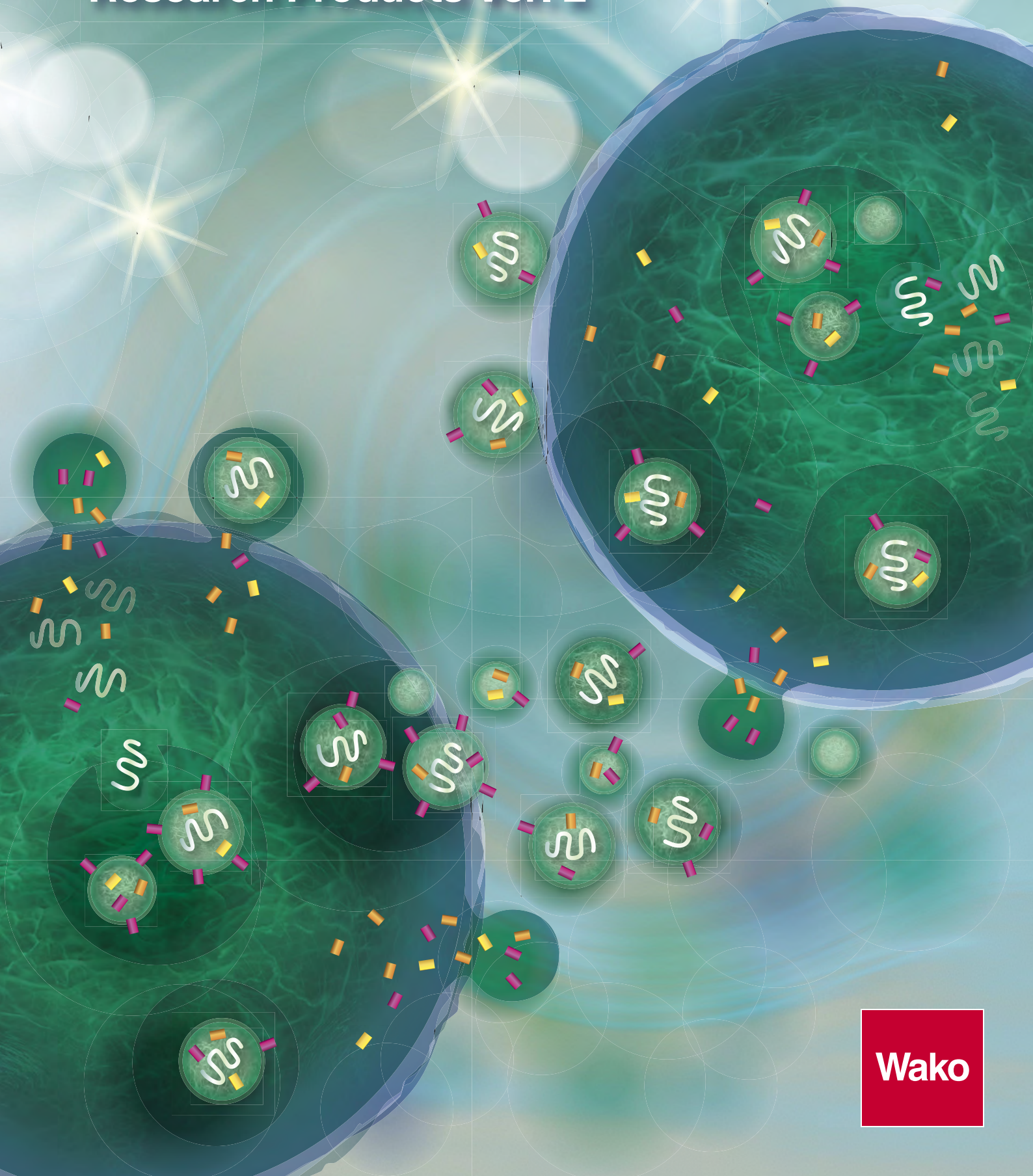
FUJIFILM
Value from Innovation

Exosome

Research Products Ver. 2



Wako

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What are exosomes?

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In recent years, research of extracellular vesicles (EVs) has been advancing at an accelerating pace. While the number of scientific articles on EVs published in 2011 was approximately two hundreds, the number increased to more than one thousand in 2016 and involvement of EVs in various physiological functions and pathogenic mechanisms has been suggested. Although EVs are roughly classified into at least two categories: exosomes derived from endosomes and microvesicles derived from plasma membrane, it is difficult to strictly separated them from each other by differential centrifugation, the technique most frequently used for purification of EVs at present, and the EVs not sedimenting at 10,000×g are called "small EVs" (mainly composed of exosomes) for convenience.[1] Exosomes are small membrane vesicles (approximately 30-100 nm in diameter) secreted by various cells and present in most body fluids (*e.g.*, blood, urine, and spinal fluid) and cell culture liquids. Exosomes, membrane vesicles surrounded by a lipid bilayer, are generated within intracellular vesicles called "multi-vesicular endosomes" and released into the extracellular space by fusion of multi-vesicular endosomes with the cell membrane. Exosomes contain proteins from secretory cells, including those of endosome origin (*e.g.*, ESCRTs), those involved in intracellular transport (*e.g.*, Rab GTPase), and those of cell membrane origin (*e.g.*, CD63 and CD81), as well as RNAs. Exosomes also contain the cell membrane of secretory cells and lipids from the endosome membrane (cholesterol and sphingomyelin, etc.).[2] Although exosomes had long been considered to be involved in release of unnecessary cell contents, exosomes are recently attracting attentions of researchers as new mediators of cell-cell communication transporting biomolecules such as lipids, proteins, and RNAs in vivo. In addition to clarification of physiological or pathophysiological functions of exosomes, research aiming at clinical application of these functions is rapidly in progress, particularly focusing on diagnostic and therapeutic application as well as development of biomarkers.

Current exosome research covers almost all research areas in biomedical science (immunology, neuroscience, oncology, endocrinology, and cardiovascular research). For example, exosomes derived from immune cells have been shown to contain antigen peptide/MHC complexes and various antigens, which suggests a possibility that exosomes might regulate various immune responses such as activation/inactivation of immune cells in addition to the exchange of antigenic information between immune cells.[3] In the nervous system, exosomes have been found to be involved not only in regulation of neural circuits[4] but also in extracellular release of proteins causing various neurodegenerative diseases for subsequent transmission to other cells, a process that might be deeply involved in disease progression.[5] Exosomes released by cancer cells contain many biomolecules related to angiogenesis and immune evasion, suggesting that they might contribute to construction of microenvironment optimal for cancer cell growth and promotion of cancer progression.[6] In addition, the expression profile of adhesion molecules on the surface of exosomes from cancer cells has been shown to determine the destination of cancer metastasis.[7] Recently, exosomes released from adipocytes have been reported to regulate gene expression in the liver.[8] Furthermore, while many viruses leave cells by utilizing the pathway for exosome production, bacteria and parasites infecting cells are likely to regulate activities of bacteria/parasites infecting other cells via exosomes.[9, 10]

Most of the above-mentioned functions are mediated by secretory cell-derived biomolecules located within exosomes. In particular, since mRNAs and miRNAs of secretory cell origin were identified in exosomes, potential involvement of exosomes in horizontal transmission of gene expression information between cells has been attracting great research interest.[11] Since these RNAs are encapsulated within the lipid bilayer membrane of exosomes, they are not susceptible to degradation by RNase and remain intact in blood or other body fluid. Exosomes incorporated into target cells fuse with the endosome membrane to release encapsulated RNAs into the cytosol of target cells. Once released into the cytosol, mRNAs are translated into proteins while miRNAs suppress translation of target genes. Thus, exosomes regulate gene expression within target cells. A single exosome is considered to contain more than several ten-thousands of proteins and more than several thousands of mRNAs and miRNAs. The composition of these biomolecules may vary depending on the type and conditions of a secretory cell which originally harbored the exosome. In addition, the composition of proteins, mRNAs, and miRNAs within an exosome is different from that within the original secretory cell, which suggests the existence of a mechanism selectively loading exosome-specific proteins and mRNAs/miRNAs into exosomes. Such specific composition of exosome RNAs makes

them attractive candidates for biomarkers and targets for therapeutic development. While mRNAs within exosomes incorporated into target cells are capable of inducing expression of functional proteins, most miRNA within exosomes are present as precursors of functional miRNA and their physiological significance is under extensive investigation. Thus, since exosomes contain a wide variety of proteins, RNAs, and lipids, construction of an exosome database "ExoCarta" is currently ongoing through claassification by cell types. Furthermore, while large scale analysis of exosomes utilizing cutting-edge methodologies in proteomics, transcriptomics, and system biology are separately conducted in laboratories all over the world, EV plugin for FunRich (a stand-alone functional enrichment analysis tool) is distributed aiming at a common and integrated analysis tool. Sharing information among researchers in a wide variety of research fields is essential for promotion of future exosome research.

[Development of exosome-based therapeutic/diagnostic methods]

In parallel with clarification of exosome functions, efforts to develop therapeutic methods applying exosome functions are being continued in recent years. For example, exosomes released from blood fibrocytes (a population of mesenchymal progenitors) accelerate wound healing by stimulating angiogenesis and inducing migration and proliferation of keratinocytes. Proangiogenic, anti-inflammatory miRNAs as well as a miRNA promoting collagen deposition within these exosomes are reportedly involved in this process.[12] In addition, exosomes released from dendritic cells in patients with cancer contain a variety of cancer cell-derived proteins and induce intense activation of cancer cell-specific cytotoxic T lymphocytes. Development of cancer immunotherapy based on this mechanism is currently in the early phase of clinical research.[13] On the other hand, suppression of exosomal functions involved in pathogenesis has also been attempted. For example, apoptosis-inducing TNF-α is accumulated at high concentrations in exosomes released from synovial fibroblasts in patients with rheumatoid arthritis and exacerbates the pathology of rheumatoid arthritis.[14] In addition, since cancer cell-derived exosomes contain molecules related to cancer progression and neuron-derived exosomes contain molecules related to neurodegenerative diseases as described above, inhibition or removal of these exosomes may potentially suppress onset of these diseases. Advancement of future research is expected to clarify exosome functions and expand indications of clinically applied exosomes, thereby realizing utilization of exosomes for the treatment of various diseases. Furthermore, delivery of drugs such as siRNAs and anticancer agents to target cells using exosomes has been attempted. Since various cell adhesion molecules are expressed on the surface of exosome membrane and the expression profile of these molecules has been found to determine the target cells for exosome delivery, application of this property to development of a new drug delivery system (DDS) is expected.[15]

Exosomes are extremely stable in body fluids, and the exosome lipid bilayer membrane encapsulating proteins and RNAs within vesicles protects them from degradation. Furthermore, exosomes remain relatively intact even in body fluid specimens stored for a long time after collection and are therefore considered as new and promising laboratory biomarkers for diseases. While correlations between exosomes and various diseases have been investigated, cancer cell-derived exosomes released into blood are recently attracting research interest due to difference in constituents from normal cell-derived exosomes and a correlation between constituents of cancer cell-derived exosomes and cancer progression has been extensively investigated as a potential tool for early cancer diagnosis.[16] In addition, exosomes in urine are expected as a new diagnostic marker for renal, prostate, and bladder diseases, while exosomes in cerebrospinal fluid as a new marker for brain tumor and neurodegenerative diseases.

