE CONJUGATES FOR PERMANENT MOUNTING

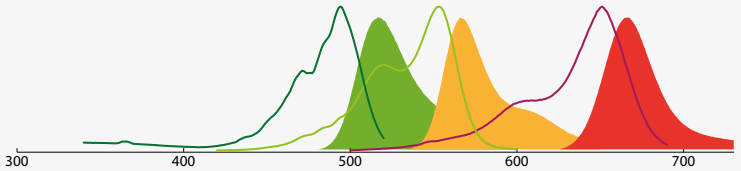


Figure 60. Excitation (line) and emission (solid) spectra of Cy2 (green), Cy3 (yellow) and Cy5 (red). Peak heights were normalized after the spectra were obtained with an M-series spectrofluorometer system from Photon Technology International, Inc.

Fluorophore	Excitation Peak (nm)	Emission Peak (nm)
Cyanine, Cy™2	492	510
Indocarbocyanine, Cy™3	550	570
Indodicarbocyanine, Cy™5	650	670

As shown in the accompanying table, Cy2, Cy3 and Cy5 are available conjugated to a selection of secondary antibodies for multiple labeling, purified IgG controls, and streptavidin. Figure 60 illustrates the discrete spectra that provide effective separation for multiple labeling.

A larger selection of Cy3-conjugated antibodies can be found in tables of whole IgG (pages 46-71) and F(ab')$_2$ fragment (pages 74-85) affinity-purified secondary antibodies. Cy3 is bright and photostable in both aqueous and non-polar media, making it a more versatile fluorophore than Cy2 and Cy5.

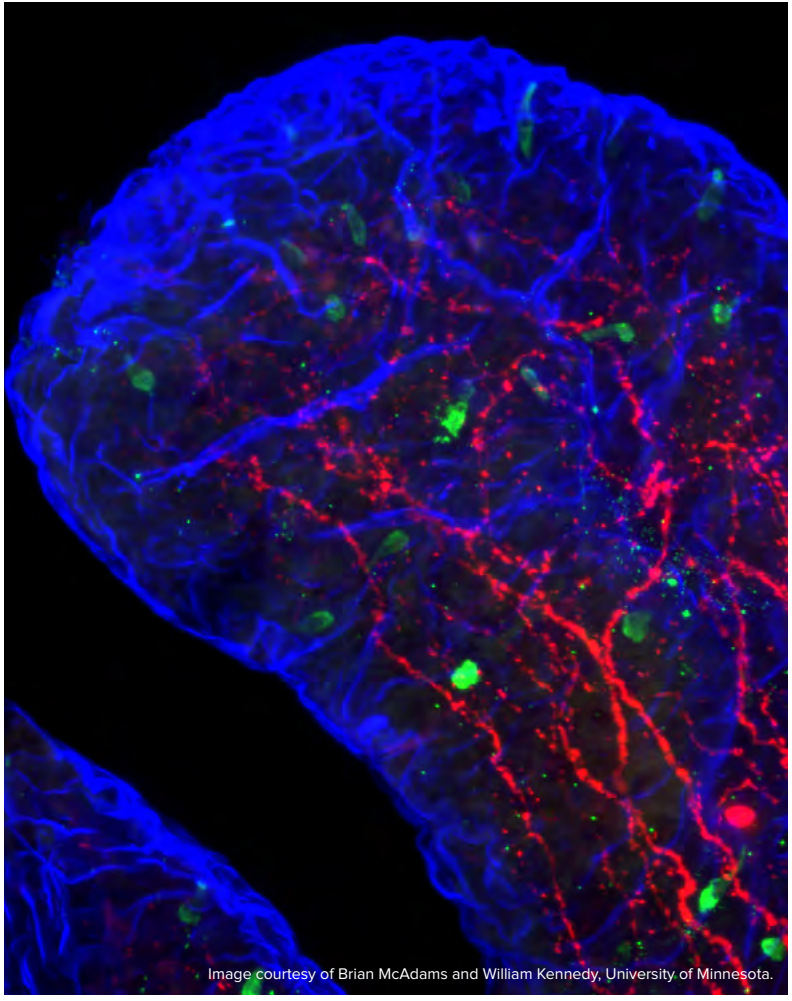


Image courtesy of Brian McAdams and William Kennedy, University of Minnesota.

CYANINE CONJUGATES

	Antibody Description	Cyanine Cy™2 A=492, E=510	Cyanine Cy™3 A=550, E= 570	Cyanine Cy™5 A=650, E=670
	ANTI-CHICKEN			
DONKEY	Donkey Anti Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	703-225-155 0.5 mg	703-165-155 0.5 mg	703-175-155 0.5 mg
	ANTI-GOAT			
DONKEY	Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	705-225-147 0.5 mg	705-165-147 0.5 mg	705-175-147 0.5 mg
	ANTI-GUINEA PIG			
DONKEY	Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	706-225-148 0.5 mg	706-165-148 0.5 mg	706-175-148 0.5 mg
	ANTI-HUMAN			
DONKEY	Donkey Anti-Human IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Ms, Rb, Rat, Shp Sr Prot) (ML)	709-225-149 0.5 mg	709-165-149 0.5 mg	709-175-149 0.5 mg
	ANTI-MOUSE			
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot) (ML)	715-225-150 0.5 mg	715-165-150 0.5 mg	715-175-150 0.5 mg
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot) (SP) (ML)	715-225-151 0.5 mg	715-165-151 0.5 mg	715-175-151 0.5 mg
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot) (ML)	115-225-146 1.5 mg	115-165-146 1.5 mg	115-175-146 1.5 mg
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot) (SP) (ML)	115-225-166 0.5 mg	115-165-166 0.5 mg	115-175-166 0.5 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

	Antibody Description		Cyanine Cy™2 A=492, E=510	Cyanine Cy™3 A=550, E= 570	Cyanine Cy™5 A=650, E=670
	ANTI-MOUSE				
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-225-071 1.5 mg	115-165-071 1.5 mg	115-175-071 1.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 1 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-225-205 0.5 mg	115-165-205 0.5 mg	115-175-205 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2a specific (min X Hu, Bov, Rb Sr Prot)	ML	115-225-206 0.5 mg	115-165-206 0.5 mg	115-175-206 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 2b specific (min X Hu, Bov, Rb Sr Prot)	ML	115-225-207 0.5 mg	115-165-207 0.5 mg	115-175-207 0.5 mg
GOAT	Goat Anti-Mouse IgG, Fc_γ subclass 3 specific (min X Hu, Bov, Rb Sr Prot)	ML	115-225-209 0.5 mg	115-165-209 0.5 mg	115-175-209 0.5 mg
GOAT	Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot)	ML	115-225-075 1.0 mg	115-165-075 1.0 mg	115-175-075 1.0 mg
	ANTI-RABBIT				
DONKEY	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	ML	711-225-152 0.5 mg	711-165-152 0.5 mg	711-175-152 0.5 mg
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot)	ML	111-225-144 1.5 mg	111-165-144 1.5 mg	111-175-144 1.5 mg
	ANTI-RAT				
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot)	ML	712-225-150 0.5 mg	712-165-150 0.5 mg	712-175-150 0.5 mg
DONKEY	Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, **Ms**, Rb, Shp Sr Prot)	SP ML	712-225-153 0.5 mg	712-165-153 0.5 mg	712-175-153 0.5 mg

CYANINE CONJUGATES

	Antibody Description	Cyanine Cy™2 A=492, E=510	Cyanine Cy™3 A=550, E= 570	Cyanine Cy™5 A=650, E=670
	ANTI-RAT			
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot) (ML)	112-225-143 1.5 mg	112-165-143 1.5 mg	112-175-143 1.5 mg
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Rb Sr Prot) (SP) (ML)	112-225-167 0.5 mg	112-165-167 0.5 mg	112-175-167 0.5 mg
GOAT	Goat Anti-Rat IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-225-071 1.5 mg	112-165-071 1.5 mg	112-175-071 1.5 mg
GOAT	Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-225-075 1.0 mg	112-165-075 1.0 mg	112-175-075 1.0 mg
	ANTI-SHEEP			
DONKEY	Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	713-225-147 0.5 mg	713-165-147 0.5 mg	713-175-147 0.5 mg
	Streptavidin	016-220-084 1.0 mg	016-160-084 1.0 mg	016-170-084 1.0 mg

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

IMMUNOGOLD COMPLEXES

ImmunoGold reagents offer excellent tissue penetration due to their small particle size (Dixon, 2015). ImmunoGold colloidal gold reagents are available for transmission (TEM) and scanning electron microscopy (SEM) (EM Grade 6, 12 and 18 nm), or for brightfield microscopy or immunoblotting (LM Grade 4 nm).

The EM Grade is distinguished from other commercial preparations by separation of monomeric particles from small aggregates using density gradients. The resulting monomeric colloidal gold-protein complexes are suitable for multiple labeling protocols, as different proteins can be labeled with different size particles. EM Grade complexes are also suitable for light microscopy. They are provided in sterile-filtered buffer containing stabilizers and a preservative.

