Wako Product Update *Bio-No.1*

Cell Science Research

ALPHABETICAL INDEX

for quantitative determination of βAmyloid peptide 40 and 42

β Amyloid ELISA Kits

Alzheimer's Disease (AD) is characterized by the presence of extracellular senile plaques (SPs) and intracellular neurofibrillary tangles (NFT) in the brain. The major protein component of SPs is β Amyloid peptide (Aβ) 40 and 42(43). Aβ42 is more prone to aggregate than Aβ. Therefore the initial Aβdeposition begins with Aβ42(43) but not with Aβ40. Aβ42(43)-positive and Aβ40-negative plaques may represent early-stage diffuse type SPs, and Aβ40-positive plaque appears in the advanced stage, especially more often in the cored portion of the mature plaque.

In these kits, we use the monoclonal antibodies which specifically detect Aβ. Therefore these kits are designed to be used for the quantitative determination of Aβ in samples such as tissue culture medium, tissue homogenate, CSF and plasma.

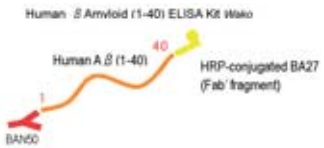
[Features]

1. These kits are designed to be used for the quantitative determination of Aβ in samples such as tissue culture medium, tissue homogenate, CSF and plasma.
2. These kits use the monoclonal antibodies that were developed by Takeda Pharmaceutical Company, Ltd.
   BAN50: Specifically detects the N-terminal of Aβ (1-16)
   BNT77: Specifically detects the Aβ (11-28) of Aβ
   BA27: Specifically detects the C-terminal of Aβ 40
   BC05: Specifically detects the C-terminal of Aβ 42

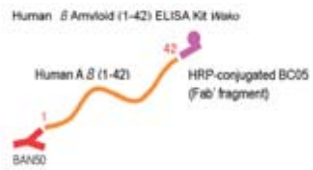
[Kit Contents]	(Keep at 2~10℃)
1) MAb-coated Microtiter Plate	1 plate
2) Standard Solution	2 vials × 2 mL
3) Standard Diluent	1 vial × 30 mL
4) Wash Solution (20 ×)	1 vial × 50 mL
5) HRP-conjugated MAb Solution	1 vial × 12 mL
6) TMB Solution	1 vial × 12 mL
7) Stop Solution	1 vial × 12 mL
8) Plate Seal	3 sheets

[Principle]

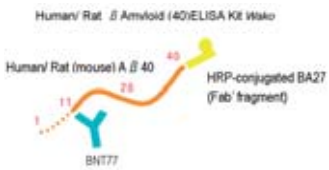
1. Human β Amyloid (1-40) ELISA Kit *Wako*



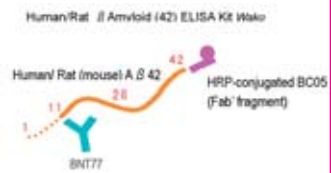
2. Human β Amyloid (1-42) ELISA Kit *Wako*



3. Human/ Ratβ Amyloid (40)ELISA Kit *Wako*



4. Human/Rat β Amyloid (42) ELISA Kit *Wako*



Description	Wako Cat. # (Pkg. Size)	βAmyloid, measured						Dynamic Range (pmol/L)
		human Aβ(1-40)	human Aβ(1-42)	rat(mouse) Aβ(1-40)	rat(mouse) Aβ(1-42)	Aβ(x-40)	Aβ(x-42)	
Human β Amyloid (1-40) ELISA Kit *Wako* ❶ [BAN50/BA27(Fab')]	292-62301 (96 tests)	●	–	–	–	–	–	1.0~100
Human β Amyloid (1-40) ELISA Kit *Wako* II* ❷ [BAN50/BA27(Fab')$_2$] *Achieved stable antigen-antibody reaction*	298-64601 (96 tests)	●	–	–	–	–	–	1.0~100
Human β Amyloid (1-42) ELISA Kit *Wako* [BAN50/BC05 (Fab')]	298-62401 (96 tests)	–	●	–	–	–	–	1.0~100
Human β Amyloid (1-42) ELISA Kit *Wako* High-Sensitive** ❸ [BAN50/BC05 (Fab')]	296-64401 (96 tests)	–	●	–	–	–	–	0.1~20.0
Human/Rat β Amyloid (40) ELISA Kit *Wako* ❹ [BNT77/BA27(Fab')]	294-62501 (96 tests)	●	–	●	–	●	–	1.0~100
Human/Rat β Amyloid (40) ELISA Kit *Wako* II* ❺ [BNT77/BA27(Fab')$_2$] *Achieved stable antigen-antibody reaction*	294-64701 (96 tests)	●	–	●	–	●	–	1.0~100
Human/Rat β Amyloid (42) ELISA Kit *Wako* [BNT77/BC05(Fab')]	290-62601 (96 tests)	–	●	–	●	–	●	1.0~100
Human/Rat β Amyloid (42) ELISA Kit *Wako* High-Sensitive** ❻ [BNT77/BC05 (Fab')]	292-64501 (96 tests)	–	●	–	●	–	●	0.1~20.0

*: Improved β Amyloid (1-40) and (x-40)*** Detection Kits
These products are detection kits that achieve more stable antigenantibody reactions by using F(ab')$_2$ fragment of the labeled antibody BA27, which recognizes the C-terminal of Aβ40, while the nonspecific binding is remaining to be reduced. Therefore, the stability in washing solution is improved. In Human/Rat kit, background is reduced.

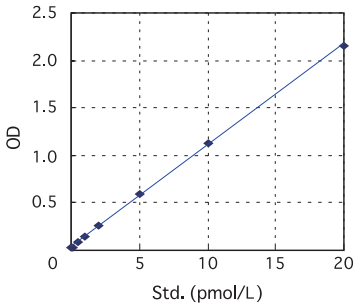
: Highly Sensitive β Amyloid (1-42) and (x-42)* Detection Kits
These ELISA kits detecting β amyloid (1-42) and (x-42) that ensure highly sensitive detection. These β amyloids are indicated to have a correlation with Alzheimer's disease. Detection sensitivity of the kits is about 10 times higher than that of the conventional products and the dynamic range is from 0.1 to 20.0 pmol/L. Fab' fragment of the labeled antibody is used as well so that the nonspecific binding is reduced.

*** Ab (x-40) and (x-42) are Aβ peptide modified or cleaved at the N-terminal.

Standard Curve

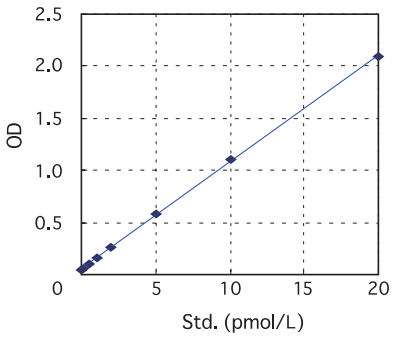
Human β Amyloid (1-42) ELISA Kit *Wako*, High-Sensitive (3)

Std. (pmol/L)	Mean(n=3) (OD at 450nm)	CV (%)
0	0.023	2.55
0.1	0.035	2.86
0.5	0.083	1.20
1.0	0.142	1.46
2.0	0.266	0.65
5.0	0.591	2.72
10.0	1.132	3.08
20.0	2.159	2.20



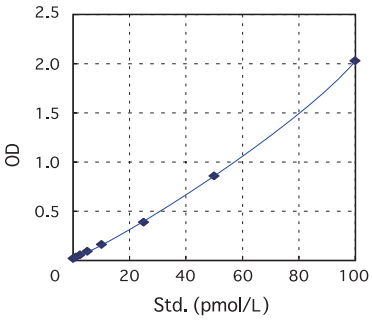
Human/Rat β Amyloid (42) ELISA Kit *Wako*, High-Sensitive (6)

Std. (pmol/L)	Mean(n=3) (OD at 450nm)	CV (%)
0	0.046	1.26
0.1	0.056	2.74
0.5	0.097	2.39
1.0	0.154	0.99
2.0	0.264	1.31
5.0	0.582	1.07
10.0	1.099	0.48
20.0	2.092	1.01



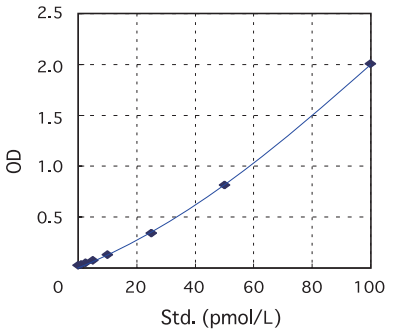
Human β Amyloid (1-40) ELISA Kit *Wako* II (2)

Std. (pmol/L)	Mean(n=3) (OD at 450nm)	CV (%)
0	0.019	2.99
1.0	0.033	1.73
2.5	0.054	0.00
5.0	0.093	2.49
10.0	0.162	6.67
25.0	0.388	7.70
50.0	0.859	9.14
100.0	2.031	0.67



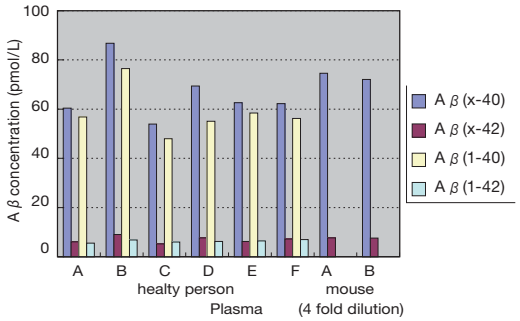
Human/Rat β Amyloid (40) ELISA Kit *Wako* II (5)

Std. (pmol/L)	Mean(n=3) (OD at 450nm)	CV (%)
0	0.024	16.61
1.0	0.032	1.79
2.5	0.047	2.13
5.0	0.073	0.79
10.0	0.130	0.55
25.0	0.340	0.74
50.0	0.814	1.72
100.0	2.005	1.37



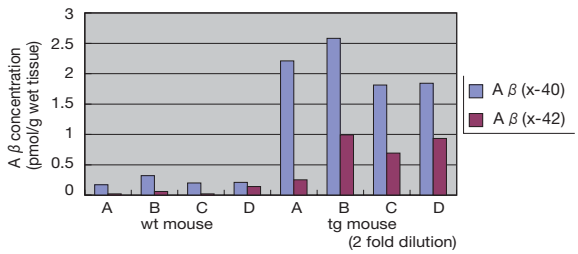
Reaction time for HRP-conjugated antibody is 2 hours.

Application Data [1] human and mouse plasma



Blood was collected using vacuum blood collection tube containing EDTA-2K. Plasma was separated by centrifugation at 5000 × g for 15 minutes at 4 ℃ and stored at −80 ℃ until used.
The plasma sample was diluted 4-fold with Standard Diluent in the Kit and measured. (A**β** (1-40) and A**β** (×-40) were measured by Human **β** Amyloid (1-40) ELISA Kit *Wako* II (Cat.# 298-64601, 2) and Human/Rat **β** Amyloid (40) ELISA Kit *Wako* II (Cat.#294-64701, 5))

[2] mouse brain tissue



The hemisphere of 12-month mouse (J20) was extracted with 2 mL of Tris Saline and stored frozen at -20 ℃ until used. The brain sample was diluted 2-fold with Standard Diluent in the Kit and measured. A trace quantity of A**β** could be detected not only in the transgenic mice (tg) but in the wildtype mice (wt).

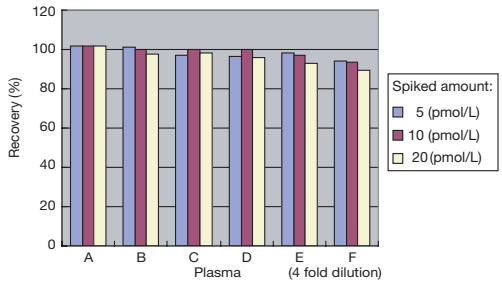
(Data provided by Prof. Iwatsubo and Instructor Hashimoto, Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, University of Tokyo)

Specificity (n=4)

(%)

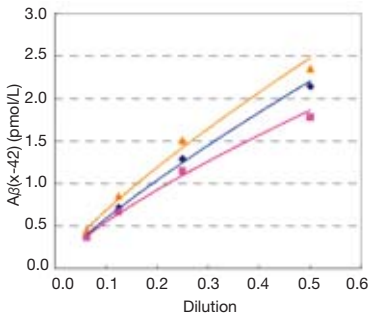
Synthetic Peptide	Human Aβ(1-40) Kit II (Cat. #298-64601) 2	Human Aβ(1-42)Kit, High-Sensitive (Cat. #296-64401) 3	Human/Rat Aβ(40) Kit II (Cat. #294-64701) 5	Human/Rat Aβ(42) Kit, High-Sensitive (Cat. #292-64501) 6
Human Aβ(1-40)	100.0	≦0.1	100.0	≦0.1
Human Aβ(1-42)	≦0.1	100.0	≦0.1	100.0
Human Aβ(1-43)	≦0.1	13.5	≦0.1	12.7
Rat(Mouse) Aβ(1-40)	0.2	≦0.1	156.0	≦0.1
Rat(Mouse)Aβ(1-42)	0.3	0.5	0.3	156.0

Spike Recovery (n=3)



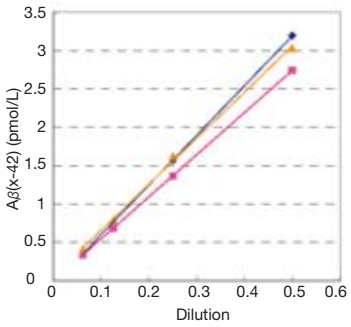
Human β Amyloid (1-42) ELISA Kit, High-Sensitive ❸

Sample	Dilution	Measured value (pmol/L)
◆ plasma A (EDTA2K)	1/2	2.143
	1/4	1.287
	1/8	0.715
	1/16	0.388
■ plasma B (EDTA2K)	1/2	1.780
	1/4	1.146
	1/8	0.668
	1/16	0.363
▲ plasma C (EDTA2K)	1/2	2.349
	1/4	1.503
	1/8	0.849
	1/16	0.445



Human / Rat β Amyloid (42) ELISA Kit, High-Sensitive ❻

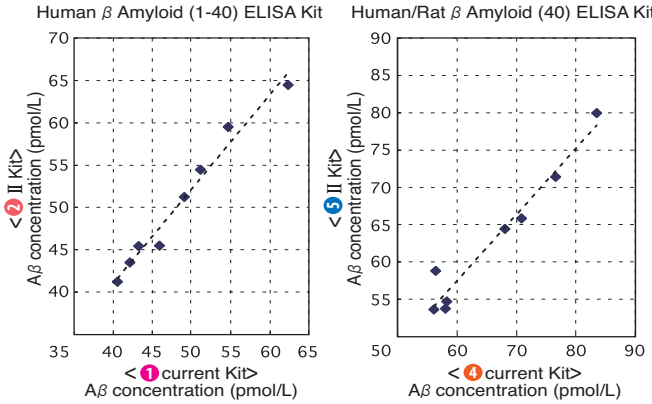
Sample	Dilution	Measured value (pmol/L)
◆ plasma A (EDTA2K)	1/2	3.193
	1/4	1.566
	1/8	0.736
	1/16	0.377
■ plasma B (EDTA2K)	1/2	2.743
	1/4	1.361
	1/8	0.686
	1/16	0.335
▲ plasma C (EDTA2K)	1/2	3.033
	1/4	1.627
	1/8	0.780
	1/16	0.409



II Kit-conventional kit Correlation

(pmol/L)

Sample	Human β Amyloid (1-40) ELISA Kit		Human/Rat β Amyloid (40)ELISA Kit	
	II Kit (❷) (F(ab')$_2$-HRP)	the conventional kit (❶) (Fab'-HRP)	II Kit (❺) (F(ab')$_2$-HRP	the conventional kit (❹) (Fab'-HRP)
plasma A	45.9	45.5	56.4	58.8
plasma B	49.1	51.2	68.1	64.4
plasma C	62.4	64.4	83.5	79.9
plasma D	40.5	41.2	56.1	53.6
plasma E	42.1	43.5	58.0	53.7
plasma F	43.3	45.4	58.3	54.7
plasma G	54.7	59.5	76.7	71.4
plasma H	51.2	54.4	70.9	65.8



[References]

1) Suzuki N., Cheung TT., Cai XD., Odaka A., Otvos L. Jr., Eckman C., Golde TE. And Younkin SG: *Science*, **264**, 1336 (1994).
2) Iwatsubo T., Odaka A., Suzuki N., Mizusawa N. and Ihara Y.: *Neuron*, **13**, 45 (1994).
3) Asami-Odaka A., Ishibashi, Y., Kikuchi T., Kitada C. and Suzuki N.: *Biochemistry*, **34**, 10272 (1995).
4) Fukumoto H., Tomita T., Matsunaga H., Ishibashi Y., Saido T.C. and Iwatsubo T.: *Neuroreport*, **10**, 2965 (1999).
5) Scheuner D., Eckman C., Jensen M., Song X., Citron M., Suzuki N., Bird TD., Hardy J., Hutton M., Kukull W., Larson E., etc.: *Nature Med.*, **2**, 864 (1996).
6) Kosaka T., Imagawa M., Seki K., Arai H., Sasaki H., Tsuji S., Asami-Odaka A., Fukushima T., Imai K. and Iwatsubo T.: *Neurology*, **48**, 741 (1997).

Histostaining of Alzheimer's Diseased Brain Tissues – Distinctive Histostaining of Aβ40 and Aβ42 plaques

Amyloid β-Protein Immunohistostain Kit

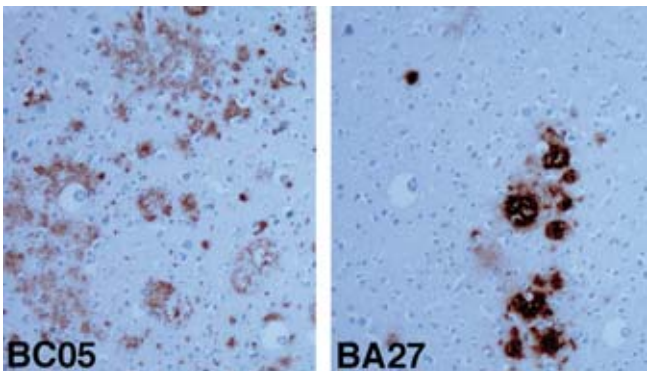
Wako Cat. No. 299-56701 50 tests
Keep at 2-10℃

[Features]

1. Distinctive histostaining of Aβ40 and 42 plaques in Alzheimer's diseased brain tissues
2. High sensitivity histo-Immune detection of Aβ plaques in tissue sections with low background

[Kit Contents (50 tests)]

1. Blocking Serum	1 bottle ×10 mL
2. Anti Mouse IgG (H+L), Goat, Conjugated	1 bottle ×10 mL
3. ABC Solution (Streptavidin-biotin-peroxidase Complex)	1 bottle ×10 mL
4. Formic Acid (90%)	1 bottle ×15 mL
5. Anti Amyloid β-Protein (1-40), MAb, Clone # BA27	1 bottle × 7 mL
6. Anti Amyloid β-Protein (1-42), MAb, Clone # BV05	1 bottle × 7 mL
7. Trypsin, Cryst	1 bottle × 50 L



Immunostaining of senile plaques in the consecutive sections of the brain affected with Alzheimer's Disease.
Left : Aβ42-staining using Anti Aβ42 Ab (Clone #BC05);
Right : Aβ40- staining using Anti Aβ40 Ab (Clone #BA27)
(provided by Dr. Iwatsubo, Univ. of Tokyo)

for microRNA Research

Anti Human Ago2, Monoclonal Antibody (Clone No. 4G8)

Wako Cat. No. 011-22033 (50 µL); 015-22031 (100 µL) <for Immunochemistry>
Keep at 2~10℃

☞ *Please see the page #22.*

Anti Mouse Ago2, Monoclonal Antibody (Clone No. 2D4)

Wako Cat. No. 014-22023 (50 µL); 018-22021 (100 µL) <for Immunochemistry>
Keep at 2~10℃

☞ *Please see the page #23.*

Study for Cholesteryl Ester Transfer Protein (CETP)

Cholesteryl ester tranfer protein(CETP) is one of the lipid transfer proteins, which mediates the transfer of cholesteryl ester (CE), triglyceride (TG) and phospholipids between lipoproteins.[1] CETP facilitates the transfer of CE from high-density lipoprotein(HDL) to very-low-density lipoprotein(VLDL) and low-density lipoprotein(LDL), and also the transfer of TG from VLDL and LDL to HDL.[2] The clinical significance of CETP has been controversial.[3)~7)] Wako supplies two kind of CETP monoclonal antibody as well as the ELISA Kit, which is based on the sandwich enzyme immunoassay measures CETP mass.

Anti Human CETP, Monoclonal Antibody (Clone No. CETP-4)*

Wako Cat. No. 010-21241 (100 µg) <for Immunochemistry>
Keep at -80℃

This monoclonal antibody (Clone No. CETP-4) inhibits cholesteryl ester tranfer protein (CETP) activity. The 10μg/mL antibody solution can entirely inhibit the CETP activity in the same quantity of normal serum.

