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MicroRNA
Research Products

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microRNA Research Products

Contents

Front cover: Tomari Lab., The University of Tokyo, Japan

microRNA and Ago-Subfamily Proteins

microRNA (miRNA) are small functional RNA molecules (about 22 nucleotides) which act as post-transcriptional regulators of gene expression. They serve many functions within biological organisms (Ref. 1), and more than 1,000 types are known to exist in humans and mice. Worldwide there are many efforts to identify new types of microRNA with unknown functions and elucidate functions of those involved in disease. Recently it has been reported that microRNA exist not only within cells, but also blood and other bodily fluids. They are now garnering attention as possible clinical markers for cancer and other diseases (Ref. 2).

In cells, microRNA pass through many steps before being incorporated into a multiprotein complex called a RISC (RNA-induced silencing complex). After binding with the main component of Ago-subfamily proteins, they bind with mRNA targets and either cleave them or inhibit translation, thus regulating gene expression (Fig. 1) (Ref. 3, 4, 5). The Ago-subfamily of proteins are characterized by containing both a PAZ domain and a PIWI domain (Fig. 2). There are four types in humans (hAgo1 to hAgo4), and although each expression levels differ from each them in each cell lines (Fig. 3) (Ref. 6). The most commonly expressed Ago-subfamily protein is Ago2, which has unique "Slicer" activity which directly cleaves target RNA. Thus, Ago2 is considered to play an important role in microRNA pathways (Ref. 7, 8, 9, 10, 11, 12, 13). Anti-Ago antibodies are used for immunoprecipitation of RISC, allowing recovery of both microRNA and their mRNA targets. This immunoprecipitation method is vital for elucidating the various functions of microRNA (Ref.14, 15, 16, 17, 18, 19, 20, 21).

We offer a full selection of antibodies for various human and mouse Ago-subfamily proteins, as well as immunoprecipitation kits for them. These tools allow comprehensive analysis of microRNA and mRNA contained in RISC in cells and tissue, as well as Ago-bound microRNA in blood.

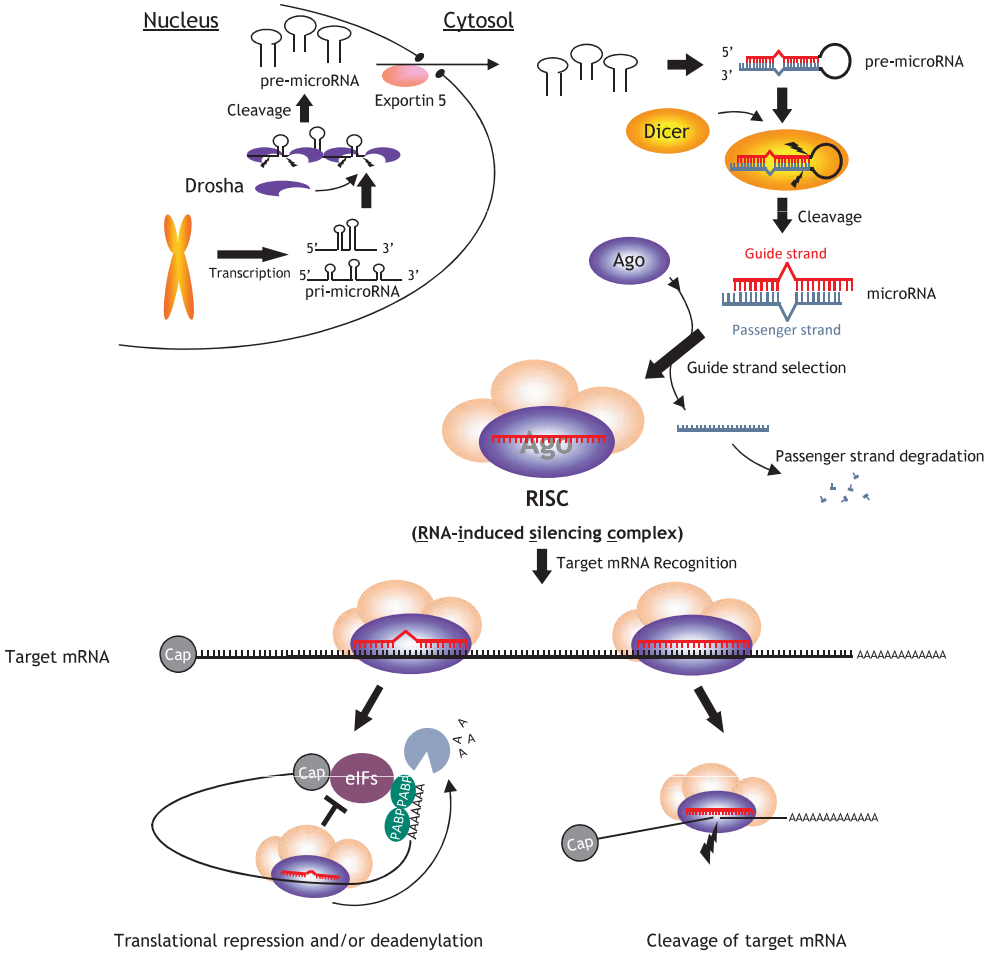


Figure 1 Molecular mechanism of RNA silencing

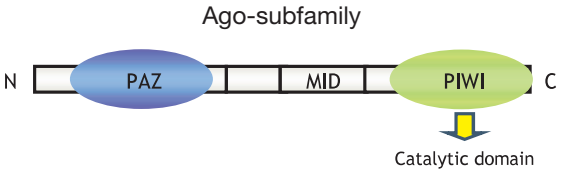


Figure 2 PAZ domain and PIWI domain

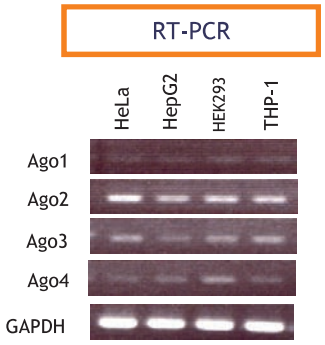
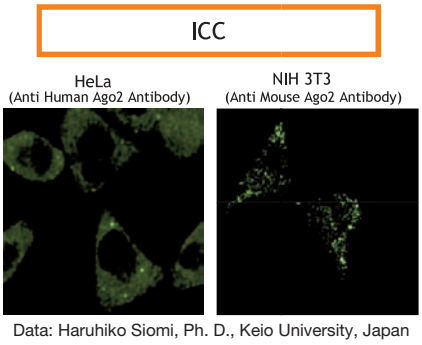


Figure 3 Expression comparison of hAgo1–hAgo4



Data: Haruhiko Siomi, Ph. D., Keio University, Japan

Figure 4 Localization of Ago2

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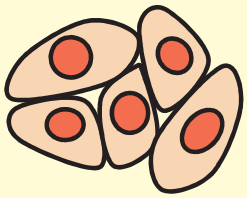
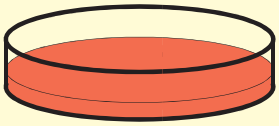
1. microRNA Experiment Flow

We offer a microRNA Isolation Kit series containing monoclonal antibodies for various Ago-subfamily proteins and immunoprecipitation methods (Ago IP) for each of these. We also offer tools such as the ISOGEN® series for purification of total RNA from samples or the microRNA Extractor® SP Kit, all allowing purification of fractions containing microRNA from samples depending on research needs. Finally, we offer additional powerful tools to support microRNA research, such as tools to clone cDNA from purified microRNA or target mRNA, electrophoresis tools, and microarray analysis services.



Cell or tissue sample



Conditioned culture medium sample

Plasma or serum sample

Purification for microRNA/target mRNA

【Related products for Ago-immunoprecipitation】

Anti Ago Monoclonal Antibody series
microRNA Isolation Kit series

【Related products for total RNA purification】

microRNA Extractor® SP kit
ISOGEN® II
ISOGEN®
ISOGEN®-LS

Analysis for purified microRNA/target mRNA

【Related products for electrophoresis p.19】

SuperSep™ Urea
Small RNA Marker
CLEAR STAIN Ag

〈Related products for cDNA cloning p.21〉

microRNA Cloning Kit
Target mRNA Cloning Kit

〈Microarray Analysis p.22〉

3D-Gene® (Toray) analysis service

2. Summary of Ago Immunoprecipitation

We offer a full selection of human and mouse antibodies for Ago-subfamily proteins as well as the microRNA Isolation Kit series based on immunoprecipitation (Ago IP) using these antibodies. These tools offer the following benefits over traditional total RNA purification.

Benefit 1 ➡ **Concentration of Ago-bound microRNA**

Improvement of detection sensitivity of microRNA incorporated in RISC and suppress detection of non-specific detection of microRNA not incorporated in RISC.

Example Comparing populations of Ago IP RNA fractions and total RNA fractions from HeLa cells

Starting with HeLa cells, we used a micro-array (*3D-Gene*®, Toray) to compare populations of RNA obtained with immunoprecipitation from monoclonal antibodies specifically recognizing each Ago subfamily protein (Ago1 to Ago4) (Ago IP RNA fraction) with microRNA contained in a total RNA fraction prepared using ISOGEN (AGPC) (Fig. 1). Results showed that more microRNA was concentrated in each Ago IP RNA fraction than the total RNA fraction (excluding the Ago4 IP RNA fraction), yielding a stronger signal (Fig. 2). One signal was strongly detected in the total RNA fraction while remaining largely absent in the Ago IP RNA fraction, suggesting that microRNA not incorporated into RISCs were non-specifically detected in the total RNA fraction.

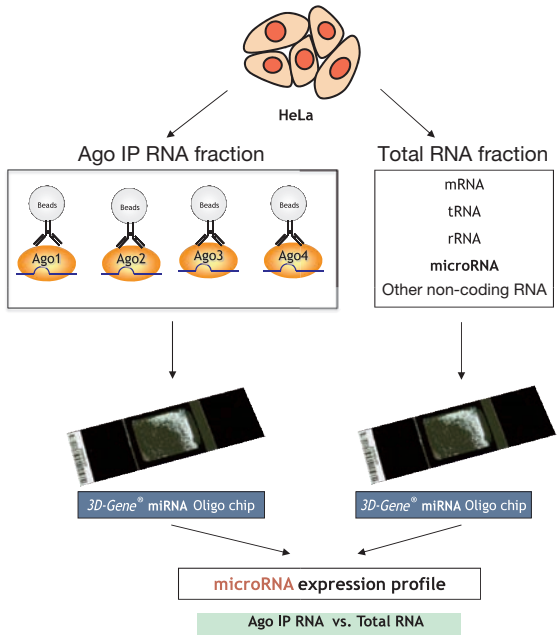


Figure 1 Experiment flow

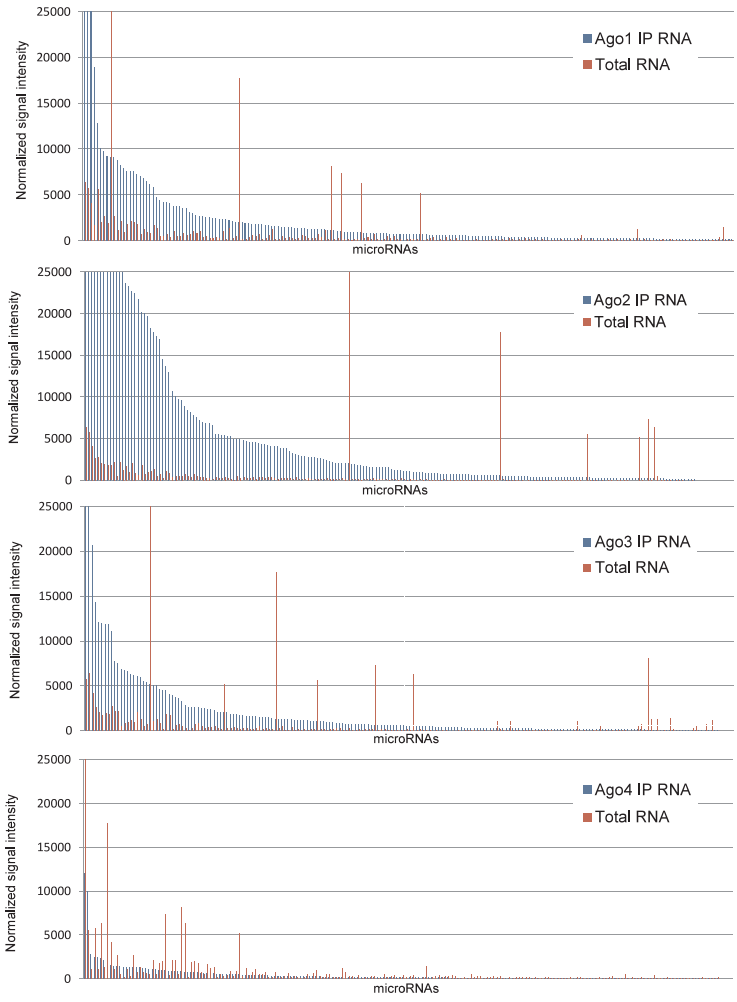


Figure 2 microRNA signal strength comparison between each Ago IP RNA fraction and total RNA fraction

Benefit 2 ➡ Concentration of RISC-binding target mRNA

RNA fractions obtained by Ago IP method are applicable to analysis of target mRNA by cDNA cloning, qPCR, microarray, Next-generation DNA sequencer because target mRNA is concentrated.