[Issues and future perspectives of exosome research]

Although many studies on roles of exosomes have already been reported, experiments providing evidence for these reported phenomena use highly concentrated exosomes purified from body fluids and cell culture supernatants. Accordingly, whether these phenomena actually occur in vivo remains unclear. The sole approach for clarification of physiological actions of exosomes is to clarify the mechanism of exosome release and physiological phenomena induced by exosome release stimulation/inhibition through modulating the mechanism, which is expected to result in further advancement in exosome research. Another important issue to be addressed in future research and development is in vivo kinetics of exosomes (*i.e.*, which exosomes are directed to which target cells).

Conventional methods for exosome purification mainly involved ultracentrifugation and various commercial purification kits using polyethylene glycol (PEG) precipitation technique. However, exosome preparations obtained by these methods contain large amounts of contaminants and careful analysis is required to determine whether experimental results obtained are actually due to actions of exosome constituents *per se*. Furthermore, ultracentrifugation requiring cumbersome manipulation has several disadvantages including inconsistent recovery interfering quantitative analysis and requirement for an expensive instrument not compatible with high-throughput analysis. Conducting exosome research under these circumstances is difficult and development of technology for easy purification of exosomes at a high purity is urgently needed. We focused on Tim4, an exosome receptor expressed on macrophages, and prepared "Tim4 magnetic beads" by immobilizing the extracellular region of Tim4 on magnetic beads.[17] Since Tim4 binds to phosphatidylserine (PS), a phospholipid on the surface of exosome membrane, in a calcium ion-dependent manner, bound exosomes are released from these beads with an elution buffer containing ethylenediaminetetraacetic acid (EDTA), a chelating agent, to obtain highly purified intact exosomes. In fact, when exosomes released from human leukemia cells were purified by the Tim4-affinity method and compared for purity with exosomes purified by ultracentrifugation and PEG precipitation, the Tim4-affinity method yielded exosome preparations with

exosome-specific proteins each exhibiting a band intensity over 10-100 times higher than that obtained by other methods and almost free from non-exosome contaminants, thereby demonstrating reproducible recovery of high-purity exosomes. As a result, many previously unidentified exosome proteins and RNAs could be identified from exosome preparations obtained by this method. Furthermore, application of the strong binding affinity of Tim4 toward exosomes realized high-sensitivity detection and assay of exosomes by enzyme-linked immunosorbent assay (ELISA) and fluorescence-activated cell sorting (FACS). On the other hand, while only crude preparations of microvesicles were conventionally obtained because differential centrifugation was the sole purification technique available, the Tim4-affinity method realized purification of microvesicles at a high purity as well. Details of these techniques are described in this guidebook. We expect that usefulness of these Tim4-affinity-based techniques will be appreciated in the world and greatly contribute to clarification of the original physiological functions of exosomes and microvesicles.

In addition to difficulties in detection and isolation of exosomes, the existence of various classification systems for exosomes and resulting lack of consensus among investigators regarding which method should be used for purification of the extracellular vesicles to be called "exosomes" make interpretation of experimental data and confirmation of reproducibility difficult. To overcome such situation, the International Society for Extracellular Vesicles (ISEV) has recently been established to nurture a global community of EV researchers and "Minimal Information for Studies of EVs" (MISEV) Guidelines has been published as international standard that investigators who intend to start EV research should consult.[18, 19] In addition, as a method for avoiding such confusion, EV-TRACK knowledge database has been constructed to record experimental conditions employed in individual EV-related articles.[20] On the other hand, as EV research has attracted global research interest, a number of large-scale research projects have been launched in various countries. In the United States, National Health Institute (NIH) has initiated a strategic large-scale project "Extracellular RNA Communication" and special interest groups on EV research have been organized at prestigious international conferences such as Gordon Conference and Keystone Symposia since 2016. In Europe, research covering EV has already been conducted as a part of CANCER-ID project supported by "Innovative Medicines Initiative (IMI)," a public-private partnership (PPP) for research and development of medicines. In Japan, EV research has been selected as one of the Research and Development Strategic Objectives in 2017 established by the Ministry of Education, Culture, Sports, Science and Technology Japan and acceleration of future research in this field is expected. In any case, development of solid research methodologies and techniques that constitute the basis of EV research is essentially required for future advancement in this research field, and we expect that the Tim4-affinity method will grow up to be one such technique.

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PS affinity method

Introduction

Exosomes are membrane vesicles (30-100 nm in diameter) released from various cells and shown to function as transmitters of encapsulated nucleic acid (mRNA, microRNA) and proteins between remote cells. Their roles as a communication tool in cell-cell signal transduction and as a potential biomarkers for various diseases including cancer have recently been attracting research interest.[1, 2] Accordingly, exosome research has been spreading across a wide variety of research areas in recent several years. Nevertheless, experimental techniques currently available for exosome research are still under development and many issues remain to be improved.

For example, among conventional techniques for exosome purification, ultracentrifugation and polymer precipitation (commercially available kits) have been shown to yield exosome preparations with large amounts of contaminants that seriously interfere with subsequent experiments. On the other hand, antibody-based affinity method and density gradient centrifugation are capable of purifying highly purified exosomes but are incapable of yielding intact exosomes, thus it is difficult to analyze their original physiological functions of exosomes.

Furthermore, Western Blotting and ELISA widely used for exosome detection have several disadvantages including the requirement of relatively large amounts of exosomes and difficulties in the detection of low-expression marker proteins.

Accordingly, we have developed a new exosome analysis tool to resolve various problems in experimental techniques for exosome research as mentioned above. This new technique is described below. Data on isolation of high-purity extracellular vesicles using MagCapture™ Exosome Isolation Kit PS are presented on pages 8-14, while data on high-sensitivity detection of extracellular vesicles using PS Capture™ Exosome ELISA Kit are shown on pages 17-20 and 22-24.

A new method for exosome purification using phosphatidylserine and Tim4

The exosome membrane contains proteins and lipids derived from secretory cells. Among those components, phosphatidylserine (PS) is known to be oriented inside the cell membrane of intact cells by the enzymatic activity of lipid flippase, and be known to be exposed also on the outer surface of exosome membrane.[3] In addition, T-cell immunoglobulin domain and mucin domain-containing protein 4 (Tim4), the receptor involved in phagocytosis of apoptotic cells by macrophages, is known to bind PS via the IgV domain located within the extracellular region in a calcium ion-dependent manner.[4]

Based on these findings, we developed a new and unprecedented method for exosome purification using Tim4-immobilized magnetic beads (capable of capturing exosomes in samples such as culture supernatants and serum in the presence of calcium ions and releasing them by elution with a buffer supplemented with a chelating reagent) in collaboration with Professor Rikinari Hanayama (Department of Immunology, Kanazawa University Graduate School of Medical Sciences) and successfully constructed an exosome purification kit based on these magnetic beads.[5] **This exosome purification kit, MagCapture™ Exosome Isolation Kit PS, has realized easy purification of intact exosomes with higher purity than that obtained by any conventional methods for exosome purification.** It is currently being established as a new exosome purification method replacing ultracentrifugation, the conventional gold standard.

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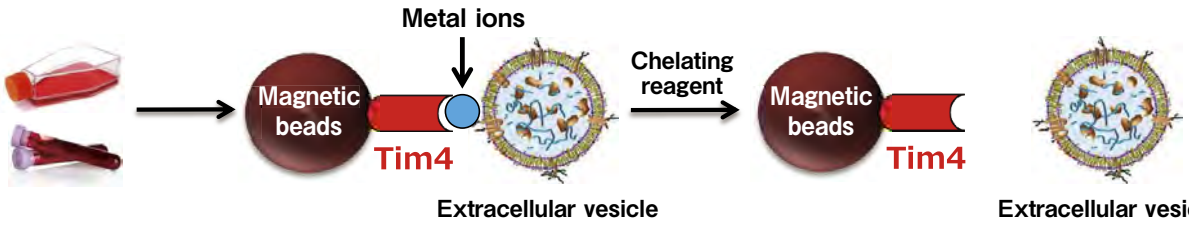
5) Nakai, W. *et al*. A novel affinity-based method for the isolation of highly purified extracellular vesicles. *Sci. Rep*. **6**, 33935 (2016).

A novel affinity-based method for phosphatidylserine (PS) on the surface of extracellular vesicles



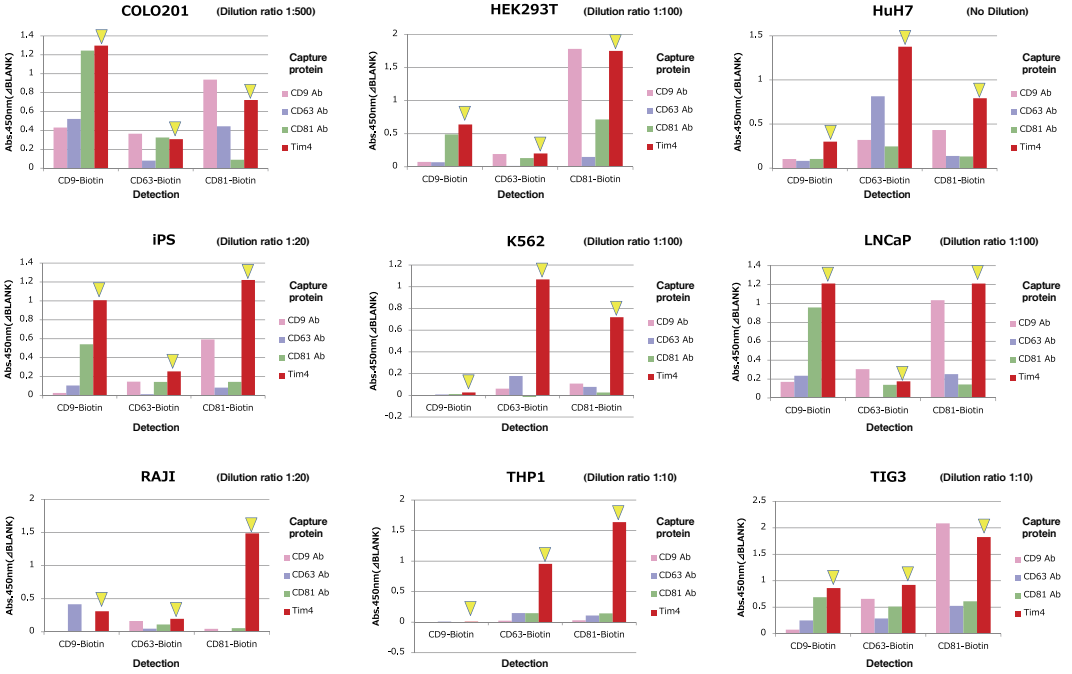
Extracellular vesicles are captured by phosphatidylserine (PS)-binding proteins and metal ions, then captured extracellular vesicles are eluted with a chelating reagent.

PS affinity method

Comparison of capture ability between Tim4 and anti-exosomal marker antibody

Microplate wells, pre-coated with anti-CD antibodies (CD9, CD63, CD81) and Tim4 were incubated with nine types of pretreated ※1 cell culture supernatants. Captured exosomes on the plate were detected using biotinylated anti-CD antibodies (CD9, CD63, CD81).

※1 Pretreatment condition: 10,000 × g, 30 min.



< Flowchart >

・Anti-CD antibodies (CD9, CD63, CD81)
・PS-ELISA (Tim4)

↓

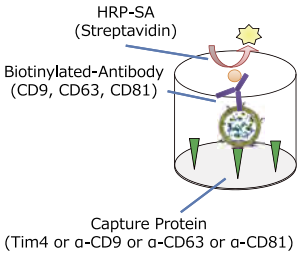
Immobilization

↓

Measuring exosomes in nine types of cell culture supernatants

↓

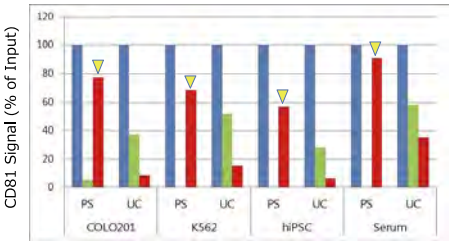
Detected by each antibody



➡ Compared with anti-exosomal marker antibodies, Tim4 was able to efficiently capture exosomes derived from a wide range of cell types.