Appearance: prepared in PBS containing 0.05% sodium azide.
Concentration: 1mg/mL
Specificity: Specific to human CETP
Antibody Titer: 1: 10,000+ (ELISA)

Anti Human CETP, Monoclonal Antibody (Clone No. CM5,a-27)*

Wako Cat. No. 017-21251 (100 µg) <for Immunochemistry>
Keep at -80℃

This monoclonal antibody, specifically recognizes SDS-treated CETP, is applicable to immunostaining on nitrocellulose membrane

Appearance: prepared in PBS containing 0.05% sodium azide.
Concentration: 1mg/mL
Specificity: Specific to human CETP
Antibody Titer: 1: 10,000+ (ELISA)

***: ELISA kit using both antibodies can be supplied upon request (minimum order: 10 kits).**

CUSTOM-ORDERED SUPPLY
Human CETP ELISA Kit *Wako*

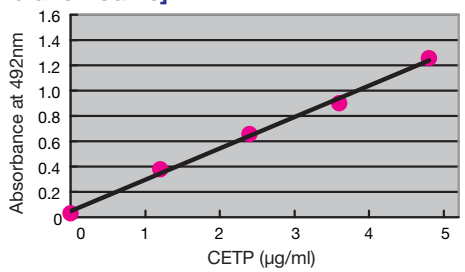
Wako Cat. No. 290-65401 (for 72 tests)
<for Immunochemistry>

[Features]
1. Easy determination of human serum CETP
2. High linearity by 4.8 µg/mL
3. 4-hour procedure

[Procedure]

- **[1 hr]** Sample Pretreatment
- **[1 hr]** Reaction with 1st Ab on each well ▶ Wash
- **[1 hr]** Reaction with 2nd Ab ▶ Wash
- **[30 min]** Reaction with Substrate Soln. ▶ Stop the reaction
- Measure the absorbance (490~492nm)

[Calibration Curve]



[References]

1) Tall, A. R.: "Plasma lipid transfer proteins.", *J. Lipid Res.* , **27**, 361-7 (1986).
2) Morton, R. E. and Zilversmit, D.B.: "Inter-relationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma", *J. Biol. Chem.* , **258**, 11751-7 (1983).
3) Fielding, C. J. and Havel, R. J.: "Cholesteryl ester transfer protein: friend or foe?", *J . Clin. Invest.* , **97**, 2687 (1996).
4) Inazu, A., Brown, M. L., Hesler, C. B., Agellon, L. B., Koizumi, J., Tanaka, K., Maruhama, Y., Mabuchi, H. and Tall, A. R.: "Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation"*N, . Engl. J. Med.* , **323**, 1234-8 (1990).
5) Tall, A. R.: "Plasma cholesteryl ester transfer protein", *J. Lipid Res.* , **34**, 1255-74 (1993).
6) Zhong, S., Sharp, D. S., Grove, J. S., Bruce, C., Yano, K., Curb, J. D. and Tall, A. R.: "Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels"*J, . Clin. Invest .*, **97**, 2917-23 (1996).
7) Marotti, K. R., Castle, C. K., Boyle, T. P., Lin, A. H., Murray, R. W. and Melchior, G. W.: "Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein", *Nature*, **364**, 73 (1993).

Antibodies against Macrophage/ Microglia-specific Protein Iba1*

*** Iba1: ionized calcium binding adapter molecule 1**

Anti Iba-1 polyclonal antibody, Rabbit, for Immunocytochemistry

Wako Cat. No. 019-19741 (50 µg (100 µL)) <for Immunochemistry>
Keep at -20℃; Working Concentration: 1 ~ 2 µg/mL (Immunocytochemistry)

Anti Iba-1 polyclonal antibody, Rabbit, for Western Blotting

Wako Cat. No. 016-20001 (50 µg (100 µL)) <for Immunochemistry>
Keep at -20℃; Working Concentration: 0.5 ~ 1 µg/mL (Western Blot)

Calcium ions are known to be one of the most important signal mediators in all cells including central nervous system (CNS) cells. Calcium ions exert their signaling activity through association with various calcium binding proteins, many of which are classified into a large protein family, the EF hand protein family.
Iba1 is a 17-kDa EF hand protein that is specifically expressed in macrophages/ microglia and is upregulated during the activation of these cells.
Wako distributes rabbit polyclonal antibodies were raised against a synthetic peptide corresponding to the Iba1 carboxy-terminal sequence, which was conserved among human, rat and mouse Iba1 protein sequences. These antibodies are specifically reactive to microglia/ macrophages, are appropriate for immuno-double staining of brain tissues and cell culture in combination with monoclonal antibody to GFAP, which specifically reacts to astrocyte.

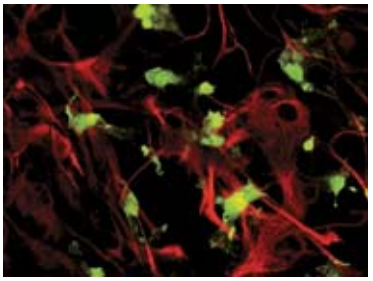


Figure :
Immuno-double staining of rat primary mixed culture cells
Green: Iba1, which reacts to anti Iba1 antibody (Wako Cat. #019-19741)
Red: astrocyte, which reacts to anti GFAP, monoclonal antibody
(Data was provided by Dept. of Neurochemistry, National Institute of Nueroscience (Japan).)

[Specificity]
Specific to microglia and macrophages, but not cross-reactive with neurons and astrocytes.
Reactive with human, mouse and rat Iba1.

Research for Olfactory Nerve

Anti Olfactory Marker Protein, Goat [Anti OMP]

Wako Cat. No. 544-10001 (100 µL) <for Immunochemistry>
Keep at -20℃

Olfactory Marker Protein (OMP) is soluble acid protein expressed in mature olfactory nerve. This goat antiserum is highly specific for mature olfactory neurons and their axons and terminals in tissue sections of many vertebrate species including rodents, humans, marsupials and amphibia.

Working Dilutions:
Western Blot: ~ 1 : 50,000
Immunocytochemistry: 1 : 200 (paraffin embedded material) ~ 1 : 50,000 (Vectastain-Elite with fixed floating sections)

Preparation: Goat antiserum to OMP (100µL) is diluted 1:1 with glycerol containing 0.05% sodium azide to facilitate shipment at ambient temperature.

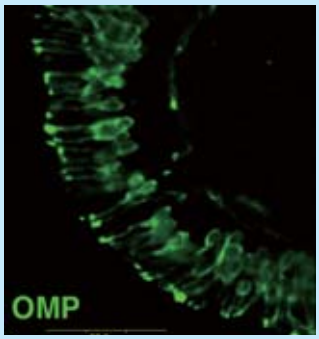


Figure :
Immunofluorescence staining of adult mouse olfactory epithelium with goat anti-OMP (Wako Cat. # 544-10001).
Green : OMP staining was visualized with Cy2 (Jackson Immuno Research).
Data was provided by Dr. Frank L. Margolis and Dr. Jae Hyung Koo, Department of Anatomy and Neurobiology, School of Medicine, University of Maryland.

[References]

1) Baker, H. *et al.* : *J. Comp. Neurol.*, **285**, 246 (1989).
2) Buiakova, O. I. *et al.* : *Genomics*, **20,** 452 (1994).
3) Cummings, D. M. *et al.* : *J. Comp. Neurol.*, **421**, 362 (2000).
4) Keller, A. and Margolis, F. L. : *J. Neurochem.*, **24**, 1101 (1975).
5) Koo, J. H. *et al.* : *J. Neurochem.*, **90**, 102 (2004).
6) Koo, J. H. *et al.* : *J. Comp. Neurol.*, **487**, 1 (2005).
7) Rama Krishna, N. S. *et al.* : *Brain Res.*, **593**, 295 (1992).
8) Verhaagen, J. *et al.* : *J. Neurosci. Res.*, **26**, 31 (1990).

Study for Human Aging Brain

Anti Phosphorylated α-Synuclein, Monoclonal Antibody (Clone No. pSyn #64)

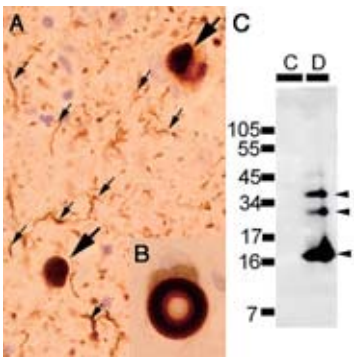
Wako Cat. No. 014-20281 (50 µL) <for Immunochemistry>

Keep at -20℃

α-Synuclein in Lewy bodies (LBs) which are pathognomonic for Parkinson's disease (PD) and dementia with Lewy bodies (DLB) contains the phosphorylated at Ser129. We have launched an antibody which specifically reacts with human α-Synuclein with a phosphorylated Ser129 residue and does not react to human α-Synuclein. This antibody is applicable to immunohistochemical and biological studies on the locations of LB-related pathology.

Subclass : Mouse IgG_1
Specificity : Specific for human α-Synuclein with a phosphorylated Ser129.
No cross-react with human α-Synuclein.
Working Dilution : 1 : 1000 ~ 1 : 10000 (Western blot and Immuhistochemistry)

[Immunohistochemistry of synucleinopahty lesions and Western blot analysis]



A : Temporal neocortex of DLB brains were immunostained with anti Phosphorylated α-Synuclein. Big arrow (➹) and mini-arrow (➶) indicate LBs and Lewy neurites, respectively.
B : Brainstem LBs in pigmented neurons of the substantia nigra in PD.
C : Western blot analysis of α-synuclein differentially extracted with urea from cerebral cortices of a patient with DLB (D) and a normal control (C) individual probed with monoclonal antibody pSyn#64 (Anti Phosphorylated α-Synuclein). This antibody strongly reacted with the urea-soluble phosphorylated α-synuclein (◀) in DLB brains.

[References]
1) Fujiwara, H., *et al.*: *Nature Cell Biology*, **4**, 160, (2002)
2) Saito, Y., *et al.*: *J. Neuropathol Exp Neurol*, **62**, 644 (2003)

[Immunohistochemistry of DLB]

<Materials>
1. Normal Goat Serum for blocking
2. Biotinylated Anti-Mouse IgG
3. ABC solution (Wako Cat. #017-15881)
4. Formic Acid (abt. 99 %)(Wako Cat. #066-00461 (100 mL))
5. Anti Phosphorylated α-Synuclein (Wako Cat. #014-20281)

<Procedure>

deparaffinized section
↓
← Formic Acid Treatment for 5 min.
← Wash for 5 min.
← Wash with PBS-Tween for 2 min.
← 0.05 % Trypsin Treatment at 37 ℃ for 15 min.
← Wash for 5 min. × 2
← Blocking at 37 C for 30 min.
← Anti Phosphorylated α-Synuclein (× 2000) at 37 ℃ for 1 hour
← Wash with 0.01M PBS-Tween for 2 min. × 5
← Biotinylated Antibody, at 37 ℃ for 1 hour
← Wash with 0.01 M PBS-Tween × 3
← ABC Solution at 37 ℃ for 30 min.
← Wash with 0.01 M PBS-Tween × 3
↓
Colored by DAB Reagent

Research for Neurontransmitter and Alzheimer's disease

Anti Substance-P, Rabbit, affinity purified IgG fraction

Wako Cat. No. 016-13911 (1 mL) <for Immunochemistry>

Keep at -20℃

Substance P is a neuropeptide which is widely distributed in the periphery and the central nervous system, where it is co-localised with other neurotransmitters such as serotonin or dopamine and where it acts as a neuromodulator. Substance P has been proposed to play a role in the antiopathology of asthma, inflammatory bowel disease, emesis, psoriasis, as well as neuropsychiatric disorders including pain syndromes (e.g. migraine and fibromyalgia) and affective disorders, anxiety disorders, schizophrenia and Alzheimer's disease.

Anti Substance P is isolated from rabbit antiserum against Substance P, and consists of the IgG fraction in 20 mM PBS solution (pH 7.2). The product is treated with Protein A column and affinity purified using Sepharose column.

Appearance: frozen
Protein content: 100 µL IgG/mL (A_{280nm})
Specificity: Does not cross-react with enkephalin, endorphin, or bradykinin
Antibody titer: 1 : 10,000 (EIA method)

[References]
1) Viamontes, G.I., *et al.*: "Antibodies to thymopoietin following implantation of paper disks derivatized with synthetic Cys-thymopoietin.", *J. Immunol. Meth.*, **94**, 13-17 (1986)
2) Hokfelt, T., *et al.*: "Dense plexus of substance P immunoreactive nerve terminals in eminentia medialis of the primate hypothalamus.", *Proc. Nat. Acad. Sci*, **75**, 1013~ 15 (1978).

Autophagy Research

Anti SQSTM1/A170/p62, Rabbit

Wako Cat. No. 018-22141 (100 µL) <for Immunochemistry>

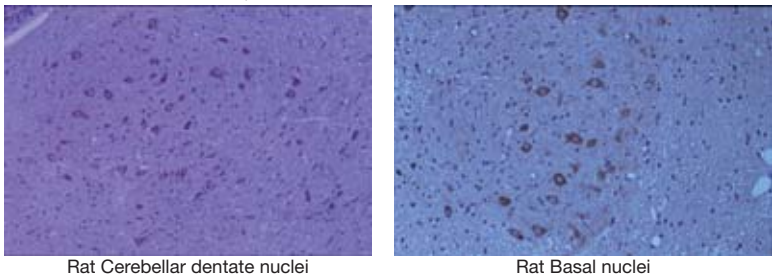
Keep at -20℃

Sequestosome 1 (SQSTM1) / A170 (mouse) / p62 (human) / ZIP (rat), a ubiquitin-binding protein, expresses oxidative stress-dependently. Abnormality of SQSTM1 leads to bone metabolic disorder, obesity and Type II diabetes. SQSTM1 was reported to bind LC3, which regulates autophagosome formation. The protein has attracted the attention of researchers because it is believed to induce a protein from ubiquitin-proteosome system (UPS) to lysosome-dependent macroautophagy (autophagy) system, which are two major intracellular pathways for protein degradation.
Wako has launched the mouse SQSTM1 (A170) rabbit antiserum, which is applicable to Western blot, immunohistochemistry and immunofluorescence.

Preparation: The antiserum is diluted with an equal amount of PBS and absorbed with *E. coli* proteins.
Immunogen: recombinant murine SQSTM1 (A170) (AA254-333) containing T7 tag at the N-terminal end and His tag at the carboxy-terminal end
Specificity: Specific for mouse and rat SQSTM1 (A170 / ZIP). Slightly reactive with human SQSTM1 (p62).
Working Dilution: Western blot 1 : 200 ; Immunohistochemistry 1 : 1,000 ; Immunofluorescence 1 : 1,000

APPLICATION-1

◆ **Immunohistochemistry**



Rat Cerebellar dentate nuclei　　Rat Basal nuclei

Figure: Brain tissues were fixed with 4% paraformaldehyde and embedded with paraffin. The 6µm sections were stained with avidin-biotin-peroxidase method.
Primary Antibody: Anti SQSTM1/A170/p62 (Wako Cat. #018-22141) 1 : 1,000
Secondary Antibody: Anti rabbit IgG, biotin conjugated

APPLICATION-2

◆ **Western blot**

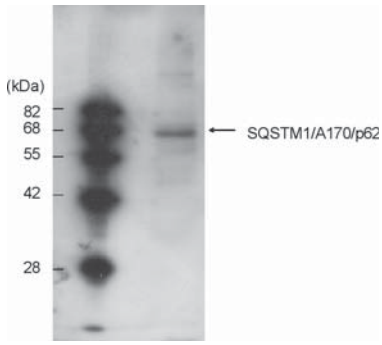


Figure: Western blot of cultivated mouse vascular smooth-muscle cell lysate (20µg)
Antibody: Anti SQSTM1/A170/p62 (Wako Cat. #018-22141) 1:200
Data was provided from Prof. Ishii, Tsukuba University (Japan)

[References]

1) Ishii, T., Yanagawa, T., Kawane, T., Yuki, K., Seita, J., Yoshida, H. and Bannai, S. : "Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages", *Biochem. Biophys. Res. Commun.*, **226**, 456-60 (1996).
2) Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S. and Yamamoto, M.: "Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages", *J. Biol. Chem.*, **275**, 16023-9 (2000).
3) Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., Hamazaki, J., Nishito, Y., Ishii, T., Kobayashi, A., Yamamoto, M., Yue, Z., Uchiyama, Y., Kominami, E. and Tanaka, K.: "Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice", *Cell*, **131**, 1149-63 (2007).
4) Nakaso, K., Kitayama, M., Ishii, T., Bannai, S., Yanagawa, T., Kimura, K., Nakashima, K., Ohama, E.. and Yamada, K. : "Effects of kainate-mediated excitotoxicity on the expression of rat counterparts of A170 and MSP23 stress proteins in the brain", *Brain Res. Mol. Brain Res.*, **69**, 155-63 (1999).

Research for NO

Anti soluble Guanylate Cyclase (sGC), MAb (Clone: mAB3221)

Wako Catalog No. 019-17801 (20 µg (40 µL)) <for Immunochemistry>

Keep at -20℃

Soluble guanylate cyclase (sGC), a hemoprotein, is the primary nitric oxide (NO) receptor in higher eukaryotes that catalyzes the conversion of guanosine 5'-triphosphate (GTP) to 3,5'-cyclic guanosine monophosphate (cGMP) and pyrophosphate (PPi) in the presence of Mg^{2+}. The binding of NO to sGC leads to a several hundred-fold increase in cGMP synthesis.

Prepared from culture supernatant and prepared in glycine-Tris solution (pH 7.4). Contains no preservatives and stabilizers.
Isotype: IgG1
Specifically reacts with rat, bovine and human sGC, and strengthens in the reactivity on activation of sGC by NO, probably, due to the conformational changes of the enzyme and its associated antibody-antigen complex.
Working Dilution :
Westernblot 1 : 5,000 ; Immunofluorescence 1 : 250

[Reference]

Tsuyama, S., *et al.*, *FEBS Lett.*, **455**, 291 (1999).

Anti soluble Guanylate Cyclase (sGC), MAb, NO insensitive (Clone: mAB28131)

Wako Catalog No. 017-18201 (20 µg (40 µL)) <for Immunochemistry>
Keep at -20℃

Prepared from culture supernatant and prepared in glycine-Tris solution (pH 7.4). Contains no preservatives and stabilizers.

Isotype: IgG_1
Specifically reacts with rat, bovine and human β-subunit of sGC, but not strengthened in the reactivity on activation of sGC by NO.
Working Dilution:
Westernblot 1 : 5,000; Immunofluorescence 1 : 250

Antibodies against Vesicular Glutamate Transporters

Anti Rat VGLUT-1, Rabbit

Wako Catalog No. 010-19771 (50 µg (100 µL)) <for Immunochemistry>
Keep at -20℃

Anti Rat VGLUT-2, Rabbit

Wako Catalog No. 017-19781 (50 µg (100 µL)) <for Immunochemistry>
Keep at -20℃

L-Glutamate is an excitatory chemical transmitter that plays an essential role in neuronal plasticity, behavior, learning and memory in the central nervous system. On the other hand, VGLUTs play an essential role in glutamate signal output through vesicular storage of L-glutamate. Three kinds of VGLUTs have been identified so far. Recent studies have demonstrated that VGLUTs are also expressed in peripheral cells such as stomach, intestines, pancreas and testes. In particular, the discovery that glutamate is co-localized with glucagon in secretory granules in_cells of islets of Langerhans has been noted for new mechanism of blood glucose control. Anti Rat VGLUT, Rabbit can detect glutamatergic central nerves, peripheral nerves and nonneural cells.
Both antibodies are applicable to immunocytochemistry, immunoelectron microscopy and Western blotting.

Anti Rat VGLUT1, Rabbit

Raised against the GST fusion peptide encoding G 509-S 560 of the cytosolic regions of VGLUT1
Specificity: Specific to rat, mouse, human and bovine VGLUT-1.
No reactive to VGLUT-2
Working Conc.: Immunofluorescence 1 : 1,500

Anti Rat VGLUT2, Rabbit

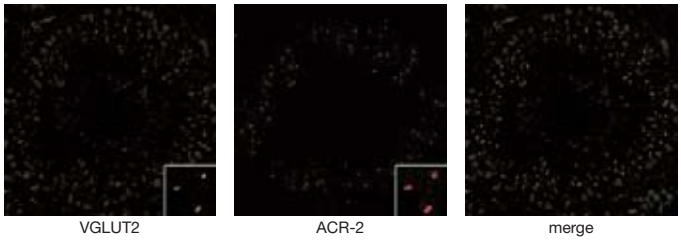
Raised against the GST fusion peptide encoding G 500-Y 582 of cytosolic regions of VGLUT2
Specificity: Specific to rat, mouse, human and bovine VGLUT-2.
No reactive to VGLUT-1
Working Conc.: Immunofluorescence 1 : 1,500

Application [1]: Immunofluorescence

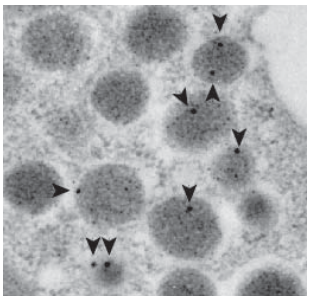
VGLUT1 is co-localized with glucagon in_cells of islets of Langerhans.