For light microscopy, silver enhancement is commonly used for signal amplification of immunogold staining. A detailed protocol for silver enhancement, using easily prepared reagents, is available at www.jacksonimmuno.com. Silver enhancement kits are also commercially available. LM Grade colloidal gold-protein complexes can also be used for electron microscopy, though small aggregates could contribute to non-uniform labeling. These products are freeze-dried in buffer with stabilizers and a preservative. After rehydration, they may be frozen in aliquots for extended storage.

For more information about EM please see page 43.

IMMUNOGOLD COMPLEXES

	Antibody Description	4 nm Gold (LM Grade)	6 nm Gold (EM Grade)	12 nm Gold (EM Grade)	18 nm Gold (EM Grade)
	ANTI-CHICKEN				
DONKEY	Donkey Anti-Chicken IgY (IgG) (H+L) (min X Bov, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	703-185-155 0.5 ml	703-195-155 0.3 ml	703-205-155 0.3 ml	703-215-155 0.3 ml
	ANTI-GOAT				
DONKEY	Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	705-185-147 0.5 ml	705-195-147 0.3 ml	705-205-147 0.3 ml	705-215-147 0.3 ml
	ANTI-GUINEA PIG				
DONKEY	Donkey Anti-Guinea Pig IgG (H+L) (min X Bov, Ck, Gt, Sy Hms, Hrs, Hu, Ms, Rb, Rat, Shp Sr Prot) (ML)	706-185-148 0.5 ml	706-195-148 0.3 ml	706-205-148 0.3 ml	706-215-148 0.3 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species. (ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation).

	Antibody Description	4 nm Gold (LM Grade)	6 nm Gold (EM Grade)	12 nm Gold (EM Grade)	18 nm Gold (EM Grade)
	ANTI-HUMAN				
GOAT	Goat Anti-Human IgG (H+L) (min X Bov, Hrs, Ms Sr Prot)	109-185-088 1.0 ml	109-195-088 0.5 ml	109-205-088 0.5 ml	109-215-088 0.5 ml
GOAT	Goat Anti-Human IgG, Fc_γ fragment specific (min X Bov, Hrs, Ms Sr Prot)	109-185-098 1.0 ml			
	ANTI-MOUSE				
DONKEY	Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Shp Sr Prot) (ML)		715-195-150 0.3 ml	715-205-150 0.3 ml	715-215-150 0.3 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, Sw Sr Prot) (ML)	115-185-146 1.0 ml	115-195-146 0.5 ml	115-205-146 0.5 ml	115-215-146 0.5 ml
GOAT	Goat Anti-Mouse IgG (H+L) (min X Hu, Bov, Hrs, Rb, **Rat** Sr Prot) (SP) (ML)	115-185-166 0.5 ml	115-195-166 0.3 ml	115-205-166 0.3 ml	115-215-166 0.3 ml
GOAT	Goat Anti-Mouse IgG, Fc_γ fragment specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-185-071 1.0 ml	115-195-071 0.5 ml	115-205-071 0.5 ml	115-215-071 0.5 ml
GOAT	Goat Anti-Mouse IgG + IgM (H+L) (min X Hu, Bov, Hrs Sr Prot)	115-185-068 1.0 ml	115-195-068 0.5 ml	115-205-068 0.5 ml	115-215-068 0.5 ml
GOAT	Goat Anti-Mouse IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	115-185-075 1.0 ml	115-195-075 0.5 ml	115-205-075 0.5 ml	115-215-075 0.5 ml

IMMUNOGOLD COMPLEXES

	Antibody Description	4 nm Gold (LM Grade)	6 nm Gold (EM Grade)	12 nm Gold (EM Grade)	18 nm Gold (EM Grade)
	ANTI-RABBIT				
DONKEY	Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot) (ML)	711-185-152 0.5 ml	711-195-152 0.3 ml	711-205-152 0.3 ml	711-215-152 0.3 ml
GOAT	Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot) (ML)	111-185-144 1.0 ml	111-195-144 0.5 ml	111-205-144 0.5 ml	111-215-144 0.5 ml
	ANTI-RAT				
GOAT	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, Rb Sr Prot) (ML)	112-185-143 1.0 ml	112-195-143 0.5 ml	112-205-143 0.5 ml	112-215-143 0.5 ml
	Goat Anti-Rat IgG (H+L) (min X Hu, Bov, Hrs, **Ms**, Rb Sr Prot) (SP) (ML)	112-185-167 0.5 ml	112-195-167 0.3 ml	112-205-167 0.3 ml	112-215-167 0.3 ml
	Goat Anti-Rat IgM, μ chain specific (min X Hu, Bov, Hrs Sr Prot) (ML)	112-185-075 1.0 ml		112-205-075 0.5 ml	
	ANTI-SHEEP				
DONKEY	Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot) (!) (ML)	713-185-147 0.5 ml	713-195-147 0.3 ml	713-205-147 0.3 ml	713-215-147 0.3 ml
	ANTI-HORSERADISH PEROXIDASE				
GOAT	Goat Anti-Horseradish Peroxidase	123-185-021 1.0 ml	123-195-021 0.5 ml	123-205-021 0.5 ml	123-215-021 0.5 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.
(ML) Multiple Labeling (see Multiple Labeling on pages 35-36 for an explanation). (SP) Caution: See page 10 (min X ... Sr Prot) before selecting an antibody adsorbed against closely related species.

ADDITIONAL ANTIBODY SPECIFICITIES

ADDITIONAL ANTIBODY SPECIFICITIES

Peroxidase-Anti-Peroxidase (PAP) Soluble Immune Complexes

PAP soluble immune complexes are prepared by the method of Sternberger et al. (J Histoche. Cytochem. 1970. 12, 315). Theoretically, they consist predominantly of two anti-HRP antibodies in soluble complex with three molecules of HRP.

PAP soluble complexes are normally used at 25-50 µg/ml. Whole antisera against IgG (H+L) are recommended for bridging PAP to primary antibodies. Antisera against IgG (H+L) or against the $F(ab')_2$ fragments of IgG will also bridge PAP to IgM primary antibodies (such as IgM monoclonal antibodies) by virtue of common light-chain recognition. Normal serum (5% v/v) from the same host species as the bridging antibody is suggested as a blocking solution to minimize non-specific binding. All PAP soluble complexes are freeze-dried at 20 mg/ml.

Endogenous peroxidase activity: PAP soluble complexes, as well as other immunoperoxidase reagents, are not recommended for tissues or cells in which endogenous peroxidase activity is difficult to suppress. In such cases, other immunoenzyme reagents may be used. Alternatively, anti-horseradish peroxidase conjugated with other enzymes or fluorophores may be used to enhance signal and reduce background, since the final signal does not depend on the enzyme activity of peroxidase, but on the antigenicity of horseradish peroxidase.

PEROXIDASE-ANTI-PEROXIDASE (PAP) SOLUBLE IMMUNE COMPLEXES

Description	Code Number	Concentration	Fill Size
Goat PAP	123-005-024	20 mg/ml	0.25 ml
Mouse PAP	223-005-024	20 mg/ml	0.25 ml
Rabbit PAP	323-005-024	20 mg/ml	0.25 ml

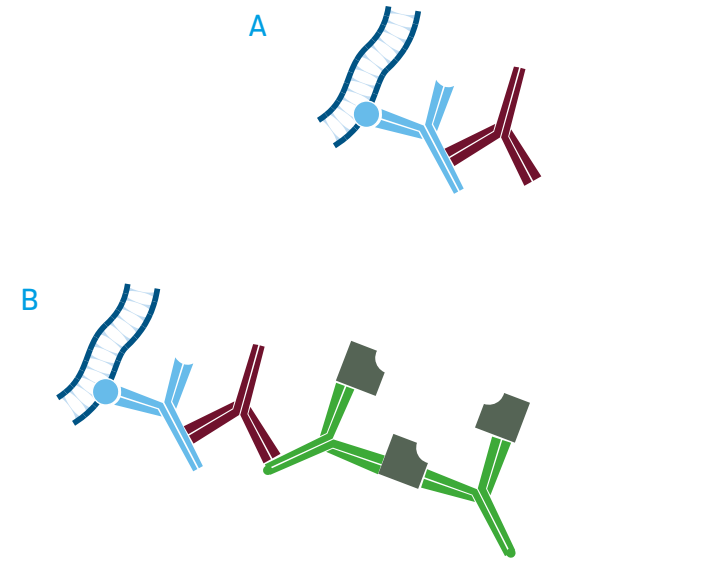


Figure 61: A. Primary antibody is bound to antigen, and a bridging antibody has been added to link the primary antibody with corresponding PAP complex. B. PAP complex (three molecules of HRP complexed with two molecules of anti-HRP) has been captured by bridging antibody.

ADDITIONAL ANTIBODY SPECIFICITIES

Antibodies in this section include those directed against the haptens biotin, fluorescein and digoxin, and against horseradish peroxidase (HRP). Peroxidase-anti-peroxidase soluble immune complexes are also found in this section.