Example Concentration of target mRNA of liver-specific microRNA(miR-122)

Either miR-122 or the control of luciferace siRNA (Luc siRNA) were delivered to HepG2, a liver cancer cell line with low expression of miR-122, then Ago2 IP RNA (using anti-Ago2 monoclonal antibody 4G8) and total RNA (using ISOGEN) purified from both samples. Quantitative PCR was then used to compare (Fig. 1) quantities of Aldo A mRNA as a miR-122 target (normalized with GAPDH mRNA) in both cell types. Results show that the quantity of Aldo A mRNA decreased by RISC cleavage, in the total RNA fraction while Aldo A mRNA in the Ago2 IP RNA fraction was concentrated (Fig. 2).

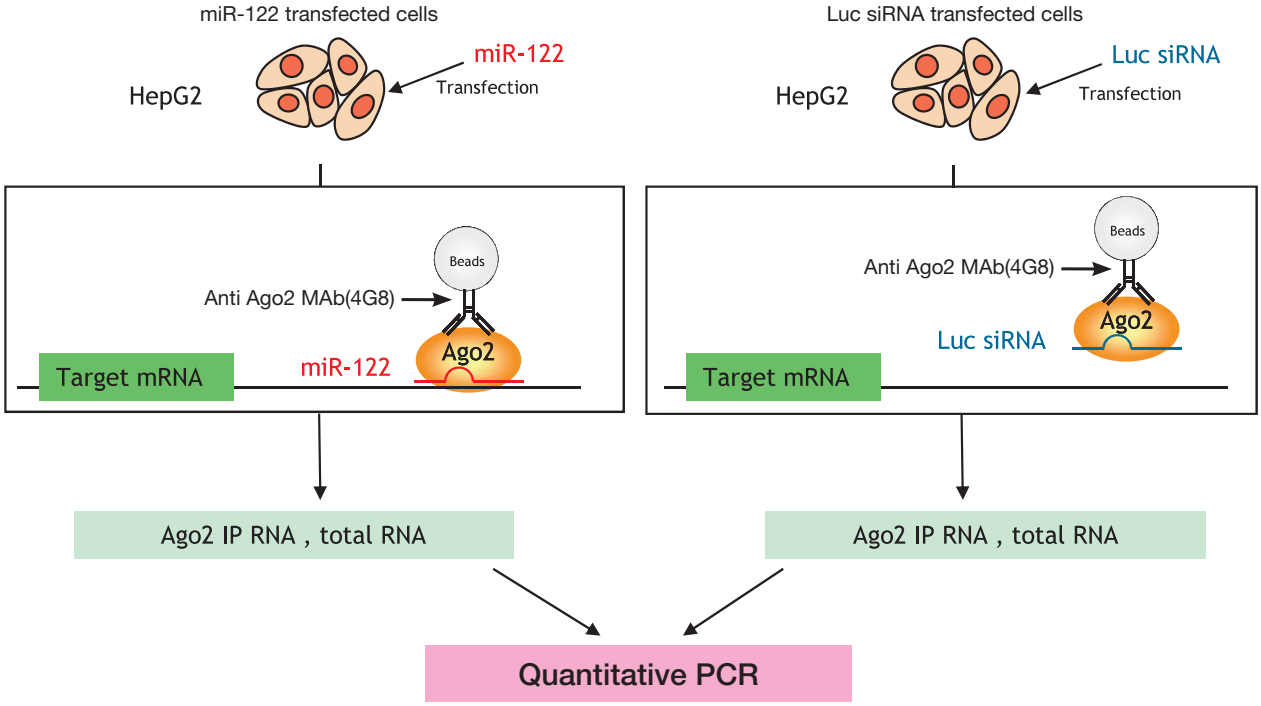


Figure 1 Experiment flow

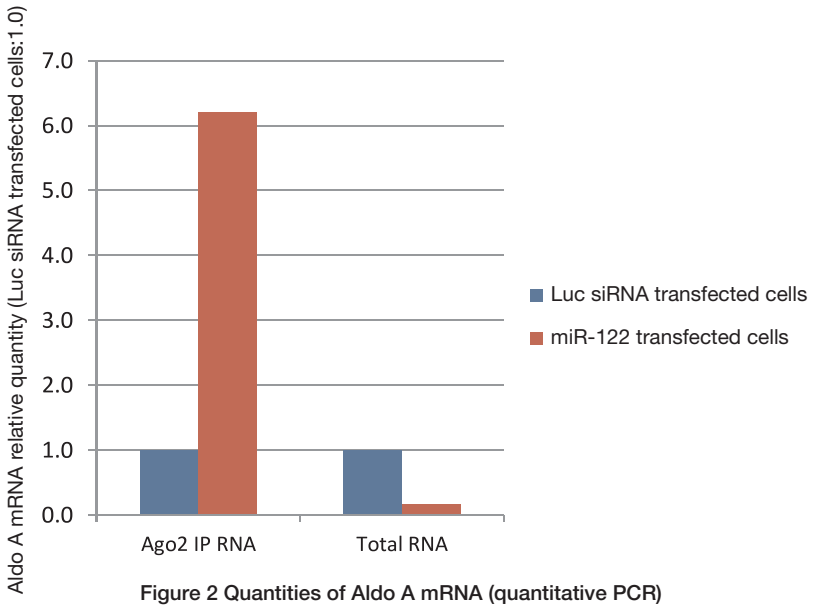


Figure 2 Quantities of Aldo A mRNA (quantitative PCR)

Benefit 3 ➡ Concentration of free Ago-bound microRNA from blood samples

Blood is known to include microRNA contained in exosomes, HDL-bound microRNA, and Ago-bound microRNA. Ago immunoprecipitation method allows for specific isolation of Ago-bound microRNA, and it can be used for biomarker searches and other uses.

Example 1 Isolation of Ago2-bound microRNA from human plasma

Quantitative PCR (Ct value) was used to measure 12 types of arbitrarily-selected microRNA in pooled plasma of healthy individuals from immunoprecipitated Ago2 IP RNA (using anti-Ago2 monoclonal antibody 4G8) and total RNA (using microRNA Extractor® SP Kit) (Fig. 1). Comparison reveals that most microRNA detected in the total RNA sample was also detected in the Ago2 IP RNA fraction, while types such as miR-22 and miR-92a were highly concentrated in the Ago2 IP RNA fraction (low Ct value compared to total RNA) (Fig. 2).

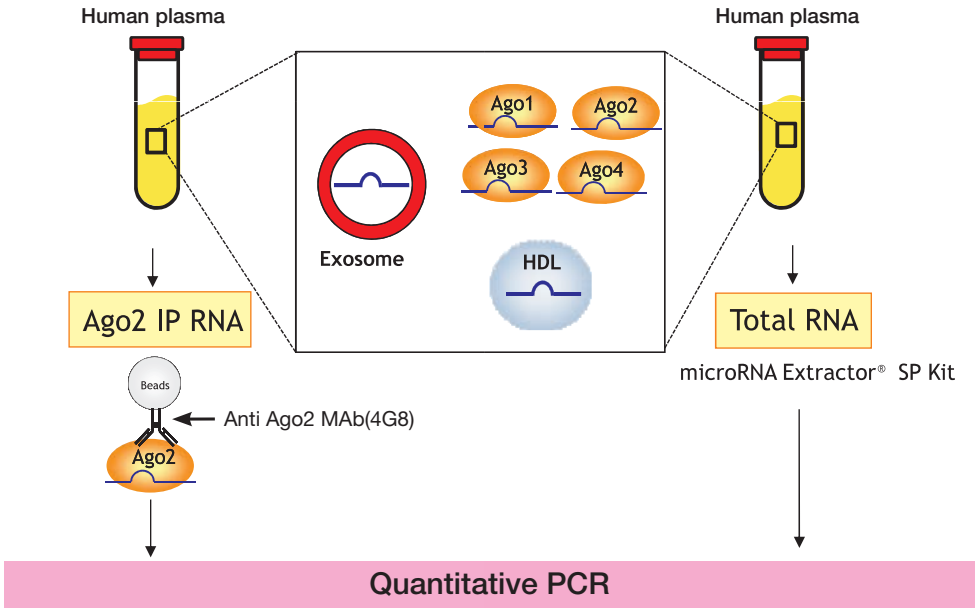


Figure 1 Experiment flow

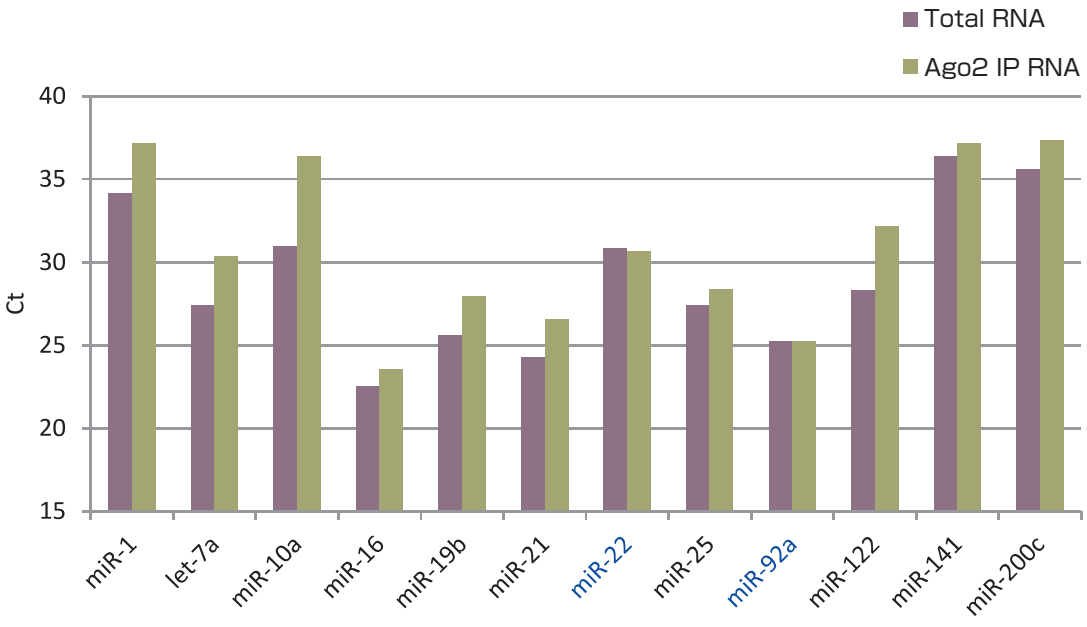


Figure 2 Comparison of microRNA quantities (Ct value)(quantitative PCR)

Example 2 Isolation of each Ago-bound microRNA from human plasma

Quantitative PCR was used to compare microRNA (miR-92a and miR-122) contained in immunoprecipitated RNA (Ago 1, 2, 3, 4 IP RNA) using anti-Ago (Ago1 to Ago4) monoclonal antibodies from pooled plasma of healthy individuals (Fig. 1). Results show microRNA present in other Ago IP RNA fractions in addition to the Ago2 IP RNA fraction, with liver-specific miR-122 highly concentrated in the Ago3 IP RNA fraction (Fig. 2).