Comparison of recovery efficiency with ultracentrifugation

From cell culture supernatant samples of various cell type and human serum samples obtained by centrifugation at 10,000 × g, exosomes were isolated and purified by PS affinity method and ultracentrifugation method. Then, purified exosomes were measured for signals of CD81 using PS Capture™ Exosome ELISA Kit. From absorbance of each sample, the amount of residual exosomes in a post-purification sample and the recovery rate of exosomes were determined for comparison.



PS: PS affinity method (as instructed in the kit protocol)
UC: Ultracentrifugation (2 runs, 110,000 × g for 70 minutes)
Sample volume: 1 mL (without concentration)

➡ The PS affinity method was found to be more efficient in recovering exosomes from various cell lines and serum than the ultracentrifugation method.

MagCapture™ Exosome Isolation Kit PS

Introduction and Features

【Introduction】

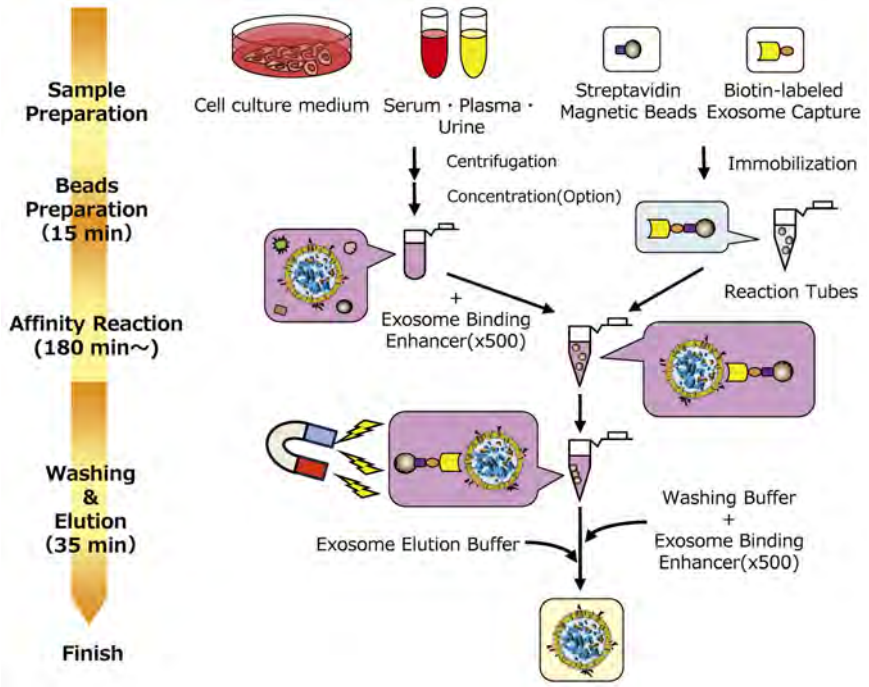
MagCapture™ Exosome Isolation Kit PS adopts a novel affinity purification method using magnetic beads and phosphatidylserine (PS)-binding protein (PS affinity method). This kit can easily isolate high purity exosomes and other EVs from cell culture medium and body fluids at high yield by a normal microcentrifuge. If higher purity exosomes are needed, please use the supernatant obtained by 10,000 × g centrifugation as the sample. This kit enables the isolation of exosomes and other EVs as intact forms because the captured EVs are eluted from magnetic beads with the metal-chelating reagent at neutral pH. The isolated intact exosomes and other EVs can be used for various applications including electron microscopic analysis, nanoparticle tracking analysis, administration of EVs and analysis of molecular constituents such as proteins, lipids, or nucleic acids.

【Product photo】

【Features】

- Purify High Purity and Intact Extracellular Vesicles
- Isolate Extracellular Vesicles from most Biofluids including Serum, Plasma, and Urine
- Enable High Reproducibility and Stable Yield
- Introduce Easy Operation and High-Throughput Purification (No ultracentrifugation required)

Outline of Procedure of MagCapture™ Exosome Isolation Kit PS



【Kit contents】

This kit includes 7 components.

Kit composition (2 purifications)	Quantity
(1) Streptavidin Magnetic Beads	120 μL×1 tube
(2) Biotin-labeled Exosome Capture	20 μL×1 tube
(3) Exosome Capture Immobilizing Buffer	7 mL×1 bottle
(4) Exosome Binding Enhancer (× 500)	100 μL×1 tube
(5) Washing Buffer	30 mL×1 bottle
(6) Exosome Elution Buffer	1 mL×1 bottle
(7) Reaction Tube	4 tubes

Kit composition (10 purifications)	Quantity
(1) Streptavidin Magnetic Beads	600 μL×1 tube
(2) Biotin-labeled Exosome Capture	100 μL×1 tube
(3) Exosome Capture Immobilizing Buffer	35 mL×1 bottle
(4) Exosome Binding Enhancer (× 500)	500 μL×1 tube
(5) Washing Buffer	75 mL×2 bottles
(6) Exosome Elution Buffer	5 mL×1 bottle
(7) Reaction Tube	22 tubes

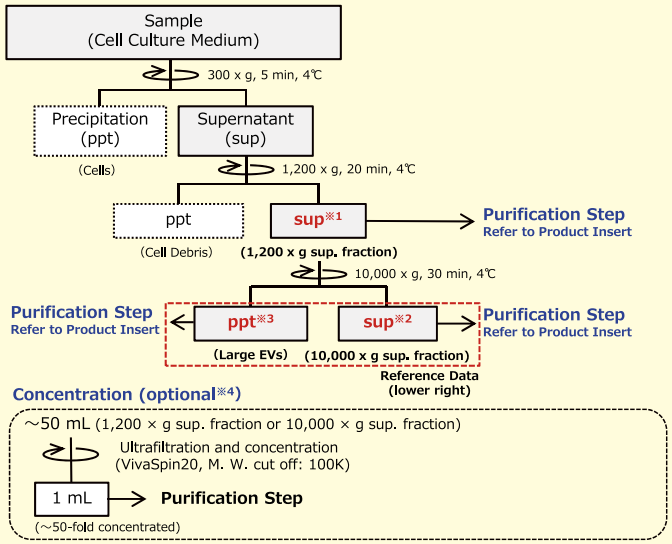
*Used Exosome Capture-immobilized beads can be recycled for "Repeated extraction of extracellular vesicles from the same sample" and "Purification of extracellular vesicles from culture supernatant sample of the same lot and body fluid sample of the same lot". Recycling is up to 4 times.

Preprocessing protocols for various samples

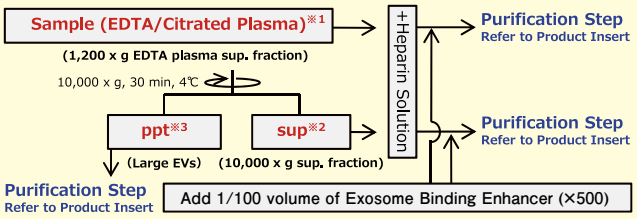
This is the section to prepare samples. When exosomes and other large EVs (microvesicles) are needed, prepare 1,200 × g supernatant※1 as a sample.

Additionally, when highly purified exosomes are needed, prepare 10,000 × g supernatant※2 as a sample. This protocol for sample preparation is set for cell culture medium, serum, and plasma. When other body fluids are used, please examine the appropriate preprocessing protocol by referring to the protocol for serum and plasma.

Cell Culture Medium

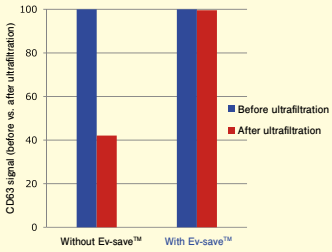


EDTA Plasma and Citrated Plasma

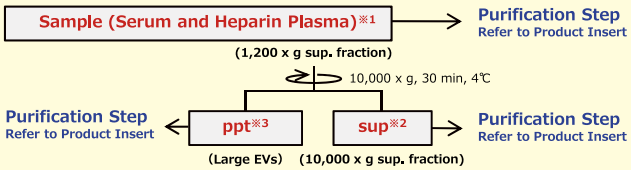


Points to be considered when cell culture medium is concentrated by ultrafiltration

Concentration by ultrafiltration has been known to lead to the decreased amount of extracellular vesicles owing to their adsorption to the column.
Addition of EV-Save™ Extracellular Vesicle Blocking Reagent is recommended to prevent loss of extracellular vesicles associated with the concentration.※5



Serum and Heparin Plasma



Filtration of supernatant obtained by centrifugation at 10,000 ×g

To remove Large EVs such as apoptotic vesicles and microvesicles from a sample, use of a centrifugal filter unit (Millipore, Ultrafree-MC, GV 0.22 μm sterile, Product Code: UFC30GV0S) on the "supernatant" obtained from the sample by centrifugation at 10,000 ×g for 30 minutes is recommended. The filtrate can be used as a test sample.

※1 When **exosomes and large EVs** are needed, use 1,200 × g sup. fraction as sample.
※2 When **exosomes** are needed, use 10,000 × g sup. fraction as sample.
※3 When **Large EVs** are needed, use ppt of Large EVs obtained by centrifugation at 10,000 x g as sample after suspending it with TBS.
※4 The concentration step is an option when using a large volume (~ 50 mL) of cell culture supernatant as a sample for purification. However, since recovery efficiency improves, please perform it as much as possible.
※5 Because EV-Save™ contains polymers, its use is not recommended for preparation of proteomic analysis samples.

Note : a volume of large or small sample

■ In the case of large volume

The concentration of the sample is recommended when using a large volume (~50 mL) of cell culture supernatant as a sample for purification. Since recovery efficiency improves, please perform it as much as possible.

■ In the case of small volume

Add the appropriate volume of TBS into samples to reach the volume of 0.5 mL to obtain the better mixture of the Exosome Capture-immobilized beads with the sample. (Example: 100-200 μL → 500 μL)

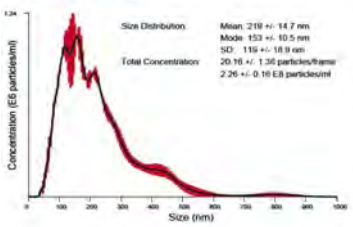
Reference data : NTA of exosomes and large EVs

1 mL of K562 cell culture supernatant (collected after stimulating exosome secretion with monensin sodium salt) was centrifuged at 10,000×g to isolate supernatant and precipitate (suspended with 1 mL of TBS) fractions.

Then, extracellular vesicles were purified from both fractions with MagCapture™ Exosome Isolation Kit PS and analyzed using NanoSight LM10.

10,000 × g precipitate

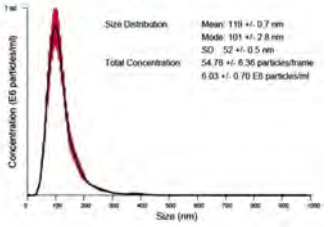
Large EVs



Size Distribution :
Mean : 219 ± 14.7 nm

10,000 × g supernatant

Exosome



Size Distribution :
Mean : 119 ± 0.7 nm

Application Data

Analysis of extracellular vesicles purified from culture supernatant samples

〈Sample Preparation Method〉

MagCapture™ Exosome Isolation Kit PS

Exosomes were recovered from 1 mL of pretreated (10,000×g, 30 min.) cell culture supernatant of K562 cells (serum-free medium or 10% exosome-depleted FBS※1 included medium) by using PS affinity method's standard protocol (reaction time: 3 hours)

Ultracentrifugation

10 mL of pretreated (10,000×g, 30 min.) cell culture supernatant of K562 cells (serum-free medium or 10% exosome-depleted FBS※1 included medium) were ultracentrifuged at 110,000×g, 70 min., and the precipitates were suspended with TBS. Then, the suspension was ultracentrifuged again and the precipitated pellet was recovered as exosome sample.