Anti-Biotin, Anti-Fluorescein and Anti-Digoxin antibodies

Monoclonal Mouse Anti-Biotin, Anti-Fluorescein and Anti-Digoxin are available in a wide range of conjugate options for direct detection of their target molecules, which are commonly used as tags on proteins and nucleic acids. If signal amplification is desired, these antibodies can be used unconjugated, followed by conjugated anti-mouse IgG (H+L) (see examples below using Mouse Anti-Biotin).

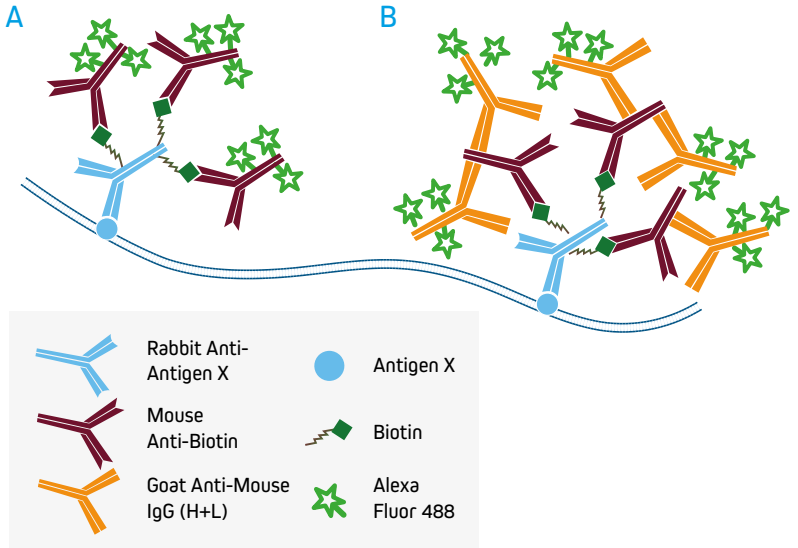


Figure 62: A. Direct detection of biotinylated primary antibodies with conjugated Mouse Anti-Biotin. B.Signal enhancement with conjugated Anti-Mouse IgG (H+L).

Detection with Anti-HRP antibodies

Affinity-purified anti-horseradish peroxidase (HRP) may be used to detect HRP or to enhance signal by binding to HRP-conjugated molecules. Anti-HRP also may be used to convert an HRP conjugate into a different signal as illustrated in the example below using ImmunoGold-complexed anti-HRP. Goat Anti-HRP is also available conjugated to alkaline phosphatase and fluorescent probes, providing additional flexibility to the detection method.

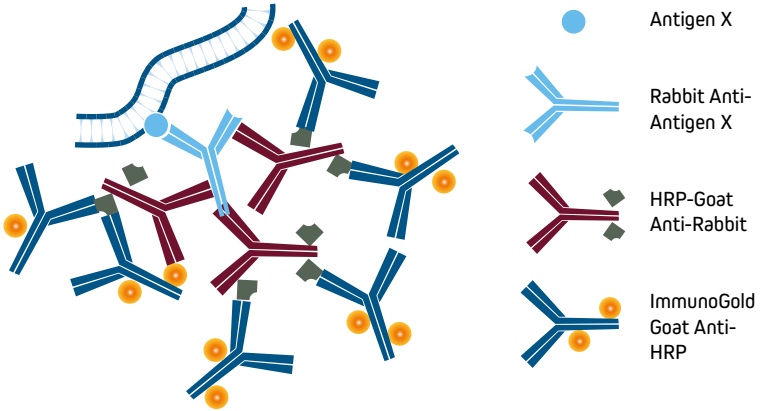


Figure 63. In this example, ImmunoGold-complexed Goat Anti-HRP binds to an HRP-conjugated secondary antibody. ImmunoGold Anti-HRP with silver enhancement has been used in place of the HRP substrate diaminobenzidine (DAB) to create less diffused images on tissue sections labeled with HRP reagents (Gee et al., J. Histochem. Cytochem. 1991, 39, 863, Roth et al., Methods in Lab. Invest. 1992. 67, 263).

ADDITIONAL ANTIBODY SPECIFICITIES

	Antibody Description	Unconjugated	Horseradish Peroxidase	Alkaline Phosphatase	Biotin-SP (long spacer)	DyLight™ 405 A=400, E=421	Coumarin AMCA A=350, E=450
	ANTI-DIGOXIN						
MOUSE	IgG Fraction Monoclonal Mouse Anti-Digoxin	200-002-156 1.0 mg	200-032-156 0.5 ml	200-052-156 0.5 ml	200-062-156 0.5 ml	200-472-156 0.5 mg	200-152-156 0.5 mg
	ANTI-BIOTIN						
MOUSE	IgG Fraction Monoclonal Mouse Anti-Biotin	200-002-211 1.0 mg	200-032-211 0.5 ml	200-052-211 0.5 ml		200-472-211 0.5 mg	200-152-211 0.5 mg
	ANTI-FLUORESCEIN						
MOUSE	IgG Fraction Monoclonal Mouse Anti-Fluorescein	200-002-037 1.0 mg	200-032-037 0.5 ml	200-052-037 0.5 ml	200-062-037 0.5 ml	200-472-037 0.5 mg	200-152-037 0.5 mg
	ANTI-HORSERADISH PEROXIDASE						
GOAT	Goat Anti-Horseradish Peroxidase	123-005-021 2.0 mg		123-055-021 1.0 ml	123-065-021 2.0 ml	123-475-021 1.5 mg	123-155-021 2.0 mg
RABBIT	Rabbit Anti-Horseradish Peroxidase	323-005-021 2.0 mg		323-055-021 1.0 ml	323-065-021 1.5 ml		323-155-021 1.5 mg

Alexa Fluor® 488 A=493, E=519	Fluorescein FITC A=492, E=520	Cyanine Cy™3 A=550, E= 570	Rhodamine Red™-X A=570, E=590	Alexa Fluor® 594 A=591, E=614	Alexa Fluor® 647 A=651, E=667	Alexa Fluor® 680 A=684, E=702	Alexa Fluor® 790 A=792, E=803
200-542-156 0.5 mg	200-092-156 0.5 mg	200-162-156 0.5 mg	200-292-156 0.5 mg	200-582-156 0.5 mg	200-602-156 0.5 mg	200-622-156 0.3 mg	200-652-156 0.3 mg
200-542-211 0.5 mg	200-092-211 0.5 mg	200-162-211 0.5 mg	200-292-211 0.5 mg	200-582-211 0.5 mg	200-602-211 0.5 mg	200-622-211 0.3 mg	200-652-211 0.3 mg
200-542-037 0.5 mg		200-162-037 0.5 mg	200-292-037 0.5 mg	200-582-037 0.5 mg	200-602-037 0.5 mg	200-622-037 0.3 mg	200-652-037 0.3 mg
123-545-021 1.5 mg	123-095-021 2.0 mg	123-165-021 2.0 mg	123-295-021 2.0 mg	123-585-021 1.5 mg	123-605-021 1.5 mg	123-625-021 0.5 mg	123-655-021 0.5 mg
323-545-021 1.0 mg	323-095-021 1.5 mg	323-165-021 1.5 mg	323-295-021 1.5 mg	323-585-021 1.0 mg	323-605-021 1.0 mg		

STREPTAVIDIN

STREPTAVIDIN

Introduction

Streptavidin is a tetrameric bacterial protein isolated from *Streptomyces avidinii* providing 4 high-affinity biotin binding sites (Figure 64). Comparisons of apo and liganded streptavidin crystal structures by Weber et al. (1989) showed that affinity is conferred by multiple hydrogen bonds and van der Waals interactions, which in conjunction with polypeptide loops confine the biotin in streptavidin's interior. The result is one of the strongest non-covalent bonds found in nature, with a femtomolar dissociation constant (Kd ~10^{-15}). Unlike egg-white avidin, which has a net positive charge at neutral pH and contains about 7% carbohydrate, streptavidin has almost no net charge at neutral pH, does not contain carbohydrate, and exhibits lower non-specific background.

Because of its binding characteristics, streptavidin is commonly employed for immunotechniques requiring signal amplification using biotinylated reagents.

Jackson ImmunoResearch streptavidin conjugates are recommended for use with Biotin-SP-conjugated affinity-purified secondary antibodies and ChromPure™ proteins, as well as with any biotinylated primary or secondary antibody, or oligonucleotide.

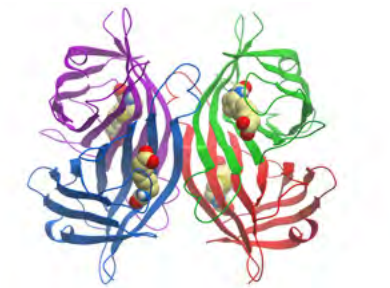


Figure 64: Ligand bound Streptavidin homotetramer showing each asymmetric unit binding to individual biotin molecules. Assembly generated using EMBL PISA:PQS PDB: STP1. Image generated Molsoft ICM browser.