VGLUT2 is co-localized with glucagon in L-cells in the mucosa of ileum.

VGLUT2 is localized in the acrosome.



Application [2]: Immunoelectron microscopy



Double immunoelectron microscopy in_cells of islets of Langerhans. Glucagon (5nm) and VGLUT2 (15nm) (arrowheads) are co-localized with secretory granules.
Photo by Dr. Mitsuko Hayashi (Yale Univ.)

[References]

1) Moriyama, Y. and Hayashi, M. : *TRENDS Pharmacol. Sci. electric version*, **24**, 511 (2003).
2) Morimoto, R., Hayashi, M., Yatsushiro, S., Otsuka, M., Yamamoto, A. and Moriyama, Y. : *J. Neurochem.*, **84**, 382 (2003).
3) Hayashi, M., Morimoto, R., Yamamoto, A. and Moriyama, Y. : *J. Histochem. Cytochem.*, **51**, 1375 (2003).
4) Hayashi, M., Yamada, H., Uehara, S., Morimoto, R., Takeda, J., Yamamoto, A, and Moriyama, Y. : *J. Biol. Chem.*, **277**, 1966 (2003).
5) Hayashi, M., Otsuka, M., Morimoto, R., Muroyama, A., Uehara, S., Yamamoto, A., and Moriyama Y. : *Diabetes*, **52**, 2066 (2003).

Apoptosis Detection Kit by TUNEL method

Apoptosis *in situ* Detection Kit *wako*

Wako Cat. No. 298-60201 (40 tests) <for Apotosis Research >
Keep at -20℃

The kit is based on TUNEL [Terminal deoxynucleotidyl Transferase(TdT)-mediated dUTP nick end labeling] procedure, that is the addition of fluorescein -dUTP to 3' -terminals of apoptotically fragmented DNA with TdT followed by immunochemical detection using anti-fluorescein antibody conjugated with horseradish peroxidase (POD) and DAB as a substrate

[Features]

1. **Rapid detection can be performed.**
   The whole process from the de-paraffinizing step to the microscopic examination can be completed in about 2 hours.
2. **Complicated preparations of various reagents are not needed.**
   The kit contains the essential reagents required for detection of apoptosis.
3. **The kit shows a clear positive image with low background.**

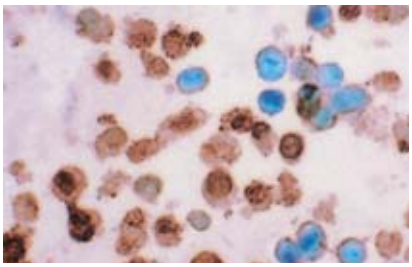
[Applicable samples]

- paraffin-embedded tissue sections
- frozen tissue sections
- neutralized formalin-fixed culture cells

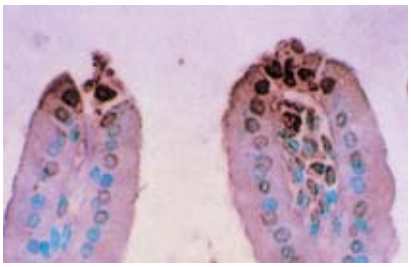
[Kit Contents] (approx. 1cm^2 × 40 reactions)

Protein Digestion Enzyme	**1 vial × 1 mL**
TdT	**1 vial × 40 µL**
TdT Substrate Solution	**1 vial × 4.4 mL**
100 × POD-Conjugated Antibody	**1 vial × 44 µL**
DAB Solution	**1 vial × 4.4 mL**
DAB Enhancer	**1 vial × 200 µL**
DNase I	**1 vial × 4 µL**
10 × DNase I Reaction Buffer	**1 vial × 40 µL**

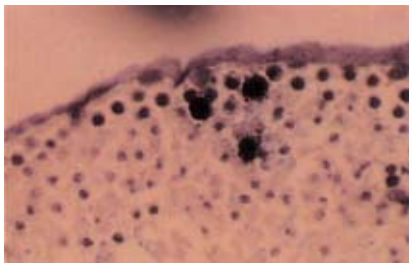
[TUNEL Staining]



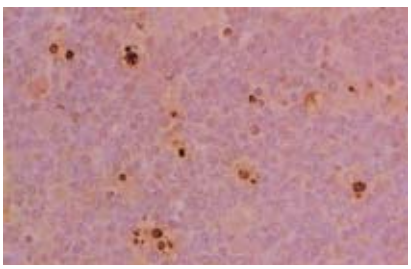
Cultured cell CHO-K1: after Apoptosis induction (CPZ treatment) (× 400)
Nuclei: Methyl Green Staining



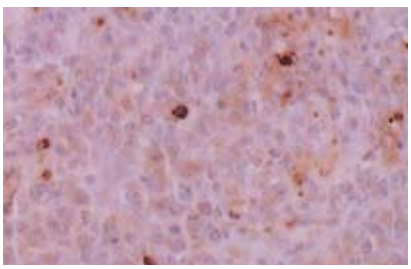
Rat small intestine (× 400)
Nuclei: Methyl Green Staining



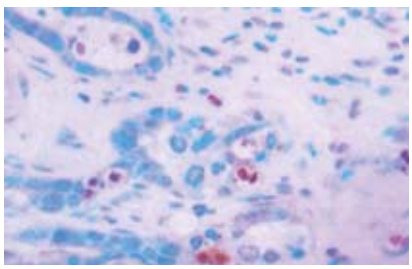
Rat testicle: DAB Intensifying Staining (× 200)



Human T cell lymphoma: HE Staining (× 200)



Human B cell lymphoma: HE Staining (× 200)



Human gastric cancer (× 200)
Nuclei: Methyl Green Staining

Description	Catalog No.	Package Size
Apoptosis *in situ* Detection Kit *wako*	298-60201	40 tests

Related Products

Description	Catalog No. (Pkg. Size)	Storage
Deoxyribonuclease I (DNase I) (a kit content of Apopsis *in situ* Detection Kit)	293-56601 (4 × 1 cm^2)	Keep at -20℃
Lemosol® <limonene-based solvent as a xylene substitute>	122-03991 (1 L)	Keep at RT
Lemosol® A <terpene-based solvent as a xylene substitute>	120-04411 (1 L)	Protect from Light
Softmount <a mounting reagent containing LemosolR A>	199-11311 (250 mL)	Protect from Light
1 × PBS (-) Powder (0.01 mol/L, pH 7.2~7.4)	162-19321 (for 1 L × 20)	Keep at RT
Methyl Green Solution (0.5 w/v%)	138-12701 (100 mL)	Keep at 2~10℃

for semi-quntifying apoptotic cells in culture cells using TUNEL

Apoptosis Screening Kit *Wako*

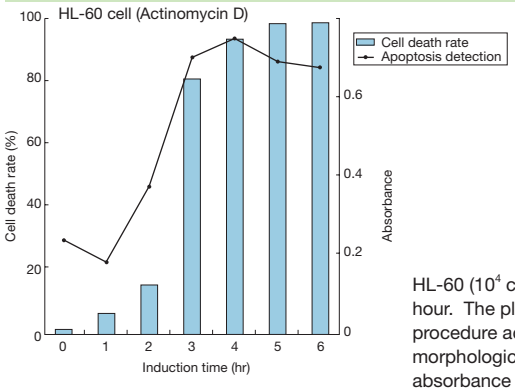
Wako Cat. No. 296-60001 (96 tests)

Keep at -20℃ <for apoptosis research>

Apoptosis Screening Kit Wako is designed to semi-quantify apoptotic cells in culture cells grown in a microtiter plate using the TUNEL (Terminal deoxynucleotidyl Transferase (TdT)-mediated dUTP nick en labeling) procedure. Fluorescein-labeled fragmented DNA by the TUNEL procedure is immunochemically quantified by horseradish peroxidase (POD)-conjugated antibody and a chromogenic substrate on a microplate. This kit provides all the essential reagents for the assay.

[Features]

1. Rapid detection can be performed in 3 hours.
2. Tedious preparations of various reagents are not needed.



HL-60 (10^4 cells) were seeded in each well and 1μg/mL of actinomycin D was added every one hour. The plate was incubated sequentially and the absorbance was measured. Then, the detection procedure according to the package insert was carried out. Cell death rate was calculated by morphologic observation of the cells under a microscope and the correlation between the rate and the absorbance was examined.

[Kit Contents]

1) Fixation Solution	1 vial × 640μL
2) Permeabilizing Solution	1 vial × 19.2 mL
3) TdT 1 vial × 20μL	1 vial × 20μL
4) TdT Substrate Solution	1 vial × 4.8 mL
5) Hydrogen peroxide	1 vial × 340μL
6) 500x POD-conjugated Antibody	1 vial × 20μL
7) Antibody Diluent	1 vial × 9.6 mL
8) Chromogenic Substrate	5 tablets × 63 mg
9) Chromogenic Substrate Buffer	1 vial × 10 mL
10) Stop Solution	1 vial × 9.6 mL
11) Sterilized Microtiter Plate (96 wells)	1 plate

Apoptosis Inducer

Camptothecin, 98.0+% (HPLC)

Wako Cat. No. 038-18191 (100 mg); 034-18193 (500 mg)

Keep at 2~10℃ <for Biochemistry>

An alkaloid contained in Nothapodytes foetida and Camptotheca acuminata. It has a quinoline skeleton but, biosynthetically, it is a resembling compound of indole alkaloid.

This product is a reversible inhibitor of DNA topoisomerase I and it binds to topoisomerase-DNA complex leading to stabilization. It exhibits antileukemic and antitumor activities and inhibits activation of HIV-1 by Tat. It exhibits cytotoxicity to tumorigenetic cells but not to non-tumorigenetic cells. It also induces apoptosis in HL-60 cells and mouse thymus cells.

Anticancer irinotecan (Topotecin) is one of its derivatives.

Apoptosis Inducing Bioprobe

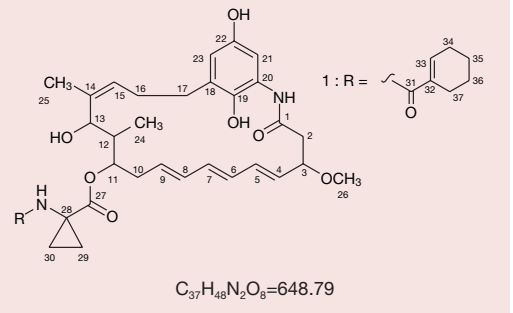
Cytotorienin A, from *Streptomyces* sp.

039-18241 (100 μg)

Keep at -20℃ <for Biochemistry>

A unique bioprobe, cytotrienin A induces apoptosis (or programmed cell death) in human promyelocytic leukemia HL-60 cells at a low concentration (10 ng/mL).

Solubility : Soluble in methanol (0.1 mg/mL)



[References]

1) Kakeya, H., Zhang, H., Kobinata, K. Onose, R., Onozawa, C., Kudo T. and Osada, H., "Cytotrienin A, a Novel Apoptosis Inducer in Human Leukemia HL-60 Cells", *J. Antibiotics*, **50** (4), 370-372 (1997)

2) Zhang, H., Kakeya, H. and Osada H., "Novel Triene-ansamycins, Cytotrienins A and B, Inducing Apoptosis on Human Leukemia HL-60 Cells", *Tetrahedron Letters*, **38** (10), 1789-1792 (1997)

3) Kakeya, H., Onose, R., and Osada, H., "Caspase-mediated Activation of a 36-kDa Myelin Basic Protein Kinase during Anticancer Drug-induced Apoptosis", *Cancer Research*, **58**, 5888-4894 (1998).

4) Watabe, M., Kakeya, H., Onose, R., and Osada, H., "Activation of MST/Krs and c-Jun N-terminal Kinases by Different Signaling Pathways during Cytotrienin A-induced Apoptosis", *J. Biol. Chem.*, **275**, 8766-8771 (2000).

for Lymphocyte Separation

Iron Powder, from Iron Carbonyl, 99.0+% (Titration)

Wako Cat. No. 098-02222 (25 g) <for Lymphocyte Separation>
Keep at RT

The product is a very fine uniform iron powder used for macrophage elimination.
CAS No. 7439-89-6

Appearance:	Grayish black, powder
Particle Size:	approx. 6 μm
Bulk specific gravity:	3~4 g/mL
Solubility:	Soluble in dil.HCl, dil.H_2SO_4, dil.HNO_3 with producing hydrogen gas.

[References]

(1) Lee, K. C., *et al*.: "Requirement for accessory cells in the antibody response to T cell-independent antigens in vitro", *Eur. J. Immunol.*, **6**, 63 (1976)
(2) Thierfelder, S.: "A METHOD FOR THE ISOLATION OF HUMAN LYMPHOCYTES", *Vox Sang*, **9**, 447-54 (1964)

for T-cell Separation

Nylon Fiber

Wako Cat. No. 146-04231 (5 × 2 g); 142-04233 (100 g) <for T-cell Separation>
Keep at RT

Appearance: White~slightly pale yellow, fibrous
Divalent cations (as Ca^{2+}): 10+ μg/g
Solubility: Soluble in dil.HCl, dil.H_2SO_4, dil.HNO_3 with producing hydrogen gas.

A simple method for the preparation of highly enriched, unselected and unaltered populations of T cells has been described by Julius. By packing nylon wool fiber into columns, efficient isolation of T cells from antisera without B cell contamination is possible. Wako has improved upon the same method, offering nylon wool fiber which is free of all toxic substances, in addition, pre-washed, pre-packaged nylon wool fiber comes ready to use in sterilized 10cc-columns, saving hours of preparation. Prepackaged columns also eliminate variability in column packing, insuring consistent cell elution rates.

Nylon Fiber Columns

Nylon Fiber Column T

Wako Cat. No. 147-06721 (10 syringes × 0.5 g) <for mouse T-cell Separation>
Keep at RT

Nylon Fiber Column T (L-Type)

Wako Cat. No. 143-07041 (10 syringes × 1 g) <for human, rabbit and rat T-cell Separation>
Keep at RT

We offer two sizes of Nylon Fiber Column T; Nylon Fiber Column T is for separation of T-cells of mouse lymphocytes and the L-type is for that of a large amount of suspended solution, especially rat splenic lymphocytes, and peripheral blood lymphocytes of rabbit and human.

[Features]

1. Simple procedure.
2. High reproducibility.
3. Sterilized.
4. Using a high quality nylon fiber.
Cell Recovery:
13 ~ 25% (mouse) when Nylon Fiber Column T was used.
25 ~ 35% (rat), 20 - 30% (human) and 25 ~ 30% (rabbit) when Nylon Fiber Column T (L-Type) was used.
B-cell contamination: **Less than 15%**

[Reference]

Julius, M.G., E. Simpson, and R.A. Herzenberg: "A rapid method for the isolation of functional thymus-derived murine lymphocytes", *Eur. J. Immun*., **3**, 645-649 (1973).

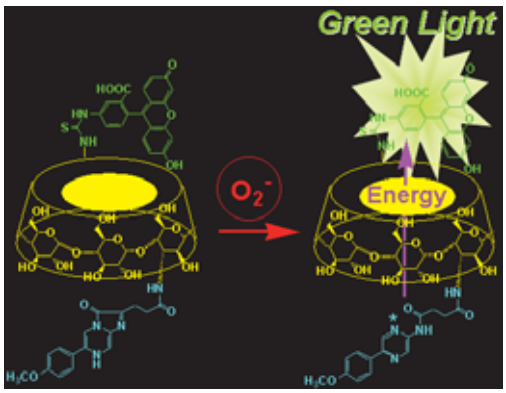
Green Chemiluminescent probe for superoxide anions

Green Chemiluminescent CD

Wako Catalog No. 075-05111 (1 mg)
Keep at -20℃

Green Chemiluminescent CD is a highly sensitive chemiluminescence probe, which was developed by Dr. Teranishi of Mie University, Japan. This probe reacts with superoxide anion and produces luminescence at long wavelengths. Therefore the luminescence remains nearly unaffected by biomaterials. Additionally, this probe is more sensitive than other probes which produce luminescence at long wavelengths.

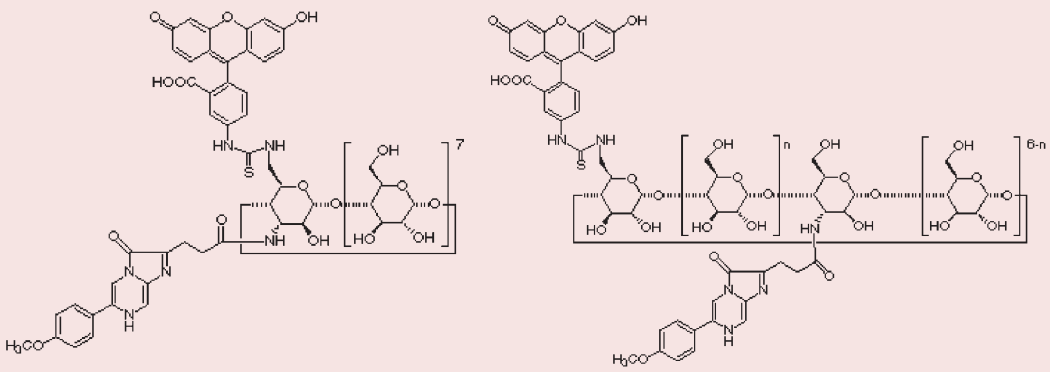
<Pathway of superoxide-induced chemiluminescence>



[Features]
1. High luminescence intensity
2. Luminescence at long wavelengths (530 nm)

[Solubility]
Soluble to hot methanol : H_2O (1:1) containing 0.1% TFA.

[Reference]
Teranishi, K. and Nishiguchi, T. : *Anal. Biochem.*, **325**, 185 (2004).



(1) (2) n=0~6
This product is mixture of 8 kinds of position isomers consisting of (1) and (2)

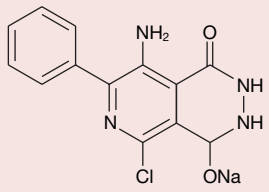
L-012 Sodium Salt, 98.0+% (HPLC)

[8-Amino-5-chloro-7-phenylpyrido [3,4-d] pyridazine-1,4-(2H,3H) dione sodium salt]

Wako Catalog No. 120-04891 (100mg)
Keep at -20℃, Solid

L-012, which is a highly sensitive chemiluminescent (CHL) probe, is more active than luminol. L-012 reacts with various types of reactive oxygen species generated by activated neutrophils in human blood and oral cavity, and from peritoneal cavity of the rat. This product can be applied to any other EIA that uses horseradish peroxidase to improve sensitivity.

[References]
1) "Improved Enzyme Immunoassay for Human Basic Fibroblast Growth Factor Using A New Enhanced Chemiluminescence System", Ii, M., *et al.*, *Biochem. Biophys. Res. Comm.*, **193** (2), 540-545 (1993)
2) "A New Sensitive Chemiluminescence Probe, L-012, for Measuring The Production of Superoxide Anion by Cells", Nishinaka, Y., *et al.*, *Biochem. Biophys. Res. Comm.*, **193** (2), 554-559 (1993)
3) "Analysis of Reactive Oxygen Species Generated by Neutrophils Using a Chemiluminescence Probe L-012", Imada, I., *et al.*, *Analytical Biochemistry*, **271**, 53-58 (1999)



$C_{13}H_9ClN_4NaO_2$=311.68

	Cell Permeability	Description (Grade) Physical data, etc.	Wako Cat. No. (Pkg. Size)	Note
Thiol/Selenol selective probe	×	**BES-Thio** (for Cellbiology) $C_{28}H_{18}N_2O_{11}S$ = 590.51 <Fluorescence> λex: 495 nm; λem: 530 nm	025-15481 (1 mg)	Keep at RT ☞ See the page #15.
H_2O_2 Probes	○ <Detection of cell-derived H_2O_2>	**BES-H_2O_2-Ac** *1, 94.0+% (HPLC) (for Cellbiology) [3'-*O*-Acetyl-6'-*O*-pentafluorobenzenesulfonyl-2'-7'-difluorofluorescein] $C_{28}H_{11}F_7O_8S$ = 640.44 Solubility: Soluble in DMSO, DMF and acetonitrile <Fluorescence> λex: 485 nm; λem: 530 nm	029-15381 (1 mg)	Keep at RT ☞ See the page #16.
	×	**BES-H_2O_2** (cell-impermeant) (for Cellbiology) $C_{26}H_9O_7F_7S$ = 598.40 Solubility: Soluble in DMSO <Fluorescence> λex: 485 nm; λem: 530 nm	021-16201 (1 mg)	
Superoxide selective probe	○ <Detection of cell-derived superoxide>	**BES-So-AM** *2, 98.0+ % (HPLC) (for Cellbiology) $C_{31}H_{19}O_{13}NF_4S$ = 721.54 <Fluorescence> λex: 505 nm; λem: 544 nm	021-15601 (1 mg)	Keep at RT ☞ See the page #17.
	×	**BES-So** (cell-impermeant) (for Cellbiology) $C_{28}H_{15}O_{11}NF_4S$ = 649.48 Solubility: Soluble in DMSO <Fluorescence> λex: 505 nm; λem: 544 nm	028-16211 (1 mg)	

*1: Previous name for BES-H_2O_2-AC: BES-H_2O_2 ; *2: Previous name for BES-So-AM: BES-So

Fluorescent Bioprobe for Visualization of PSA in Living Cells	**DAMPAQ-22** (for Cellbiology) $C_{31}H_{32}O_4N_4S$ = 556.68 CAS No. 519183-48-3 <Fluorescence> λex: 320 nm; λem: 510 nm	049-30761 (2 mg)	Keep at RT ☞ See the page #32.
Mg^{2+}-Selective Fluoroionophore	**KMG-20-AM** (for Cellbiology) $C_{19}H_{19}NO_6$ = 357.36 <Fluorescence> λex: 440 nm; λem: 500~530 nm	110-00711 (1 mg) 116-00713 (5 mg)	Keep at -20℃ ☞ See the page #18.