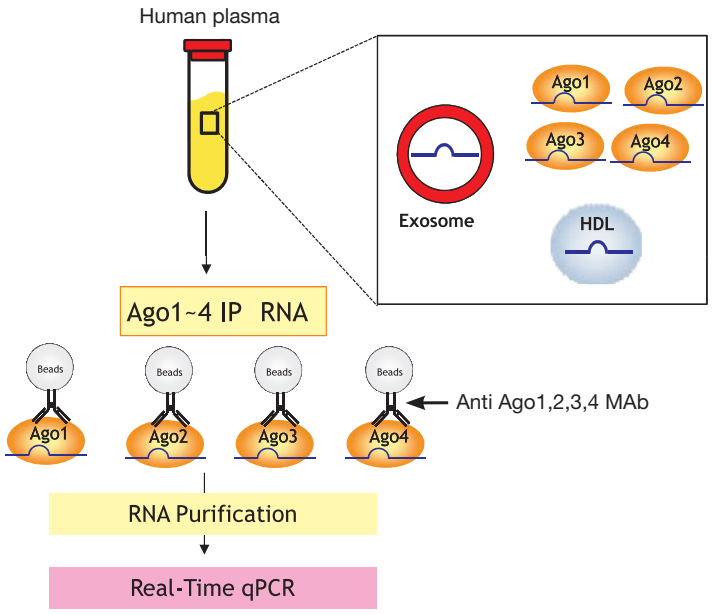


Figure 1 Experiment flow

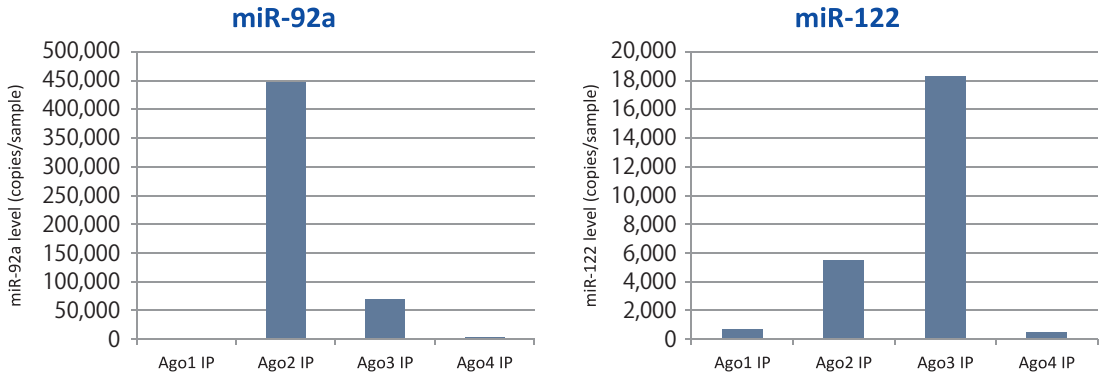


Figure 2 Comparison of microRNA quantities from each Ago IP RNA fractions (quantitative PCR)

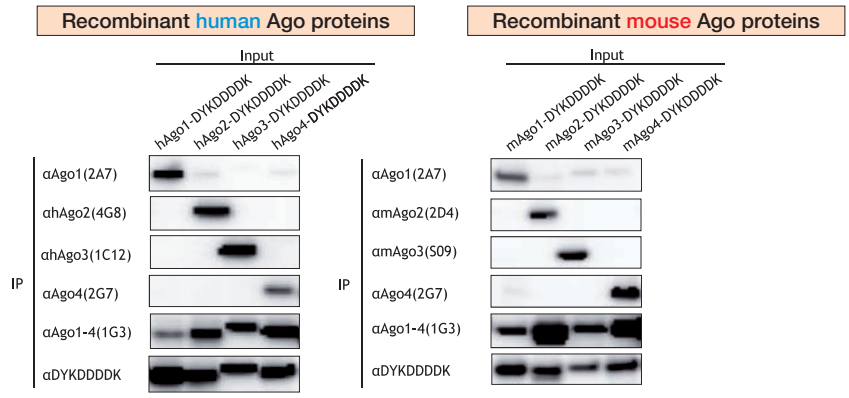
3. Anti-Ago Monoclonal Antibody Series

We offer a full line of mouse monoclonal antibodies both for Western blot detection and immunoprecipitation recognizing various human and mouse Ago-subfamily proteins. These tools allow selective immunoprecipitation of various Ago-subfamily proteins from human and mouse samples in order to obtain microRNA bonded to each. Proteins obtained can also be confirmed with Western blot.

Antigen	Cross-reactivity	Description	Clone No.	Subclass	Application	Concentration
Ago1	Human, Mouse	Anti Ago1, Monoclonal antibody	2A7	$IgG_{2a\cdot k}$	IP, ICC, RIP	1.0mg/mL
		Anti Ago1, Monoclonal antibody	1F2	$IgG_{2a\cdot k}$	WB	
Ago2	Human	Anti Human Ago2, Monoclonal antibody	4G8	IgG_1	IP, WB ICC, RIP	
	Mouse	Anti Mouse Ago2, Monoclonal antibody	2D4	IgG_1	IP, WB ICC, RIP	
Ago3	Human	Anti Human Ago3, Monoclonal antibody	1C12	IgG_1	IP, RIP	
		Anti Ago3, Monoclonal antibody	6-107	$IgG_{1\cdot k}$	WB	
	Mouse	Anti Mouse Ago3, Monoclonal antibody	S09	$IgG_{1\cdot k}$	IP, RIP	
		Anti Mouse Ago3, Monoclonal antibody	S011	$IgG_{1\cdot k}$	WB	
Ago4	Human, Mouse	Anti Ago4, Monoclonal antibody	2G7	IgG_{2b}	IP, RIP	
		Anti Ago4, Monoclonal antibody	2B2	IgG_1	WB	
Ago1–4	Human, Mouse	Anti Ago1–4, Monoclonal antibody	1G3	IgG_1	IP, WB	

Immunoprecipitation of recombinant Ago proteins

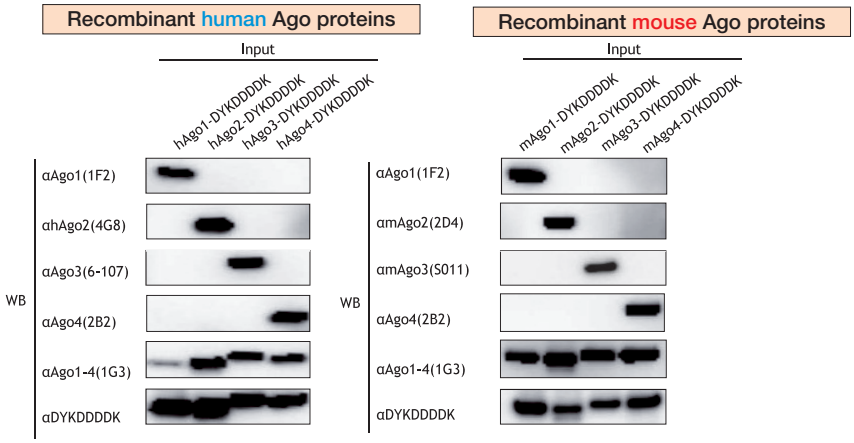
We evaluated immunoprecipitation performance of each antibody using human and mouse recombinant Ago proteins (DYKDDDDK tag was fused to C-terminal). Each anti-Ago1 to anti-Ago4 antibody specifically immunoprecipitated each recombinant Ago protein, demonstrating that anti-Ago1 to anti-Ago4 antibodies are capable of immunoprecipitation of all Ago-subfamily proteins.



[Detection] Primary antibody: Anti-DYKDDDDK Antibody(1E6)
Secondary antibody: Anti-mouse IgG, Peroxidase Conjugated

Western blot analysis of recombinant Ago proteins

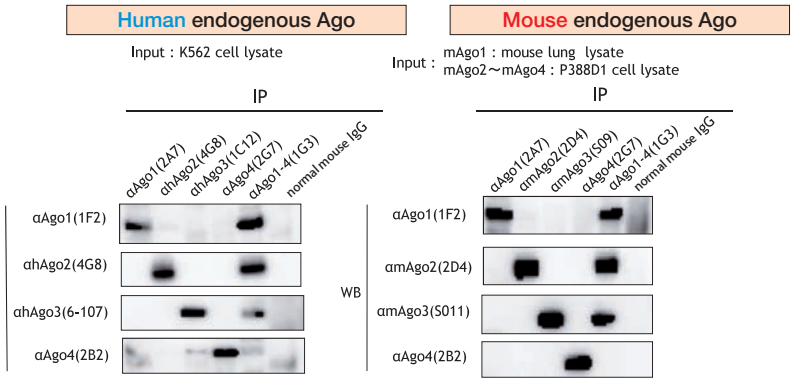
We evaluated Western blot performance of each antibody using human and mouse recombinant Ago proteins (DYKDDDDK tag was fused to C-terminal). Each anti-Ago1 to anti-Ago4 antibody specifically detected each recombinant Ago protein, demonstrating that anti-Ago1 to anti-Ago4 antibodies are capable of detection of all Ago-subfamily proteins.



[Detection] Primary antibody: Each Anti-Ago Monoclonal Antibody
Secondary antibody: Anti-mouse IgG, Peroxidase Conjugated

Endogenous Ago protein immunoprecipitation and Western blot analysis

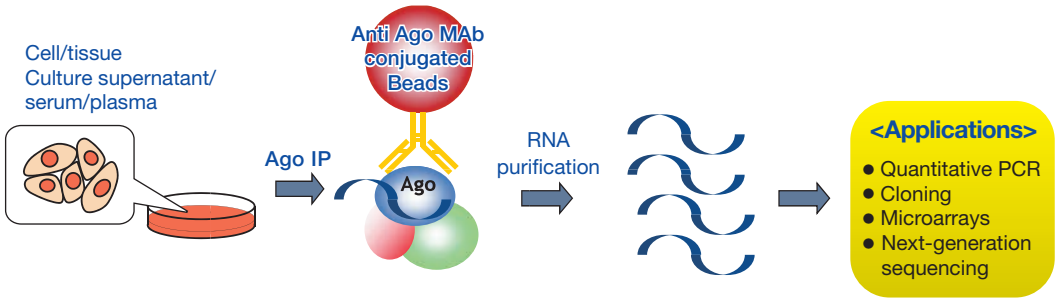
We immunoprecipitated and then used Western blot analysis for detection of endogenous Ago proteins using anti-human Ago antibodies from human K562 cells and anti-mouse Ago antibodies from mouse P388D1 cells or mouse lung tissue. Each anti-Ago1 to anti-Ago4 antibody specifically immunoprecipitated each endogenous Ago protein and used for Western blot analysis, indicating that anti-Ago1 to anti-Ago4 antibodies are capable of immunoprecipitation of endogenous Ago1 to Ago3.



[Detection] Primary antibody: Each Anti-Ago Monoclonal Antibody
Secondary antibody: Anti-mouse IgG, Peroxidase Conjugated

4. microRNA Isolation Kit Series

We offer a microRNA Isolation Kit series to simplify an entire range of procedures from Ago immunoprecipitation to RNA purification. This kit series allows recovery of Ago-bound microRNA and target mRNA from both human and mouse cells and tissues, and free Ago-bound microRNA from culture supernatant, serum, and plasma. Obtained RNA can then be analyzed by quantitative PCR, microarrays, and next-generation sequencing. This series includes MagCapture™ microRNA Isolation Kits, which adopt magnetic beads, and microRNA Isolation Kits, which adopt silica-based beads. Features and benefits of each product are described below.



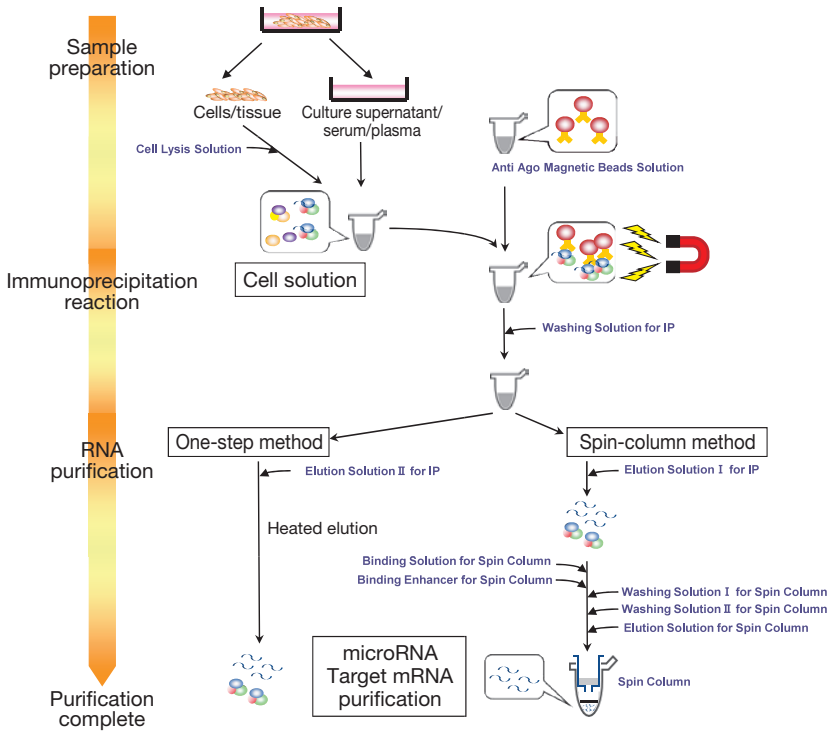
4-1. MagCapture™ microRNA Isolation Kit (magnetic beads)

We offer four types of MagCapture™ microRNA Isolation Kits: three types contain magnetic beads immobilized to anti human Ago2 antibody (4G8), anti mouse Ago2 antibody (2D4), or anti-Ago1-4 antibody (1G3), and one type contain with Protein G magnetic beads which can immobilize an optional anti Ago subfamily antibodies. All of these kits adopt magnetic beads for easy immunoprecipitation. After immunoprecipitation, two protocols for the RNA purification step are selectable: One-step method for obtaining high-yield microRNAs by a simple procedure or Spin column method for obtaining high-purity microRNAs.