Signal amplification with Streptavidin

A number of signal amplification techniques are possible with Jackson ImmunoResearch secondary antibodies (see page 136). Streptavidin is offered for signal enhancement (Figure 65) as a superior technique to the avidin-biotin-HRP complex (ABC) method. Compared with the ABC method, HRP-conjugated streptavidin is more stable, gives less background, and is more sensitive as reported by Shi et al. (1988) and Milde et al. (1989). The increased sensitivity may be due to enhanced tissue penetration and less steric hindrance, since nominal molecular weights for all components of HRP-conjugated Streptavidin total less than 200 kDa, considerably lower than the weight of ABC.

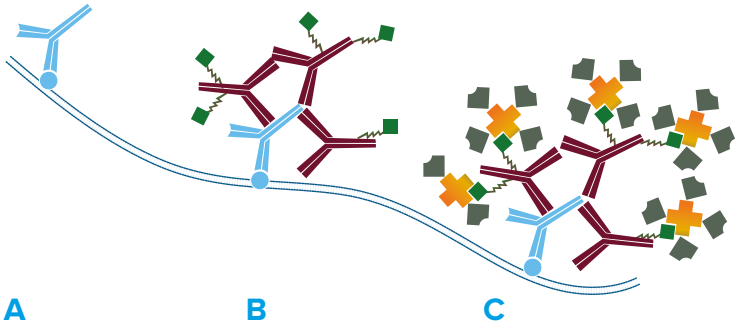


Figure 65: Labeled streptavidin biotin method (LSAB). A. Primary antibody detects target antigen. B. Biotinylated secondary antibody recognizes primary antibody. C. HRP-conjugated streptavidin binds biotinylated secondary antibody, enhancing the signal compared with HRP-conjugated secondary antibody.

Jackson ImmunoResearch offers a comprehensive list of fluorophores and enzymes conjugated to streptavidin for use in enzyme immunoassays, immunohistochemistry, flow cytometry, in situ hybridization, and immunoblotting procedures. Most streptavidin products are freeze-dried in buffer containing stabilizers and preservative. Exceptions are unconjugated streptavidin (freeze-dried from a sodium chloride solution), HRP-conjugated streptavidin (freeze-dried without preservative) and alkaline phosphatase-conjugated streptavidin (sterile-filtered liquid with preservative).

References

Weber, PC., Ohlendorf, DH., Wendoloski, JJ., Salemme, FR. (1989) Structural origins of high-affinity biotin binding to streptavidin. *Science*., 1989 Jan 6;243(4887):85-8.

Shi, ZR., Itzkowitz, SH., Kim, YS., (1988) A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma tissues. *Journal of Histochemistry & Cytochemistry* Vol 36, Issue 3, pp. 317 - 322

Milde, P., Merke, J., Ritz, E., Haussler, MR., Rauterberg, EW., (1989) Immunohistochemical detection of 1,25-dihydroxyvitamin D3 receptors and estrogen receptors by monoclonal antibodies: comparison of four immunoperoxidase methods. *Journal of Histochemistry & Cytochemistry* Vol 37, Issue 11, pp. 1609 - 1617

STREPTAVIDIN

Streptavidin	Code Number	Size
Unconjugated	016-000-084	1.0 mg
	016-000-113	5.0 mg
	016-000-114	10.0 mg

Fluorophore Conjugates	Amax (nm)	Emax (nm)	Code Number	Size
DyLight™405	400	421	016-470-084	1.0 mg
Aminomethylcoumarin AMCA	350	450	016-150-084	1.0 mg
Cy™2	492	510	016-220-084	1.0 mg
Alexa Fluor® 488	493	519	016-540-084	1.0 mg
Fluorescein, DTAF*	492	520	016-010-084	1.0 mg
Cy™3	550	570	016-160-084	1.0 mg
Phycoerythrin, R-PE	488	580	016-110-084	1.0 ml
Rhodamine Red™-X	570	590	016-290-084	1.0 mg
Alexa Fluor® 594	591	614	016-580-084	1.0 mg
Allophycocyanin, APC	650	660	016-130-084	0.5 ml
Alexa Fluor® 647	651	667	016-600-084	1.0 mg
Cy™5	650	670	016-170-084	1.0 mg
Peridinin-Chlorophyll-Protein, PerCP	488	675	016-120-084	0.5 ml
Alexa Fluor® 680	684	702	016-620-084	0.5 mg
Alexa Fluor® 790	792	803	016-650-084	0.5 mg

Enzyme Conjugates	Code Number	Size
Horseradish Peroxidase	016-030-084	1.0 mg
Alkaline Phosphatase	016-050-084	1.0 mg

* DTAF and FITC contain the same fluorescein molecule, and have identical peaks of excitation and emission. However, DTAF is brighter than FITC when conjugated to streptavidin.

BLOCKING AND CONTROLS

BLOCKING AND CONTROLS

Blocking reagents and controls may be required for experimental protocols depending on the immunotechniques undertaken. Here we detail a selection of common problems solved with appropriate blocking, diluents or control reagents.

Western blotting

Problem	Solution	Indicated Product
Background (non-specific signal obscuring bands of interest)	Use appropriate blocking reagent to block membrane prior to incubating with primary.	Normal serum (page 147) (5% v/v) from the host species of the labeled antibody, or BSA (IgG- and protease-free) (page 148).
	Avoid using milk or BSA if primary antibodies are derived from **goat, horse** or **sheep.**	5% (v/v) normal serum from the host species of the labeled antibody.
Detection of reduced immunoprecipitating (IP) antibody at 50 or 25 kDa	To avoid detecting IP antibody heavy chains at 50 kDa, use conjugated anti-light chain specific antibody. See pages 108-111 for a more detailed explanation.	Anti-light chain specific antibodies (pages 108-111).
	To avoid detecting IP antibody light chains at 25 kDa, probe blot with conjugated anti-IgG, Fc fragment after blocking with monovalent Fab fragment anti-Fc. See page 28 for a more detailed explanation.	For anti-Fc specific antibodies, see whole IgG antibodies (pages 46-71). Fab fragments (pages 100-103).

Table 7: Optimal blocking for Western blotting. For more information on troubleshooting Western blots see our guide online.

IHC/ICC/IF

Problem	Solution	Indicated Product
Confirmation that primary antibody binding is due to antigen specificity	To demonstrate specific binding of the primary antibody, use an **isotype negative control** (non-specific IgG from the same species as the primary antibody).	ChromPure™ proteins (pages 149-154).
Background (general)	Block endogenous binding sites which may interact with experimental reagents.	Normal serum from host of the labeled antibody (page 147).
Background (homologous Ig recognition)	Block endogenous immunoglobulins.	Fab fragments (pages 87, 94-97).
Multiple label primary antibodies from same host species	Utilize Fab fragments in suggested protocols to accomplish multiple labeling.	Fab fragments (pages 88-97).
	Immunolabel primary antibody prior to incubation.	FabuLight (pages 98-103).
Endogenous enzymes	Inactivate endogenous peroxidase with hydrogen peroxide.	See our guide to selecting control, diluent and blocking reagents online.
	Use levamisole to inactivate endogenous phosphatases.	
Endogenous biotin	Block endogenous biotin.	Incubate with streptavidin, followed by free biotin.
Ionic interactions	Include detergent in buffers, optimize salt concentrations and pH.	Tween®-20 and/or Triton™ X-100.

Table 8: Optimal blocking for IHC/ICC/IF.

Flow cytometry

Problem	Solution	Indicated Product
Background from antibodies binding Fc receptors.	Block Fc receptors.	Normal serum from host of the labeled antibody (pages 147) For more information see Technical - flow cytometry pages 39-40.
	Use $F(ab')_2$ format secondary antibody to avoid entrapment by Fc receptors.	$F(ab')_2$ secondary antibodies (pages 74-85).
Confirmation that primary antibody binding is due to antigen specificity	Use an **isotype negative control** (non-specific IgG from the same species as the primary antibody) to demonstrate specific binding of the primary antibody.	ChromPure™ purified proteins (pages 149-154).

Table 9: Optimal blocking for Flow cytometry.

ELISA

Problem	Solution	Indicated Product
Background	Use an appropriate blocking reagent to block wells prior to incubating with the primary antibody.	Normal serum (5% v/v) from the host species of the labeled antibody (pages 147), or BSA (IgG-free and protease-free) page 148.
No Signal	Use a positive control to demonstrate activity of the labeled secondary antibody: coat with primary antibody isotype and detect directly with secondary.	ChromPure proteins (pages 149-154).

Table 10: Optimal blocking for ELISA.

ChromPure™ purified proteins from normal serums - experimental controls and blocking reagents. (pages 149-154)

ChromPure proteins are primarily used as experimental controls for either primary or secondary antibodies. They are available conjugated to a range of fluorescent dyes and reporter enzymes, allowing the isolation of signal derived from non-specific interactions. ChromPure purified proteins may also be used as blocking reagents for Western blotting, IHC, and IF.