Thiol / Selenol selective fluorescent probe

BES-Thio

Several chemiluminescent and fluorescent reagents are known as a thiol group detection reagent, and used for detection of thiol groups or measurement of cholinesterase activity.

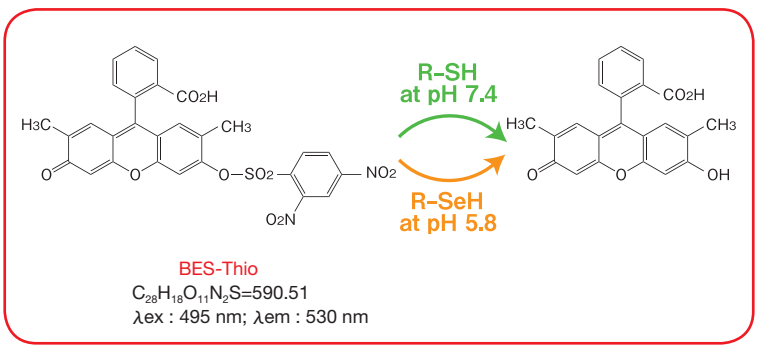
Most of these reagents have a low hydrophilicity, so separate reaction steps, enzyme and detection reactions, are required.

BES-Thio has a high hydrophilicity and can be used in aqueous solution. This feature makes it easy to measure the enzyme activity such as cholinesterase using acetylthiocholine or butyrylthiocholine as a substrate.

Furthermore, by pH adjustment, BES-Thio can also detect selenol groups, which sulfur (S) in thiol group is substituted by selenium (Se), and can be used for selenoprotein detection reagent.

[Features]

1. **High hydrophilicity**
2. **Respond to thiol at pH 7.4**
3. **Respond to selenol at pH 5.8**



BES-Thio
$C_{28}H_{18}O_{11}N_2S$=590.51
λex : 495 nm; λem : 530 nm

[References]

1) Maeda, H., Matsuno, H., Ushida, M., Katayama, K., Saeki, K. and Itoh, N.: *Angew. Chem. Int. Ed.*, **44**, 2922 (2005).
2) Maeda, H., Katayama, K., Matsuno, H. and Uno, T.: *Angew . Chem. Int. Ed.*, **45**, 1810 (2006).
3) Maeda, H: *Wako Jun-yaku Jiho*, **73** (3), 2 (2005) (written in Japanese).

Highly Selective Fluorescent Probe for Hydrogen Peroxide

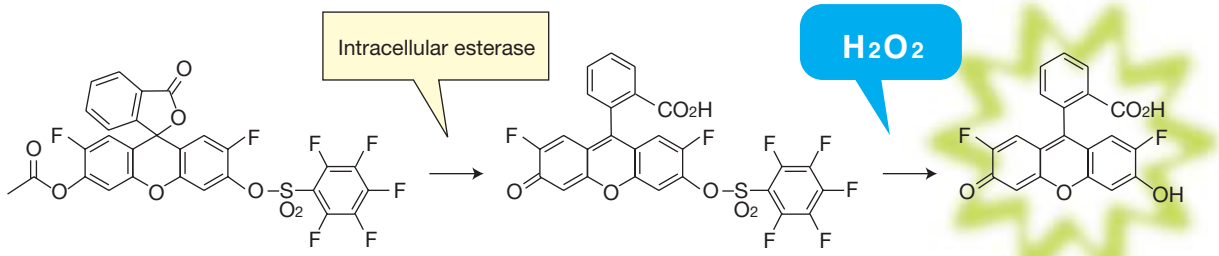
BES-H_2O_2-Ac (cell-permeant), BES-H_2O_2 (cell-impermeant)

Reactive oxygen species (ROS) such as superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO\cdot$) are important mediators of pathological processes in various diseases. 2',7'-Dichlorofluorescein (DCFH) and its diacetyl derivative have been widely used as fluorescent probes for measuring cell-derived H_2O_2, but these compounds suffer from the major drawback that they are poorly selective toward H_2O_2.

Wako has launched two kinds of BES-H_2O_2, which are probes for cell-derived H_2O_2 and cell-impermeat H_2O_2 with high selectively. BES-H_2O_2-Ac is applicable to clarifying cell response as well as dynamic function of H_2O_2 with diseases.

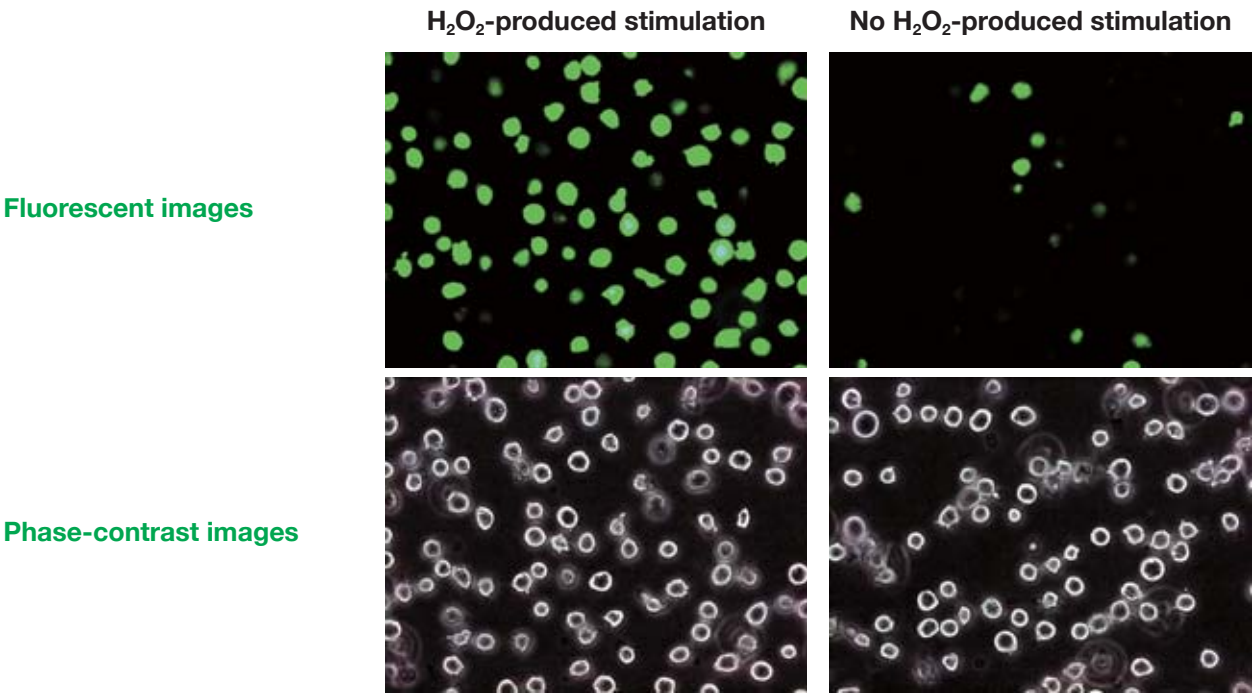
[Features]

1. Highly selectivity toward H_2O_2
2. Applicable to Molecular Imaging



BES-H_2O_2-Ac (Cell-permeant)
$C_{28}H_{11}O_8F_7S$ = 640.44
Cell-derived H_2O_2 Detectable; Permeable to cell membrane
λex : 485 nm; λem : 530 nm

BES-H_2O_2 (Cell-impermeant)
$C_{26}H_9O_7F_7S$ = 598.40
λex : 485 nm; λem : 530 nm



Fluorescent images of Jurkat T cells with BES- H_2O_2 -Ac and the same-field phase-contrast images
Jurkat T cells were cultured in a medium with 50μM BES-H_2O_2-Ac for 1 hour. Then, one group of them was cultured in a medium with 5mM butyric acid (H_2O_2-produced stimulation), whereas the other in a medium without butyric acid (No H_2O_2-produced stimulation), each for 1 hour. (*Courtesy: Professor Hatsuo Maeda, PhD., School of Pharmacy, Hyogo Univ. of Health Sciences*)

[References]

1) Maeda, H., Fukuyasu, Y., Yoshida, S., Fukuda, M. Saeki, K.. Matsuno, H., Yamauchi, Y., Yoshida, K., Hirata, K. and Miyamoto, K. : *Angew. Chem. Int. Ed.*, **43**, 2389 (2004).
2) Maeda, H., Matsuura, S., Nishida, M., Senba, T.,Yamauchi, Y. and Ohmori, H. : *Chem. Pharm. Bull.*, **49**, 294 (2001).

Superoxide selective fluorescent probe
BES-So-AM (cell-permeant), BES-So (cell-impermeant)

Since superoxide ($O_2^{-\cdot}$) is a reactive oxygen having weak cytotoxicity, it is getting attention as a molecule positioned on the uppermost stream side of various reactive oxygen species.

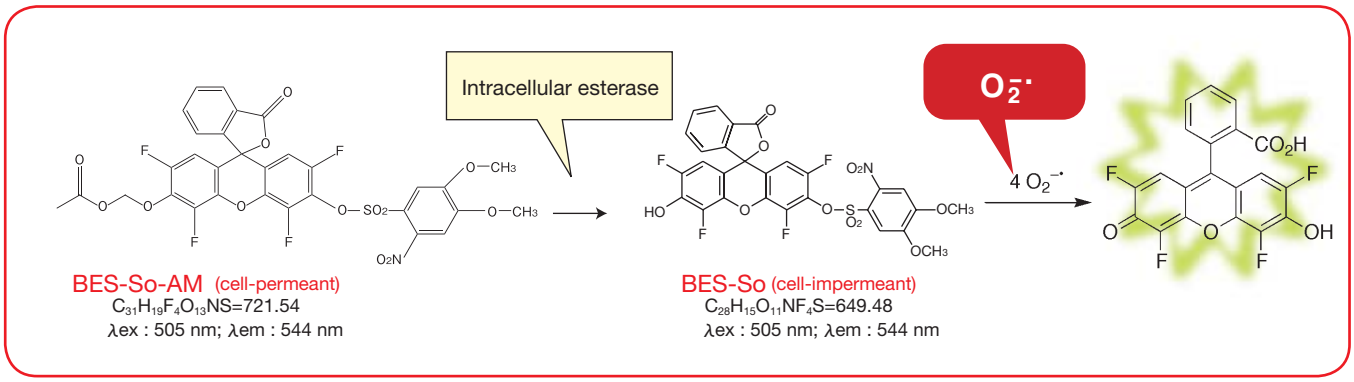
$O_2^{-\cdot}$ is detected based on different chemiluminescence and fluorescence methods. Among these detection methods, hydroethidine is commonly used but existing probes including hydroethidine are pointed out to have low selectivity for $O_2^{-\cdot}$. BES-So shows a fluorescence by non-redox-reaction dependent mechanism, and indicates high selectivity for $O_2^{-\cdot}$.

[Features of BES-So-AM]

After cellular uptake, deacetoxymethyl compound by the action of cellular esterase responds to $O_2^{-\cdot}$.

[References]

1) Maeda, H., Yamamoto, K., Nomura, Y., Kohno, I., Hafsi, L., Ueda, N., Yoshida, S., Fukuda, M., Fukuyasu, Y., Yamauchi, Y. and Itoh, N.: *J. Am. Chem. Soc.*, **127**, 68 (2005).
2) Maeda, H. *et al.*: *Chem. Eur. J.*, **13**, 1946 (2007)

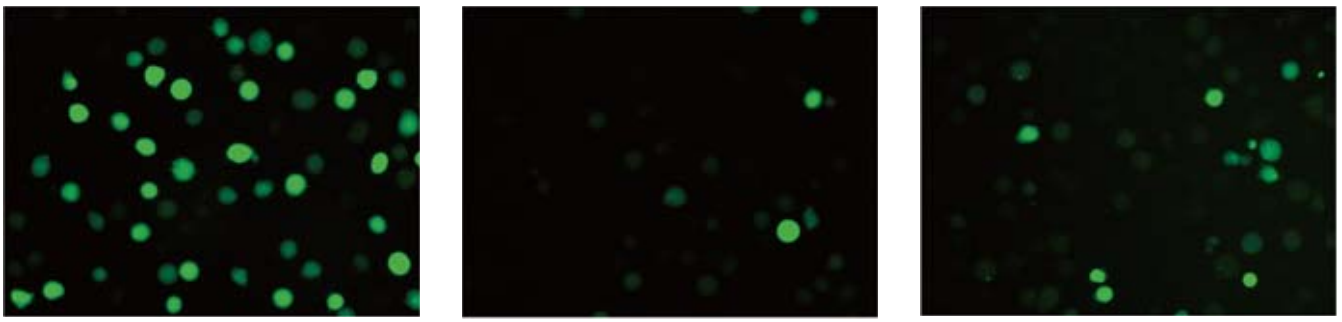


BES-So-AM (cell-permeant)
$C_{31}H_{19}F_4O_{13}NS$=721.54
λex : 505 nm; λem : 544 nm

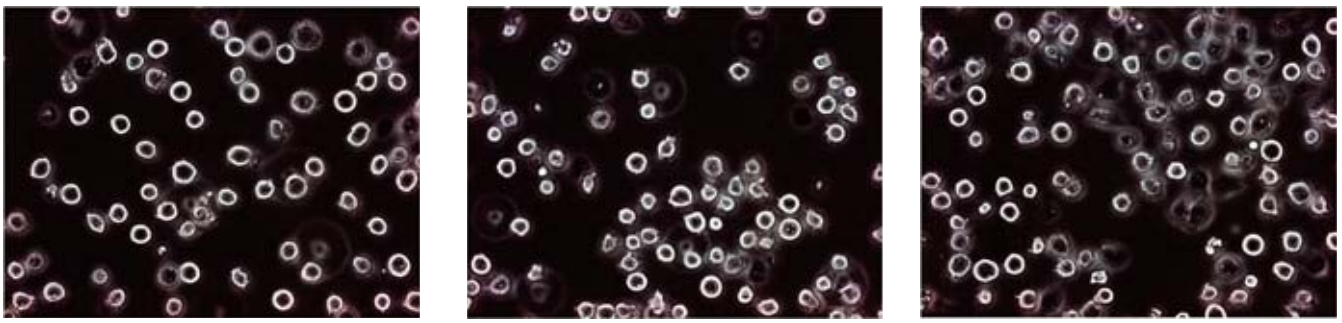
BES-So (cell-impermeant)
$C_{28}H_{15}O_{11}NF_4S$=649.48
λex : 505 nm; λem : 544 nm

$O_2^{-\cdot}$-produced stimulation (①)
No $O_2^{-\cdot}$-produced stimulation (②)
$O_2^{-\cdot}$-produced stimulation with $O_2^{-\cdot}$-scavenger (③)

Fluorescent images



Phase-contrast images



Fluorescent images of Jurkat T cells with BES-So-AM and the same-field phase-contrast images

Jurkat T cells were cultured in a medium with 33μM BES-So-AM at 37℃ for 1 hour. Then, one group (①) was cultured in the medium with 4 mM butyric acid ($O_2^{-\cdot}$-produced stimulation), whereas the other group (②) in a medium without butyric acid (no $O_2^{-\cdot}$-produced stimulation) at 37℃ for 1 hour. A group (③) was cultured in a medium with 33μM BES-So-AM and Tiron (super-oxide scavenger), and then 5 mM butyric acid was added in the medium.

(Courtesy: Professor Hatsuo Maeda, PhD, School of Pharmacy, Hyogo University of Health Sciences)

Fluorescent Bioprobe for Visualization of Puromycin-Sensitive Aminopeptidase (PSA) in Living Cells

DAMPAQ-22

Wako Catalog No. 049-30761 (2 mg) <for Cellbiology>
Keep at RT

☞ See the page #32.

Mg^{2+}-selective Fluoroionophore

KMG-20-AM

Wako Catalog No. 110-00711 1 mg
Wako Catalog No. 116-00713 5 mg
Keep at -20℃

Dynamic distribution of Mg^{2+} in living cells can be done due to selective recognition of Mg^{2+} by KMG-20-AM. KMG-20-AM is much less reactive to Ca^{2+} than Mg^{2+}.
KMG-20-AM enables accurate measurement of Mg^{2+} because it has very much low affinity to Ca^{2+} compared to Mg^{2+}.
Appearance: Brown, powder
Assay (HPLC): 95+ %

[Features]

1. **Mg^{2+}-imaging without interference of Ca^{2+}**
2. **Precise observation of Mg^{2+} distribution by Fluorescent Microscopy**
3. **Direct observation of Mg^{2+} ion dynamics in living cells**

Fluorescent imaging

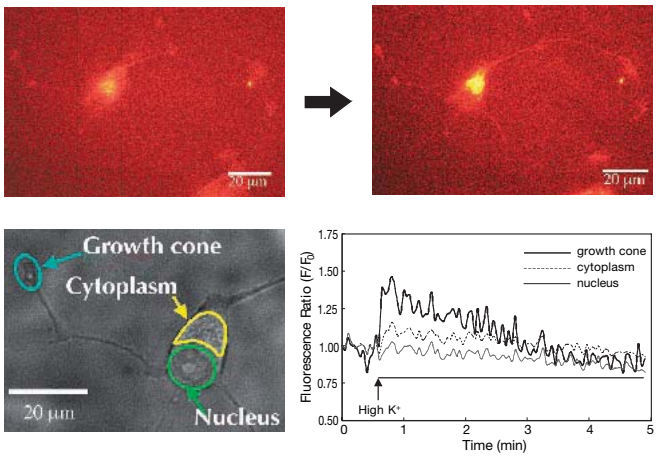


Figure : Dynamics of Mg^{2+} probe (KMG-20-AM) in neuron by addition of K^+

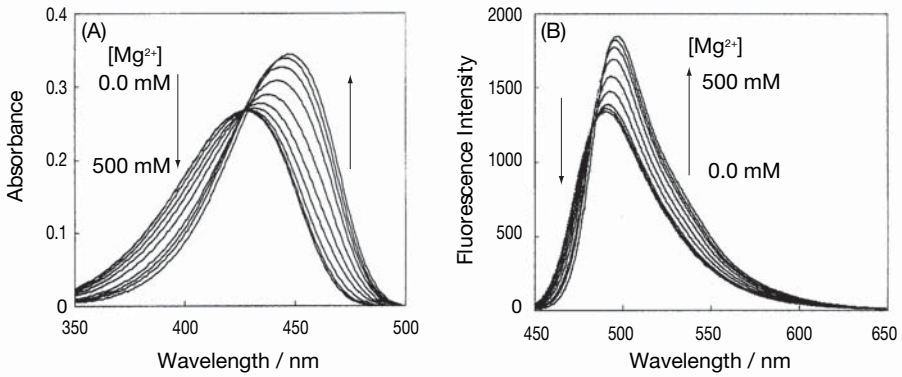
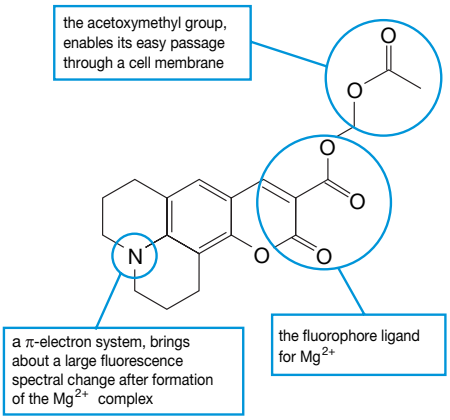


Figure : Absorption spectra (A) and fluorescence spectra (B) of 10.0 μM KMG-20-AM before and after the addition of $MgCl_2$ at 37℃ in 10.0 mM HEPES, 120.0 mM KCl, 20.0 mM NaCl (pH 7.2). $[MgCl_2]$=0, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 mM. Excitation at 445 nm for the fluorescence measurements.



KMG-20-AM
$C_{19}H_{19}NO_6$ = 357.36
λex : 440 nm; λem : 500 ~ 530 nm

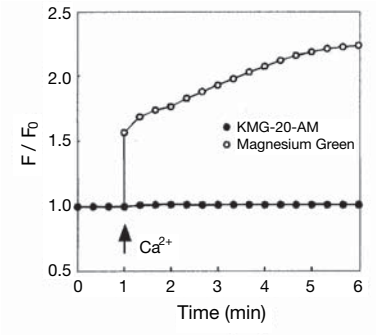


Figure : Responses of fluorescence intensity of KMG-20-AM and Magnesium Green for Ca^{2+}. Arrow indicates the timing of 10 μM $CaCl_2$ addition ($[Ca^{2+}]$ increased from 140 to 850 nm).