Kit contents
(Example: Human Ago2, 10 reactions)

1) Anti-Human Ago2 Magnetic Beads Solution	600µL×1 tube
2) Cell Lysis Solution	20mL×1 tube
3) Washing Solution for IP	40mL×1 tube
4) Elution Solution I for IP	500µL×1 tube
5) Elution Solution II for IP	500µL×1 tube
6) Binding Solution for Spin Column	2mL×1 tube
7) Binding Enhancer for Spin Column	100µL×1 tube
8) Washing Solution I for Spin Column	3mL×1 tube
9) Washing Solution II for Spin Column	4mL×1 tube
10) Elution Solution for Spin Column	1mL×1 tube
11) Spin Column/Collection Tube	10 tubes

Outline



Description	Antibody clone no.	Animal	Compatible samples	Recovered RNA	IP B/F separation method	RNA purification method
MagCapture™ microRNA Isolation Kit, Human Ago2	4G8	Human, monkey, dog	Cell, tissue, serum, plasma	microRNA and target mRNA	Magnetic separation	Spin column or one-step
MagCapture™ microRNA Isolation Kit, Mouse Ago2	2D4	Mouse, rat, dog				
MagCapture™ microRNA Isolation Kit, Ago 1-4	1G3	Human, monkey, dog, mouse, rat		microRNA		
MagCapture™ microRNA Isolation Kit, Protein G	Any	Depends on antibody	Cell, tissue	Depends on antibody		Spin column

Recovering microRNA from human cell strains

We used MagCapture™ microRNA Isolation Kit, Human Ago2 and a previous product to obtain microRNA from human K562 cells (1×10^7 cells), then compared quantities of microRNA obtained with denaturing polyacrylamide gel electrophoresis (silver staining) (Fig. 1) and quantitative PCR (miR-92a quantity) (Fig. 2). Results showed that the two products yielded equivalent quantities of microRNA.

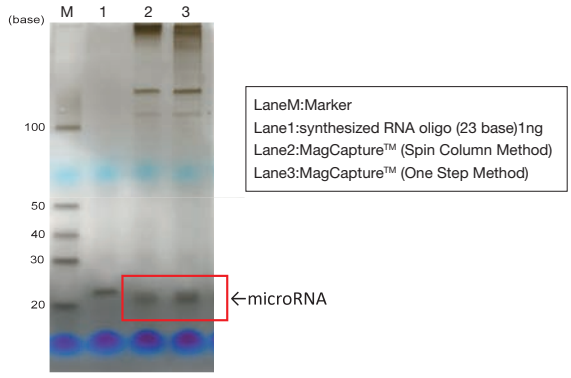


Figure 1 Detection of purified microRNAs from human K562 cells by silver staining

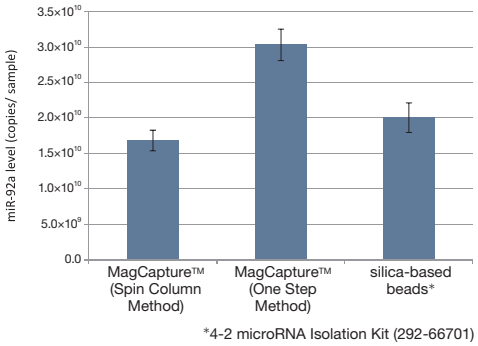


Figure 2 Comparison of recovery quantities of purified microRNAs from human K562 cells

Recovering free Ago2-bound microRNA from human plasma

We used MagCapture™ microRNA Isolation Kit, Human Ago2 and a previous product to obtain microRNA from pooled plasma of healthy individuals, then compared quantities obtained with quantitative PCR (miR-92a quantity). As shown below, using the spin column method with the MagCapture™ resulted in approximately equivalent yield to our previous product, while the one-step method demonstrated greater quantities of microRNA recovered compared to our previous product.

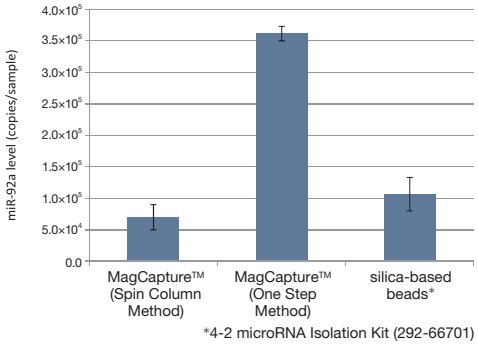


Figure 3 Comparison of recovery quantities of purified microRNAs from human plasma

Recovering target mRNA of microRNA

Either miR-122 or the control of luciferase siRNA (Luc siRNA) were delivered to HepG2, a liver cancer cell line with low expression of miR-122, then immunoprecipitated Ago2 IP RNA obtained using either MagCapture™ microRNA Isolation Kit, Human Ago2 or our previous product. Total RNA was prepared with ISOGEN. Quantitative PCR was then used to compare quantities of miR-122 (Fig. 4) and its target mRNA (Aldo A corrected with GAPDH mRNA) (Fig. 5). Results showed that miR-122 and Aldo A mRNA were concentrated in both MagCapture™ and our previous kit, while the former also allowed recovery of microRNA's target mRNA. In contrast, total RNA obtained with ISOGEN showed decreased quantities of Aldo A mRNA due to cleavage accompanying increase of miR-122.

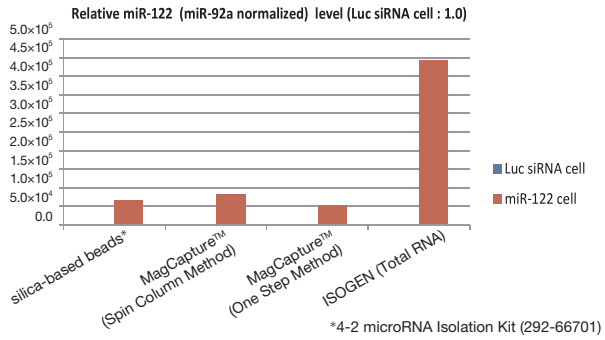


Figure 4 Comparison of recovery quantities of miR-122

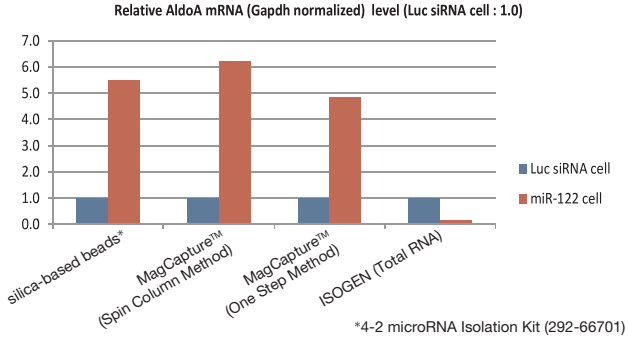
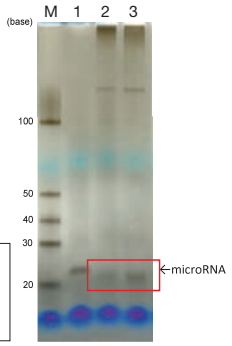


Figure 5 Comparison of recovery quantities of target mRNA

MagCapture™ microRNA Isolation Kit, Mouse Ago2

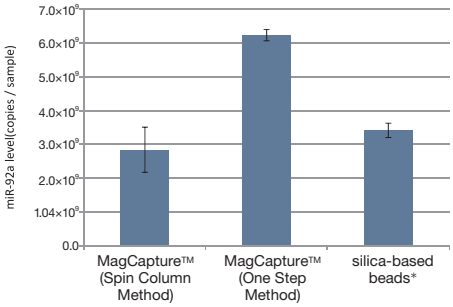
Recovering microRNA from mouse cell strains

We used MagCapture™ microRNA Isolation Kit, Human Ago2 and a previous product to obtain microRNA from mouse P388D1 cells (2×10^7 cells), then compared quantities of microRNA obtained with denaturing polyacrylamide gel electrophoresis (silver staining) (Fig. 1) and quantitative PCR (miR-92a quantity) (Fig. 2). Results showed that the two products yielded equivalent quantities of microRNA.



LaneM:Marker
Lane1:synthesized RNA oligo (23 base)1ng
Lane2:MagCapture™ (Spin Column Method)
Lane3:MagCapture™ (One Step Method)

Figure 1 Detection of purified microRNAs from mouse P388D1 cells by silver staining

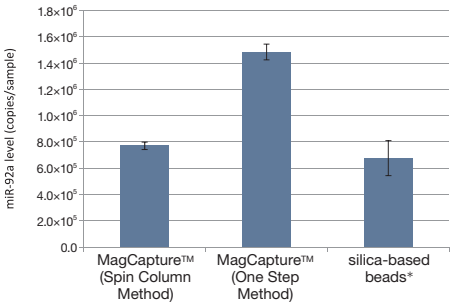


*4-2 microRNA Isolation Kit (292-67301)

Figure 2 Comparison of recovery quantities of purified microRNAs from mouse P388D1 cells

Recovering free Ago2-bound microRNA from rat plasma

We used MagCapture™ microRNA Isolation Kit, Mouse Ago2 and a previous product to obtain microRNA from 200 µL rat plasma, then compared quantities obtained with Quantitative PCR (miR-92a quantity). As shown below, using the spin column method with the MagCapture™ resulted in approximately equivalent yield to our previous product, while the one-step method demonstrated greater quantities of microRNA recovered compared to our previous product.



*4-2 microRNA Isolation Kit (292-67301)

Figure 2 Comparison of recovery quantities of purified microRNAs from mouse plasma

MagCapture™ microRNA Isolation Kit, Ago 1-4

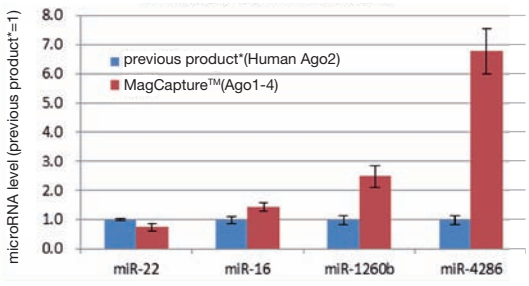
Recovering Ago1-4-bound microRNA

We used a microarray (*3D-Gene*®, Toray) to compare microRNA expression profile of RNA fractions obtained from HeLa cells by immunoprecipitation of anti-Ago1 to anti-Ago4 antibodies. While large quantities of miR-22 and miR-16 were incorporated into Ago2, large quantities of miR-1260b and miR-4286 were incorporated into Ago1, Ago3, and Ago4 (see table below).

Based on these results, we decided to study whether MagCapture™ could recover microRNA binding Ago1, Ago3, or Ago4 at high levels, which was difficult with our previous product (microRNA Isolation Kit, Human Ago2). Both products were used to recover microRNA from HeLa cells (1×10^7 cells), and quantitative PCR used to measure quantities of miR-22, miR-16, miR-1260b, and miR-4286 recovered, using miR-92a as an internal standard. Results showed equivalent recovery between the two products of miR-22 and miR-16, which are shown present in Ago2 in high levels, while MagCapture™ microRNA Isolation Kit, Ago1-4 recovered miR-1260b and miR-4286 at higher levels than the previous product (see below). These results show that the new MagCapture™ microRNA Isolation Kit, Ago1-4 is capable of the detection of not only microRNA binding Ago2, but also in Ago1, Ago3, and Ago4.

	Signal intensity				Signal ratio		
Name	Ago1 IP	Ago2 IP	Ago3 IP	Ago4 IP	Ago2/Ago1	Ago2/Ago3	Ago2/Ago4
hsa-miR-22	3521.4	21696.5	3771.2	292.4	6.16	5.75	74.20
hsa-miR-16	6886.3	28527.2	5013.4	582.5	4.14	5.69	48.97
hsa-miR-1260b	2060.3	540.9	1270.6	1977.9	0.26	0.43	0.27
hsa-miR-4286	678.8	202.4	2019.2	471.2	0.30	0.10	0.43

Table 1 Result of microarray analysis



*4-2 microRNA Isolation Kit (292-66701)

Figure 1 Comparison of quantities of each microRNAs from HeLa cells

Recovering microRNA from human and mouse cell lines

MagCapture™ microRNA Isolation Kit, Ago1-4 and our previous product (microRNA Isolation Kit, Human Ago2 or Mouse Ago2) were used to recover microRNA from human K562 cells (1×10^7 cells) and mouse P388D1 cells (2×10^7 cells). Quantitative PCR (miR-92a quantity) comparisons shown below indicate that MagCapture™ recovered more microRNA more than the previous product.