Normal Serums (page 147)

Normal serums are obtained from non-immunized animals and consequently do not detect any specific antigen. Normal serum is recommended for use as a blocking agent to reduce background from non-specific, conserved- sequence, and/or Fc-receptor binding.

Gamma Globulins

Gamma globulins are derived from non-immunized animal serums and have been further purified by salt fractionation, ion-exchange chromatography, and gel filtration. Gamma globulins can be used as blocking reagents and as controls. A product list can be found on our website.

Bovine Serum Albumin (IgG-Free, Protease Free) (page 148)

Bovine serum albumin (BSA) is used extensively as a carrier protein to dilute antibodies and as a general protein blocking agent in immunoassays and immunodetection protocols.

Monovalent Fab Fragments (pages 94-97)

Fab fragments can enable the blocking of endogenous immunoglobulins to reduce background staining and for multiple labeling assays when primary antibodies are derived from the same host species . Learn more with our blocking protocols on pages 86-93.

BLOCKING AND CONTROLS

Cautions: When using a labeled secondary antibody for detection, never block with normal serum or IgG from the host species of the primary antibody. If immunoglobulins in normal serum bind to the specimen of interest, they will be recognized by the labeled secondary antibody, resulting in higher background.

Bovine serum albumin (BSA) and dry milk, both commonly used for blocking, may contain bovine IgG. With the exception of bovine anti-goat IgG, many secondary antibodies such as **anti-bovine, anti-goat, anti-horse and anti-sheep** will react strongly with bovine IgG. Therefore, use of BSA or dry milk for blocking or diluting these antibodies may significantly increase background and/or reduce antibody titer. For blocking, use normal serum (5% v/v) from the host species of the labeled secondary antibody.

NORMAL SERUMS

Applications

Normal serum diluted to 5% (v/v) in PBS is strongly recommended as a blocking reagent to reduce background from non-specific, conserved-sequence and/or Fc-receptor binding. Best results are obtained with diluted normal serum from the same host as the labeled antibody, as a separate incubation step before addition of the primary antibody.

Preparation and format

Normal serums (from non-immunized animals) are lipid extracted to improve clarity, dialyzed against phosphate buffered saline (PBS) containing sodium azide, and freeze-dried.

For more information see pages 144-146.

Normal Serums	Code Number	Size
Alpaca	028-000-001 028-000-121	2 ml 10 ml
Bovine	001-000-001 001-000-121	2 ml 10 ml
Cat	002-000-001 002-000-120	2 ml 5 ml
Chicken	003-000-001 003-000-120	2 ml 5 ml
Dog	004-000-001 004-000-120	2 ml 5 ml
Donkey	017-000-001 017-000-121	2 ml 10 ml
Goat	005-000-001 005-000-121	2 ml 10 ml
Guinea Pig	006-000-001 006-000-120	2 ml 5 ml

Normal Serums	Code Number	Size
Syrian Hamster	007-000-001 007-000-120	2 ml 5 ml
Horse	008-000-001 008-000-121	2 ml 10 ml
Human	009-000-001 009-000-121	2 ml 10 ml
Mouse	015-000-001 015-000-120	2 ml 5 ml
Rabbit	011-000-001 011-000-120	2 ml 5 ml
Rat	012-000-001 012-000-120	2 ml 5 ml
Sheep	013-000-001 013-000-121	2 ml 10 ml
Swine	014-000-001 014-000-121	2 ml 10 ml

BOVINE SERUM ALBUMIN (IgG-FREE, PROTEASE-FREE)

Applications

Bovine Serum Albumin (BSA) is used extensively as a carrier protein to dilute antibodies and as a general protein blocking agent in immunoassays. For more information on blocking and diluents see pages 144-146.

JIR Bovine Serum Albumin is verified to be IgG- and protease-free, alleviating many problems associated with commonly available preparations.

Note: Most commercial preparations of BSA, including some of the highest purity grades, contain contaminating bovine IgG that may become an antigen for cross-reacting secondary antibodies. This is particularly common when using **anti-bovine IgG, anti-goat IgG** (with the exception of bovine anti-goat IgG), **anti-horse IgG**, or **anti-sheep IgG**, but may occur with other antibodies that cross-react with **bovine IgG**. The result of these interactions may be loss of desired antibody activity, loss of antibody stability, and/or increased background.

Secondary antibody activity may be lost if the antibody is diluted in BSA that contains contaminating bovine IgG. Background may derive from sticky soluble immune complexes in the antibody diluent, or from bovine IgG found in a BSA blocking solution which becomes a target for labeled secondary antibodies. Even small amounts of contaminating IgG may create these problems, due to high concentrations of BSA in many protocols.

Format

IgG-free BSA is supplied as a pure protein, freeze-dried from deionized water. Please inquire about availability of larger sizes.

BOVINE SERUM ALBUMIN (IgG-FREE, PROTEASE-FREE)

Description	Code Number	Fill Size
Bovine Serum Albumin (IgG-Free, Protease-Free)	001-000-161	10 g
	001-000-162	50 g
	001-000-173	250 g

CHROMPURE™ PURIFIED PROTEINS FROM NORMAL SERUMS

ChromPure™ is our trade name for highly purified proteins from the serum of non-immunized animals. The purified immunoglobulins in this section do not represent antibodies directed against known antigens.

Preparation

ChromPure proteins are prepared by a variety of methods, including ion-exchange, gel-filtration, hydrophobic, dye-ligand, metal-affinity, Protein A, and immunoaffinity chromatographies. Enzyme digestion is used to generate $F(ab')_2$ (pepsin), and Fab and Fc (papain) fragments from highly purified whole molecules (see page 8, Figure 1).

Purity

No contaminating whole molecules or undesired fragments are observed at a protein concentration of 20 mg/ml when tested by immunoelectrophoresis against anti-whole serums, anti-immunoglobulins (class specific), or anti-fragment specific antisera.

Applications

ChromPure proteins are ideal for use as experimental controls (isotype controls). For more detailed information about experimental controls please see pages 144-147.

Note: ChromPure proteins are not intended for use as immunogens to produce monospecific antibodies, nor should they be used as molecular weight markers.

Format

Unconjugated ChromPure proteins are supplied as sterile-filtered liquids without stabilizers or preservative. *Conjugated* ChromPure proteins are freeze-dried with stabilizers and a preservative, with the exception of peroxidase conjugates, which do not contain a preservative.

Protein	Conjugate	Code Number	Fill Size
Bovine IgG, whole molecule	Unconjugated	001-000-003	10.0 mg
	Alexa Fluor® 488	001-540-003	1.0 mg
	Alexa Fluor® 594	001-580-003	1.0 mg
	Alexa Fluor® 647	001-600-003	1.0 mg
	Biotin	001-060-003	1.0 mg
	Cy™3	001-160-003	1.0 mg
	DyLight® 405	001-470-003	1.0 mg
	FITC	001-090-003	1.0 mg
	HRP	001-030-003	1.0 mg
Bovine IgG, Fc fragment	Unconjugated	001-000-008	2.0 mg
Bovine IgG, Fab fragment	Unconjugated	001-000-007	2.0 mg
Cat IgG, whole molecule	Unconjugated	002-000-003	10.0 mg
Chicken IgY (IgG), whole molecule	Unconjugated	003-000-003	5.0 mg
	Alexa Fluor® 488	003-540-003	1.0 mg
	Alexa Fluor® 594	003-580-003	1.0 mg
	Alexa Fluor® 647	003-600-003	1.0 mg
	Biotin	003-060-003	1.0 mg
	Cy3	003-160-003	1.0 mg
	FITC	003-090-003	1.0 mg
	HRP	003-030-003	1.0 mg

CHROMPURE™ PURIFIED PROTEINS FROM NORMAL SERUMS

Protein	Conjugate	Code Number	Fill Size
Chicken IgY (IgG), Fc fragment	Unconjugated	003-000-008	1.0 mg
Chicken IgY (IgG), Fab fragment	Unconjugated	003-000-007	2.0 mg
Dog IgG, whole molecule	Unconjugated	004-000-003	10.0 mg
Donkey IgG, whole molecule	Unconjugated	017-000-003	10.0 mg
	Alexa Fluor® 488	017-540-003	1.0 mg
	Alexa Fluor® 594	017-580-003	1.0 mg
	Alexa Fluor® 647	017-600-003	1.0 mg
	Alexa Fluor® 680	017-620-003	0.5 mg
	Alexa Fluor® 790	017-650-003	0.5 mg
	Biotin	017-060-003	1.0 mg
	BV421™	017-670-003	0.25 ml
	BV480™	017-680-003	0.25 ml
	Cy™2	017-220-003	1.0 mg
	Cy™3	017-160-003	1.0 mg
	Cy™5	017-170-003	1.0 mg
	DyLight ™ 405	017-470-003	1.0 mg
	FITC	017-090-003	1.0 mg
	HRP	017-030-003	1.0 mg