[References]

1) Nagashima, H., Tohda, K., Matsunari, Y.,Tsunakwa, Y., Watanabe, K., Inoue, H. andSuzuki, K.: *Anal. Lett.*, **23**, 1993(1990).
2) Suzuki, K., Watanabe, K., Matsumoto, Y.,Kobayashi, M., Sato, S., Siswanta, D. andHisamoto, H.: *Anal. Chem.*, **67**, 324(1995).
3) Suzuki, Y., Saito, N., Komatsu, H., Citterio,D., Kitamura, Y., Kubota, T., Oka, K. andSuzuki, K.: *Anal. Sci.*, **17**, i1451(2001).
4) Suzuki, Y., Komatsu, H., Ikeda, T., Saito, N., Araki, S., Citterio, D., Hisamoto, H., Kitamura,Y., Kubota, T., Nakagawa, J., Oka, K. and Suzuki, K.: *Anal. Chem.*, **74**, 1423(2002).
5) Haugland, R. P.: "Handbook of FluorescentProbes and Research Products, 7thed. "*,Molecular Probes Inc.*
6) Kubota, T., Tokuno, K., Nakagawa, J., Kitamura, Y., Ogawa, H., Suzuki, Y., Suzuki,K. and Oka, K.: *Biochem. Biophys. Res.Commun.*, **303**, 332(2003).

microRNA " Specific" Purification Kit

microRNA Isolation Kit, Human Ago2

Wako Catalog No. 292-66701 (10 Reactions) <for Genetic Research>

microRNA Isolation Kit, Human Ago2 is patent pending. (11, 30, 2007)

microRNA Isolation Kit, Mouse Ago2

Wako Catalog No. 292-67301 (10 Reactions) <for Genetic Research>

microRNA Isolation Kit, Mouse Ago2 is patent pending. (11, 30, 2007)

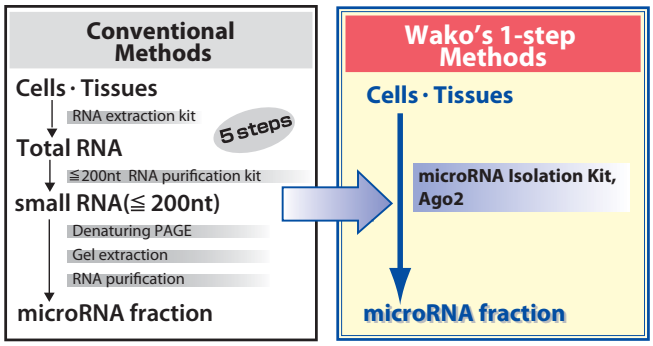
microRNA Isolation Kit, Ago2 can prepare high purity fractions of microRNA, which are bound with Argonaute2 (Ago2) protein, based on immunoprecipitation method by using a high affinity monoclonal antibody against Ago2.
The purified microRNA fraction will contain very little contaminated degradation fragments of rRNA and tRNA.
These kits will highly improve the microRNA cloning efficiency compared with conventional microRNA purification method.

[Features]

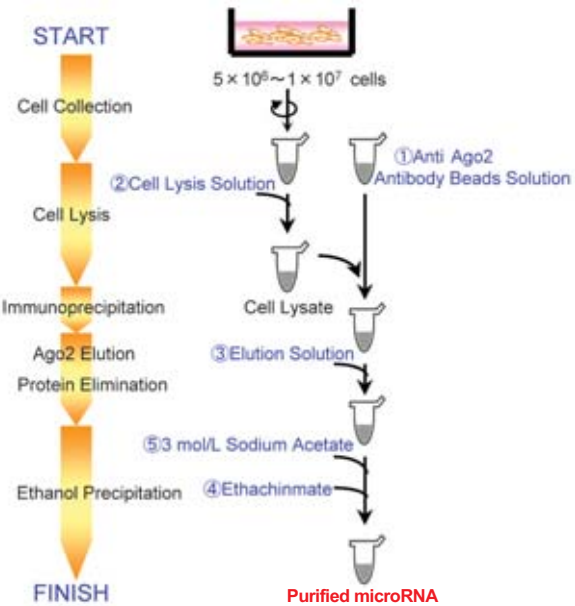
1. High purification performance of microRNA
2. Ago2 Specific
3. Little contamination of other RNAs
4. High efficiency of microRNA cloning

[Comparison with conventional method]

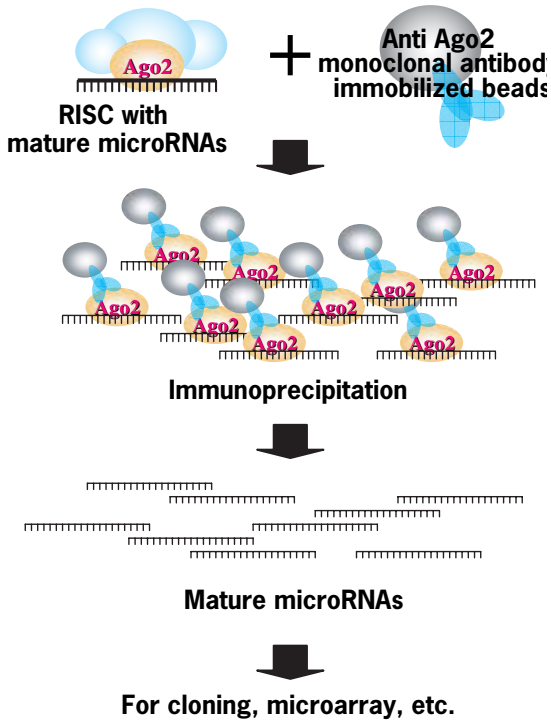
	Conventional method	Wako's 1-step Method
Total RNA extraction	necessary	unnecessary
small RNA (≦ 200nt) purification		
Denaturating PAGE		
Gel extraction from denaturating PAGE		
Gel purification from denaturating PAGE		



[Outline of procedure]



[Simple procedure]



[Cloning of purified microRNA from HeLa cells]

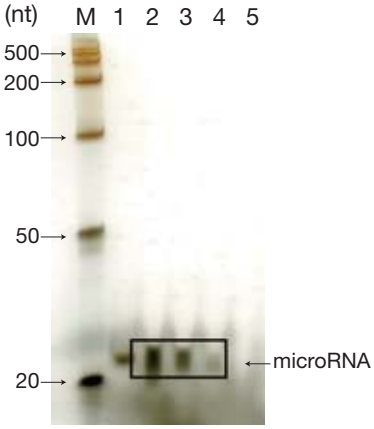
High efficiency of microRNA cloning by using this kit followed by using microRNA Cloning Kit *Wako*.

[Kit Contents (10 reactions)]

1) Anti Ago2 Antibody Beads Solution	500µL × 1 vial
2) Cell Lysis Solution	50mL × 1 vial
3) Elution Solution	500µL × 1 vial
4) Ethachinmate	30µL × 1 vial
5) 3 mol/L Sodium Acetate	400µL × 1 vial

microRNA Isolation Kit, Human Ago2

Wako Catalog No. 292-66701 (10 Reactions)



Lane M : Molecular weight makar
Lane 1 : Single strand RNA (22nt) 1ng
Lane 2 : HeLa
Lane 3 : HepG2 (Human)
Lane 4 : HEK293
Lane 5 : P388D1 (Mouse)

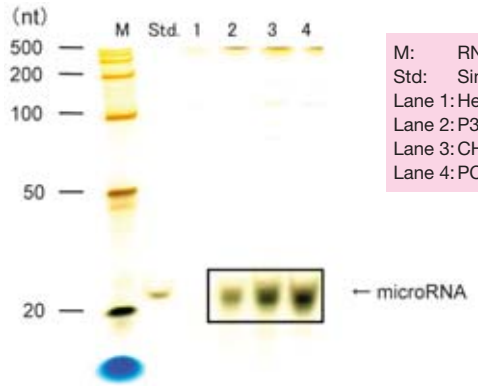
Figure. 1 Purification of microRNA fractions by using microRNA Isolation Kit, Human Ago2. The purified microRNA fractions from HeLa cells were specifically detected by Urea-PAGE. Cell number is approximately 5×10^6.

microRNA Isolation Kit, Mouse Ago2

Wako Catalog No. 292-67301 (10 Reactions)

for purification of rodents microRNA

[Purification of microRNA fractions from several rodent cell lines]



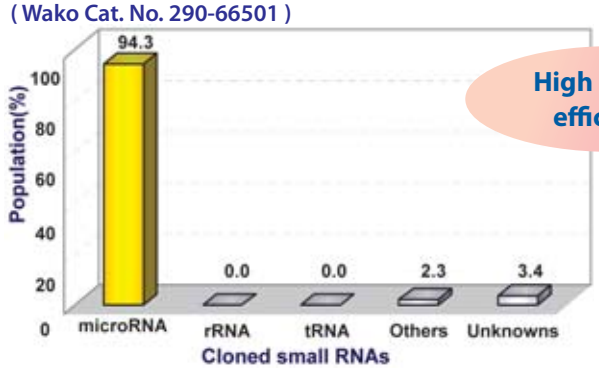
M: RNA Molecular weight makar
Std: Single strand RNA(22nt) 1ng
Lane 1: HeLa (Human) (Negative control)
Lane 2: P388D1 (Mouse)
Lane 3: CHO-K1 (Chinese Hamster)
Lane 4: PC-12 (Rat)

Figure. 2 Urea-PAGE pattern of purified RNA by using microRNA Isolation Kit, Mouse Ago2 (Wako catalog #292-67301). The purified microRNA fractions from cultured rodent cell lines(P388D1, CHO-K1, PC-12) were detected by silver stain. Cell number of each cell line is approximately 5×10^6. The applied volume per lane is half of isolated sample by this kit.

[Cloning of purified microRNA from P388D1 cells]

High efficiency of microRNA cloning by the combination use of microRNA Cloning Kit *Wako*

(Wako Cat. No. 290-66501)



High cloning efficiency

Figure 3 : Cloning efficiency of microRNA from P388D1 cell lysate. The presence ratio of microRNA was more than 90%. Others indicated cDNAs which were listed in miRBase of other organism species. Unknowns indicated cDNAs which were found in genome sequence, but not listed in miRBase. The contents of cloned microRNA are indicated on Table 1.

Table 1. The contents of cloned microRNA.

microRNA	The number of clone
mmu-miR-92a	40
mmu-miR-23a	21
mmu-miR-25	5
mmu-miR-315	2
mmu-miR-31	2
mmu-miR-23b	2
mmu-miR-22	2
mmu-miR-21	2
mmu-let-7d	2
mmu-miR-652	1
mmu-mir-423	1
mmu-miR-132	1
mmu-miR-18a	1
Total	82

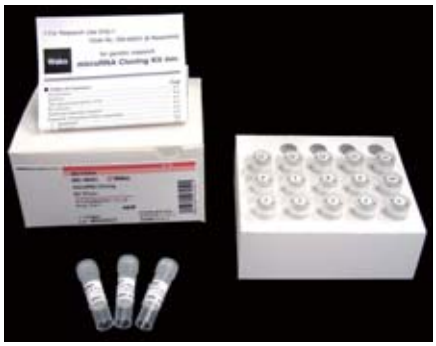
[Procedure of microRNA cloning]

1) The microRNA fraction was prepared by microRNA Isolation Kit, Mouse Ago2.
2) The cDNA encoding microRNA was synthesized by microRNA Cloning Kit *Wako* and inserted it into T-vector.
3) The 96 transformants of *E. coli* were randomly selected from selection LB agar medium.
4) Inserted cDNA sequences were determined by DNA sequencer and colleted sequences by using data base of Sanger miRBase.

"High Efficiency" microRNA Cloning Kit

microRNA Cloning Kit *Wako*

Wako Catalog No. 290-66501 (8 Reactions) <for Genetic Research>



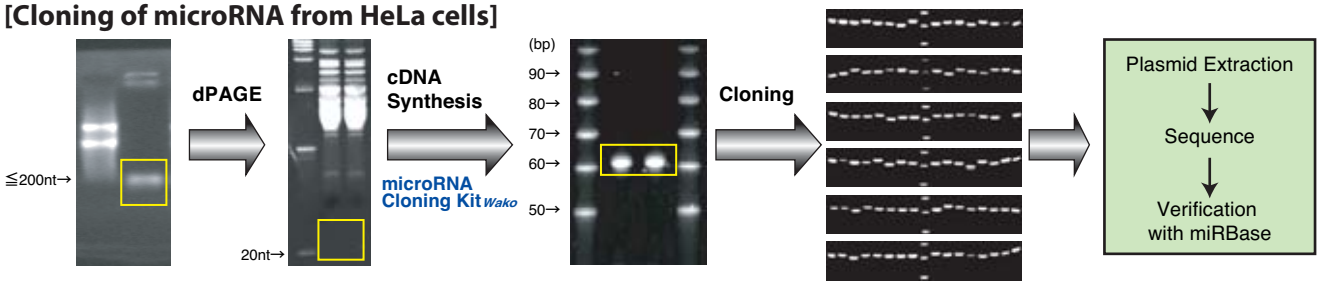
microRNA Cloning Kit Wako is patent pending. (1, 10, 2007)

The microRNA Cloning Kit *Wako* can prepare the cDNA encoding microRNA. The cloning procedure will be completed within 1.5 days after preparation of microRNA fraction.
This kit is supported by shrimp alkaline phosphatase (SAP), thermostable single strand DNA ligase (, which is selling separately: Wako Cat. #292-65101 (500 units); #298-65103 (200 units)), and original modified adaptors.
The cloning efficiency using this kit is improved higher than that of the conventional methods, which used bacterial alkaline phosphatase and T4 RNA ligase.

[Features]

1. High cloning efficiency
2. Cloning of secondary structured microRNA
3. High reproducibility of microRNA Cloning

[Cloning of microRNA from HeLa cells]



[Procedure of microRNA cloning]

1) Preparation of total RNA from HeLa cells (1×10^7 cells) by ISOGEN (Nippon Gene #315-02504, 10mL).
2) Preparation of small RNA fraction, less than 200nt, from total RNA by microRNA Isolation Kit (Bio Chain Institute Inc. catalog #KS341025).
3) Separation of microRNA fraction by denaturing PAGE.
4) Collection of the gels of 20~23nt region after electrophoresis.
5) Cloning by using microRNA Cloning Kit *Wako* (Wako catalog #290-66501).
6) Construction of the plasmids harboring cDNA encoding microRNA and transformation of *E. coli*.
7) Random selection of the 96 transformed *E. coli* from selection LB agar medium.
8) Determination and verification of the cDNA sequences by using Sanger miRBase.

Table 1. Contents of cloned microRNA species

microRNA species	The number of clone
hsa-miR-23a	38
hsa-miR-92a	16
hsa-miR-22	5
hsa-miR-25	5
hsa-miR-23b	3
hsa-miR-19b	1
hsa-miR-21	1
hsa-miR-210	1
Total	70

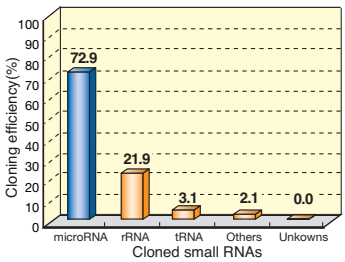
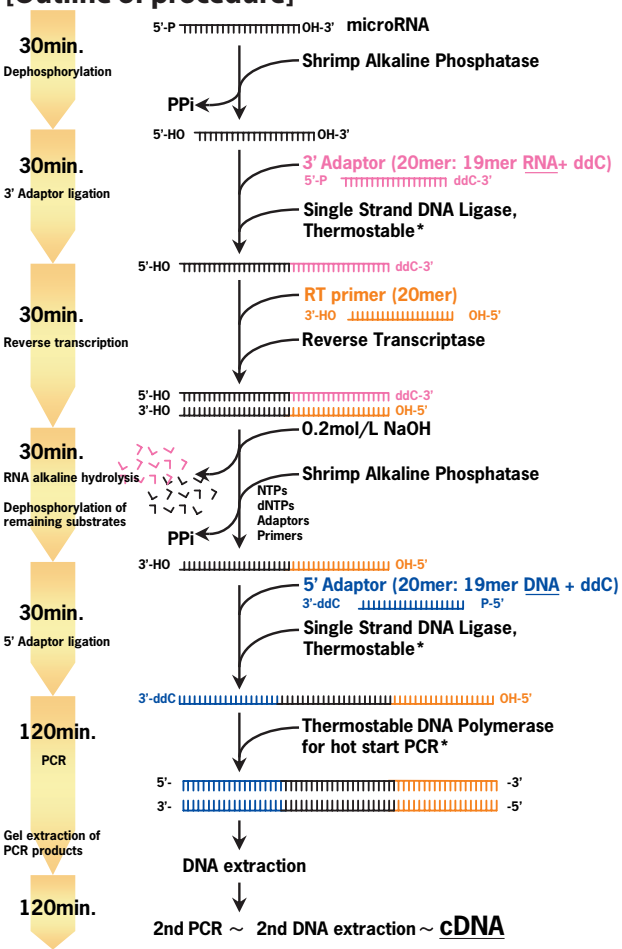


Figure. 1 Cloning efficiency of microRNA from HeLa cell lysate.
The cloning efficiency of microRNA was more than 70%. Others indicate that isolated cDNA sequences were not matched miRBase. Unknowns indicate that isolated cDNA sequences were not matched human genome sequence. The contents of cloned microRNA species are indicated on Table 1.

[Outline of procedure]



for Immunoprecipitation, Western Blot, Immunocytochemistry

Anti Human Ago2, Monoclonal Antibody (Clone No. 4G8)

Wako Cat. #011-22033 (50µL); 015-22031 (100µL)

Keep at 2~10℃ <for Immunochemistry>

Argonaute2(Ago2) was isolated as one of the main components of RISC (RNA-induced silencing complex). Ago2 captures siRNA and microRNA which are working as a guide molecule for interaction with target mRNAs in RNAi pathway. In this pathway, Ago2 catalyzes the nicking of target mRNAs and binding between RISC and target mRNAs. This monoclonal antibody is not only used for western blot and immunocytochemistry (ICC), but also immunoprecipitation (IP) of hAgo2.

[Features]

1. For IP, ICC, Western Blot
2. Specific reactivity with human Ago2 protein
3. For purification of RNA captured by RISC

Concentration (protein): Indicated on the label.
Formulation: 0.09% Sodium Azide, 10% Glycerol with 1 × TBS, pH7.4.
Subclass: IgG1
Antigen: Recombinant human Ago2
Storage: 2~10°C in the dark. Avoid the freeze and thaw.

Application	Working Dilution
Western Blot	1 : 100 - 1 : 200
Immunoprecipitation	1 : 50
Immunocytochemistry	1 : 20 - 1 : 50

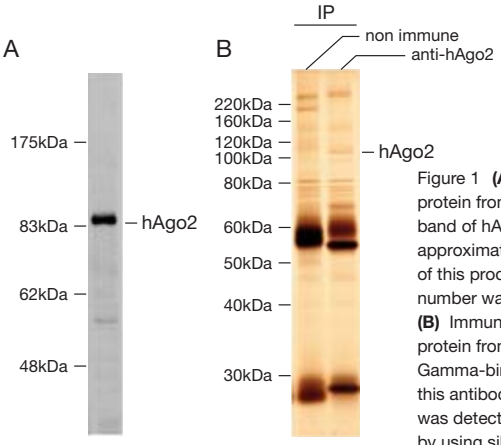
Organisms	Human	Mouse	Hamster	Rat
Cell	HeLa	P388D1	CHO	SCC-131
Western Blot	O	X	X	X
Immunoprecipitation	O	X	X	X
Immunocytochemistry	O	X	X	X
microRNA Purification	O	X	X	X

[References]

1) Qi, H. H., *et al.*: *Nature*, **455** (7211), 421 (2008)
2) Miyoshi, K., *et al.*: *Methods Mol. Biol.*, **442**, 29 (2008)
3) Azuma-Mukai A., *et al.*: *Proc. Natl. Acad. Sci. U. S. A.*, **105**(23), 7964 (2008)

APPLICATION DATA

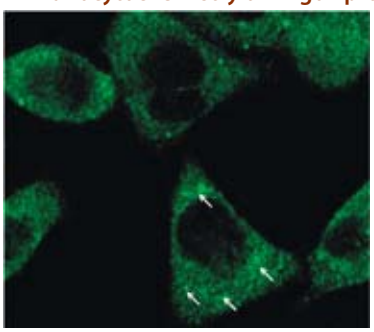
Immunoprecipitation of hAgo2 protein from HeLa cell line



(Provided by Haruhiko Siomi, PhD and Mikiko Siomi, PhD)

Figure 1 **(A)** Western blot of hAgo2 protein from HeLa cell lysate. The band of hAgo2 protein was detected in approximately 100kDa. Working dilution of this product was 1/100 dilution. Cell number was 5-10 × 10^7 cells.
(B) Immunoprecipitation of hAgo2 protein from HeLa cell lysate by using Gamma-bind beads immobilized with this antibody. The band of hAgo2 protein was detected in approximately 100kDa by using silver staining. Cell number was 5 × 10^6 cells.

Immunocytochemistry of hAgo2 protein of HeLa cell line



(Provided by Haruhiko Siomi, PhD and Mikiko Siomi, PhD)

Figure 2 Immunocytochemistry of hAgo2 protein of HeLa cell line by using 1/50 diluted this antibody. hAgo2 protein was localized in P-body, indicated by arrows, and cytoplasm.