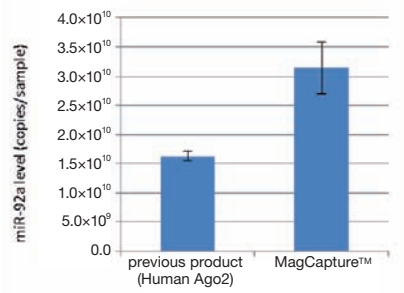


Figure 2 Comparison of recovery quantities of purified microRNAs from human K562 cells

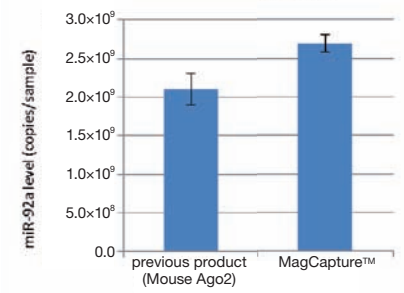


Figure 3 Comparison of recovery quantities of purified microRNAs from mouse P388D1 cells

Recovering free Ago-binding microRNA from human and rat plasma

MagCapture™ microRNA Isolation Kit, Ago1-4 and our previous product (microRNA Isolation Kit, Human Ago2 or Mouse Ago2) were used to recover microRNA from 200 µL human and rat plasma. Quantitative PCR (miR-92a quantity) comparisons shown below indicate that MagCapture™ recovered microRNA more than the previous product.

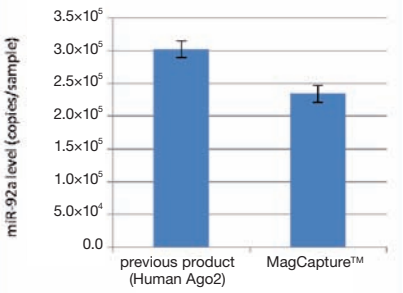


Figure 4 Comparison of recovery quantities of purified microRNAs from human plasma

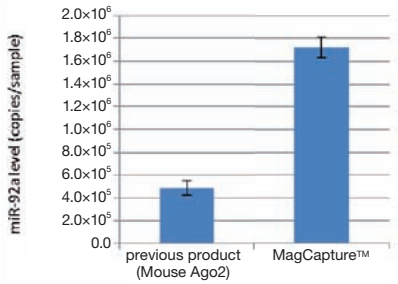


Figure 5 Comparison of recovery quantities of purified microRNAs from rat plasma

Recovering microRNA from various animal cell lines

MagCapture™ microRNA Isolation Kit, Human Ago2, Mouse Ago2, and Ago1–4 were used to immunoprecipitate microRNA from K562 cells, monkey COS-7 cells, dog MDCK cells, mouse P388D1 cells, and rat SCC-131 cells (each: 2×10^6 cells). On the other hand, ISOGEN was used to obtain total microRNA fractions from the same cell samples.
As a result of comparison of relative value of miR-92a quantities between each Ago IP fractions and total RNA fractions, MagCapture™ recovered microRNAs from all cell types.

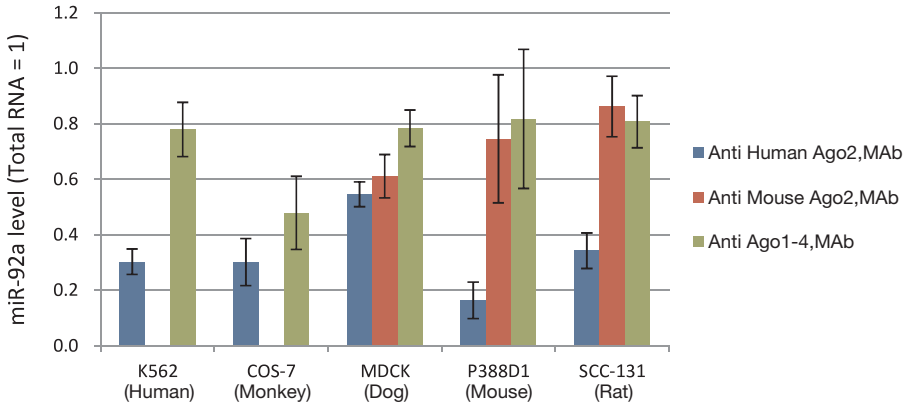
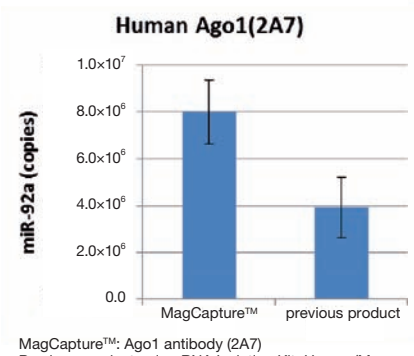


Figure 6 Comparison of recovery quantities of purified microRNAs from each cells

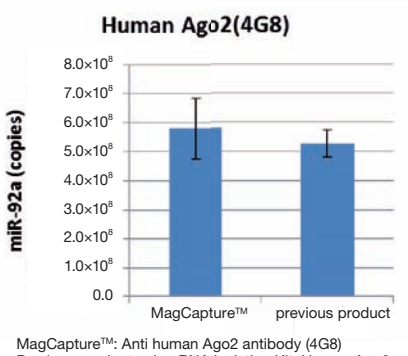
Recovering microRNA from human and mouse cell lines (product comparison)

MagCapture™ microRNA Isolation Kit, ProteinG (using anti-Ago1 (2A7), anti-Ago2 (4G8), and anti-Ago3 (1C12) antibodies) and our previous products were used to obtain microRNA from human K562 cells (1×10^7 cells). Figure 1, 2, 3 shows quantitative PCR (miR-92a quantity) comparisons of quantities of microRNA recovered. Next, MagCapture™ (using mouse anti-Ago2 antibodies (2D4) and our previous product were used to obtain microRNA from mouse P388D1 cells (2×10^7 cells) (Fig. 4). Results show that MagCapture™ with an optional anti-Ago antibody enables microRNA recovery superior to that of our previous product.



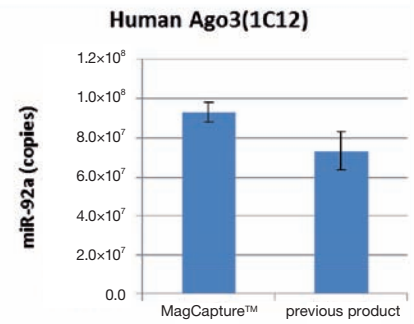
MagCapture™: Ago1 antibody (2A7)
Previous product: microRNA Isolation Kit, Human/Mouse Ago1 (291-70201)

Figure 1 Comparison of recovery quantities of purified microRNAs
Immobilized antibody: Anti-Human Ago1(2A7)



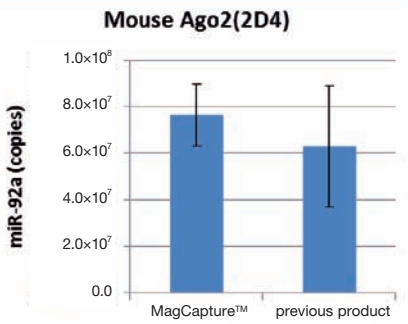
MagCapture™: Anti human Ago2 antibody (4G8)
Previous product: microRNA Isolation Kit, Human Ago2 (292-66701)

Figure 2 Comparison of recovery quantities of purified microRNAs
Immobilized antibody: Anti-Human Ago2(4G8)



MagCapture™: Anti human Ago3 antibody (1C12)
Previous product: microRNA Isolation Kit, Human Ago3 (297-70301)

Figure 3 Comparison of recovery quantities of purified microRNAs
Immobilized antibody: Anti-Human Ago3(1C12)

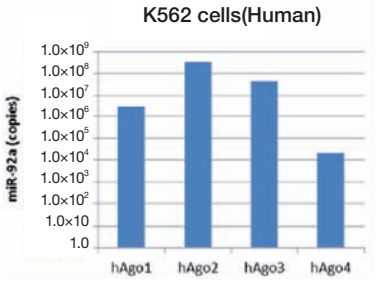


MagCapture™: Anti mouse Ago2 antibody (2D4)
Previous product: microRNA Isolation Kit, Mouse Ago2 (292-67301)

Figure 4 Comparison of recovery quantities of purified microRNAs
Immobilized antibody: Anti-Mouse Ago2(2D4)

Recovering different types of Ago-binding microRNA from human and mouse cell lines

MagCapture™ microRNA Isolation Kit, ProteinG (using anti-Ago1 (2A7), anti-Ago2 (4G8), anti-Ago3 (1C12), and anti-Ago4 (2G7) antibodies) were used to obtain microRNA from human K562 cells (1×10^7 cells). Quantities of microRNA were measured with denaturing polyacrylamide gel electrophoresis (silver staining) and quantitative PCR (miR-92a quantity) (Fig. 5). Next, MagCapture™ (using anti-Ago1 (2A7), anti-Ago2 (2D4), anti-Ago3 (S09), and anti-Ago4 (2G7) antibodies) was used to obtain microRNA from mouse P388D1 cells (2×10^7 cells). Quantities of microRNA were measured with denaturing polyacrylamide gel electrophoresis (silver staining) and quantitative PCR (miR-92a quantity) (Fig. 6). Results show that MagCapture™ with an optional anti-Ago antibody enables recovery of each Ago-binding microRNA.



hAgo1: Anti Ago1 monoclonal antibody (2A7)
hAgo2: Anti human Ago2 monoclonal antibody (4G8)
hAgo3: Anti human Ago3 monoclonal antibody (1C12)
hAgo4: Anti Ago4 monoclonal antibody (2G7)

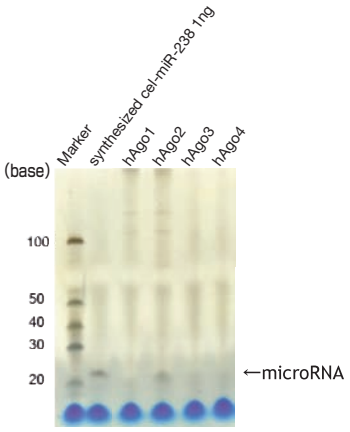
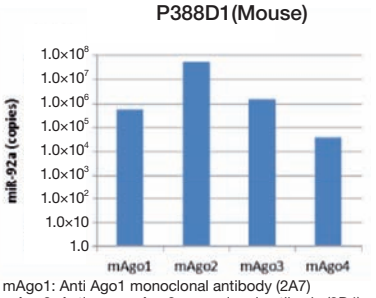


Figure 5 Comparison of recovery quantities of purified microRNAs from human K562 cells and detection by silver staining



mAgo1: Anti Ago1 monoclonal antibody (2A7)
mAgo2: Anti mouse Ago2 monoclonal antibody (2D4)
mAgo3: Anti mouse Ago3 monoclonal antibody (S09)
mAgo4: Anti Ago4 monoclonal antibody (2G7)

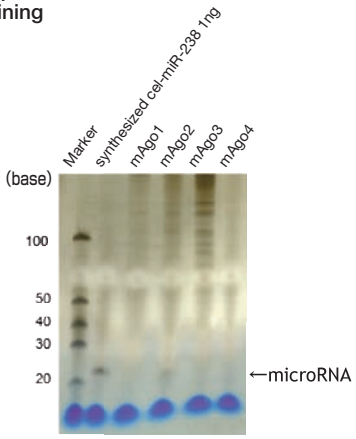
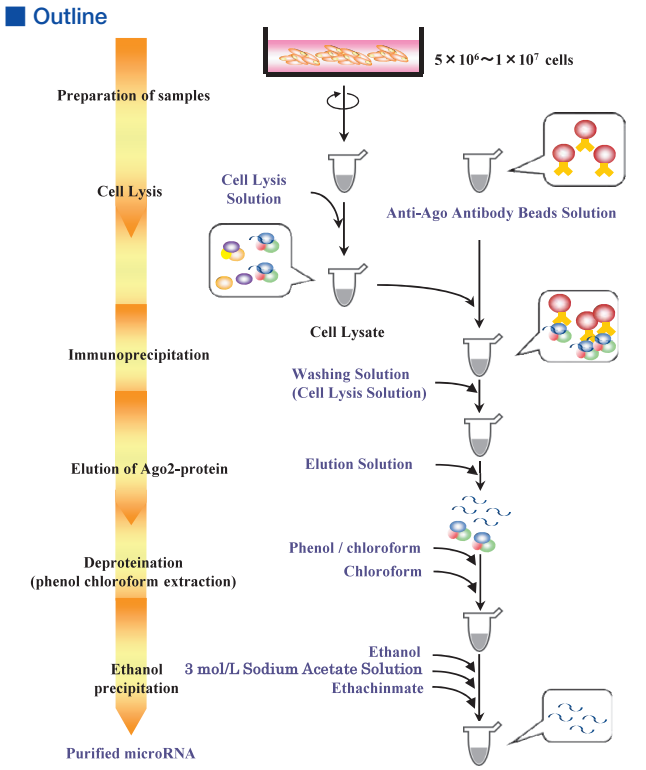


Figure 6 Comparison of recovery quantities of purified microRNAs from mouse P388D1 cells and detection by silver staining

4-2. microRNA Isolation Kit (silica-based beads)

Our microRNA Isolation Kit uses anti-Ago antibodies conjugated to silica-based polymer beads with low non-specific absorbency for centrifuge immunoprecipitation. Post-immunoprecipitation RNA purification steps are performed by phenol/chloroform and ethanol precipitation. We offer a lineup of four types: anti-Ago1 antibody (2A7), anti human Ago2 antibody (4G8), anti mouse Ago2 antibody (2D4), and anti human Ago3 antibody (1C12).