Polymer-based precipitation

Exosomes were collected from 1 mL of pretreated (10,000×g, 30 min.) cell culture supernatant of K562 cells (serum-free medium or 10% exosome-depleted FBS※1 included medium) by Supplier A's product in accordance with manual (Precipitation time: overnight).

※1···Centrifuged for 2 hours at 110,000 × g, and the supernatant was collected so as not to take up the precipitate.

Analysis of exosomes using NanoSight

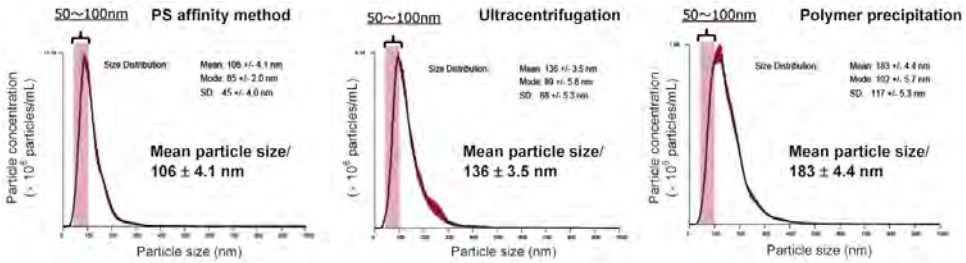
The NanoSight series of instruments utilize Nanoparticle Tracking Analysis (NTA) to visualize Brownian motion of nanoparticles in solution for analysis of size and concentration of nanoparticles. Even when nanoparticles in solution constitute either a mixture of various substances or a polydisperse system with varying particle size, these instruments are capable of determining the particle count by particle size range using video images of the Brownian motion of nanoparticles obtained by visualization technology.

Here, exosomes purified from cell culture supernatant by affinity adsorption using either MagCapture™ Exosome Isolation Kit PS, ultracentrifugation, or polymer precipitation were analyzed using NanoSight LM10.

NanoSight LM10

[Description and results of experiment]

Exosomes obtained by different techniques were separately diluted to an appropriate concentration with ultrapure water and analyzed for particle size and concentration using NanoSight (Figure 1). Results demonstrate that the PS affinity method preferentially concentrates particles with a diameter corresponding to those of exosomes (50-100 nm), indicating a high purity of the exosome preparation obtained.



(Sci Rep. 2016 Sep 23;6:33935. Nakai W et al.)

Figure 1. Analysis of exosomes obtained by different techniques using NanoSight LM10

Electron microscopic analysis of exosomes

[Description and results of experiment]

Electron microscopy is capable of obtaining magnified images of measurement objects using electron beams. Since electron beams as an electromagnetic wave have extremely short wavelengths, electron microscopy has the advantage that it is capable of morphological observation at a magnification far higher than that of light microscopy. Accordingly, this technique is used for observation and analysis of a wide variety of samples ranging from metals/polymer materials to biological tissues from rats and mice as well as hydrated compounds such as plants and food.

Here, electron microscopic analysis of exosomes was performed using a transmission electron microscope (TEM) (Figure 2).

[Samples used]

(1) Sample purified by the PS affinity method
Sample: COLO201 cell culture supernatant, 10 mL
Isolation method: MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
Particle counts: 3.69 ×10^{10} particles/mL

(2) Sample purified by ultracentrifugation
Sample: COLO201 cell culture supernatant, 10 mL
Isolation method: Ultracentrifugation
Particle counts: 1.68 ×10^{10} particles/mL

<Photographic data of exosomes>
Hanaichi Ultrastructure Research Institute

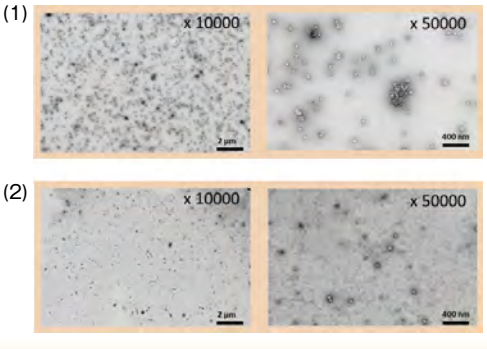
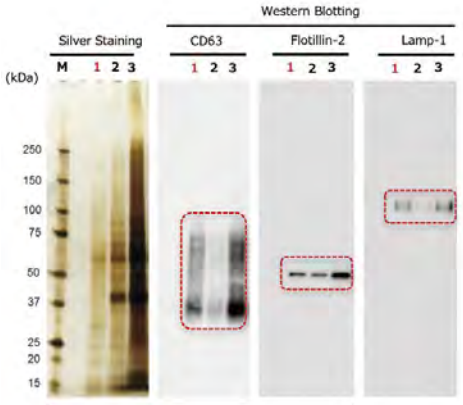


Figure 2. Results of electron microscopic analysis

Comparing the recovery amount and purity of exosomes (serum-free)

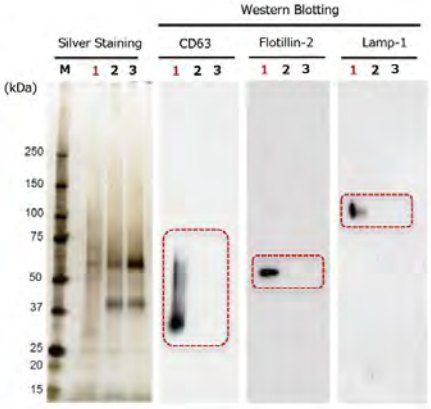
Exosomes were collected from cell culture supernatant of K562 cells (serum-free medium) by MagCapture™, ultracentrifugation and polymer-based precipitation. The recovery amount and purity were analyzed by silver staining and Western Blot with anti-CD63, anti-Flotillin-2, and anti-Lamp-1 antibodies.

Comparison of recovery amount
(Recovery amount from 150 μL cell culture supernatant)



Lane 1: MagCapture™
Lane 2: Ultracenrifugation
Lane 3: Polymer-based precipitation[Supplier A]

Comparison of purity
(Amount of marker proteins per 200 ng of total protein)



With MagCapture™, the recovery performance of exosomes is excellent and the amount of contaminant of proteins is very low, thus the balance of purity and recovery amount is the best.

■ Detection antibody
Anti-CD63 mouse mAb (MEM-259)

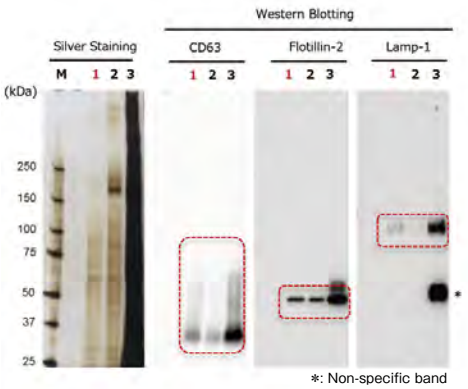
Anti-Flotillin-2 mouse mAb
(29/Flotillin-2), BD Bioscience

Anti-Lamp-1 mouse mAb
(25/Lamp-1), BD Bioscience

Comparing the recovery amount and purity of exosomes (10% exosome-depleted FBS)

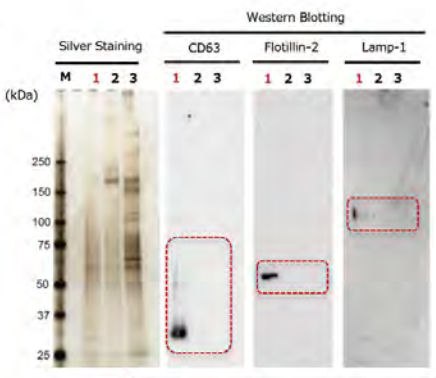
The exosomes were collected from cell culture supernatant of K562 cells (10% exosome-depleted FBS included medium) by MagCapture™, ultracentrifugation and polymer-based precipitation. The recovery amount and purity were analyzed by silver staining and Western Blot with anti-CD63, anti-Lamp-1, and anti-Flotillin-2 antibodies. Furthermore, collected samples by each method were analyzed by mass spectrometry and compared the percentage of human-derived peptides from K562 cells.

Comparison of recovery amount
(Recovery amount from 150 μL cell culture supernatant)



*: Non-specific band

Comparison of purity
(Amount of marker proteins per 200 ng of total protein)



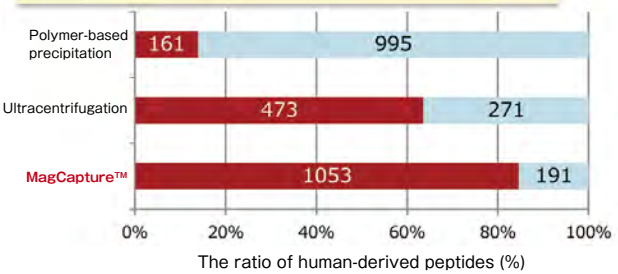
Lane 1: MagCapture™
Lane 2: Ultracentrifugation
Lane 3: Polymer-based precipitation[Supplier A]

■ Detection antibody
Anti-CD63 mouse mAb (MEM-259)

Anti-Flotillin-2 mouse mAb
(29/Flotillin-2), BD Bioscience

Anti-Lamp-1 mouse mAb
(25/Lamp-1), BD Bioscience

Comparing the percentage of human-derived peptides identified by MASS analysis



With MagCapture™, high purity exosomes are recovered even from culture medium with FBS, thus MASS analysis can be done with low background.

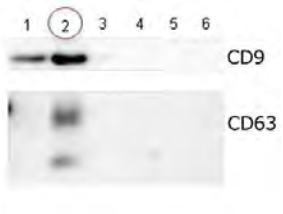
MASS analysis data was provided by Dr. R. Hanayama at Graduate School of Medicine, Kanazawa University and Dr. W. Nakai at iFReC Osaka University.

Application Data

Analysis of extracellular vesicles purified from serum/plasma/urine samples

Comparing the yield of exosomes isolated from human normal serum

Exosomes were isolated from normal human serum by using MagCapture™, ultracentrifugation and affinity method with antibody against surface antigen of exosome, followed by Western Blot with the anti-CD9 and anti-CD63 antibodies.



Lane 1: Ultracentrifugation
Lane 2: MagCapture™
Lane 3: Exosome Isolation reagent with antibody against CD9 [Supplier A]
Lane 4: Exosome Isolation reagent with antibody against CD63 [Supplier A]
Lane 5: Exosome Isolation reagent with antibody against CD81 [Supplier A]
Lane 6: Exosome Isolation reagent with antibodies [Supplier A]
(antibody beads-mixture of CD9, CD63, CD81 & EpCAM)

The yield of exosomes by MagCapture™ is higher than ultracentrifugation and the antibody-based method.

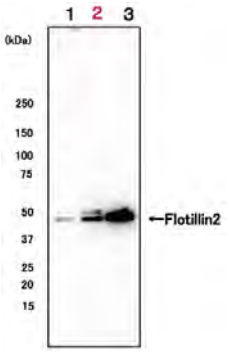
■ Detection antibody
Anti-CD9 rabbit pAb, System Bioscience

Anti-CD63 rabbit pAb, System Bioscience

■ About data of WB
Each signal corresponds to 150 μL of the serum sample. 15 μL of eluate and 5 μL of 4×SDS sample buffer were mixed and applied all in each well.