Specificity of anti hAgo2, Monoclonal Antibody (4G8)

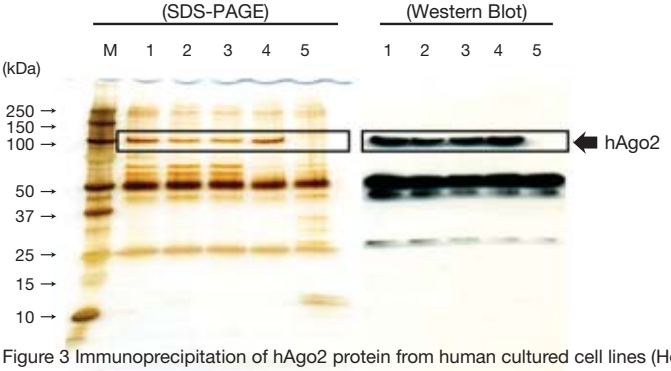
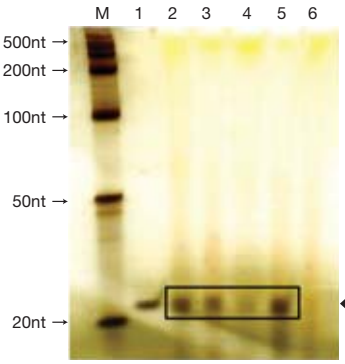


Figure 3 Immunoprecipitation of hAgo2 protein from human cultured cell lines (HeLa, HepG2, HEK293, THP-1) and mouse cultured cell line (P388D1) by using 20µL 10% Protein G slurry immobilized with 10µg this antibody. The bands of hAgo2 protein was detected in approximately 100kDa by using silver staining and western blot. Cell number was 5×10^6 cells.
<Lane M: MW marker; Lane 1: HeLa (Human); Lane 2: HepG2 (Human); Lane 3: HEK 293 (Human); Lane 4: THP-1 (Human); Lane 5: P388D1 (Mouse)>



Lane M : Molecular weight marker
Lane 1 : Single strand RNA (22nt) 1ng
Lane 2 : HeLa (Human)
Lane 3 : HepG2 (Human)
Lane 4 : HEK293 (Human)
Lane 5 : THP-1 (Human)
Lane 6 : P388D1 (Mouse)

Figure 4 Purification of microRNA fraction from immunoprecipitated hAgo2 protein. The purified microRNA fraction from human cultured cell lines (HeLa, HepG2, HEK293, THP-1) were specifically detected by Urea-PAGE. Cell number of each cell line is 5×10^6 cells. The applied volume per lane is half of 10µL of final solution prepared with an IP.

for Immunoprecipitation, Western Blot, Immunocytochemistry

Anti Mouse Ago2, Monoclonal Antibody (Clone No. 2D4)

Wako Cat. #014-22023 (50µL); 018-22021 (100µL)

Keep at 2~10℃ <for Immunochemistry>

Argonaute2 (Ago2) was isolated as one of the main components of RISC (RNA-induced silencing complex). Ago2 captures siRNA and microRNA which are working as a guide molecule for interaction with target mRNAs in RNAi pathway. In this pathway, Ago2 catalyzes the nicking of target mRNAs and binding between RISC and target mRNAs. This monoclonal antibody is not only used for western blot and immunocytochemistry (ICC), but also immunoprecipitation (IP) of mAgo2.

[Features]

1. For IP, ICC, Western Blot
2. Cross reactivity with Ago2 protein of rat & hamster
3. For purification of RNA captured by RISC

Concentration (protein): Indicated on the label.
Formulation: 0.05% Sodium Azide, 10% Glycerol with 1 × TBS, pH7.4.
Subclass: IgG1
Antigen: Synthesized peptide of N terminal mouse Ago2.
Storage: 2~10℃ in the dark. Avoid the freeze and thaw.

[Reference]

Mishima, T., *et al.*: *Reproduction*, Sep. 4, [E pub ahead of print]

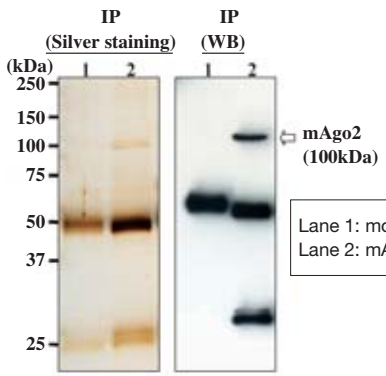
Application	Working Dilution
Western Blot	1 : 200 - 1 : 1,000
Immunoprecipitation	5~10µg / IP
Immunocytochemistry	1 : 100 - 1 : 500

Organisms	Mouse	Hamster	Rat	Human
Cell	P388D1 NIH-3T3	CHO	SCC-131	NCI-H460
Western Blot	O	O	O	X
Immunoprecipitation	O	O	O	X
Immunocytochemistry	O (NIH-3T3)	NT	NT	X
microRNA Purification	O (P388D1)	O	O	X

NT : Not Tested.

APPLICATION DATA

Immunoprecipitation of mAgo2 protein from P388D1 cell line



Lane 1: mouse IgG IP fraction
Lane 2: mAgo2 antibody(2D4) IP fraction

Figure 1. Immunoprecipitation of mAgo2 protein from P388D1 cell line by using 20µL 10% Protein G slurry immobilized with 5µg this antibody (2D4). The band of endogenous mAgo2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

Immunocytochemistry of mAgo2 protein of NIH-3T3 cell line

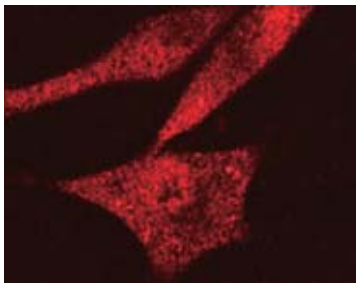
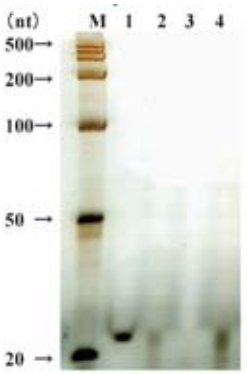


Figure 2. Immunocytochemistry of mAgo2 protein of NIH-3T3 cell line by using 1/300 diluted this antibody. mAgo2 protein was localized in cytoplasm.

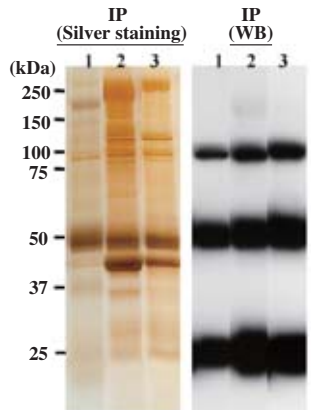
microRNA purification



Lane M : Molecular weight marker
Lane 1 : Single strand RNA (22nt) 1ng
Lane 2 : hAgo2 antibody(4G8) NCI-H460 IP fraction
Lane 3 : mouse IgG P388D1 IP fraction
Lane 4 : mAgo2 antibody(2D4) P388D1 IP fraction

Figure 3. Immunoprecipitation of mAgo2 protein from P388D1 cell line by using 20µL 10% Protein G slurry immobilized with 5µg this antibody. The purified microRNA fraction from P388D1 cell lines were specifically detected by Urea-PAGE. Cell number was 5 ×10^6 cells.

Immunoprecipitation of Ago2 from other rodent cell lines



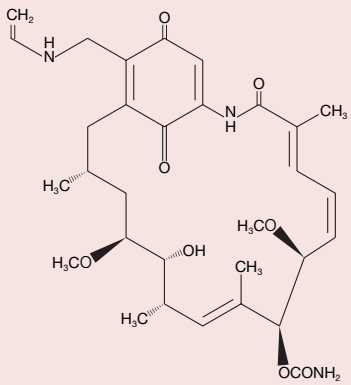
Lane 1: NIH-3T3 (Mouse) IP fraction
Lane 2: SCC-131(Rat) IP fraction
Lane 3: CHO (Hamster) IP fraction

Figure 4. Immunoprecipitation of Ago2 protein from NIH-3T3(Mouse), SCC-131(Rat) and CHO(Hamster) cell line by using 20µL 10% Protein G slurry immobilized with 5µg this antibody (2D4). The band of endogenous mAgo2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

17-AAG [17-(Allylamino)-17-desmethoxygeldanamycin; Allylaminogeldanamycin]

Wako Cat. No. 012-20101 (1 mg) <for Cellbiology>
Keep at -20℃

Potent, less toxic derivative of geldanamycin. Inhibits the essential ATPase activity of HSP90. apoptosis with antitumor activity.



$C_{31}H_{43}N_3O_8$ = 585.69, CAS No. 75747-14-7

Appearance: Red ~ dark purple, crystalline powder ~ powder
Solubility: Soluble in DMSO (10mg/mL) or methanol (10mg/mL)

[References]

1) Zhou, P, *et al.*: "ErbB2 degradation mediated by the co-chaperone protein CHIP", *J. Biol. Chem.*, **278**, 13829-37 (2003)
2) Villa, R. *et al.*: "Inhibition of telomerase activity by geldanamycin and 17-allylamino, 17-demethoxygeldanamycin in human melanoma cells", *Carcinogenesis*, **24**, 851-9 (2003)
3) Vasilevskaya, I. A., *et al.*: "Geldanamycin and its 17-allylamino-17-demethoxy analogue antagonize the action of Cisplatin in human colon adenocarcinoma cells: differential caspase activation as a basis for interaction", *Cancer Res.*, **63**, 3241-6 (2003)
4) Kamal, A., *et al.*: "A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors", *Nature*, **425**, 407-10 (2003)

ADP Ribosyltransferase C3, from *Clostridium botulinum*

Wako Cat. No. 011-14441 (10 µg) <for Biochemistry>
Keep at -20℃

Neurotoxin; Botulinum neurotoxin C_3 with no toxicity

Source: *Clostridium botulinum*
Appearance: Lyophilized
Solubility: Soluble in ethanol, methanol and acetone.
Principle: P2 fraction + [α-^{32}P]NAD $\xrightarrow{\text{ADP-ribosyltransferase C3}}$ [^{32}P]ADP-rebose-P2 fraction + Nicotinamide
Activity: Approximately 1 pmol/mg P2/μg C_3
Unit Definition: An amount of ADP-ribose required for the formation of substrate (P2 fraction) 1 mg by 1 μg of ADP-ribosyltransferase C_3

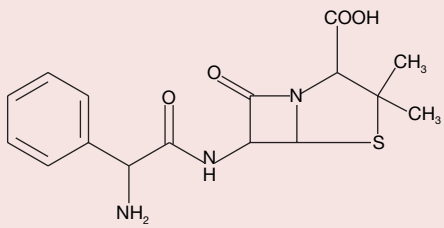
[References]

1) Ohashi, Y. and Narumiya, S.: "ADP-ribosylation of a Mr 21,000 membrane protein by type D botulinum toxin", *J. Biol. Chem.*, **262**, 1430-3 (1987)
2) Morii, M. *et al.*: "Immunochemical identification of the ADP-ribosyltransferase in botulinum C1 neurotoxin as C3 exoenzyme-like molecule", *J. Biochem.*, **107**, 769-75 (1990)
3) Narumiya, S., *et al.*: "Subcellular distribution and isoelectric heterogeneity of the substrate for ADP-ribosyl transferase from Clostridium botulinum", *Biochem. Biophys. Res. Commun.*, **150**, 1122-30 (1988)

Antibiotics/Folate Metabolism related Substances

Ampicillin

[Anhydrous] Wako Cat. No. 017-10381 (5 g); 015-10382 (25 g) <for Biochemistry>
[Standard; anhydrous] Wako Cat. No. 017-20531 (200 mg) <for HPLC>
[Sodium Salt] Wako Cat. No. 010-10371 (5 g); 016-10373 (10 g); 018-10372 (25 g) <for Biochemistry>
Keep at -20℃



(Anhydrous) $C_{16}H_{19}N_3O_4S$ = 349.40
CAS No. 69-53-4

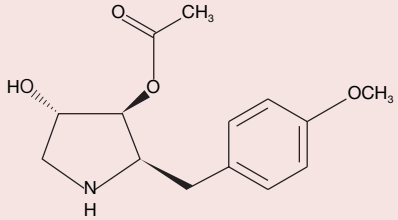
Antibiotics/Folate Metabolism related Substances
An antibiotic which is a synthetic penicillin used in studies on dysentery and urinary tract infections.
It has a broad antibacterial spectrum and is active against Gram-positive and Gram-negative bacteria.
It is also used for checking one of the properties of Ames test strain, i.e. the existence of drug resistance factor plasmid PKM101.

Appearance: [Anhydrous & the standard] White ~ slightly yellow, powder
[Na Salt] White, crystalline powder ~ powder
Assay (HPLC): [Anhydrous] 96.0+%; [Anhydrous standared] 98+%
Potency (calculated on the dehydrous basis): [Sodium Salt] 850+μg/mg
Solubility: [Anhydrous] Soluble in MeOH or water. Slightly soluble in EtOH. Practically insoluble in ether.
[Na Salt] Freely soluble in water. Sparingly soluble in EtOH.

Anisomycin, 96.0+ % (HPLC)

Wako Cat. No. 017-16861 (10 mg); 013-16863 (50 mg); 011-16864 (250 mg) <for Biochemistry>
Keep at -20℃

Antibiotic. Activator of p38 and MAP kinases. Synergistic with growth factors and phorbol esters to superinduce cFos and cJun, by acting as a potent signalling agonist. Induces apoptosis in the human monoblastoid cell line. Used in the eradication of bean mildew. Inhibits other pathogenic fungi in plants.



$C_{14}H_{19}NO_4$ = 265.3, CAS No. 22862-76-6

Source: Isolated from *Streptomyces griseolus*
Appearance: White ~ slightly yellow, crystalline powder ~ powder
Solubility: Soluble in DMSO (25mg/mL), 100% ethanol, methanol or ethyl acetate (10mg/mL)

Aristeromycin

Wako Cat. No. 015-09691 (5 mg) <for Biochemistry>
Keep at -20℃

The product is an antibiotic which inhibits the growth of plant pathogens such as Xanthomonas oryzae and Piricuraria oryzae.
Carbocyclic nucleoside antibiotic. It inhibits the synthesis of AMP in mammalian cells and S-adenosylhomocysteine hydrolase activity.

Source: *Streptomyces citricolor*
Appearance: White ~ grayish white, powder or mass
Solubility: Soluble in water and *N*, *N*-dimethylformamide

$C_{11}H_{15}N_5O_3$ = 265.27, CAS No. 19186-33-5

Bafilomycin A1, 95.0+% (HPLC)

Wako Cat. No. 023-11641 (100µg); 029-11643 (1 mg) <for Biochemistry>
Keep at -20℃

Bafilomycin A1, isolated from *Streptomyces* sp., is a macrolide antibiotic which inhibits vacuolar-type H^+-ATPases with a high degree of specificity.
Bafilomycin A1 thus serves as an ideal tool for distinguishing among the different types of ATPases that exist in eukaryotic cells. Among them,vacuolar-type ATPase (V-ATPases) are widely distributed in the central vacuolar system, consisting of endosomes, trans-Golgi network, lysosomes, and secretion granules. While V-ATPases are extremely sensitive to the antibiotic and are affected by nanomolar concentrations, F-ATPases (F1F0-type) are unaffected, and P-ATPases (E1E2-type) are only moderately affected at the same concentration.

Source: *Streptomyces griseus*
Appearance: White, Crystals ~ powder or film
Solubility: Soluble in DMSO, ethanol and ethyl acetate. Slightly soluble in water.

$C_{35}H_{58}O_9$ = 622.84, CAS No. 88899-55-2

[References]

1) Bowman, E. J., Siebers, A. and Altendorf, K.: *Proc. Natl. Acad. Sci., U.S.A.*, **85**, 7972 (1988)
2) Moriyama, Y. and Nelson, N.: *J. Biol. Chem.*, **264**, 18445 (1989)
3) Nelson, N. and Taiz, L.: *Trends Biochem. Sci.*, **14**, 113 (1989)
4) Hanada, H., Moriyama, Y., Maeda, M. and Futai, M.: *Biochem. Biophys. Res. Commun.*, **170**, 873 (1990)
5) Moriyama, Y. and Futai, M.: *J. Biol. Chem.*, **265**, 9165 (1990)
6) Moriyama, Y., Maeda, M. and Futai, M.: *J. Biochem.*, **108**, 689 (1990)
7) Sundquist, K., Lakkakorpi, P., Wallmark, B. and Vaeaenaenen, K.: *Biochem. Biophys. Res. Commun.*, **168**, 309 (1990)
8) Umata, T., Moriyama, Y., Futai, M. and Mekada, E.: *J. Biol. Chem.*, **265**, 21940 (1990)
9) Oda, K., Nishimura, Y., Ikehara, Y., Kato, K.: *Biochem. Biophys. Res. Commun.*, **178**, 369 (1991)
10) Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M. and Tashiro, Y.: *J. Biol. Chem.*, **266**, 17707 (1991)
11) Werner, G., Hagenmaier, H., Drautz, H., Baumgartner, A. and Zaehner, H.: *J. Antibiot.*, (*Tokyo*), **37**, 110 (1984)

Bleomycin Hydrochloride

Wako Cat. No. 028-07801 (10 mg) <for Biochemistry>
Keep at 2~10℃

This is an anticancer antibiotic acting on various cancers. It inhibits DNA synthesis in *Escherichia coli*, HeLa cells and Ehrlich cancer cells and, moreover, inhibits their cell divisions at a low concentration.

Appearance: White ~ pale yellow, powder or small mass
Potency: 1,400 ~ 2,000 µg/mg (calculated on the dried basis)
Solubility: Soluble in water. Slightly soluble in ethanol. Practically insoluble in ether.

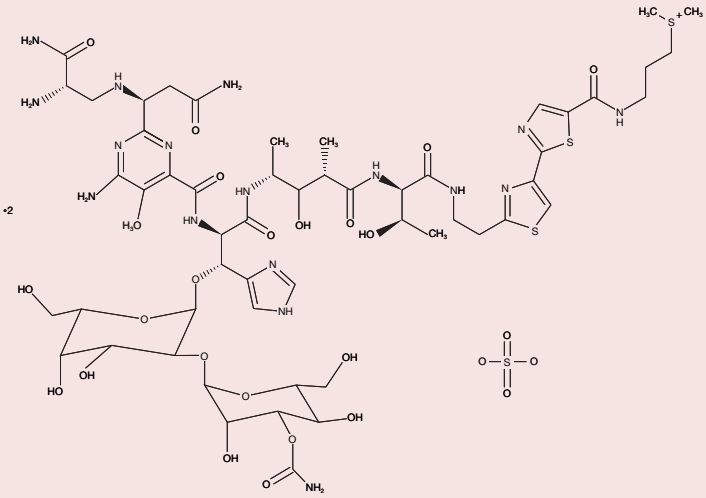
CAS No. 67763-87-5

Bleomycin Sulfate

Wako Cat. No. 027-15941 (10 mg); 023-15943 (50 mg)
< for Pharmacology Research >
Keep at 2~10℃

anticancer antibiotic

Appearance:	White ~ pale brown, Crystalline powder ~ powder
Potency:	1,500+ IU/mg (calculated on the dried basis)



CAS No. 9041-93-4

α-Bungarotoxin

Wako Cat. No. 026-07961 (1 mg) < for Biochemistry >
Keep at 2~10℃

Neurotoxin which binds irreversibly to motor endplate of acetylcholine receptors; prevents opening of nicotinic receptor-associated ion channels

Source:	*Bungarus multicinctus*
Appearance:	White, lyophilized

CAS No. 11032-79-4

[Reference]

Mebs, D. and Lee, C. Y.: *Biochem. Biophys. Res. Comm.*, **44**, 711 (1971)

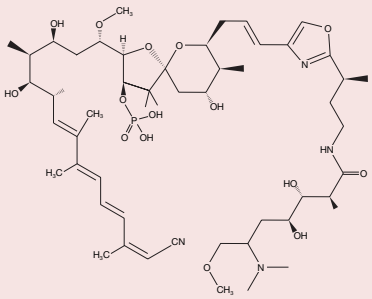
Marine Natural Product - Protein Phosphatase Inhibitor

Calyculin A, 95.0+ % (HPLC)

Wako Cat. No. 038-14453 (10 µg); 032-14451 (100µg) < for Biochemistry >
Keep at -20℃

Inhibitor of protein phosphatases types 1 and 2A; marine toxin, potent tumor promotor.

Source:	*Disodermia calyx*
Appearance:	White ~ pale yellow, Crystals ~ powder or film.
Solubility:	Soluble in methanol or ethanol. Insoluble in water.