Outline



Kit contents (Example: Human Ago2, 10 reactions)

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

Description	Antibody clone no.	Animal	Compatible samples	Recovered RNA	IP B/F separation method	RNA purification method
microRNA Isolation Kit, Human/Mouse Ago1	2A7	Human, mouse	Cells, tissue	microRNA and target mRNA	Centrifuge	Phenol/ chloroform
microRNA Isolation Kit, Human Ago2	4G8	Human, monkey, dog				
microRNA Isolation Kit, Mouse Ago2	2D4	Mouse, rat				
microRNA Isolation Kit, Human Ago3	1C12	Human				

Application Data

The microRNA Isolation Kit, Human Ago2 was used to purify microRNAs from human HeLa, HepG2, and HEK293 cell lines (5 × 10^6 cells) and mouse P388D1 cell lines (5 × 10^6 cells). The purified microRNAs were detected by silver staining (311-03961, CLEAR STAIN Ag, 20stains) after Urea-PAGE. As a result, the microRNA Isolation Kit, Human Ago2 purified microRNAs specifically from human HeLa, HepG2, and HEK293 cell lines. In addition, the microRNA Isolation Kit, Mouse Ago2 was used to purify microRNAs from human HeLa cell lines (5 × 10^6 cells) and rodent P388D1, CHO, PC-12 cell lines (5 × 10^6 cells). The purified microRNAs were detected by the previous described method. As a result, the microRNA Isolation Kit, Mouse Ago2 purified microRNAs specifically from rodent P388D1, CHO, PC-12 cell lines.

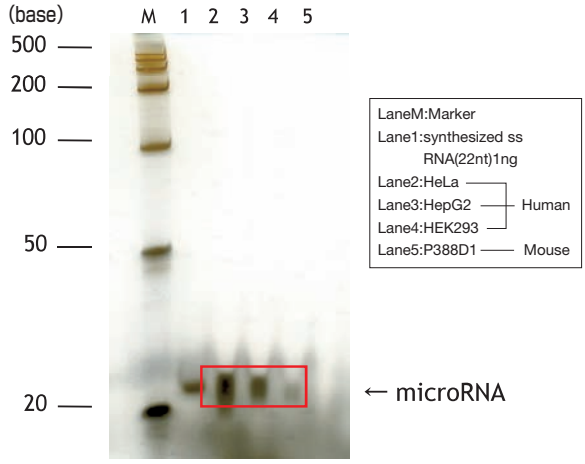


Figure 1 Detection of microRNA purified with Human Ago2 kit by silver staining.

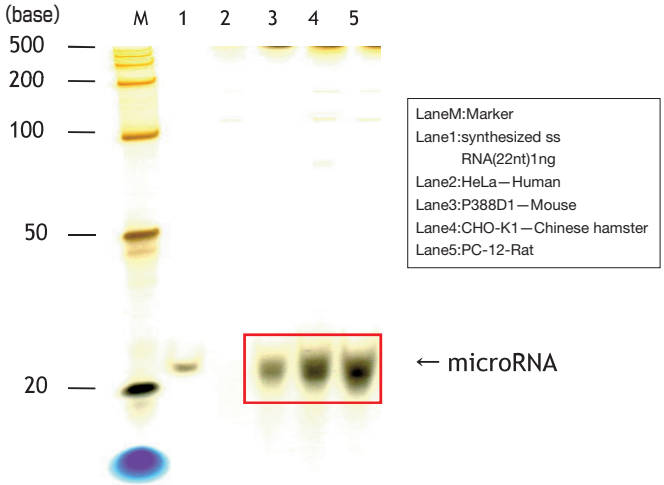


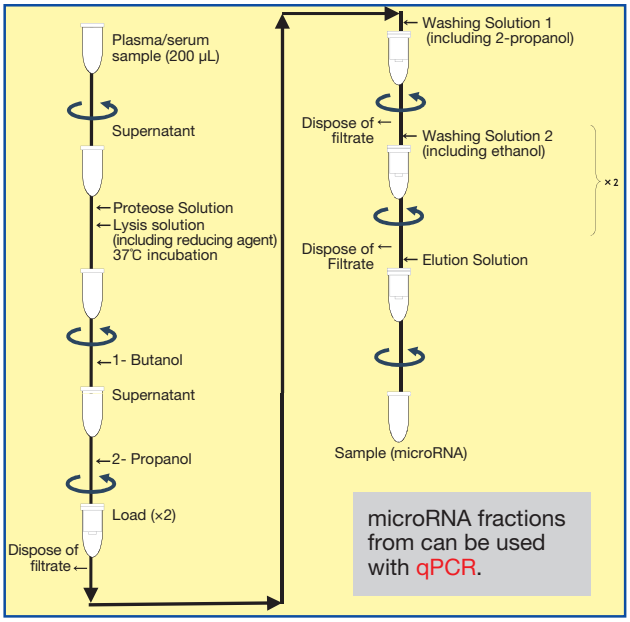
Figure 2 Detection of microRNA purified with Mouse Ago2 kit by silver staining.

5. Total RNA Purification

5-1. microRNA Extractor® SP Kit

This kit is for extraction of microRNA from serum or plasma of humans and animals. It enables microRNA extraction without use of phenol, chloroform, and other hazardous reagents required in previous methods.

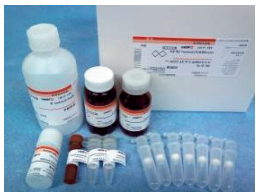
Protocol flow



Kit contents

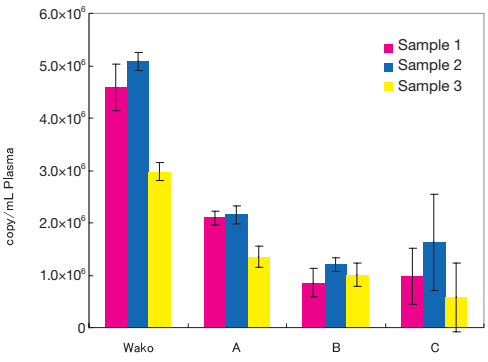
Lysis Solution	20 mL x 1
Reducing reagent	60 µL x 1
Protease Solution	600 µL x 1
Enhancer	600 µL x 1
Washing Solution 1	12 mL x 1
Washing Solution 2	22.5 mL x 1
Elution Solution	4 mL x 1
Spin column/2 mL tube	50 sets

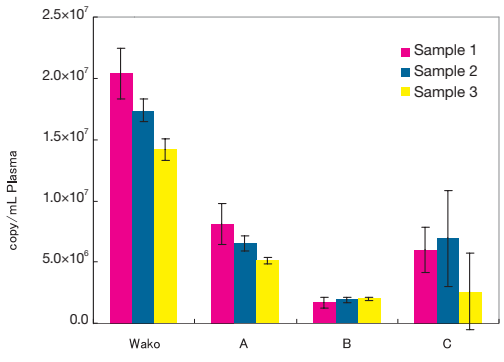
The following necessary reagents are not included.
1-Butanol (code No.022-16035)
2-Propanol (code No.168-21675)
Ethanol (code No.054-07225)



For plasma coagulation, please use EDTA or citric acid. Using heparin inhibit PCR detection after RNA extraction.

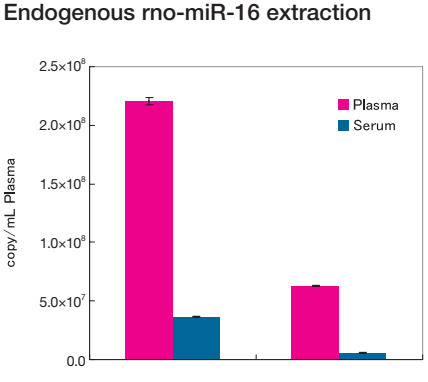
Extraction from normal human plasma





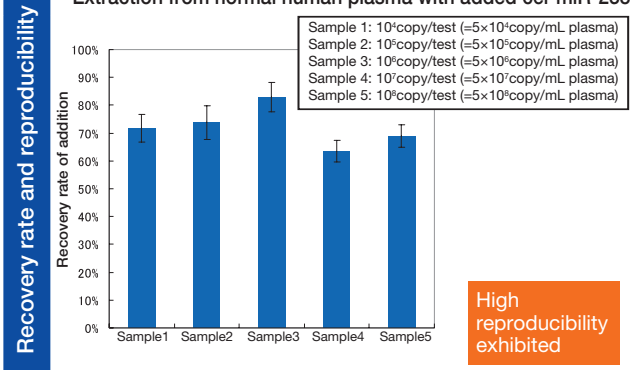
Higher extraction rates observed than from previous methods

Extraction from rat plasma and serum



Extraction from animal experiments possible

Recovery rate and reproducibility



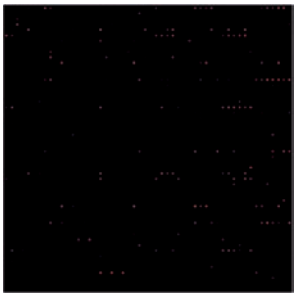
High reproducibility exhibited

Code No.	Description	Package size
295-71701	microRNA Extractor® SP Kit	50 Extractions

5-2. ISOGEN® II

ISOGEN® II is a reagent for extracting total RNA or small RNA from animal tissue or cultured cells. It is a uniform fluid containing phenol and guanidine that can isolate RNA in a single step through interactions with cellular components. There is not require to separate the liquid phase with chloroform as previous products (ISOGEN® or ISOGEN®-LS).

ISOGEN®



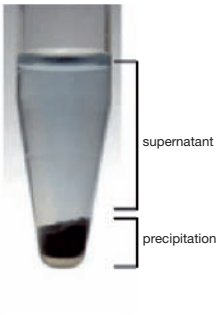
Effective spots: 638 microRNA

ISOGEN® II

Effective spots: 688 microRNA

Total RNA was extracted with ISOGEN® and ISOGEN® II, then analyzed with a *3D-Gene*® Human microRNA Oligo chip (Toray).

Results show ISOGEN® II efficacy in small RNA extraction.

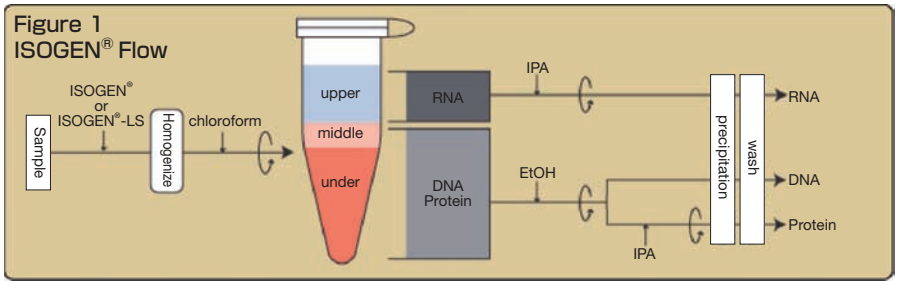


	Code No.	Description	Package size
Ref	317-07363	ISOGEN® II	10ml
Ref	311-07361		100ml

5-3. ISOGEN®　5-4. ISOGEN®-LS

ISOGEN® and ISOGEN®-LS enable the isolation of undamaged RNA in high yield about 1 hour without contaminating DNA or protein. Therefore, Northern analysis or dot blot hybridization can be performed without DNase or other processing (Fig. 1).