Isolating exosomes from human EDTA-plasma

Exosomes were isolated from EDTA-plasma, EDTA-plasma (buffer-exchanged), and serum by using MagCapture™, followed by Western Blot with the anti-Flotillin-2 antibody.



1: EDTA-plasma
2: EDTA-plasma (buffer-exchanged)
3: Serum

By exchanging buffer by ultrafiltration, exosomes can be isolated more efficiently.

■ Detection antibody
Anti-Flotillin-2 mouse mAb (29/Flotillin-2), BD Bioscience

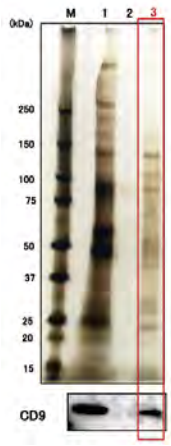
■ Sample volume
1 mL each

■ About data of WB
Each signal corresponds to 150 μL of various samples. 15 μL of eluate and 5 μL of 4×SDS sample buffer were mixed and applied all in each well.

Comparing the yield of exosomes isolated from human normal urine

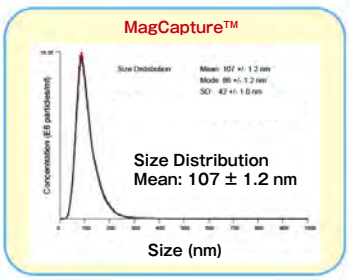
Exosomes were isolated from normal human urine by using MagCapture™, ultracentrifugation and polymer-based precipitation, followed by Western Blot with the anti-CD9 antibody.

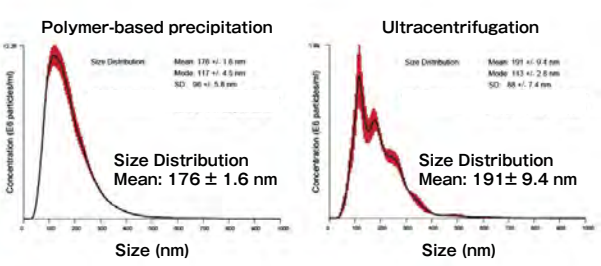
Comparison of recovery amount of marker protein



Lane 1: Polymer-based precipitation
Lane 2: Ultracentrifugation
Lane 3: MagCapture™

Comparion of NTA





High purity exosomes can be isolated from urine sample.

■ Detection antibody
Anti-CD9 rabbit pAb, System Bioscience

■ Sample volume
1 mL each

■ About data of WB
Each signal corresponds to 150 μL of the urine sample. 15 μL of eluate and 5 μL of 4×SDS sample buffer were mixed and applied all in each well.

Application Data

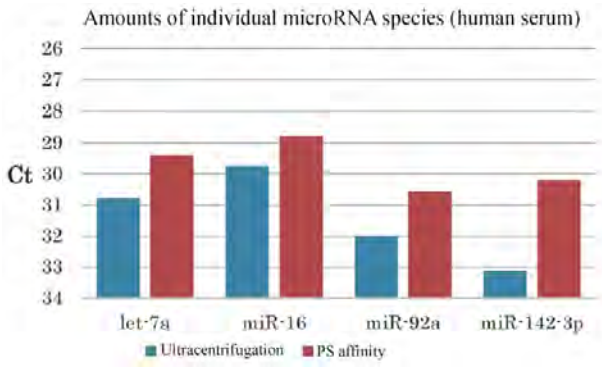
Comparison of microRNA and mRNA recovery amount from exosomes derived from normal human serum

Experimental data

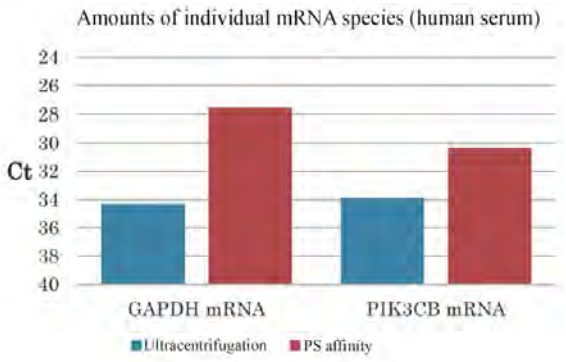
(1) Comparison of microRNA and mRNA recovery amount from exosomes prepared by different techniques

After isolation of exosomes from normal human serum samples by ultracentrifugation and the PS affinity method, RNA was recovered using microRNA Extractor SP Kit (Code No. 295-71701). microRNA (let-7a, miR-16, miR-92a, miR-142-3p) and mRNA (GAPDH, PIK3CB) were determined by quantitative PCR and compared it using Ct values.

■ Amounts of individual microRNA species



■ Amounts of individual mRNA species

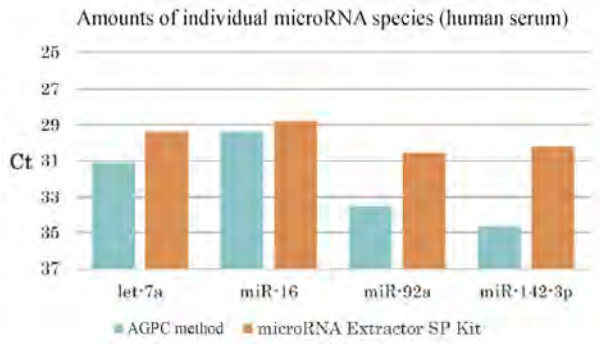


microRNA and mRNA were recovered more efficiently from exosomes isolated by the PS affinity method than those isolated by ultracentrifugation.

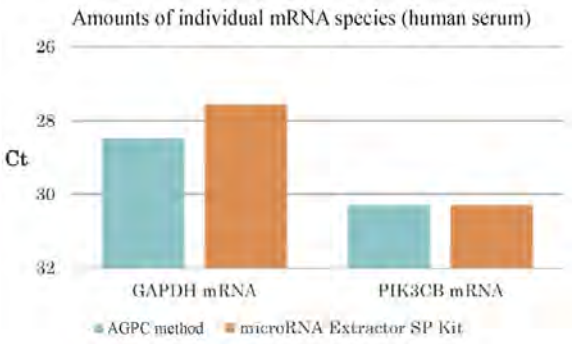
(2) Comparison of RNA extraction methods from the PS affinity fractions

After isolation of exosomes from normal human serum samples by the PS affinity method, RNA was recovered using microRNA Extractor SP Kit (Code No. 295-71701) or acid guanidinium thiocyanate-phenol-chloroform extraction (AGPC method). microRNA (let-7a, miR-16, miR-92a, miR-142-3p) and mRNA (GAPDH, PIK3CB) were determined by quantitative PCR and compared it using Ct values.

■ Amounts of individual microRNA species



■ Amounts of individual mRNA species



microRNA and mRNA were recovered more efficiently from exosomes using microRNA Extractor SP Kit than using AGPC method.

[Related product]

■ microRNA Extractor SP Kit

This kit is intended for extraction of total RNA including microRNA from human and animal serum/plasma. It extracts microRNA at a high efficiency without using the deleterious substance such as phenol and chloroform that were indispensable for conventional methods for RNA extraction.

Code No.	Description	Package Size	Storage
295-71701	microRNA Extractor SP Kit	50 purifications	2 ~ 10℃

Application Data

Proteomic Analysis of Exosomes

<Description and results of experiment>

Exosomes were purified from K562 cell culture supernatant containing 10% exosome-depleted FBS using either the PS affinity method, ultracentrifugation, or polymer precipitation. The obtained exosome samples were separated by 10% polyacrylamide gel electrophoresis and the individual whole protein bands were cut out. After in-gel digestion, proteins were identified by liquid chromatography mass spectrometry (LC-MS). Proteins identified in exosome preparations purified by the 3 different methods (n=3 for each method) were also compared for Pair-wise correlations.

Comparison of the top 10 proteins identified by MASS analysis

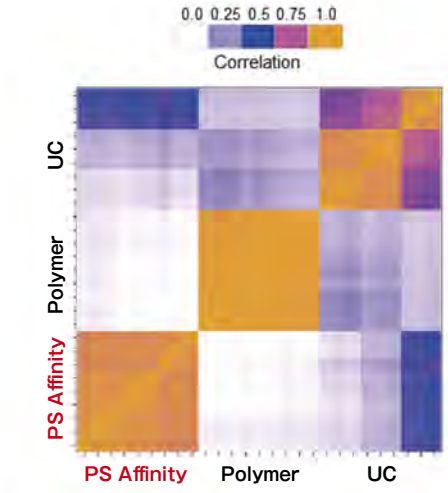
White columns: human protein derived from EVs
Gray columns: bovine protein contaminants derived from FBS
Red color: marker proteins of EVs

Sample:
K562 cell culture Sup.
(10% exosome-depleted FBS included)

	PS Affinity	Ultracentrifugation	Polymer Precipitation
1	Heat shock cognate 71 kDa protein	DNA-dependent protein kinase catalytic subunit	Complement C3
2	Annexin A6	Transferrin receptor protein 1	Alpha-2-macroglobulin
3	Transferrin receptor protein 1	Serum albumin	Fibronectin
4	V-type proton ATPase catalytic subunit A	ATP-dependent RNA helicase A	Serum albumin
5	Flotillin-2	Tubulin beta-5 chain	Thrombospondin-1
6	Programmed cell death 6-interacting protein	Heat shock cognate 71 kDa protein	Complement C4
7	4F2 cell-surface antigen heavy chain	Fatty acid synthase	Alpha-1-antiproteinase
8	Annexin A1	4F2 cell-surface antigen heavy chain	Apolipoprotein B-100
9	Kinase D-interacting substrate of 220 kDa	U5 small nuclear ribonucleoprotein helicase	Hemoglobin fetal subunit beta
10	Annexin A2	Tubulin beta-4B chain	Tubulin beta-5 chain

While proteins of bovine serum origin are present in the greater amount of exosome preparations obtained by polymer precipitation, more exosome marker proteins are identified in exosome preparations obtained by the PS affinity method.

Comparison of Pair-wise correlation



Sci Rep. 2016 Sep 23;6:33935. Nakai W et al.

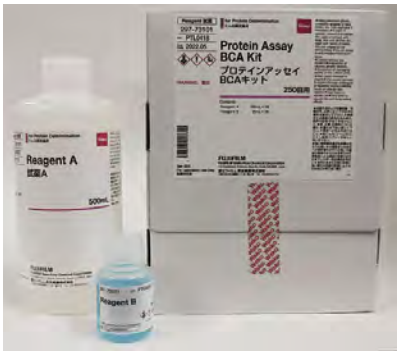
Sample:
K562 cell culture Sup.
(10% exosome-depleted FBS included)

	Reproducibility
Ultracentrifugation (UC)	△
Polymer Precipitation	○
PS Affinity	○

Polymer precipitation and the PS affinity method exhibit high intra-method correlation, while the intra-method correlation for ultracentrifugation is a little bit lower.
Inter-method correlations between different methods are relatively low. The different purification methods might yield different exosome populations.

Application Data

Assay of protein concentration in exosomes using Protein Assay BCA Kit



Protein Assay BCA Kit, capable of assaying total protein concentration in solution using bicinchoninic acid (BCA), is the most widely used protein assay kit. It is based on the principle of reduction of Cu^{2+} to Cu^{+} by protein under basic conditions. Chelate formation between Cu^{+} and BCA generates a purple-colored chelate. Since the purple color becomes more intense in proportion with protein concentration, the protein concentration is determined by comparing this absorbance at 562 nm with a standard curve of absorbance from varying bovine serum albumin (BSA).