Protein	Conjugate	Code Number	Fill Size
Donkey IgG, $F(ab')_2$ fragment	Unconjugated	017-000-006	2.0 mg
	Alexa Fluor® 488	017-540-006	1.0 mg
	Alexa Fluor® 647	017-600-006	1.0 mg
	APC	017-130-006	0.5 ml
	Biotin	017-060-006	1.0 mg
	Cy3	017-160-006	1.0 mg
	FITC	017-090-006	1.0 mg
	HRP	017-030-006	1.0 mg
	PerCP	017-120-006	0.5 ml
	R-PE	017-110-006	1.0 ml
Goat IgG, Fc fragment	Unconjugated	005-000-008	1.0 mg
Goat IgG, $F(ab')_2$ fragment	Unconjugated	005-000-006	2.0 mg
	Alexa Fluor® 488	005-540-006	1.0 mg
	Alexa Fluor® 647	005-600-006	1.0 mg
	APC	005-130-006	0.5 ml
	Biotin	005-060-006	1.0 mg
	Cy3	005-160-006	1.0 mg
	FITC	005-090-006	1.0 mg
	HRP	005-030-006	1.0 mg
	PerCP	005-120-006	0.5 ml
	R-PE	005-110-006	1.0 ml
Goat IgG, Fab fragment	Unconjugated	005-000-007	2.0 mg

Protein	Conjugate	Code Number	Fill Size
Goat IgG, whole molecule	Unconjugated	005-000-003	10.0 mg
	Alexa Fluor® 488	005-540-003	1.0 mg
	Alexa Fluor® 594	005-580-003	1.0 mg
	Alexa Fluor® 647	005-600-003	1.0 mg
	Alexa Fluor® 680	005-620-003	0.5 mg
	Alexa Fluor® 790	005-650-003	0.5 mg
	APC	005-130-003	0.5 ml
	Biotin	005-060-003	1.0 mg
	BV421™	005-670-003	0.25 ml
	BV480™	005-680-003	0.25 ml
	Cy™2	005-220-003	1.0 mg
	Cy™3	005-160-003	1.0 mg
	Cy™5	005-170-003	1.0 mg
	DyLight™ 405	005-470-003	1.0 mg
	FITC	005-090-003	1.0 mg
	HRP	005-030-003	1.0 mg
	PerCP	005-120-003	0.5 ml
	R-PE	005-110-003	1.0 ml
Guinea Pig IgG, whole molecule	Unconjugated	006-000-003	10.0 mg

Protein	Conjugate	Code Number	Fill Size
Syrian Hamster IgG, whole molecule	Unconjugated	007-000-003	5.0 mg
	Biotin	007-060-003	1.0 mg
	Cy3	007-160-003	1.0 mg
	FITC	007-090-003	1.0 mg
	HRP	007-030-003	1.0 mg
Horse IgG, whole molecule	Unconjugated	008-000-003	10.0 mg
Horse IgG, $F(ab')_2$ fragment	Unconjugated	008-000-006	2.0 mg
Horse IgG, Fab fragment	Unconjugated	008-000-007	2.0 mg
Human IgG, whole molecule	Unconjugated	009-000-003	10.0 mg
	Alexa Fluor® 488	009-540-003	1.0 mg
	Alexa Fluor® 594	009-580-003	1.0 mg
	Alexa Fluor® 647	009-600-003	1.0 mg
	Biotin	009-060-003	1.0 mg
	Cy3	009-160-003	1.0 mg
	DyLight 405	009-470-003	1.0 mg
	FITC	009-090-003	1.0 mg
	HRP	009-030-003	1.0 mg

CHROMPURE™ PURIFIED PROTEINS FROM NORMAL SERUMS

Protein	Conjugate	Code Number	Fill Size
Human IgG, Fc fragment	Unconjugated	009-000-008	1.0 mg
	Alexa Fluor® 488	009-540-008	1.0 mg
	Alexa Fluor® 647	009-600-008	1.0 mg
	Biotin	009-060-008	1.0 mg
	Cy™3	009-160-008	1.0 mg
	FITC	009-090-008	1.0 mg
	HRP	009-030-008	1.0 mg
Human IgG, $F(ab')_2$ fragment	Unconjugated	009-000-006	2.0 mg
	Alexa Fluor® 488	009-540-006	1.0 mg
	Alexa Fluor® 647	009-600-006	1.0 mg
	Biotin	009-060-006	1.0 mg
	Cy3	009-160-006	1.0 mg
	FITC	009-090-006	1.0 mg
	HRP	009-030-006	1.0 mg
Human IgG, Fab fragment	Unconjugated	009-000-007	2.0 mg
	Alexa Fluor® 488	009-540-007	1.0 mg
	Alexa Fluor® 647	009-600-007	1.0 mg
	Biotin	009-060-007	1.0 mg
	Cy3	009-160-007	1.0 mg
	FITC	009-090-007	1.0 mg
	HRP	009-030-007	1.0 mg

Protein	Conjugate	Code Number	Fill Size
Human IgM (myeloma), whole molecule	Unconjugated	009-000-012	2.0 mg
	Alexa Fluor® 488	009-540-012	1.0 mg
	Alexa Fluor® 647	009-600-012	1.0 mg
	Biotin	009-060-012	1.0 mg
	Cy3	009-160-012	1.0 mg
	FITC	009-090-012	1.0 mg
	HRP	009-030-012	1.0 mg
Human Serum IgA, whole molecule	Unconjugated	009-000-011	2.0 mg
	Biotin	009-060-011	1.0 mg
	Cy3	009-160-011	1.0 mg
	FITC	009-090-011	1.0 mg
	HRP	009-030-011	1.0 mg
Human Albumin	Unconjugated	009-000-051	5.0 mg
	Alexa Fluor® 488	009-540-051	1.0 mg
	Alexa Fluor® 647	009-600-051	1.0 mg
	Biotin	009-060-051	1.0 mg
	Cy3	009-160-051	1.0 mg
	FITC	009-090-051	1.0 mg
	HRP	009-030-051	1.0 mg

Protein	Conjugate	Code Number	Fill Size
Human Transferrin	Unconjugated	009-000-050	5.0 mg
	Alexa Fluor® 488	009-540-050	1.0 mg
	Alexa Fluor® 647	009-600-050	1.0 mg
	Biotin	009-060-050	1.0 mg
	Cy™3	009-160-050	1.0 mg
	FITC	009-090-050	1.0 mg
	HRP	009-030-050	1.0 mg
Mouse IgG, whole molecule	Unconjugated	015-000-003	5.0 mg
	Alexa Fluor® 488	015-540-003	1.0 mg
	Alexa Fluor® 594	015-580-003	1.0 mg
	Alexa Fluor® 647	015-600-003	1.0 mg
	Alexa Fluor® 680	015-620-003	0.5 mg
	Alexa Fluor® 790	015-650-003	0.5 mg
	Biotin	015-060-003	1.0 mg
	Cy3	015-160-003	1.0 mg
	DyLight™ 405	015-470-003	1.0 mg
	FITC	015-090-003	1.0 mg
	HRP	015-030-003	1.0 mg
Mouse IgG, Fc fragment	Unconjugated	015-000-008	1.0 mg
	Biotin	015-060-008	1.0 mg
	Cy3	015-160-008	1.0 mg
	FITC	015-090-008	1.0 mg
	HRP	015-030-008	1.0 mg

Protein	Conjugate	Code Number	Fill Size
Mouse IgG, $F(ab')_2$ fragment	Unconjugated	015-000-006	2.0 mg
	Alexa Fluor® 488	015-540-006	1.0 mg
	Alexa Fluor® 647	015-600-006	1.0 mg
	Biotin	015-060-006	1.0 mg
	Cy3	015-160-006	1.0 mg
	FITC	015-090-006	1.0 mg
	HRP	015-030-006	1.0 mg
Mouse IgG, Fab fragment	Unconjugated	015-000-007	2.0 mg
	Biotin	015-060-007	1.0 mg
	Cy3	015-160-007	1.0 mg
	FITC	015-090-007	1.0 mg
	HRP	015-030-007	1.0 mg
Mouse Transferrin	Unconjugated	015-000-050	5.0 mg
	Alexa Fluor® 488	015-540-050	1.0 mg
	Alexa Fluor® 647	015-600-050	1.0 mg
	Biotin	015-060-050	1.0 mg
	Cy3	015-160-050	1.0 mg
	FITC	015-090-050	1.0 mg
	HRP	015-030-050	1.0 mg

CHROMPURE™ PURIFIED PROTEINS FROM NORMAL SERUMS

Protein	Conjugate	Code Number	Fill Size
Rabbit IgG, whole molecule	Unconjugated	011-000-003	10.0 mg
	Alexa Fluor® 488	011-540-003	1.0 mg
	Alexa Fluor® 594	011-580-003	1.0 mg
	Alexa Fluor® 647	011-600-003	1.0 mg
	Biotin	011-060-003	1.0 mg
	Cy™3	011-160-003	1.0 mg
	DyLight™ 405	011-470-003	1.0 mg
	FITC	011-090-003	1.0 mg
	HRP	011-030-003	1.0 mg
Rabbit IgG, Fc fragment	Unconjugated	011-000-008	1.0 mg
Rabbit IgG, $F(ab')_2$ fragment	Unconjugated	011-000-006	2.0 mg
Rabbit IgG, Fab fragment	Unconjugated	011-000-007	2.0 mg
Rat IgG, whole molecule	Unconjugated	012-000-003	10.0 mg
	Alexa Fluor® 488	012-540-003	1.0 mg
	Alexa Fluor® 594	012-580-003	1.0 mg
	Alexa Fluor® 647	012-600-003	1.0 mg
	Biotin	012-060-003	1.0 mg
	Cy3	012-160-003	1.0 mg
	DyLight 405	012-470-003	1.0 mg
	FITC	012-090-003	1.0 mg
	HRP	012-030-003	1.0 mg