Figure 1
ISOGEN® Flow



Sample			Yield
Tissue	Mouse	Liver	6–10 µg RNA/mg tissue
		Spleen	6–10 µg RNA/mg tissue
		Kidney	3–4 µg RNA/mg tissue
		Skeletal	1–1.5 µg RNA/mg tissue
		Brain	1–1.5 µg RNA/mg tissue
		Placenta	1–4 µg RNA/mg tissue
	Rat, pituitary gland, or human liver needle biopsy sample		4–8 µg RNA/mg tissue
Plant	Arabidopsis or tobacco		150–200 µg RNA/0.5–1 g weight tissue
Cultured cells	Epithelial		8–15 µg RNA/10^6 cell
	Fibroblasts		5–7 µg RNA/10^6 cell
Blood	Human or animal		7–15 µg RNA/mL blood

	Code No.	Description	Package size
Ref	315-02504	ISOGEN®	10ml
Ref	317-02503		50ml
Ref	311-02504		100ml

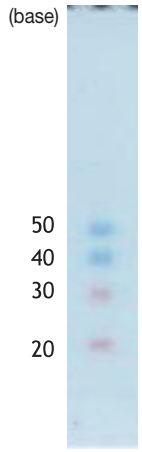
	Code No.	Description	Package size
Ref	317-02623	ISOGEN®-LS	10ml
Ref	311-02621		100ml

6. microRNA Analysis Tools

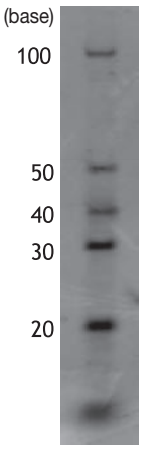
Electrophoresis

6-1. SuperSep RNA, 15%, 17-Well

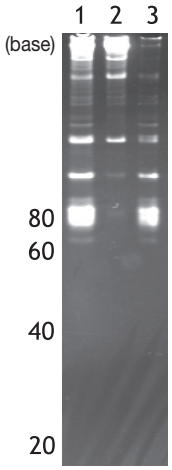
This product is an polyacrylamide gel with 4M urea. It is a ready-to-use precast gel, so it does not require steps such as dissolving urea or preparing gels. Acrylamide concentration is 15%.



Sample: RNA Prestaine Marker
Running buffer: TBE Buffer
Current: 10 mA constant

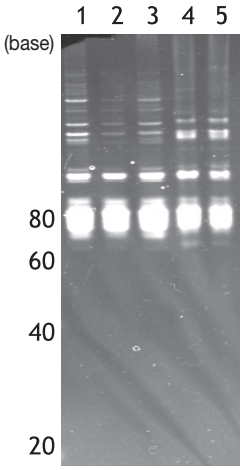


Sample: RNA Marker
Running buffer: 0.5×TBE Buffer
Current: 10 mA constant
Stain: Silver
Exposure time: 5 min



1: Total RNA 2.5µg
2: High-molecule RNA 2.5µg
3: Small RNA 250ng
EtBr staining

RNA extracted from HeLa cells according to ISOGEN II protocol before performing polyacrylamide gel electrophoresis.



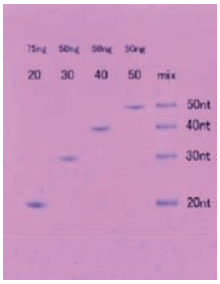
1 Mouse brain
2 Mouse liver
3 Mouse kidney
4 HeLa cells
5 Jurkat cells
Each sample: 500 ng run
EtBr stain

Small RNA extracted from various samples using ISOGEN II (with optional operations) before performing polyacrylamide gel electrophoresis.

	Code No.	Description	Package size
Ref	194-15881	SuperSep™ RNA, 15%, 17well	5 sheets

6-2. R-Mark™ Small RNA Marker (20–50 nt)

This product is consisted of four types of single-chain RNA (20, 30, 40, 50 nt). Use after mixing with RNA loading buffer.



Recommended quantity: 1 µL/lane (50 times)
Component: Water (RNase free)
Total RNA quantity: 112.5 µg/mL
RNA quantity in each band/µL: 20 nt (37.5 ng), 30 nt, 40 nt, 50 nt (25 ng each)

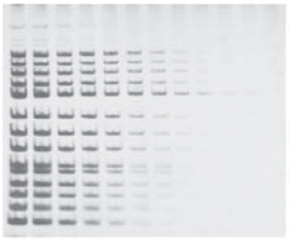
	Code No.	Description	Package size
F	182-02711	R-Mark™ small RNA Marker	50 µl

6-3. CLEAR STAIN Ag

CLEAR STAIN Ag is a silver staining kit developed especially for nucleic acids (DNA/RNA), enabling high sensitivity detection after polyacrylamide gel electrophoresis with low background noise. This method offers 50 to 100 higher sensitivity than ethidium bromide staining (measured at 302 nm), allowing detection of 20 to 50 pg DNA per band.

Kit contents (140×140×1mm 6% polyacrylamide gel, 20 stains)

1) Fixing solution (×20): CTAB, ethanol, 200 mL
2) Ammonia solution (×20): ammonia, 200 mL
3) Staining solution A (×20): silver nitrate, 200 mL
4) Staining solution A (×20): sodium hydroxide, ammonia, 200 mL
5) Developing solution A(×10): sodium carbonate, 200 ml×2 (10× concentrate)
6) Developing solution B(×20): formaldehyde, sodium thiosulfate, 200 mL
7) Preserving solution (×20): glycerol, acetic acid, 200 mL
8) Product manual



Sample: Marker 9 (ϕ X174/Hinf I digest)
Sample quantity: From left: 500, 250, 125, 63, 32, 16, 8, 4, 2, 1, 0.5, 0.25 ng/lane
Buffer: TBE
Gel size: 140×140×1 mm (6% polyacrylamide gel)

Code No.	Description	Package size
311-03961	Clear Stain Ag	20 stains

6-4. 5×TBE

Compo-nents 0.445 mol/l Tris-borate, 10 mmol/l EDTA

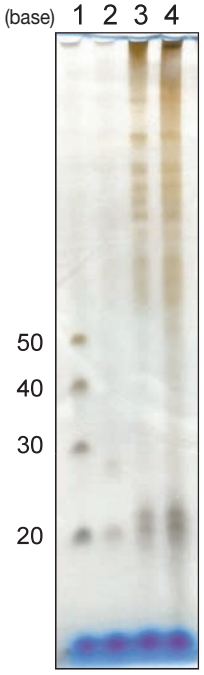
Storage Room temp.

Comments
Dilution amounts
Agarose gel electrophoresis: About 10×
Polyacrylamide gel electrophoresis: About 5×

Code No.	Description	Package size
318-90041	5×TBE	1,000 ml

Usage Example of Electrophoresis Products

MagCapture™ microRNA Isolation Kit, Human Ago1-4 was used to obtain an RNA fraction from human K562 cells (1×10^7 cells), then denaturing polyacrylamide gel electrophoresis performed with SuperSep RNA, 15%, 17-well, with CLEAR STAIN Ag used for silver staining detection. Result show the detection of microRNA of about 22 bp in the RNA fraction.



Lane 1: R-Mark™ Small RNA Marker 1µL 1/50 diluted fluid
Lane 2: Control siRNA duplex, Firefly Luciferases GL3(22mer) 0.5ng
Lane 3: MagCaputure™ microRNA Isolation Kit, Ago1-4 Spin column method
Lane 4: MagCaputure™ microRNA Isolation Kit, Ago1-4 One-step method

Gel: SuperSep™ RNA, 15%, 17-well
Running buffer: 0.5×TBE
Current: 10 mA constant for 75 min.
Stain: Clear Stain Ag (staining time: 60 min., developing time: 5 min.)

6-5. microRNA Cloning Kit *Wako*

microRNA Cloning Kit *Wako* adopt our original buffer solution which allows two reactions in the same solution: dephosphorylation by shrimp alkaline phosphatase (SAP), which is easily inactivated with heat treatment; and efficient adaptor ligation of single-strand DNA/RNA with a thermostable ligase (sold separately). This allows simple and efficient adaptor ligation reactions, enabling easy cDNA synthesis from microRNA.

Features

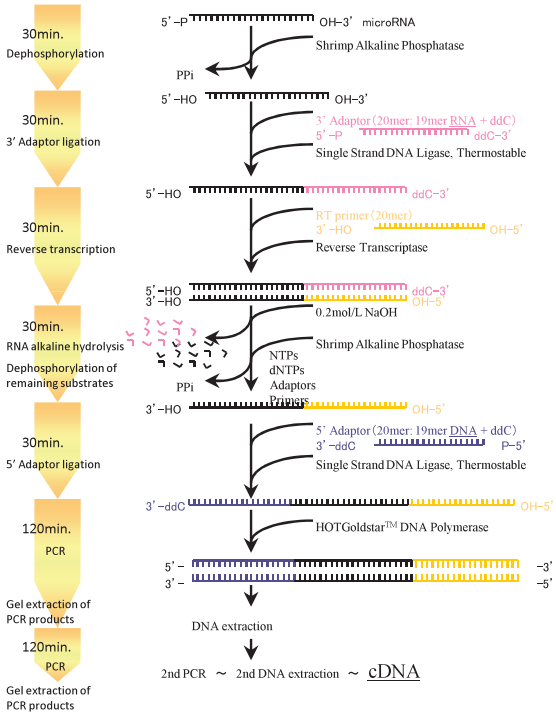
1. Highly-efficient and accurate adaptor ligation by thermostable ligase.
2. Suitable for cloning of microRNA forming secondary structures.
3. Reduced RNA operations means cDNA coding microRNA can be synthesized within 1.5 days.

Kit contents (8 reactions)

1) SAP	16µl
2) 5×SAP Buffer	64µl
3) 40×Ligation Buffer	16µl
4) RNase Inhibitor	16µl
5) 10mmol/L $MnCl_2$	16µl
6) Reverse Transcriptase	8µl
7) 10×RT Buffer	16µl
8) dNTP Mixture	112µl
9) 0.5 mol/L EDTA, pH 8.0	16µl
10) 1mol/L Tris-HCl, pH 7.5	160µl
11) Ethachinmate	24µl
12) 10mol/L Ammonium Acetate	960µl
13) 3'Adaptor(50pmol/µ L)	8µl
14) 5'Adaptor(50pmol/µ L)	8µl
15) RT Primer(50pmol/µ L)	8µl
16) 5'PCR Primer(50pmol/µ L)	16µl
17) 3'PCR Primer(50pmol/µ L)	16µl
18) Control RNA(30ng/µ L)	8µl

Please use Single Strand DNA Ligase, Thermostable Recombinant Solution (sold separately)from EPICENTRE with microRNA Cloning Kit *Wako*. Buffer components and reaction conditions are all optimized for this enzyme, which can be used in ligation reactions for both DNA and RNA (ATP-dependent). Optimal reaction temperature is 55-65℃, enabling significantly more efficient adaptor ligation of microRNA than with T4 RNA ligase.

Outline

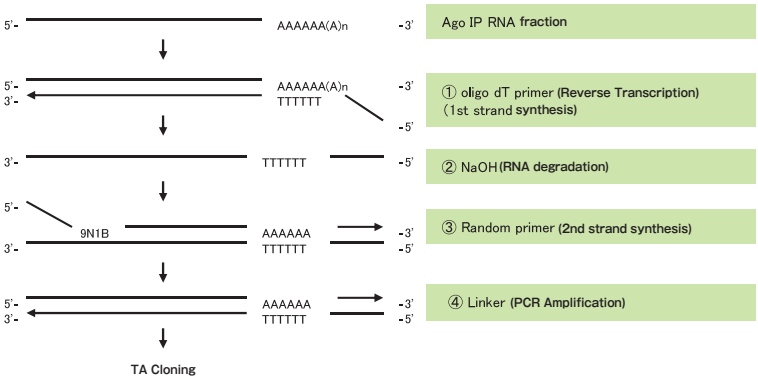


Code No.	Description	Package size
290-66501	microRNA Cloning Kit *Wako*	8 reactions

6-6. Target mRNA Cloning Kit *Wako*

Target mRNA Cloning Kit *Wako* is used for amplification of cDNA from target mRNA.
This kit is used for generating cDNA from mRNA interacting with microRNA in RNA fractions in immunoprecipitated Ago protein. DNA sequences obtained from cDNA synthesized from this mRNA and then cloned can be used for screening target mRNA candidates of microRNA. Using this kit in combination with Ago immunoprecipitation enables screening of target mRNA of microRNA not with previous database-based prediction, but with molecular biology-based methods.