$C_{50}H_{81}N_4O_{15}P$ = 1,009.17, CAS No. 101932-71-2

[Reference]

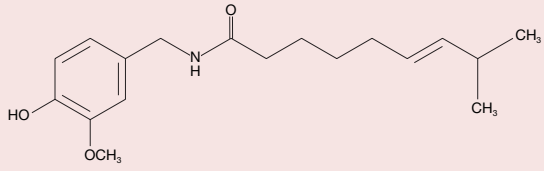
Kato, Y., Fusetani, N., Matsunaga, S., Fujita, S., Furuya, T. and Hashimoto, K.: *J. Am. Chem. Soc.*, **108**, 2780 (1986)

Capsaicin

Wako Cat. No. 039-15963 (20 mg); 033-15961 (100 mg), 90.0+ % (HPLC)
< for Biochemistry >
Wako Cat. No. 034-11351 (100 mg); 030-11353 (1 g), 60.0+ % (HPLC)
< Wako 1st Grade >
Wako Cat. No. 039-18981 (20 mg), 99.0+ % (HPLC)
< for Crude Drugs Determination >
Keep at 2~10℃

An active component contained in capsicum (CAPSICI FRUCTUS). It is a neurotoxin acting on the peptide-containing nervous system and is used as a pharmacological means to elucidate the functional roles of neuropeptides.

Appearance:	White ~ pale brown, Crystals ~ powder or mass
Solubility:	<for Biochemistry> Soluble in ethanol. Slightly soluble in water.



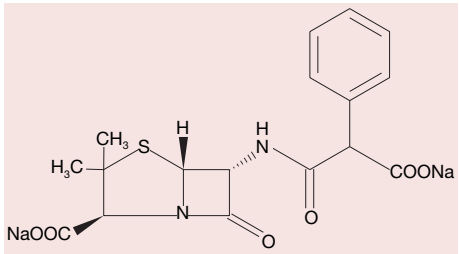
$C_{18}H_{27}NO_3$ = 305.42, CAS No. 404-86-4

Carbenicillin Sodium Salt

Wako Cat. No. 032-18954 (1 g); 038-18951 (5 g); 034-18953 (10 g) < for Cellbiology>
Wako Cat. No. 030-17671 (1 g); 036-17673 (5 g); 034-17674 (10 g) < for Biochemistry>
Keep at 2~10℃

Penicillin antibiotic acting on Gram-negative bacteria; bacterial cell-wall synthesis inhibitor

[both for Biochemistry and for Cellbiology]
Appearance: White ~ slightly greenish yellow, crystalline powder ~ powder
Potency: 770+ μg/mg (calculated on the dried basis)
Solubility: Soluble in water (0.5 g/50 mL)



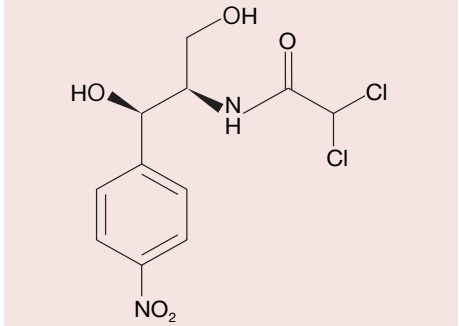
$C_{17}H_{16}N_2Na_2O_6S$= 422.36, CAS No. 4800-94-6

Chloramphenicol

Wako Cat. No. 032-19451 (5 g); 030-19452 (25 g); 038-19453 (100 g) <for Molecular Biology, *DNase and RNase tested*>
Wako Cat. No. 036-10571 (5 g); 034-10572 (25 g); 032-10573 (100 g) <for Biochemistry>
Wako Cat. No. 037-19641 (200 mg) < 98.0+ % (HPLC), Standard for HPLC>
Keep at 2~10℃

Antibiotics
The product inhibits protein synthesis in bacteria thus suppressing their proliferation (bacteriostatic effect). It exhibits a strong activity especially against Gram-negative bacilli such as Salmonellae and rickettsia such as epidemic louse-borne typhus and chigger.
This is an analogue of nitrobenzene. Although it has a wide antimicrobial spectrum, it is used only in cases where other antibiotics are ineffective or in critical conditions due to its strong adverse reactions.

Appearance: White ~ slightly yellow, crystals ~ crystalline powder
Solubility: Freely soluble in methanol and ethanol.
Slightly soluble in water and ether.



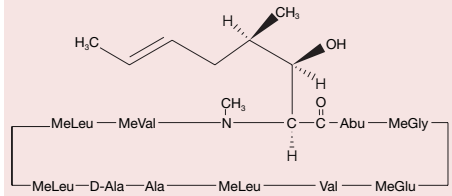
$C_{11}H_{12}Cl_2N_2O_5$= 323.13
CAS No. 56-75-7

Ciclosporin A, 97.0+ % (HPLC)

Wako Cat. No. 035-16303 (100 mg); 039-16301 (200 mg) <for Biochemistry >
Wako Cat. No. 031-18963 (50 mg); 035-18961 (200 mg) <for Cellbiology >
Keep at 2~10℃

<Pharmacologic and Physiologic Research><Autacoid>
Like hormones, autacoids exhibit strong bioactivity in small amounts, but unlike hormones they have no particular production organ, and they refer to biologically active substances expressing strong physiological effects immediately at the site of production. Chemically, they are classified into amines (such as histamine and serotonin), peptides (such as bradykinin and angiotensin) and fatty acids (such as prostaglandin).
The product is a typical immunosuppressant used for prevention of graft rejection after organ transplantation. It is a cyclic polypeptide consisting of 11 amino acids and inhibits secretion of interleukin 2 from helper T cells, which promote immune reaction, and thus inhibits immunity.

Appearance: White, crystalline powder ~ powder or mass
Solubility: Very soluble in methanol and ethanol.
Freely soluble in acetone and ether. Practically insoluble in water.



$C_{62}H_{111}N_{110}O_{12}$= 1202.61, CAS No. 59865-13-3

Vacuolar H^+-ATPase Inhibitor

Concanamycin A, 90.0+ % (HPLC)

Wako Cat. No. 036-16034 (25 μg); 032-16031 (100 μg) <for Biochemistry>
Keep at -20℃

Concanamycin A is a specific inhibitor of V-ATPases. Isolated from an antifungal biotic of the 18-membered macrocyclic lactones, Concanamycin A inhibits blastogenesis of cultured cells stimulated by Concanavalin A (Con-A). While Concanamycin A is structurally and pharmacologically similar to Bafilomycin A1. It exhibits about 10 times stronger inhibition than that by Bafilomycin A1.

Appearance: White, crystals ~ powder
Solubility: Freely soluble in chloroform, methanol, ethanol, acetone, ethyl acetate and DMSO.

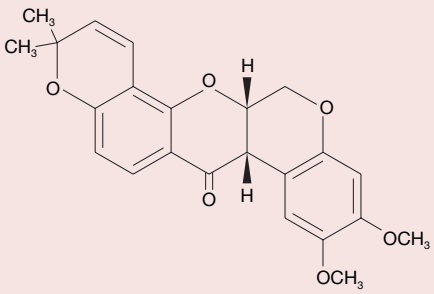
$C_{46}H_{75}NO_{14}$= 866.10, CAS No. 80890-47-7

Akt (Protein Kinase B; PKB) inhibitor

Deguelin

Wako Cat. No. 047-29211 (5 mg) <for Cellbiology>
Keep at 2~10℃

It inhibits proliferation of cells in the GM-2 stage of cell cycle. It induces apoptosis in the precancerous and cancerated cell lines. The derris root, a legume growing naturally in the South Seas and the tropics, contains a highly potent ingredient lethal to fish and insects. This is an analogue of the active component , rotenone.



$C_{23}H_{22}O_6$= 394.42, CAS No. 522-17-8

Appearance: Slightly pale yellow ~ yellow, crystalline powder ~ powder
Solubility: Soluble in acetone, dichloromethane, acetonitrile and DMSO.

[References]

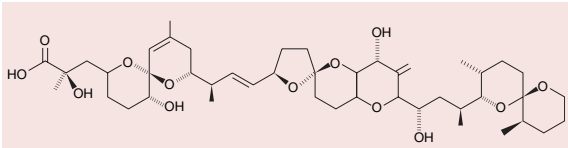
1) Chun, K. H., *et al*.: "Effects of deguelin on the phosphatidylinositol 3-kinase/Akt pathway and apoptosis in premalignant human bronchial epithelial cells", *J. Natl. Cancer Inst*., **95**, 291-302 (2003)
2) Ito, C., et. al.: "Cancer chemopreventive activity of rotenoids from Derris trifoliata", *Planta Med*., **70**, 8-11 (2004)

Marine Natural Products – Protein Phosphate Inhibitor

Dinophysistoxin-I [DTX-I]

Wako Cat. No. 042-28661 (100 µg) <for Biochemistry>
Keep at 2~10℃

Dinophysistoxin, isolated from *Halichondria okadai*, is a diarrhetic shellfish toxin with 35-methyl okadaic acid. Dinophysistoxin-1 is a potent Non-TPA* type tumor promoter and specifically inhibits protein phosphatases.
*TPA: 12-*o*-Tetradecanoyl-phorbol-13-acetate



$C_{45}H_{70}O_{13}$= 819.03, CAS No. 81720-10-7

Source: *Halichondria okadai*
Appearance: film
Solubility: Soluble in methanol, ether, acetone, ethyl acetate and chloroform.

[Reference]

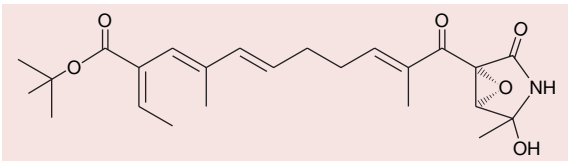
Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K. and Sugimura, T.: "Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter", *Proc. Natl. Acad. Sci. USA*, **85**, 1768 -71 (1988).

HSP60 Inhibitor

ETB [Epolactaene Tertiary Butyl Ester] (mixture of isomers)

Wako Cat. No. 051-07671 (200 µg) <for Cellbiology>
Keep at -20℃

Wako has launched a new inhibitor which were discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN) under license from RIKEN.
This product is a derivative of epolactaene isolated from Penicillium. It has a more potent cytostatic effect on human neuroblastoma cells SH-SY 5Y than that of epolactaene, and induces apoptosis. Furthermore, it has been revealed that ETB induces apoptosis in human T-lymphoma cells Jurkat. Recently, HSP60 was identified as one of ETB binding proteins. ETB binds to HSP60 to inhibit chaperone activity.



$C_{24}H_{33}NO_6$= 431.52

Appearance: White ~ slightly pale brown, crystalline powder ~ powder
Solubility: Soluble in methanol (1 vial is dissolved with 0.2 mL of methanol).

[Reference]

Nagumo, Y., Kakeya, H., Shoji, M., Hayashi, Y., Dohmae, N. and Osada, H.: "Epolactaene binds human Hsp60 Cys442 resulting in the inhibition of chaperone activity", *Biochem. J*., **387**(3), 835-40 (2005).

Bacterial Protein Synthesis Inhibitor
Gentamicin Sulfate

Wako Cat. No. 073-02971 (250 mg); 079-02973 (1 g); 077-02974 (5 g); 071-02972 (25 g) <for Biochemistry>
Wako Cat. No. 078-04981 (250 mg); 074-04983 (1 g); 072-04984 (5 g) <for Molecular Biology>
Keep at 2~10℃

This product is an antibiotic that has an antimicrobial activity against gram positive and nagative bacteria. It inhibits the initiation of protein synthesis of bacteria by acting as a ribosome to induce misreading of codons. It is a mixture of gentamicin C1, C2, and C1a.
As a reagent for molecular biology, it has been confirmed for DNase and RNase activities.

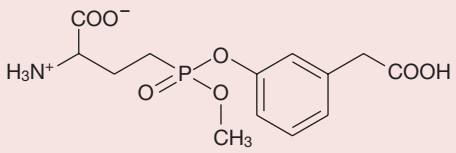
Source:	*Micromonospora purpurea*
Appearance:	White ~ slightly pale brown, powder
Potency:	590+ μg/mg (calculated on the dried basis)
Solubility:	Freely soluble in water.
	Practically insoluble in ethanol

CAS No. 1405-41-0

Highly Selective γ-Glutamyl transpeptidase (GGT) Inhibitor
GGsTop™

Wako Cat. No. 075-05471 (10 mg) <for Cellbiology>
Keep at -20℃

GGsTop™ is a highly selective γ-Glutamyl Transpeptidase (GGT) inhibitor. While acivicin [AT-125], which is widely used as a GGT inhibitor also inhibits asparagine synthetase (GA family), GGsTop™ does not inhibit the asparagine synthetase.



$C_{13}H_{18}NO_7P$ = 331.26
CAS No. 926281-37-0

[Features]

1. ***High specificity to GGT***
   Acivicin deactivates more than 90% of 100μM of *E. coli* asparagine synthetase for 2 hours. On the other hand, GGsTop™ did not deactivates even the 10mM enzyme.
2. ***High inhibitory activity to human GGT***

	to human GGT	to *E. coli* GGT
GGsTop™	51	170
Acivicin [AT-125]	0.40	4,200

Inhibitory activities of GGsTop™ and aciviin toward *E. coli* and human GGT. Each numeric value shows second-order rate constant for enzyme inactivation[1].

3. ***Low toxicity***
   On acute toxicity test, there is no toxicity with intravenously-infused GGsTop™ (30mg/kg). On the other hand, acivicin has severe toxicity to the CNS.
4. ***Chemically stable***
   The reconstituted neutral or acid aqueous solution such as 0.1% TFA solution and 0.1N HCl solution is stable for 1 month at room temperature for NMR analysis.

[Reference]

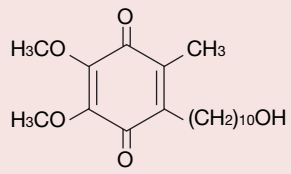
Han, L., Hiratake, J., Kamiyama, A. and Sakata, K.: "Design, synthesis, and evaluation of γ-phosphono diester analogues of glutamate as highly potent inhibitors and active site probes of γ-glutamyl transpeptidase", *Biochemistry*, **46**, 1432-47 (2007).

A synthetic analog of Coenzyme Q10
Idebenone

Wako Cat. No. 096-05001 (100 mg) <for Biochemistry>
Keep at 2~10℃

Idebenone is known to act on the central nervous system (CNS) and ameliorate cerebral apoplexy, cerebral ischemia with affective disorder, tetraplegia, and impaired passive avoidance response.

Appearance:	Yellowish red ~ orange, crystals ~ crystalline powder or mass
Solubility:	Soluble in ethanol.



$C_{19}H_{30}O$ = 338.44, CAS No. 58186-27-9

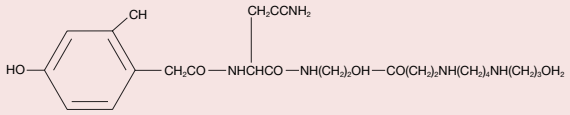
[Reference]

p.282, Pharmaceutical Handbook, the 5th edition, edited by Osaka Pharmaceutical Association.

Neurotoxin – Glutamate Receptor Selective Agonist

Joro Spider Toxin [JSTX-3], 98.0+ % (HPLC)

Wako Cat. No. 104-00051 (0.1 mg) <for Biochemistry>
Keep at 2~10℃



$C_{27}H_{47}N_7O_6$ = 565.71, CAS No. 112163-33-4

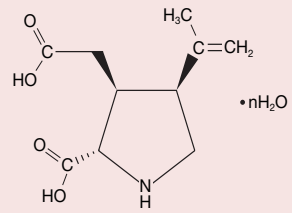
JSTX-3 is derived from the venom of *Nephila clavata* and consist of three active principles of similar chemical structure and function. Each of these components has been found to be a potent antagonist of neurotransmitter receptors. Wako offers JSTX-3, a chemical of low molecular weight which selectively inhibits excitatory synaptic transmission by blocking quisqualate-sensitive L-glutamate receptors. The high degree of specificity JSTX-3 exhibits makes it an especially valuable tool for the study of neurological disorders and for the research of excitatory neurotransmitter mechanisms.

Appearance: Lyophilized
Solubility: Soluble in water

Neurotoxin – Glutamate Receptor Selective Agonist

Kainic Acid n-Hydrate, 98.0+ % (HPLC)

Wako Cat. No. 118-00751 (10 mg) <for Biochemistry>
Keep at 2~10℃



$C_{10}H_{15}NO_4 \cdot nH_2O$ ($C_{10}H_{15}NO_4$ = 213.23)

It is an amino acid with glutamate skeleton isolated from a red algae, Digenea (Corsican weed, Digenea simplex) known as an ascaricide. This product is one of selective agonist for kainate-type glutamate receptor and has a potent CNS stimulating effet. It is used for studies on the signal transduction system via kainate cascade, neuronal apoptosis, ALS (amyotrophic lateral sclerosis), and pathological mechanism of Alzheimer's disease.

Potent Synthesis Inhibitor - Aminoglycoside Antibiotic

Kanamycin Sulfate

Wako Cat. No. 117-00341 (1 g); 113-00343 (5 g); 115-00342 (25 g); 111-00344 (100 g) <for Biochemistry>
Wako Cat. No. 113-00701 (1 g); 119-00703 (5 g); 117-00704 (100 g) <for Cell Culture>
Keep at 2~10℃

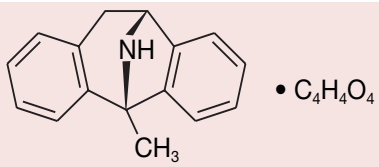
Appearance: White ~ slightly pale yellow, crystals ~ powder or mass
Potency: 600+ μg/mg (calculated on the dried basis)
Solubility: Freely soluble in water. Practically insoluble in ethanol and ether.

$C_{18}H_{36}N_4O_{11} \cdot nH_2SO_4$
($C_{18}H_{36}N_4O_{11}$ = 484.50)
CAS No. 25389-94-0

Neurotoxin - NMDA-Glutamate Receptor Antagonist

(+)-MK 801 Maleate [Dizocilpine Maleate], 98.0+ % (HPLC)

Wako Cat. No. 134-15461 (10 mg); 130-15463 (50 mg) <for Cellbiology>
Keep at 2~10℃



$C_{16}H_{15}N \cdot C_4H_4O_4$ = 337.37, CAS No. 77086-22-7

Acts by binding to a site located within the NMDA associated ion channel.
It is a non-competitive antagonist showing selectivity for NMDA-type glutamate receptor.
It binds to the pore of the ion channel, which is opened by the binding of ligands, and acts as an open-channel blocker.

Appearance: White ~ nearly white, crystals ~ powder

Marine Toxin – Actin Inhibitor

Mycalolide B, 98.0+ % (HPLC)

Wako Cat. No. 132-12081 (100 μg) <for Biochemistry>
Keep at -20℃

Inhibits actin polymerization. Mycalolide B depolymerizes F-actin by nibbling and forms a 1:1 complex with G-actin.

Source: *Mycale* sp.
Appearance: Clear film
Solubility: Soluble in methanol, ethanol and DMSO.

$C_{52}H_{74}N_4O_{17}$ = 1027.18
CAS No. 122752-21-0

Polymethoxy Flavonoids derived from Shekwasha

Nobiletin, 95.0+ % (HPLC)

Wako Cat. No. 149-07521 (10 mg) <for Biochemistry>

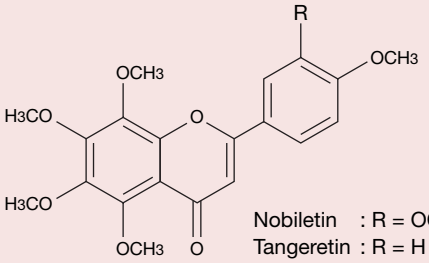
Tangeretin, 95.0+ % (HPLC)

Wako Cat. No. 208-15671 (10 mg) <for Biochemistry>

Keep at -20℃

Nobiletin and tangeretin are polymethoxy flavonoids contained in the juice of Shekwasha (Citrus depressa Hayata), a citrus fruit. These flavonoids are receiving attention for a variety of beneficial effects such as reducing elevation of blood pressure and plasma glucose levels.

[Nobiletin]
Appearance: White ~ slightly pale yellow, crystalline powder ~ powder or mass
Solubility: Soluble in ethanol, methanol and acetone.
[Tangeretin]
Appearance: White ~ nearly white, crystalline powder ~ powder
Solubility: Soluble in ethanol and methanol. Insoluble in water.



[Nobiletin] $C_{21}H_{22}O_8$ = 402.39, CAS No. 478-01-3
[Tangeretin] $C_{20}H_{20}O_7$ = 372.37, CAS No. 481-53-8

[References]

1) Rooprai, H. K. *et al.*: "Evaluation of the effects of swainsonine, captopril, tangeretin and nobiletin on the biological behaviour of brain tumour cells *in vitro*", *Neuropathol.Appl. Neurobiol.*, **27**, 29 (2001)
2) Datla, K. P. *et al.*: "Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease", *Neuroreport*, **12**, 3871 (2001)

Protein phosphatase inhibitor

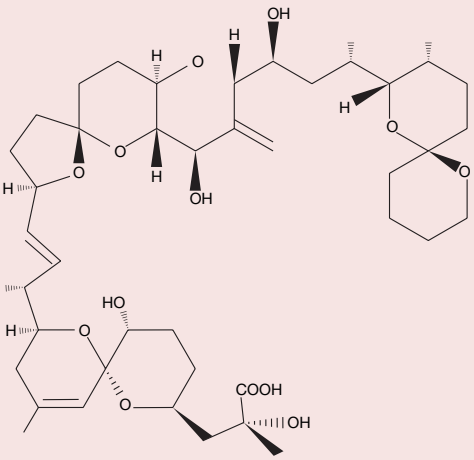
Okadaic Acid

[Okadaic Acid] Wako Cat. No. 150-01653 (25 µg); 154-01651 (100 µg) <for Biochemistry>
[Ammonium Salt] Wako Cat. No. 156-02211 (100 µg); 152-02213 (500 µg) <for Biochemistry>

Keep at 2~10℃

Okadaic acid is a causative agent of diarrhetic shellfish poisoning and a potent and specific inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A) that is isolated from the sponge *Halichondria Okadai*.