Protein	Conjugate	Code Number	Fill Size
Rat IgG, Fc fragment	Unconjugated	012-000-008	1.0 mg
Rat IgG, $F(ab')_2$ fragment	Unconjugated	012-000-006	2.0 mg
Rat IgG, Fab fragment	Unconjugated	012-000-007	2.0 mg
Rat Transferrin	Unconjugated	012-000-050	5.0 mg
	Alexa Fluor® 488	012-540-050	1.0 mg
	Alexa Fluor® 647	012-600-050	1.0 mg
	Biotin	012-060-050	1.0 mg
	Cy3	012-160-050	1.0 mg
	FITC	012-090-050	1.0 mg
	HRP	012-030-050	1.0 mg
Sheep IgG, whole molecule	Unconjugated	013-000-003	10.0 mg
	Alexa Fluor® 488	013-540-003	1.0 mg
	Alexa Fluor® 594	013-580-003	1.0 mg
	Alexa Fluor® 647	013-600-003	1.0 mg
	Biotin	013-060-003	1.0 mg
	Cy3	013-160-003	1.0 mg
	DyLight 405	013-470-003	1.0 mg
	FITC	013-090-003	1.0 mg
	HRP	013-030-003	1.0 mg
Sheep IgG, Fc fragment	Unconjugated	013-000-008	1.0 mg
Swine IgG, whole molecule	Unconjugated	014-000-003	10.0 mg

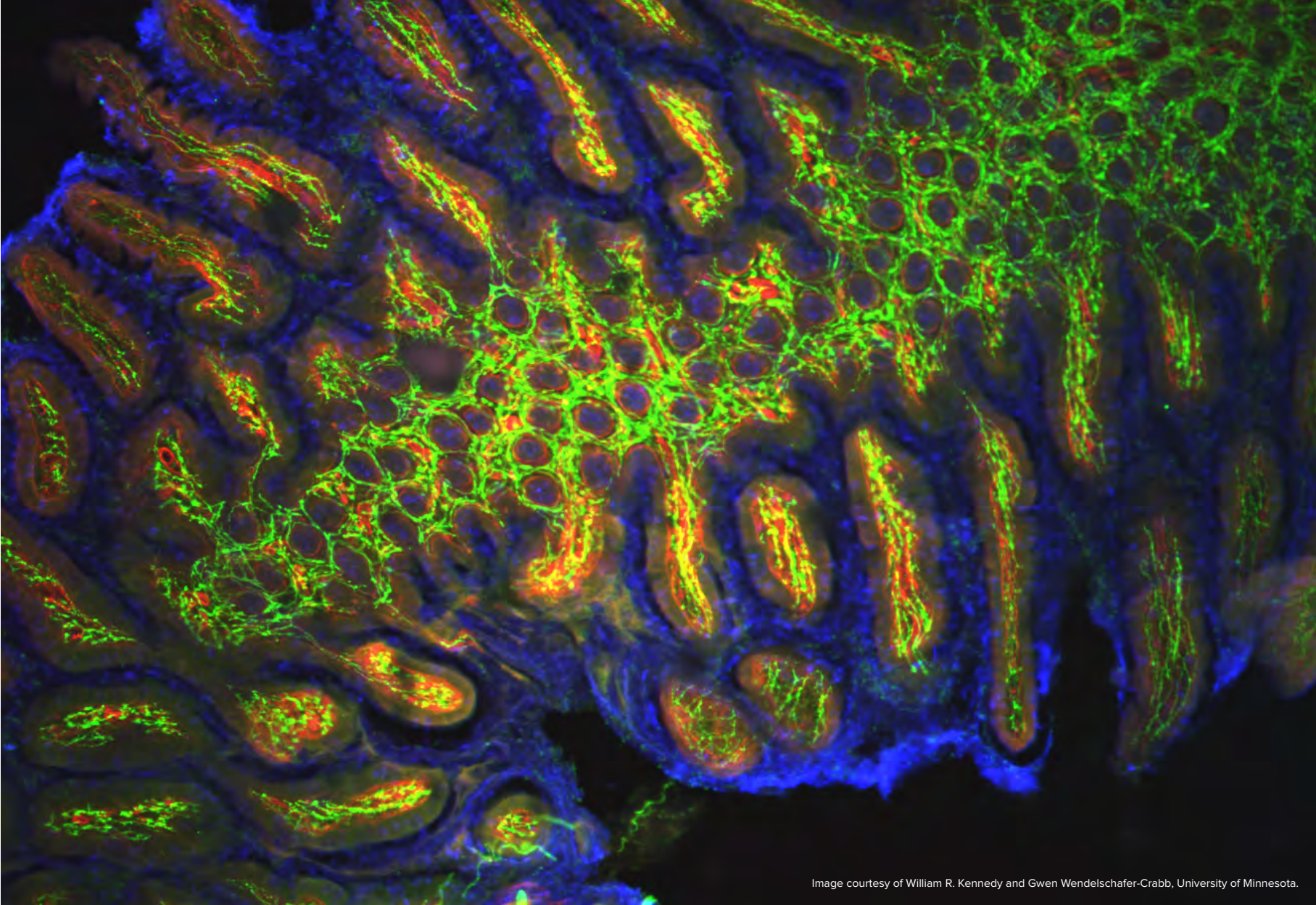


Image courtesy of William R. Kennedy and Gwen Wendelschafer-Crabb, University of Minnesota.

ANTISERA

157 Antisera to Immunoglobulins, Whole Serums and Enzymes

ANTISERA TO IMMUNOGLOBULINS, WHOLE SERUMS AND ENZYMES

Antisera against whole serums are obtained by immunizing host animals with whole serum. Antisera against IgG (H+L) and antibody fragments are generated through immunization with the appropriate molecule.

Applications

Antisera are commonly used for immunoprecipitation assays such as immunodiffusion and immunoelectrophoresis, but are not suitable for coating ELISA plates or other applications requiring affinity-purified antibodies. Antisera against whole IgG molecules (Anti-IgG (H+L)) are recommended for bridging PAP (page 136) to primary antibodies.

Antisera Purification and format

Polyclonal antisera from immunized hosts are lipid extracted to improve clarity, salt fractionated, dialyzed against phosphate buffered saline containing sodium azide, and freeze-dried. All products on this page are supplied in 2 ml volumes.

ANTISERA TO IMMUNOGLOBULINS, WHOLE SERUMS & ENZYMES

Antiserum	Host	Code Number	Fill Size
Anti-Bovine IgG (H+L) (!)	Rabbit	301-001-003	2.0 ml
Anti-Bovine Whole Serum (!)	Rabbit	301-001-001	2.0 ml
Anti-Chicken IgY (IgG) (H+L)	Donkey	703-001-003	2.0 ml
Anti-Dog IgG (H+L)	Rabbit	304-001-003	2.0 ml
Anti-Goat IgG (H+L) (!)	Donkey	705-001-003	2.0 ml
Anti-Goat Whole Serum (!)	Rabbit	305-001-001	2.0 ml
Anti-Guinea Pig IgG (H+L)	Donkey	706-001-003	2.0 ml
Anti-Guinea Pig IgG (H+L)	Goat	106-001-003	2.0 ml
Anti-Horseradish Peroxidase	Goat	123-001-021	2.0 ml
Anti-Human IgG (H+L)	Goat	109-001-003	2.0 ml
Anti-Human IgG, Fc fragment specific	Goat	109-001-008	2.0 ml
Anti-Human IgG, F(ab')$_2$ fragment specific	Goat	109-001-006	2.0 ml
Anti-Human IgM, Fc$_{5\mu}$ fragment specific	Goat	109-001-043	2.0 ml
Anti-Human Whole Serum	Goat	109-001-001	2.0 ml
Anti-Human IgG (H+L)	Rabbit	309-001-003	2.0 ml
Anti-Human IgG, Fc fragment specific	Rabbit	309-001-008	2.0 ml

Antiserum	Host	Code Number	Fill Size
Anti-Mouse IgG (H+L)	Donkey	715-001-003	2.0 ml
Anti-Mouse IgG (H+L)	Goat	115-001-003	2.0 ml
Anti-Mouse IgG, Fc fragment specific	Goat	115-001-008	2.0 ml
Anti-Mouse IgM, μ chain specific	Goat	115-001-020	2.0 ml
Anti-Mouse Whole Serum	Goat	115-001-001	2.0 ml
Anti-Mouse IgG (H+L)	Rabbit	315-001-003	2.0 ml
Anti-Mouse IgG, Fc fragment specific	Rabbit	315-001-008	2.0 ml
Anti-Rabbit IgG (H+L)	Donkey	711-001-003	2.0 ml
Anti-Rabbit IgG (H+L)	Goat	111-001-003	2.0 ml
Anti-Rabbit IgG, Fc fragment specific	Goat	111-001-008	2.0 ml
Anti-Rabbit Whole Serum	Goat	111-001-001	2.0 ml
Anti-Rat IgG (H+L)	Donkey	712-001-003	2.0 ml
Anti-Rat IgG (H+L)	Goat	112-001-003	2.0 ml
Anti-Rat IgG, Fc fragment specific	Goat	112-001-008	2.0 ml
Anti-Rat Whole Serum	Goat	112-001-001	2.0 ml
Anti-Sheep IgG (H+L) (!)	Rabbit	313-001-003	2.0 ml

(!) Warning: BSA and dry milk may contain IgG which will be recognized by this antibody. Use of BSA or dry milk to block or dilute this antibody may increase background and/or reduce secondary antibody titer.