Here, the protein concentration in an exosome preparation purified from cell culture supernatant using MagCapture™ Exosome Isolation Kit PS was determined according to the high-sensitivity protocol for Protein Assay BCA Kit.

[Description and results of experiment]

An exosome solution purified using MagCapture™ Exosome Isolation Kit PS pipetted into a 96-well plate in 25 μL aliquots and then 200 μL of a mixture of Reagent A and Reagent B included in Protein Assay BCA Kit was added to each well. After subsequent incubation at 60℃ for 30 minutes, the absorbance was measured at 560 nm (Figure 1). The result demonstrated that the protein concentration in a purified exosome preparation was able to determine using the high-sensitivity protocol for Protein Assay BCA Kit.

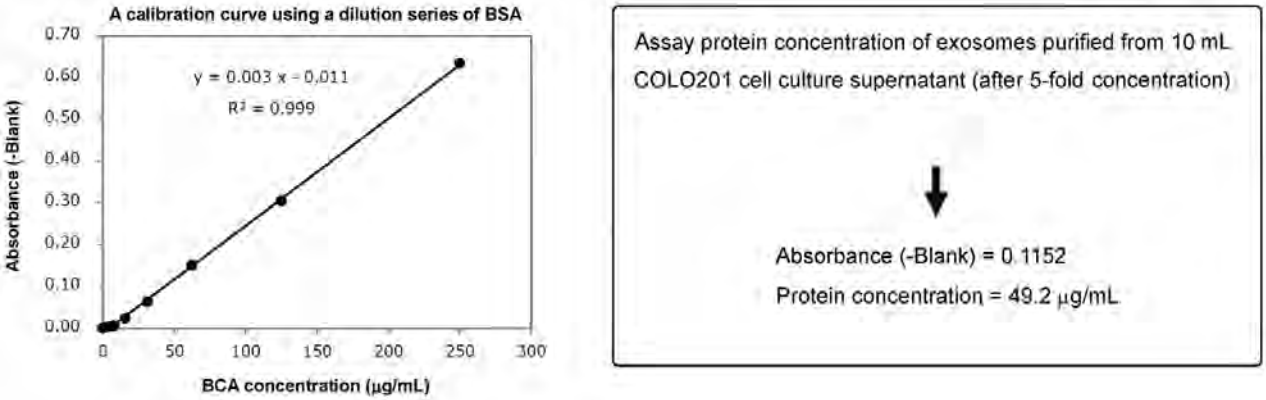


Figure 1. A BSA calibration curve determined using Protein Assay BCA Kit and assay of protein concentration of a purified exosome preparation

Code No.	Description	Package Size	Storage
297-73101	Protein Assay BCA Kit	250 assays	Room Temperature
015-25613	2 mg/mL Albumin Solution from Bovine Serum	1 mL × 10	Room Temperature

<Overview of BCA assay protocol>

Since protein concentrations of exosome preparations are rather low, use undiluted samples for BCA protein assay.

(1) Pipette 25 μL per well of standard BSA solutions (250, 125, 62.5, 31.25, 15.625 μg/mL) and a standard BLANK into a 96-well plate.
(2) Pipette 25 μL per well of a purified exosome preparation and Elution Buffer (BLANK) on a 96-well plate.
(3) Add 200 μL per well of a mixture of Reagent A and Reagent B (A: B=50: 1) of Protein Assay BCA Kit (Code: 297-73101) to each sample-containing well.
(4) Incubated the plate at 60℃ for 30 minutes.
(5) Allow the plate to cool at room temperature.
(6) Measure the absorbance at 560 nm.

Application Data

Exosome labeling and uptake assay with HeLa cells

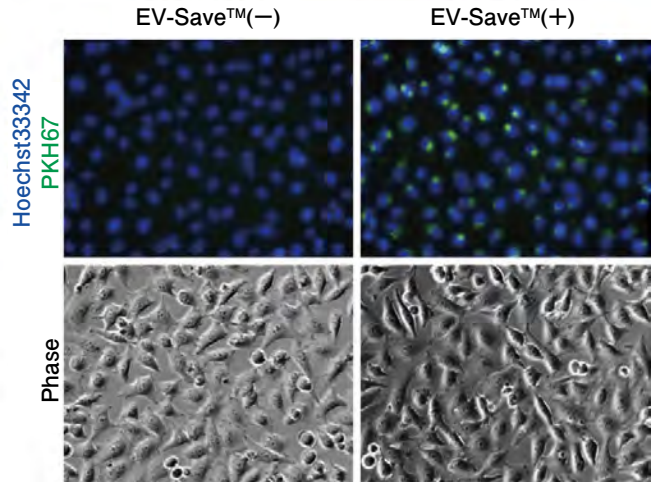
[Experimental overview]

Exosomes purified by MagCapture™ Exosome Isolation Kit PS were labeled with PKH67 (Sigma) and confirmed their ability to be incorporated into HeLa cells.

<Exosome labeling with PKH67>

1. Purify exosomes using MagCapture™ Exosome Isolation Kit PS (from COLO201 cell culture supernatant on the day of experiment).
   * To prevent loss of labeled samples due to adsorption in Step 6-7 Gel filtration, addition of EV-Save™ Extracellular Vesicle Blocking Reagent to the elution buffer included in the kit is recommended. Using the elution buffer containing EV-Save™, the sample can be prepared with the minimal loss.
2. Determine the protein concentration and particle concentration by BCA Assay and using NanoSight.
3. Dispense the exosome sample solution corresponding to 3 μg protein* to a 1.5 mL tube.
   * Prepare an appropriate amount as needed for the experiment.
4. Dissolve 2.0 μL PKH67 linker in 0.25 mL of Diluent C (provided with the PKH67 kit) - 4×Dye solution*
   * Prepare an appropriate amount as needed for the experiment.
5. Add 1/3 volume of 4×Dye solution to the exosome sample, mix, and incubate the mixture at room temperature for 5-10 minutes.
6. Equilibrate Exosome Spin Columns (MW 3000) (Thermo #4484449) with PBS according to the protocol provided with the product.
7. Apply 100 μL of each sample to each spin column equilibrated as described above* and centrifuge at 750× g for 2 minutes to separate unbound dye from labeled exosomes.
   * Because the maximum loading volume is 100 μL per column, multiple columns corresponding to the sample volume are recommended to be kept on hand where the sample amount is more than 100 μL.
8. Add the solution containing labeled exosomes to HeLa cells seeded on a dish on a preceding day. After 24 hours, perform microscopic observation and flow cytometry.
   * Adjust the number of exosomes added.

Results of microscopic observation



Results of flow cytometry

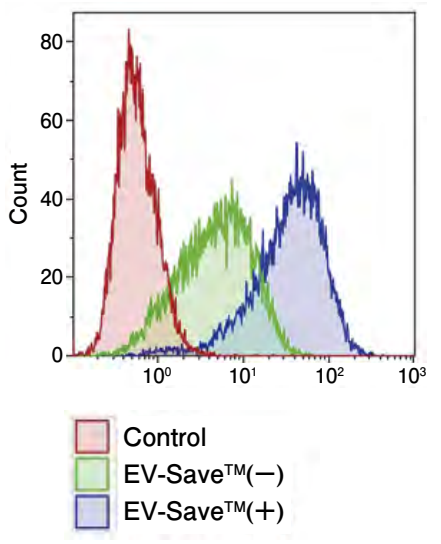


Figure 1. Observation of the uptake of PKH-labeled exosomes into HeLa cells.
Exosomes were isolated from COLO201 cell conditioned medium using MagCapture™ Exosome Isolation Kit PS (total protein 3 μg, particle number 1×10^{10}), followed by labeling using PKH67 Green Fluorescent Cell Linker Kit (Sigma). The uptake of the labeled exosomes into HeLa cells was confirmed by fluorescence microscopy (left) and flow cytometry (right).

Now you can see that PKH67-labeled exosomes are incorporated through endocytosis in both microscopy image and flow cytometry chart. Also, you can find that EV-Save™ Extracellular Vesicle Blocking Reagent added to the sample remarkably reduced the loss due to adsorption to the gel filtration column in a dye-removal step.

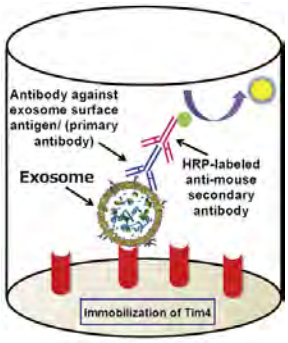
Application of the PS affinity method to ELISA

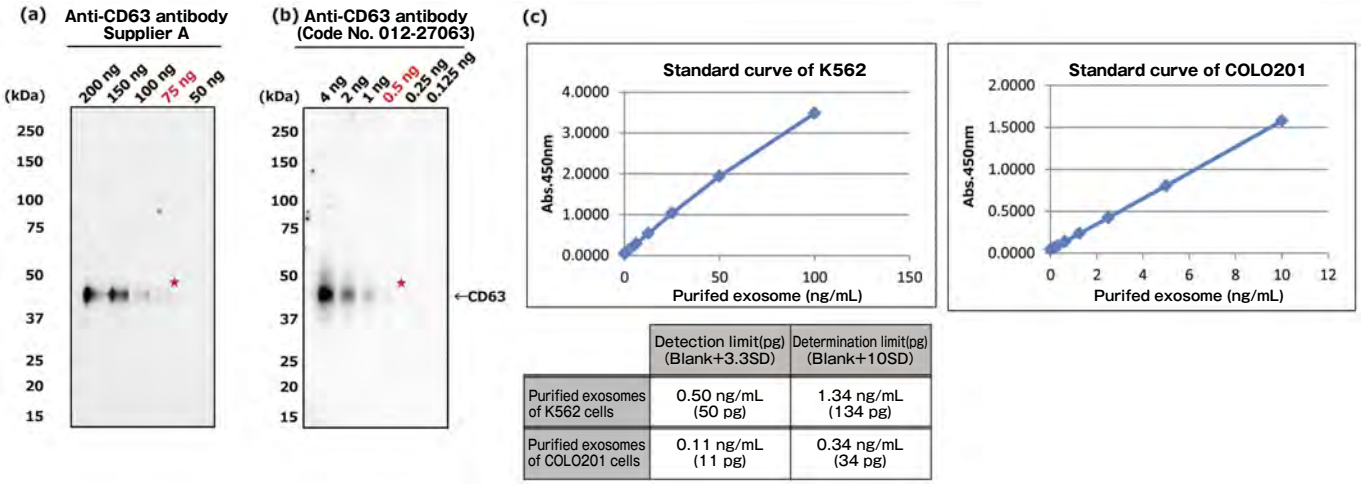
Introduction

We developed PS Capture™ Exosome ELISA Kit by applying affinity binding of Tim4 protein with exosomes. **This kit is capable of detecting exosomes at a sensitivity higher than that of conventional ELISA methods immobilization of antibodies against exosome surface markers.** Exosomes in samples such as culture supernatants and serum are captured by Tim4 protein on a dry plate in the presence of calcium ion. The captured exosomes are detected by a primary antibody against an exosome surface marker protein and a labeled secondary antibody. While a mouse anti-CD63 monoclonal antibody is provided with the kit, a user-provided mouse primary antibody against any other exosome surface marker may also be used for exosome detection.