Appearance: [Okadaic Acid] Lyophilized, film; [Ammonium Salt] Lyophilized
Assay (HPLC): [Okadaic Acid] 80.0+%
Solubility: [Okadaic Acid] Soluble in DMF, DMSO, Chloroform-methanol. Slightly soluble in water and n-hexane.
[Ammonium Salt] 100µg/100µL (water)



Okadaic Acid $C_{44}H_{68}O_{13}$ = 805.00, CAS No. 78111-17-8
Okadaic Acid Ammonium Salt
$C_{44}H_{67}N_3O_{13} \cdot NH_4$ = 822.04, CAS No. 155716-06-6

[References]

1) Tachibana, K. *et al.*: *J. Am. Chem. Soc.*, **103**, 2469-71 (1981)
2) Suganuma, M. *et al.*, "Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter", *Proc. Natl. Acad. Sci. USA*, **85**, 1768-71 (1988)
3) Ozaki, H. *et al.*: "Calcium-independent phosphorylation of smooth muscle myosin light chain by okadaic acid isolated from black sponge (Halichondria okadai).", *J. Pharmacol. Exp. Ther.*, **243**, 1167-73 (1987)

Neurotoxin; Na^+ Channel Agonist

Palytoxin, 90.0+ % (HPLC)

Wako Cat. No. 161-15131 (100 µg) <for Biochemistry>

Keep at 2~10℃

Palytoxin is a potent marine toxin which acts as a strong hemolysin, histamine releaser, inhibitor of sodium-potassium ATPase, and cation ionophore.

Appearance: Film (invisible due to very small quantity)
Solubility: Soluble in pyridine, DMSO and water. Slightly soluble in ethanol and methanol. Insoluble in chloroform, ether and acetone.

$C_{129}H_{223}N_3O_{54}$ = 2,680.14, CAS No. 77734-91-9

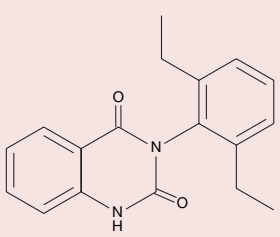
[References]

1) Uemura, D., Hirata, Y., Iwashita, T. and Naoki, H.: *Tetrahedron*, **41**, 1007 (1985).
2) Cha, J.K., Christ, W.J., Finan, J.M., Fujioka, H., Kishi, Y., Klein, L., Ko, S.S., Leder J., McWhater, W.W., Jr. Pfaff. K.-P., Yonaga, M., Uemura, D. and Hirata, Y.: *J. Am. Chem. Soc.*, **104**, 7369 (1982).

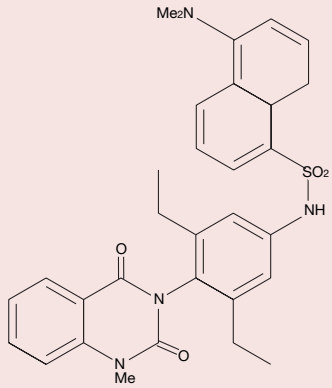
Puromycin-Sensitive Aminopeptidase (PSA) Inhibitor

PAQ-22 [3-(2,6-diethylphenyl)-2,4(1*H*,3*H*)-quinazolinedione]

Wako Cat. No. 165-23581 (10 mg) <for Cellbiology>
Keep at RT



PAQ-22
$C_{18}H_{18}O_2N_2 \cdot 294.3$
CAS No. 371968-89-7



DAMPAQ-22
$C_{31}H_{32}O_4N_4S \cdot 556.68$
CAS No. 519183-48-3
λex : 320 nm; λem : 510 nm

Fluorescent Bioprobe for Visualization of PSA in Living Cells

DAMPAQ-22

Wako Cat. No. 049-30761 (2 mg) <for Cellbiology>
Keep at RT

Puromycin-sensitive aminopeptidase (PSA), which is a neutral aminopeptidase with a substrate specificity similar to that of aminopeptidase N (APN), is distributed mainly in the brain and neurons. The physical roles/functions of PSA remain unclear. Wako has launched non-peptide, small-molecular, non-competitive PSA Inhibitor, PAQ-22 and the structurally modified fluorescent bioprobes, DAMPAQ-22. The cellular localization of PSA could be specifically visualized by the use of DAMPAQ-22[1]. These are long-awaited tools for PSA research.

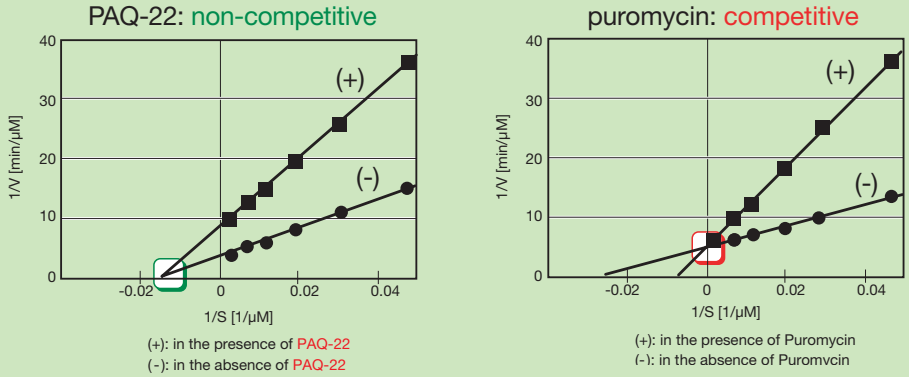


Figure 1. Lineweaver-Burk plot analysis of PSA inhibition by PAQ-22 and Puromycin.
Using living human monocytic cell MOLT-4, which is known to express PSA, PSA inhibitory activity of PAQ-22 and Puromycin was determined with an indicator, which is fluorescence generated from a fluorescent substrate Ala-MCA broken down by PSA. Lineweaver-Burk plot indicated that PAQ-22 acts as specific non-competitive inhibitor. On the other hand, Puromycin acts a competitive inhibitor. In addition, PAQ-22 and DAMPAQ-22 are easily incorporated into MOLT-4 under the general cell culture conditions.

	PSA IC50 (μmol/L)	APN IC50 (μmol/L)
PAQ-22	3.8	>100
DAMPAQ-22	4.6	N/A
Puromycin	0.6	4.8

Table 1. Aminopeptidase-Inhibitory Activity of PAQ-22 and Puromycin
PSA- and APN-inhibitory activities were assayed by the use of L-Ala-MCA with MOLT-4. PAQ-22 is inactive toward APN, indicating that PAQ-22 is specific to PSA.
(*These data were provided by Dr. Yuichi Hashimoto, Institute of Molecular and Cellular Biosciences, The University of Tokyo*)

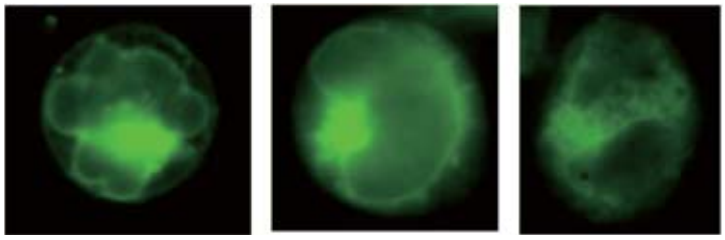


Figure 2. Fluorescent microscopic images of B16F10 mouse melanoma cells treated with 10μmol/L DAMPAQ-22 for 10 min.
(*These data were provided by Dr. Yuichi Hashimoto, Institute of Molecular and Cellular Biosciences, The University of Tokyo*)

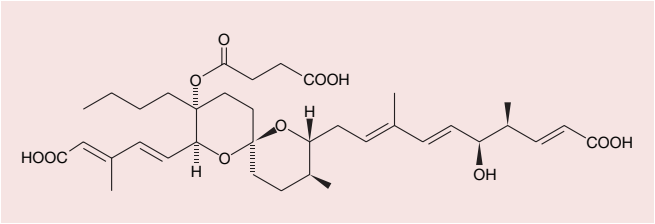
[References]

1) Kakuta, H., Koiso, Y., Nagasawa, K., Hashimoto, Y.: "Fluorescent Bioprobes for Visualization of Puromycin-Sensitive Aminopeptidase in Living Cells" *Bioorg. Med. Chem. Lett.*, **13**, 83-6 (2003)
2) Bukowska A, Tadje J, Arndt M, Wolke C, Ka¨hne T, Bartsch J, Faust J, Neubert K, Hashimoto Y, Lendeckel U.: "Transcriptional regulation of cytosol and membrane alanyl-aminopeptidase in human T cell subsets", *Biol. Chem.*, **384**, 657-65 (2003).
3) Kakuta H, Tanatani A, Nagasawa K, Hashimoto Y.: "(1*H*,3*H*)-quinazolinedione skeleton", *Chem. Pharm. Bull.*, **51**, 1273-82 (2003).
4) Sánchez-Mora´n E, Jones GH, Franklin FC, Santos JL.: "A puromycin-sensitive aminopeptidase is essential for meiosis in Arabidopsis thaliana", *Plant Cell.*, **16**, 2895-909 (2004).
5) Thielitz A, Bukowska A, Wolke C, Vetter R, Lendeckel U, Wrenger S, Hashimoto Y, Ansorge S, Gollnick H, Reinhold D.: "Identification of extra- and intracellular alanyl aminopeptidases as new targets to modulate keratinocyte growth and differentiation", *Biochem. Biophys. Res. Commun.*, **321**, 795-801 (2004).

New Protein Synthesis Inhibitor - Isoleucyl tRNA Synthesis Enzyme Inhibitor

Reveromycin A Sodium Salt

Wako Cat. No. 185-02181 (500 µg) <for Cellbiology>
Keep at -20℃



This product is an antibiotic isolated from *Streptomyces*. It targets isoleucyl-tRNA synthetase and inhibits protein synthesis in eukaryotes. It has been investigated for it antitumor and antifungal activities. However, recent studies have revealed that low-dose of reveromycin A induces cell death of activated osteoclasts, which leads to acidic environment. Thus it receives attention as a candidate for the treatment of osteoporosis / multiple myeloma.
This new inhibitor was discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN, Japan).

Source: *Streptomyces reveromyceticus* SN593
Appearance: Lyophilized

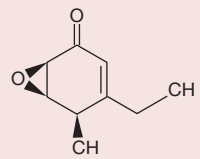
[Reference]

Woo, J. T., Kawatani, M., Kato, M., Shinki, T., Yonezawa, T., Kanoh, N., Nakagawa, H., Takami, M., Lee, K.H., Stern, P.H., Nagai, K. and Osada, H.: *Proc. Natl. Acad.Sci. USA*: **103**(12), 4729 (2006).

Apoptosis Inhibitor

RKTS-33

Wako Cat. No.182-02191 (200 µg) <for Cellbiology>
Keep at -20℃



$C_7H_8O_4$ = 156.14

This product, which was discovered by Dr. Hiroyuki Osada, Antibiotics Laboratory of Institute of Physical and Chemical Research (RIKEN) under license from RIKEN, is a derivative of epoxycylohexenone isolated from *Paecilemyces*. It has lower toxicity than epoxycylohexenone. Like epoxycylohexenone, it inhibits apoptosisi not by inhibition of perforin-dependent pathway by cytotoxic T lymphocytes but by selective inhibition of Fas ligand-dependent pathway alone.

Source: *Paecilomyces* sp.
Appearance: Lyophilized
Solubility: Soluble in ethanol

[Reference]

Mitsui, T., Miyake, Y., Kakeya, H., Hayashi, Y., Osada, H. and Kataoka, T. : *Biosci. Biotechnol. Biochem.*: **69** (10), 1923 (2005).

Potent Inhibitor of Ca^{2+} Release

Ryanodine, *from Ryania speciosa,* 98.0+% (HPLC)

Wako Cat. No. 181-02281 (1 mg); 187-02283 (5 mg) <for Cellbiology>
Keep at -20℃

$C_{25}H_{35}NO$ = 493.55; CAS No. 15662-33-6

A Ca^{2+} Channel Inhibitor Ryanodine is an alkaloid isolated from *Ryania speciosa Vahl*. It acts to increase calcium permeability by binding to the sarcoplasmic reticulum calcium channel.

[References]

1) Alexandre, F.: "Effects of ryanodine in skinned cardiac cells", *Federation Proceedings*, **44**, 2970 (1985)
2) Ito, K., Ikemoto, T., Aoki, S. and Ota, M.: "Effects of ryanodine and 9,21-didehydroryanodine on caffeine-induced contraction of rat and guinea pig aortae", *Japan. J. Pharmacol.*, **51**, 531-8 (1989)

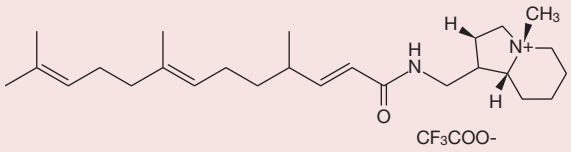
Calmodulin Inhibitor

Stellettamide A Trifluoroacetate, 95.0+ % (HPLC)

Wako Cat. No. 193-11831 (100 µg) <for Biochemistry>
Keep at -20℃, Lyophilized

Stellettamide A (ST-A) is a marine toxin isolated from a marine sponge. It is a calmodulin antagonist and inhibits Ca^{2+}-calmodulin-dependent phosphodiesterase.

Source:*Stelletta sponge*
Solubility:Soluble in methanol (100 µg/100 µL)



$C_{26}H_{45}N_2O \cdot CF_3COO$ = 514.66; CAS No. 129744-24-7

[Reference]

Abe, Y., *et al*.: "Stellettamide-A, a novel inhibitor of calmodulin, isolated from a marine sponge", *Br. J. Pharmacol*., **121**, 1309 (1997)

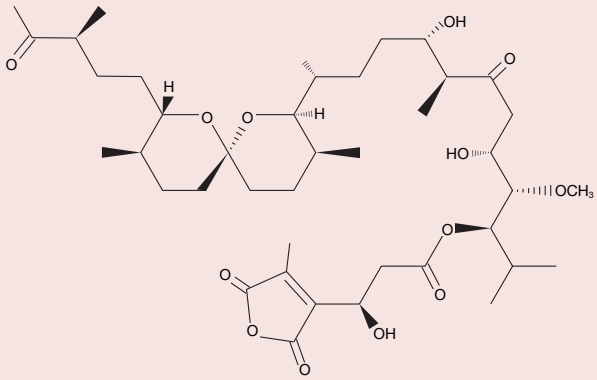
Protein Phosphatase Inhibitor

Tautomycin

Wako Cat. No. 209-12041 (100 µg) <for Biochemistry>
Keep at -20℃, Lyophilized

Tautomycin, is a highly potent and specific protein phosphatase inhibitor, induced morphological change (bleb-formation) of human myeloid leukemia K562 cells.
The appearance of blebs is inhibited by protein kinase C (PKC) inhibitors. Tautomycin acts as an activator of PKC.

Source:*Streotomyces spiroverticillatus*
Solubility:Soluble in ethanol. Practically insoluble in water.



$C_{41}H_{66}O_{13}$ = 766.97; CAS No. 109946-35-2

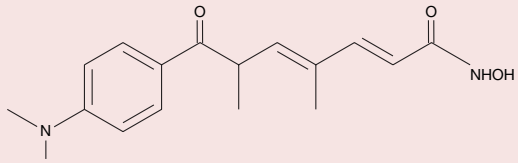
[References]

1) Hori, M., Magae, J., Han, Y-G., Hartshorne, D.J. and Karaki, H.:"A novel protein phosphatase inhibitor, tautomycin Effect on smooth muscle", *FEBS LETTERS*, **285**, 145 (1991)
2) Magae, J., Osada, H., Fujiki, H.,Saido, T., Suzuki, K., Nagai, K., Yamasaki, M. and Isono, K.: "Morphological changes of human myeloid leukemia K-562 cells by a protein phosphatase inhibitor, tautomycin", *Proc. Jpn. Acad*., **66(B)**, 209-12 (1990)
3) Kurisaki, T., *et al*.: "Morphological changes and reorganization of actinfilaments in human myeloid leukemia cells induced by a novel protein phosphatase inhibitor, tautomycin", *Cell Struct. Funct*., **18**, 33-9 (1993)
4) Ubukata, M. *et al*.: *J. Chem. Soc. Chem. Commun*., 244 (1991)
5) Ubukata, M. *et al*.: *J. Chem. Soc. Perkin Trans*., **1**, 617 (1993).

Histone Deacetylase (HDAC) Inhibitor

Trichostatin A, 99.0+ % (HPLC)

Wako Cat. No. 200-11993 (1 mg); 204-11991 (5 mg) <for Biochemistry >
Keep at -20℃



$C_{17}H_{22}N_2O_3$ = 302.37

HDAC plays a central role in chromatin structure formation associated with the nuclear distribution of DNA. There are presently 17 known types of this enzyme in mammals, which are classified into 3 classes. Also, HDAC Class III has been reported to be associated with regulation of aging and life span. HDAC inhibitors show connections with cell division cycles and differentiation, as well as with antitumor activity and apoptosis-inducing activity through the inhibition of the deacetylating activity of HDAC. They can be used for studies on cellular functions involving histone deacetylase.
Trichostatin A (TSA), a *Streptomyces* product, specifically inhibits the cell cycle of normal rat fibroblasts in the G1 and G2 phases at very low concentrations as reported by Yoshida, *et al*. TSA-induced G2-arrest induces the formation of proliferative tetraploid cells. In addition, nanomolar concentration of TSA has been shown to cause an accumulation of highly acetylated histones *in vivo*, and markedly inhibit the activity of partially purified histone deacetylase *in vitro*.
TSA appears to be a useful product for researching the multiple functions of histone acetylation in regulatory mechanisms of eukaryotic cell proliferation and differentiation.

Source: *Streptmyces Hygroscopicus*
Solubility: Soluble in ethanol and acetone. 1 mg/10 mL (methanol)

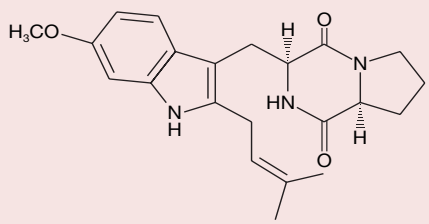
[References]

1) Yoshida, M., Beppu, T.: "Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A", *Exp. Cell. Res.*, **177**, 122-31 (1988)
2) Yoshida, M. *et al.*: "Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A", *J. Biol. Chem.*, **265**, 17174-9 (1990)
3) Dion, L.D. *et al.*: "Amplification of recombinant adenoviral transgene products occurs by inhibition of histone deacetylase", *VIROLOGY*, **231**, 201-9 (1997)

Cell Cycle Inhibitor

Tryprostatin A, from Xaspergillus fumigatus BM939

Wako Cat. No. 203-16961 (500 µg) <for Cellbiology>
Keep at -20℃, Lyophilized film



$C_{22}H_{27}N_3O_3$ = 381.47

Tryprostatin A (TPS-A) is an alkaloid antibiotic isolated from *Aspergillus*. It affects the microtubule-associated protein binding site and exhibits antitumor activity by inhibition of cell cycle progression in the M phase specifically.
This product was discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN, Japan).

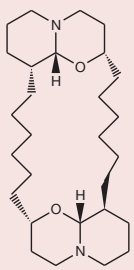
[Reference]

Usui T, Kondoh M, Cui CB, Mayumi T, Osada H.: "Tryprostatin A, a specific and novel inhibitor of microtubule assembly", *Biochem J.*, **333**, 543-8 (1998).

Membrane-Permeable Inhibitor of IP_3 Receptor

Xestospongin C, from Xestospongia sp., 90+% (HPLC)

Wako Cat. No. 244-00721 (100 µg) <for Cellbiology>
Keep at -20℃, Lyophilized form in 20 mmol/L HEPES solution (pH 7.3) containing 0.1 % BSA as a stabilizer, packaged under inert gas.



Xestospongin C
(= araguspongine E)

An Okinawan marine sponge *Xestospongia* sp., provided by Dr. Kobayashi, Osaka Univ.

A selective and membrane-permeable inhibitor of the inositol 1,4,5-triphophate (IP3) receptor-mediated Ca^{2+} release, isolated from an Okinawan marine sponge *Xestospongia* sp. A potent and highly sensitive inhibitor of IP_3 receptor with IC50 of 350 nM, which is 30 times lower than that for ryanodine-receptor.

[Reference]

Miyamoto, S., *et al.*, "Xestospongin C, a selective and membrane-permeable inhibitor of IP_3 receptor, attenuates the positive inotropic effect of α-adrenergic stimulation in guinea-pig papillary muscle", *Br J Pharmacol*. **130**, 650-4 (2000).

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