IMMUNOADSORBENT GELS

SOLID-PHASE IMMUNOADSORBENT GELS

Highly purified IgG from the serum of non-immunized animals is coupled to cyanogen bromide-activated 4% agarose gels for use in preparing affinity-purified antibodies or removing cross-reactive antibodies. Proteins are coupled at a concentration of 1 mg protein per ml of settled gel, and are packaged in phosphate buffered saline with sodium azide.

SOLID-PHASE IMMUNOADSORBENT GELS

Description	Code Number	Fill Size
Goat IgG, whole molecule	005-000-052	5.0 ml
Mouse IgG, whole molecule	015-000-052	5.0 ml
Rabbit IgG, whole molecule	011-000-052	5.0 ml

APPENDIX

Recommended storage conditions

Unconjugated AffiniPure Antibodies, Unconjugated ChromPure™ Proteins, and Gamma Globulins

Store at 2-8°C under sterile conditions. Prepare working dilution fresh each day. Expiration date: one year from date of receipt. The expiration date may be extended if test results are acceptable for the intended use.

Normal Sera and Antisera

Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. Product is stable for about 6 weeks at 2-8°C as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, aliquot and freeze undiluted product at -20°C or below. Avoid repeated freezing and thawing. Expiration date: one year from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

Fluorophore and Biotin conjugates

Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. Product is stable for about 6 weeks at 2-8°C as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, aliquot and freeze at -70°C or below. Avoid repeated freezing and thawing. Alternatively, add an equal volume of glycerol (ACS grade or better) for a final concentration of 50%, and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein concentration and dilution range by one-half. Expiration date: one year from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

Peroxidase conjugates and Peroxidase-Anti-Peroxidase Immune Complexes

(Warning: Use of sodium azide as a preservative will substantially inhibit the enzyme activity of horseradish peroxidase.) Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. Product is stable for about 6 weeks at 2-8°C as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, aliquot and freeze at -70°C or below. Avoid repeated freezing and thawing. Alternatively, add an equal volume of glycerol (ACS grade or better) for a final concentration of 50%, and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein concentration and dilution range by one-half. Expiration date: one year from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

Alkaline Phosphatase-conjugated antibodies

Store freeze-dried powder at 2-8° C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. Product is stable for about 6 weeks at 2-8°C as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, add an equal volume of glycerol (ACS grade or better) for a final concentration of 50%, and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein concentration and dilution range by one-half. Expiration date: one year from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

Alkaline Phosphatase-conjugated Streptavidin

Store at 2-8°C. Prepare working dilution fresh each day. For extended storage, add an equal volume of glycerol (ACS grade or better) for a final concentration of 50%, and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein concentration and dilution range by one-half. Expiration date: one year from the date of receipt. The expiration date may be extended if test results are acceptable for the intended use.

Fluorescent protein conjugates (R-PE, APC and PerCP)

Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. Store at 2-8°C - DO NOT FREEZE. Prepare working dilution fresh each day. Expiration date: six months from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

6, 12 and 18 nm Colloidal Gold Complexes (EM Grade)

Aliquot and freeze undiluted product at -20°C or below. Avoid repeated freezing and thawing. Prepare working dilution fresh each day. Expiration date: six months from date of receipt. The expiration date may be extended if test results are acceptable for the intended use.

4 nm Colloidal Gold Complexes

Store freeze-dried powder at 2-8°C. When ready to use, rehydrate with dH_2O to the indicated volume and centrifuge if not clear. After rehydration, aliquot and freeze undiluted product at -20°C or below. Avoid repeated freezing and thawing. Prepare working dilution fresh each day. Expiration date: one year from date of rehydration. The expiration date may be extended if test results are acceptable for the intended use.

ChromPure™ Proteins Coupled to Agarose

Store at 2-8°C. Expiration date: one year from date of receipt.
The expiration date may be extended if test results are acceptable for the intended use.

Custom bulk liquids

Refer to Product Spec Sheet.

Suggested dilution factors

The dilution factors suggested in the following table are presented as ranges because the optimal dilution is a function of many factors, such as antigen density, permeability, etc. The optimal working dilution should be determined empirically for each application.

		Application			
Product	Conjugate	ELISA	Western Blots	Histo/Cyto chemistry	Flow Cytometry
Whole IgG and $F(ab')_2$ secondary antibodies	Unconjugated	10-20 µg/ml			
Fab secondary antibodies	Unconjugated	20-40 µg/ml			
Whole IgG, $F(ab')_2$ and Fab fragment secondary antibodies	Alexa Fluor® 488, 594, 647 and Cy™3			1:100-1:800	1:100-1:800
Whole IgG secondary antibodies	Alexa Fluor® 680 and 790		1:50,000-200,000		
Whole IgG secondary antibodies	BV421™ and BV480™			1:50 -1:200	1:50 -1:200
Whole IgG, $F(ab')_2$ and Fab fragment secondary antibodies	AMCA, Cy™2, FITC, TRITC, RRX			1:50-1:200	1:50-1:200
Whole IgG secondary antibodies	Cy™5			1:100-1:400	1:100-1:400
Whole IgG, $F(ab')_2$ secondary antibodies and Streptavidin	R-PE and APC				1:50-1:200
Whole IgG, $F(ab')_2$ secondary antibodies and Streptavidin	PerCP				1:25-1:100
Whole IgG and $F(ab')_2$ secondary antibodies	Horseradish Peroxidase	1:5,000-1:100,000	1:5,000-1:100,000 (non-ECL) 1:10,000-1:200,000 (ECL)	1:500-1:5,000	

Product	Conjugate	Application			
		ELISA	Western Blots	Histo/Cyto chemistry	Flow Cytometry
Whole IgG and $F(ab')_2$ fragment secondary antibodies	Alkaline Phosphatase	1:5,000-1:50,000	1:5,000-1:50,000		
Whole IgG, $F(ab')_2$ and Fab fragment secondary antibodies	Biotin-SP (using Fluorophore-Conjugated Streptavidin)			1:200 1:1,000	1:200 1:1,000
Whole IgG and $F(ab')_2$ secondary antibodies	Biotin-SP (using enzyme-Conjugated Streptavidin)	1:20,000 - 1:400,000	1:20,000 - 1:400,000	1:500-1:5,000	1:200 1:1,000
Streptavidin	Horseradish Peroxidase	1-2 µg/ml	1-2 µg/ml 0.01-0.1 µg/ml (ECL)	1-2 µg/ml	
Streptavidin	Alkaline Phosphatase	1-2 µg/ml	1-2 µg/ml 0.1-1 µg / ml (ECL)	1-2 µg/ml	
Streptavidin	All Alexa Fluor®, DyLight™ 405, and Cy™3			0.5-2 µg/ml	0.5-2 µg/ml
Streptavidin	Cy™5			1-4 µg/ml	1-4 µg/ml
Streptavidin	All other Fluorophores			2-5 µg/ml	2-5 µg/ml

Product	Conjugate	Application			
		ELISA	Western Blots	Histo/Cyto chemistry	Flow Cytometry
4 nm Colloidal Gold-Antibody Complexes				1:20-1:200	
6 nm, 12 nm Colloidal Gold-Antibody Complexes				1:20-1:40	
18 nm Colloidal Gold-Antibody Complexes				1:10-1:20	
Peroxidase-Anti-Peroxidase (PAP)		1:5,000 - 1:50,000	1:5,000 - 1:50,000	25-50 µg/ml 1:400 -1:800	
FabuLight Anti-IgM		To complex with primary antibody in solution, use 1:1 weight ratio of Fab: primary antibody (15:1 molar ratio).			
FabuLight Anti-IgG		To complex with primary antibody in solution, use 1:1 weight ratio of Fab: primary antibody (3:1 molar ratio).			
Normal serum		5% (v/v) for blocking			
ChromPure™		10 µg/ml			

Table 11: Recommended dilution factors.

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