Principles



Kit contents (10 reactions)

1) dT(20)-RT Primer(20pmol/µL) 10µL×1
2) 10×RT Buffer 20µL×1
3) dNTP Mixture Solution (2.5mmol/l each) 20µL×1
4) RNase Inhibitor(20U/µL) 10µL×1
5) Reverse Transcriptase(200U/µL) 10µL×1
6) 0.5 mol/L EDTA Solution 20µL×1
7) 1mol/L Tris-HCl Solution 200µL×1
8) Ethachinmate 30µL×1
9) 10mol/L Ammonium Acetate Solution 480µL×1
10) 2nd Strand Synthesis Primer(20pmol/µL) 10µL×1
11) 5' PCR Primer(20pmol/µL) 10µL×1
12) 3' PCR Primer(20pmol/µL) 10µL×1
13) 5' Colony PCR Primer(5pmol/µL) 960µL×1
14) 3' Colony PCR Primer(5pmol/µL) 960µL×1

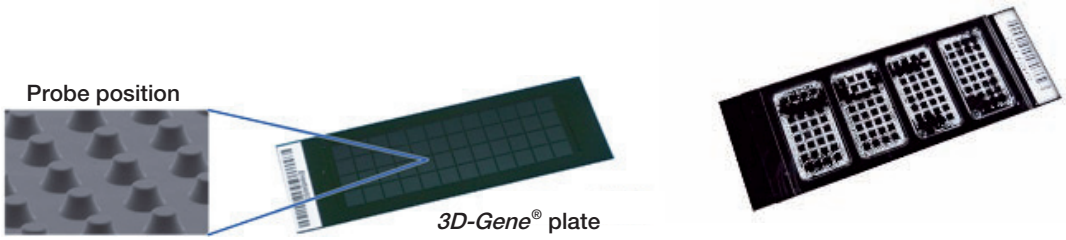
Code No.	Description	Package size
298-68001	Target mRNA Cloning Kit *Wako*	10 reactions

Microarray Analysis

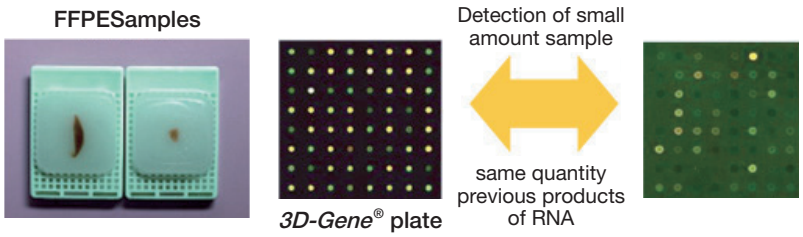
6-7. Toray *3D-Gene*®

Please send us an RNA sample, and we will perform mRNA or microRNA gene expression analysis using the *3D-Gene*® chip. We also offer consultations on statistical or biological analysis services by using analyzed data.

- High sensitivity, reproducibility, and accuracy compared to traditional methods
- Low noise through black resin and 3D pillar probe structure
- Surface covered on nano-level with dense layer of oligo DNA probes
- Promotes hybridization reaction with microbead agitation



3D-Gene® characteristics make it perfect for analysis of low-expression genes and microRNA, as well as analysis of RNA samples from small specimens, blood, or FFPE (formalin-fixed paraffin embedded).
*Our services begin at RNA extraction step for blood and FFPE samples.



Method	All human genes	All mouse genes	All rat genes	miRNA chip	Custom chip
	DNA chip	DNA chip	DNA chip	Human, mouse, rat	(Creation and analysis services)
2-color	√	√	√	–	√
1-color	√	√	√	√	√

*The microRNA chip is available only in 1-color. * Please inquire about samples FFPE (formalin fixed paraffin embedded) samples or blood samples

■ Sample

Sample	Total RNA	Tissue/cell
Sample quantity	2 µg or more	Please inquire.
Sample concentration purity	0.5 µg/µL or more (recommended)	
	OD_{260}/OD_{280}=1.8~2.0	

■ Turnaround
Turnaround is usually within 1 month, but may differ according to sample quality(In the case of Japan).

■ Price
Please inquire.

■ How to order
Please inquire through the Wako website:
http://www.wako-chem.co.jp → Reagents → Services → Microarray analysis → *3D-Gene*®

■ Notes
- Please use recommended kits for RNA extraction.
- Recommended kits differ for gene expression analysis and microRNA analysis.
- Please send RNA samples dissolved in frozen DNase/RNase-free water, and use a frozen shipping services. Inquire first to determine a delivery time to set. We cannot be responsible for shipping-related problems.
- We may not be able to accept highly infectious samples.
- Please obtain informed consent from subject for any human samples.
- We can only accept P1 level samples as determined by the Ministry of Education, Culture, Sports, Science and Technology's Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms.
- Samples fundamentally cannot be returned.
- This analysis service is intended for research use only. Please do not use for other purposes (medical diagnosis or drugs, food manufacture, testing, etc.)

7. Wako Pure Chemical Industries, Ltd. Publication list

hAgo2(4G8) MAb

Cell. 2014 Jan 16;**156**:146-157.
Inefficient SRP interaction with a nascent chain triggers a mRNA quality control pathway.

microRNA Isolation Kit human / mouse Ago1, and human Ago2

Nucleic Acids Research, 2014,**1-13**.
Novel functional small RNAs are selectively loaded onto mammalian Ago1.

microRNA Cloning Kit Wako, ssDNA Ligase, thermostable

Mol Cell. 2011 Nov 4;**44**(3):424-36.
MCPIP1 Ribonuclease Antagonizes Dicer and Terminates MicroRNA Biogenesis through Precursor MicroRNA Degradation.
Suzuki HI, Arase M, Matsuyama H, Choi YL, Ueno T, Mano H, Sugimoto K, Miyazono K.

Ago1(2A7), hAgo2(4G8), hAgo3(1C12) MAb

RNA Biol. 2011 Jan 1;**8**(1).
Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals
Argonaute association with RNA fragments of diverse origin.
Burroughs AM, Ando Y, Hoon ML, Tomaru Y, Suzuki H, Hayashizaki Y, Daub CO.,

hAgo2(4G8) MAb

PNAS 2011 Mar 29.
MicroRNA let-7 establishes expression of {beta}2-adrenergic receptors and dynamically down-regulates agonist-promoted down-regulation.
Wang WC, Juan AH, Panebra A, Liggett SB.

mAgo2(2D4) MAb

EMBO J. 2011 March 2; **30**(5): 823–834.
Small RNA-mediated regulation of iPS cell generation
Zhonghan Li,Chao-Shun Yang, Katsuhiko Nakashima, and Tariq M Rana

mAgo2(2D4) MAb

Molecular Pharmacology March 22, 2011
RISC bound siRNA is a determinant of RNAi mediated gene silencing in mice.
Wei J, Jones J, Kang J, Card A, Krimm M, Hancock P, Pei Y, Ason B, Payson E, Dubinina N, Cancilla M, Stroh M, Burchard J, Sachs A, Hochman J, Flanagan WM, Kuklin N.

mAgo2(2D4) MAb

Nature structural & molecular biology 2011, 18, 2, Feb
Genome-wide identification of Ago2 binding sites from mouse embryonic stem cells with and without mature microRNA.
Leung AK, Young AG, Bhutkar A, Zheng GX, Bosson AD, Nielsen CB, Sharp PA.

Ago1(2A7), hAgo2(4G8), hAgo3(1C12) MAb

microRNA Isolation Kit, Ago1, Human Ago2 and Human Ago3

Genome Res. August 18, 2010
A comprehensive survey of 39 animal miRNA modification events and a possible role for 39 adenylation in modulating miRNA targeting effectiveness.
Burroughs AM, Ando Y, de Hoon MJ, Tomaru Y, Nishibu T, Ukekawa R, Funakoshi T, Kurokawa T, Suzuki H, Hayashizaki Y, Daub CO.

hAgo2(4G8) MAb

Nucleic Acids Research, 2009, 1–13
Expanded RNA-binding activities of mammalian Argonaute 2.
Tan GS, Garchow BG, Liu X, Yeung J, Morris JP 4th, Cuellar TL, McManus MT, Kiriakidou M.

hAgo2(4G8) MAb

Nature structural & molecular biology 2009 Dec 16;**12**:1259
Distinct passenger strand and mRNA cleavage activities of human Argonaute proteins.Wang B, Li S, Qi HH, Chowdhury D, Shi Y, Novina CD

microRNA Isolation Kit, Human Ago2

BIOLOGY OF REPRODUCTION 2009 81, 717–729
Human Villous Trophoblasts Express and Secrete Placenta-Specific MicroRNAs into Maternal Circulation via Exosomes.
Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, Takizawa T, Shigihara T, Goto T, Izumi A, Ohkuchi A, Matsubara S, Takeshita T, Takizawa T.

hAgo2(4G8) MAb

BMC Research Notes 2009, **2**:169
A useful approach to total analysis of RISC-associated RNA.
Hayashida Y, Nishibu T, Inoue K, Kurokawa T.

hAgo2(4G8) MAb

RNA 2009, **15**:1078–1089
Mammalian GW182 contains multiple Argonaute-binding sites and functions in microRNA-mediated translational repression.
Takimoto K, Wakiyama M, Yokoyama S.

mAgo2(2D4) microRNA Isolation Kit, Human Ago2

Reproduction 2008 Dec;**136**(6):811-22.
MicroRNA (miRNA) cloning analysis reveals sex differences in miRNA expression profiles between adult mouse testis and ovary.
Mishima T, Luo SS, Ishibashi O, Kawahigashi Y, Mizuguchi Y, Ishikawa T, Mori M, Kanda T, Goto T, Takizawa T.

hAgo2(4G8) MAb

Methods Mol Biol. 2008;**442**:29-43.
In vitro RNA cleavage assay for Argonaute-family proteins.
Miyoshi K, Uejima H, Nagami-Okada T, Siomi H, Siomi MC.

hAgo2(4G8) MAb

Nature, 2008 Sep 18;**455**(7211):421-4.
Prolyl 4-hydroxylation regulates Argonaute 2 stability.
Qi HH, Ongusaha PP, Myllyharju J, Cheng D, Pakkanen O, Shi Y, Lee SW, Peng J, Shi Y.

hAgo2(4G8) MAb

PNAS 2008 June 10, vol. 105 no. 23 7964–7969
Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing.
Azuma-Mukai A, Oguri H, Mituyama T, Qian ZR, Asai K, Siomi H, Siomi MC.

hAgo2(4G8) MAb

J Hepatol. 2009 Mar;**50**(3):453-60. Epub 2008 Jul 9.
Regulation of the hepatitis C virus genome replication by miR-199a.
Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K.

and others.....

8. Products List

Other Reagents for microRNA Research

	Code No.	Description	Package size
Ref	317-07363	ISOGEN® II	10ml
Ref	311-07361		100ml
Ref	315-02504	ISOGEN®	10ml
Ref	317-02503		50ml
Ref	311-02504		100ml
Ref	317-02623	ISOGEN®-LS	10ml
Ref	311-02621		100ml
Ref	314-07513	ISOGEN® with Spin Column	10 extractions
Ref	318-07511		60 extractions
Ref	194-15881	SuperSep™ RNA, 15%, 17well	5 gels
F	182-02711	R-Mark™ small RNA Marker	50µl
	311-03961	CLEAR STAIN Ag	20 stains
	318-90041	5×TBE	1,000ml

8. Products List

Monoclonal Antibodies for microRNA Research

	Code No.	Description	Package size
Ref	018-22401	Anti Ago1, Monoclonal Antibody(1F2)	50µl
Ref	015-22411	Anti Ago1, Monoclonal Antibody(2A7)	50µl
Ref	015-22031	Anti Human Ago2, Monoclonal Antibody(4G8)	100µl
Ref	011-22033		50µl
Ref	018-22021	Anti Mouse Ago2, Monoclonal Antibody(2D4)	100µl
Ref	014-22023		50µl
Ref	018-23241	Anti Human Ago3, Monoclonal Antibody(1C12)	50µl
Ref	010-23821	Anti Human Ago3, Monoclonal Antibody(6-107)	50µl
-80	013-25491	Anti Mouse Ago3, Monoclonal Antibody(mA3-S11)	50µl
-80	016-25501	Anti Mouse Ago3, Monoclonal Antibody(mA3-S9)	50µl
-80	012-24741	Anti Ago4, Monoclonal Antibody(2B2)	50µl
-80	019-24751	Anti Ago4, Monoclonal Antibody(2G7)	50µl
-80	012-25581	Anti Ago1-4, Monoclonal Antibody(1G3)	200µl
-80	018-25583		1ml
-80	016-25584		5ml
F	017-23451	Anti PIWIL1, Monoclonal Antibody(2C12)	100µl
F	018-23981	Anti Human PIWIL2, Monoclonal Antibody(1A12)	50µl
F	015-23991	Anti Human PIWIL2, Monoclonal Antibody(3C4)	50µl

Reagent Kits for microRNA Research

	Code No.	Description	Package size
Ref	295-71701	microRNA Extractor® SP Kit	50 extractions
F	290-66501	microRNA Cloning Kit *Wako*	8 reactions
F	298-68001	Target mRNA Cloning Kit *Wako*	10 reactions
Ref	291-70201	microRNA Isolation Kit, Human/Mouse Ago1	10 reactions
Ref	292-66701	microRNA Isolation Kit, Human Ago2	10 reactions
Ref	292-67301	microRNA Isolation Kit, Mouse Ago2	10 reactions
Ref	297-70301	microRNA Isolation Kit, Human Ago3	10 reactions
Ref	295-74001	MagCapture™ microRNA Isolation Kit, Human Ago2	10 reactions
Ref	297-74201	MagCapture™ microRNA Isolation Kit, Mouse Ago2	10 reactions
Ref	293-74801	MagCapture™ microRNA Isolation Kit, Ago 1-4	10 reactions
F	299-74401	MagCapture™ microRNA Isolation Kit, Protein G	20 reactions

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit our online catalog to search for other products from Wako; http://labchem-wako.fujifilm.com/ This leaflet may contain products that cannot be exported to your country due to regulations. / Bulk quote requests for some products are welcomed. Please contact us.

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