The greatest feature of this kit is that it provides exosomes detection with higher sensitivity than that of Western Blot analysis and conventional product for exosome ELISA. First, the detection limit for exosomes in Western Blotting was examined for comparison with this kit (Figures 1a and b). Western Blot analysis of exosomes purified from COLO201 cells (of human colon adenocarcinoma origin) with an anti-CD63 monoclonal antibody detected exosomes in an amount as small as 75 ng on the protein basis. Next, the detection limits of this kit for exosomes purified from K562 cells (of human leukemia origin) and COLO201 cells were determined to be 49.9 pg and 10.9 pg, respectively, demonstrating that this kit had a detection sensitivity more than 1,000 times higher than that of Western Blotting (Figure 1c). Considering that the detection limits of conventional products for exosome ELISA range approximately several ng to several μg (refer to instruction manuals for individual products), the present results demonstrated that this kit utilizing affinity binding of exosomes to Tim4 via PS has a sensitivity more than 100 times higher than those of conventional ELISA methods involving immobilization of an antibody against an exosome surface protein marker.

Assay principle





	Detection limit(pg) (Blank+3.3SD)	Determination limit(pg) (Blank+10SD)
Purified exosomes of K562 cells	0.50 ng/mL (50 pg)	1.34 ng/mL (134 pg)
Purified exosomes of COLO201 cells	0.11 ng/mL (11 pg)	0.34 ng/mL (34 pg)

PS ELISA detected the marker proteins with 50-1,000 times higher sensitivity than WB.

Fig. 1 Comparing the detection sensitivities of Western Blot and PS ELISA

(a), (b) Result of sensitivity by Western Blot with each of anti-CD63 antibody (supplier A and Wako: Code No. 012-27063).
Sample : purified extracellular vesicles from cell culture supernatant of COLO201 cells with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
★ : detection limit by Western Blot

(C) Result of limit by PS ELISA
A standard curve was prepared using blank value of buffer and absorbance value of 2-fold serial dilution samples of extracellular vesicles purified from cell culture supernatant of K562 cells and that of COLO201 cells with MagCapture ™ Exosome Isolation Kit PS. Then, the detection limit of purified extracellular vesicles of K562 cells and COLO201 cells were calculated using its standard curve. (each dilution point: n=6, blank: n=12)

PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)

Introduction and Features

[Introduction]

The kit includes reagents for enzyme-linked immunosorbent assay (ELISA) available for a **qualitative analysis of extracellular vesicles purified from cell culture supernatant or body fluid** as well as **a quantitative analysis of extracellular vesicles in cell culture supernatant directly.** It can detect extracellular vesicles, which have any surface marker protein, with high sensitivity by using a mouse monoclonal antibody against any surface marker protein of extracellular vesicles as a primary detection antibody and HRP-conjugated anti mouse IgG antibody of the kit as a secondary detection antibody after extracellular vesicles are captured by a plate on which proteins that specifically bind with phosphatidylserine (PS) on the surface of extracellular vesicles are immobilized. As a control primary detection antibody, anti human CD63 mouse monoclonal antibody is included in the kit. By using this, human CD63 positive extracellular vesicles can be detected.

This kit can easily detect surface marker proteins of extracellular vesicles purified by MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601) with 50 to 1,000 times higher sensitivity than Western Blot. Also, extracellular vesicles in cell culture supernatant can be quantitatively measured by using extracellular vesicles purified from cell culture supernatant with MagCapture™ Exosome Isolation Kit PS as a reference standard.

[Features]

- High sensitivity (detectable at a sensitivity 50-1,000 times higher than that of WB)
- Direct qualitative/quantitative analysis of exosomes in the culture supernatant
- Capable of saving the number of exosomes used for analysis (less than 1/10-1/1,000 of the number required for WB)

[Kit contents]

Kit composition (96 reactions)	Quantity
Exosome Capture 96 Well Plate	8 well × 12 strips/1 plate
Reaction / Washing Buffer (10 ×)	50 mL × 2 vials
Exosome Binding Enhancer (100 ×)	10 mL × 1 vial
Control Primary Antibody Anti-CD63 (100 ×)	120 μL × 1 vial
Secondary Antibody HRP-conjugated Anti-mouse IgG (100 ×)	120 μL × 1 vial
TMB Solution	12 mL × 1 vial
Stop Solution	12 mL × 1 vial
Plate Seal	4 sheets
Instruction Manual	1 copy

[Product photo]

[Purpose]

(1) A qualitative analysis of extracellular vesicles purified from cell culture supernatant or body fluids

The kit provides a highly sensitive qualitative analysis of any surface marker protein of extracellular vesicles purified from cell culture supernatant or body fluids with MagCapture™ Exosome Isolation Kit PS by using a mouse monoclonal antibody against any surface marker protein of extracellular vesicles as a primary detection antibody.

(2) A quantitative analysis of extracellular vesicles in cell culture supernatant

Extracellular vesicles which are positive for any marker protein in cell culture supernatant can be quantitatively analyzed by using extracellular vesicles purified from cell culture supernatant with MagCapture™ Exosome Isolation Kit PS as a reference standard and using a mouse monoclonal antibody against any surface marker protein of extracellular vesicles as a primary detection antibody.

Note: While Anti CD63 antibody as a control primary detection antibody in the kit can detect human CD63, it cannot detect mouse, rat, and bovine CD63. When a surface marker protein other than human CD63 is required to be detected, use a mouse monoclonal antibody of interest.

Note: Since HRP-conjugated Anti mouse IgG as a secondary detection antibody of the kit can strongly react non-specifically with mouse IgG in a sample and weakly react non-specifically with human IgG and rat IgG, a quantitative analysis of serum or plasma samples including these IgGs should be avoided.

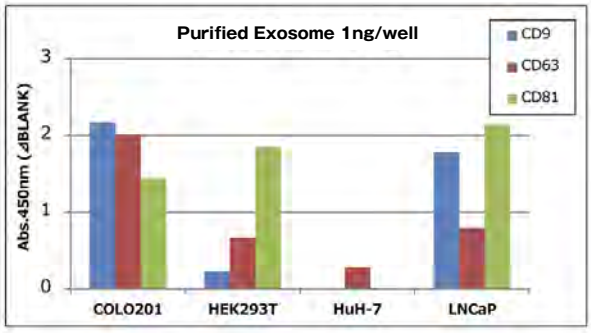
Application Data

Qualitative analysis of extracellular vesicles purified from various cell culture supernatants

Add 1 ng of extracellular vesicles purified from various cell culture supernatants to each well, and the expression level of surface marker proteins was compared by a qualitative analysis with three primary detection antibodies.

In addition, as reference comparative data, 150 ng of extracellular vesicles were purified from various cell culture supernatants and expression levels of their each surface markers were detected by Western Blot similarly. Then, qualitative analysis was conducted.

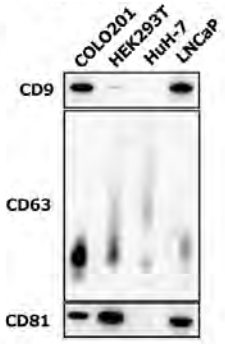
< Comparison of qualitative data per 1 ng of purified exosomes >



Expression pattern of marker proteins between ELISA and WB have a correlation.

< Reference comparative data >

Purified exosome: 150ng



■ Detection antibody

<ELISA>

Anti-CD9 mouse mAb (M-L 13), BD Bioscience

Anti-CD63 mouse mAb (H5C6), BD Bioscience

Anti-CD81 mouse mAb (JS-81), BD Bioscience

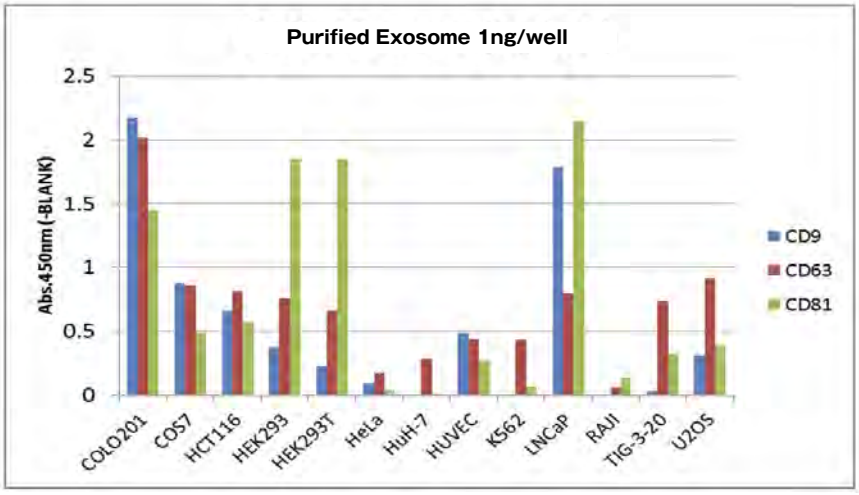
<WB>

Anti-CD9 rabbit pAb, System Bioscience

Anti-CD63 mouse mAb (8A12), CosmoBio

Anti-CD81 mouse mAb (1D6), Novus Biologicals

Reference data: Qualitative analysis of EVs purified from various cell culture supernatants



With 1 ng of extracellular vesicles purified from various cell culture supernatants to each well, expression level of surface marker proteins were detected by using three primary detection antibodies for CD9, CD63, and CD81.

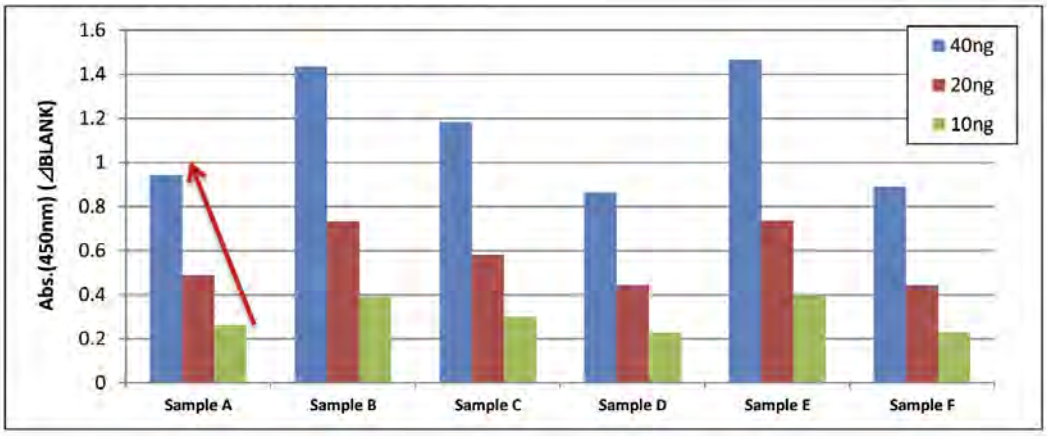
It is confirmed that expression levels of particular markers on exosomes is different between cell strains.

Application Data

Qualitative analysis of extracellular vesicles purified from human normal serum

Each of 40, 20, and 10 ng of extracellular vesicles purified from six human normal serum samples was added to a well and qualitative analysis was conducted using a control primary detection antibody against CD63 in the Kit.

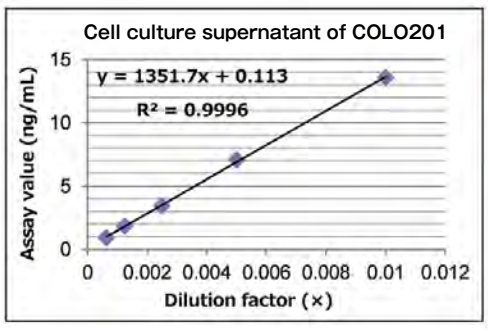
< Comparison of qualitative data of each sample >



The results showed properly linear curves in each samples.

Reference data: dilution linearity of cell culture supernatant sample

A standard curve was prepared using extracellular vesicles purified from cell culture supernatant of COLO201 cells, and then the dilution linearity of 5-step dilution samples of cell culture supernatant of COLO201 cells (1:100 to 1:1600) was evaluated.



Cell culture supernatant of COLO201 cells					
CM volume (μL)	Dilution		Assay value	Expected value	% of expected
	Ratio	Factor (×)	ng/mL	ng/mL	
0.0625	1 : 1600	0.000625	0.89	0.91	98.4
0.125	1 : 800	0.00125	1.82	1.72	105.6
0.25	1 : 400	0.0025	3.44	3.52	97.8
0.5	1 : 200	0.005	7.04	6.78	103.9
1	1 : 100	0.01	13.6	-	-

Reference standard: extracellular vesicles purified from cell culture supernatant of COLO201 cells with MagCapture™ Exosome Isolation Kit PS
Measured sample: cell culture supernatant of COLO201 cells
Primary antibody: anti-CD63 antibody in the kit

The results showed properly linear curves in each samples.

Application Data

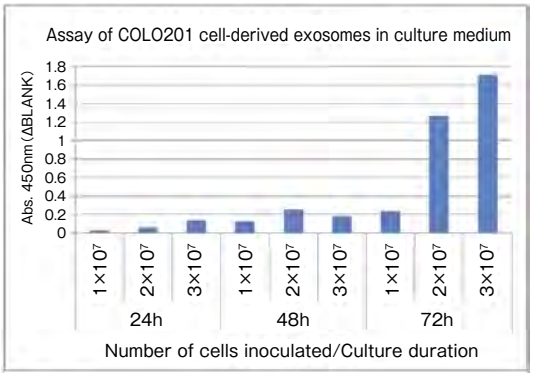
Monitoring changes of the amount of extracellular vesicles over time by the number of seeding cell and culture day

1 × 10^7, 2 × 10^7, and 3 × 10^7 COLO201 cells were separately seeded into T75 flasks and cultured for 72 hours. The small amount of culture supernatant samples were collected every 24 hours and subjected to spectrophotometric assay of CD63 using PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) and Control Primary Antibody Anti-CD63 (100 ×) included in the kit.

For the CD63 assay, 4 μL cell culture supernatant was diluted to 100 μL with Reaction/Washing Buffer (1 ×) supplemented with Exosome Binding Enhancer.

- Assay sample: 25-fold diluted COLO201 cell culture supernatant (4 μL → diluted to 100 μL)
- Number of seeding cells: 1 × 10^7, 2 × 10^7, 3 × 10^7 cells/T75 flask
- Culture duration: 24, 48, 72 hours
- Primary antibody: anti-CD63 antibody

Since it is possible to directly measure the amount of extracellular vesicles in the medium using only 4 μL of medium, this assay is recommended because an optimization of culture condition for newly culturing a cell line takes time and effort. By this method, the conditions under which the most extracellular vesicles are secreted can be easily examind.

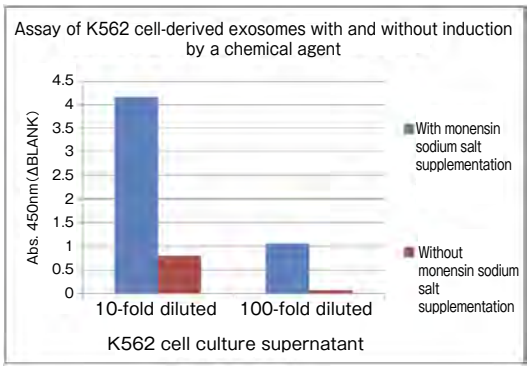


Changes in extracellular vesicle production induced by addition of chemical agent

K562 cells were seeded into T225 flasks and cultured in serum-free medium for 72 hours. Then, the culture medium was changed to serum-free medium supplemented either with or without monensin sodium salt whose final concentration is 10 μM and cultured for 24 hours. After the end of culture, culture supernatant samples were collected and subjected to spectrophotometric assay of CD63 using PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) and Control Primary Antibody Anti-CD63 (100 ×) included in the kit.

10-fold and 100-fold diluted cell culture supernatant samples were prepared by dilution of collected cell culture supernatant with Reaction/Washing Buffer (1 ×) supplemented with Exosome Binding Enhancer.

- Assay sample: K562 cell culture supernatant (cultured for 24 hours after changing to culture medium supplemented with monensin sodium salt or control culture medium)
- Primary antibody: anti-CD63 antibody



This assay requires no sample purification and just a small aliquot of culture medium collected during culture is sufficient for assay.
Since changes of the amount of exosomes in culture medium over time can be assayed and quantified, comparative assay of them is much easier than WB analysis. It's very convenient.

Application Data

Comparison of detection sensitivity of various ELISA kits using exosomes purified from COLO201 cell culture supernatant and human serum

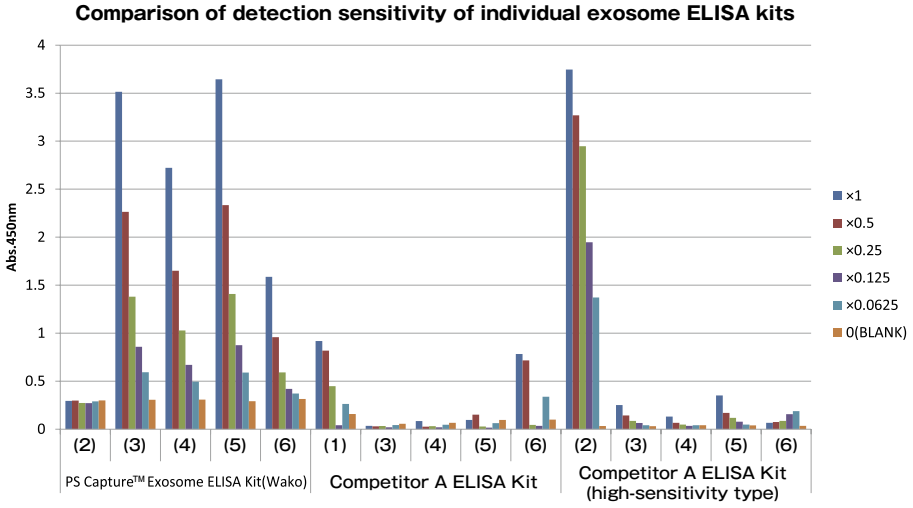
The following samples (1) to (6) were prepared and used for comparison of detection sensitivity of PS Capture™ Exosome ELISA Kit, Competitor A ELISA kit, and Competitor A ELISA kit (high-sensitivity type) with detection of CD63, an exosome marker protein.

[Samples used for comparison]

(1) Standard included in Competitor A ELISA kit
(2) Standard included in Competitor A ELISA kit (high-sensitivity type)
(3) Exosomes purified from COLO201 cell culture supernatant using MagCapture™ Exosome Isolation Kit PS
(4) Exosomes purified from COLO201 cell culture supernatant by polymer precipitation
(5) Exosomes purified from human serum using MagCapture™ Exosome Isolation Kit PS
(6) Exosomes purified from human serum by polymer precipitation

[Dilution rates and protein concentrations]

	(1)	(2)	(3)	(4)	(5)	(6)
× 1	1/16	1/1000	40 ng/mL	160 ng/mL	800 ng/mL	2000 μg/mL
× 0.5	1/32	1/2000	20 ng/mL	80 ng/mL	400 ng/mL	1000 μg/mL
× 0.25	1/64	1/4000	10 ng/mL	40 ng/mL	200 ng/mL	500 μg/mL
× 0.125	1/128	1/8000	5 ng/mL	20 ng/mL	100 ng/mL	250 μg/mL
× 0.0625	1/256	1/16000	2.5 ng/mL	10 ng/mL	50 ng/mL	125 μg/mL
0 (BLANK)	0	0	0	0	0	0



* Data under the detection limit are not indicated.

[Results]

PS Capture™ Exosome ELISA Kit detected CD63 at a sensitivity higher than those of Competitor A ELISA Kit and Competitor A ELISA Kit (high-sensitivity type). While both Competitor A ELISA Kit and Competitor A ELISA Kit (high-sensitivity type) strongly reacted with standards in their kit, their reactivity to exosomes purified by the PS affinity method was low.

These results suggested that PS Capture™ Exosome ELISA Kit was capable of detecting CD63 on the surface of exosomes more specifically and at a higher sensitivity than Competitor A ELISA Kit and Competitor A ELISA Kit (high-sensitivity type).

PS Capture™ Exosome ELISA Kit (Streptavidin HRP)

Introduction and Features

This kit is an enzyme immunoassay kit that is utilizable in the qualitative and quantitative analyses of extracellular vesicles in cell culture supernatant and body fluid samples.

This kit is capable of using antibodies of a variety of animal species and lectins for primary detection by labeling with biotin, though PS Capture™ Exosome ELISA Kit (Anti mouse IgG POD) (Code No. 297-79201) is capable of using only mouse monoclonal antibodies. Moreover, as this kit employs HRP-conjugated streptavidin for secondary detection, it exhibits low non-specific binding to blood components. Thus this kit enables sensitive detection of extracellular vesicles in a blood sample, while it is difficult to detect with PS Capture™ Exosome ELISA Kit (Anti mouse IgG POD).

【Features】

- Sensitive qualitative analysis (50 to 1000 folds more sensitive than WB in detection)
- Direct relative quantitation of extracellular vesicles in conditioned medium and body fluid specimens
- Application to detection systems using antibodies from various animal species and lectins
- Microanalysis with small amounts of extracellular vesicles
  (only 1/10 to 1/1000 of the sample amount for WB)

【Kit Contents】

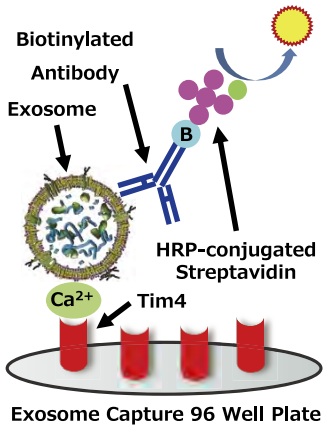
Kit composition (96 reactions)	Quantity
Exosome Capture 96 Well Plate	8 well × 12 strips/1 plate
Reaction Buffer	80 mL × 1 vial
Washing Buffer (10 ×)	100 mL × 1 vial
Exosome Binding Enhancer (100 ×)	10 mL × 1 vial
Control Biotinylated Antibody Anti-CD63 (100 ×)	120 μL × 1 vial
HRP-conjugated Streptavidin (100 ×)	240 μL × 1 vial
TMB Solution	12 mL × 1 vial
Stop Solution	12 mL × 1 vial
Plate Seal	4 sheets
Instruction Manual	1 copy

【Sample Type】

Sample	Qualitative Analyses	Quantitative Analyses
Cell culture supernatant	Analyzable	Analyzable
Body fluid	Analyzable	Analyzable

【Assay Principle】

Exosome Capture 96 Well Plate which is pre-coated with protein that specifically binds to phosphatidylserine (PS) on the surface of extracellular vesicles (EVs) capture EVs with Ca^{2+}. Then biotinylated antibody for the surface marker protein of EVs is used as a primary detection and HRP-conjugated streptavidin is used as a secondary detection.



【Product Photo】

【Purpose】

(1) A qualitative analysis of extracellular vesicles purified from cell culture supernatants and body fluids

The kit enables us to perform a high-sensitive qualitative analysis for any surface marker proteins of extracellular vesicles purified from cell culture supernatants and body fluids with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601) by using a biotinylated antibody specific for an interested surface marker protein of extracellular vesicles as a primary detection antibody.

(2) A quantitative analysis of extracellular vesicles in cell culture supernatants and body fluids

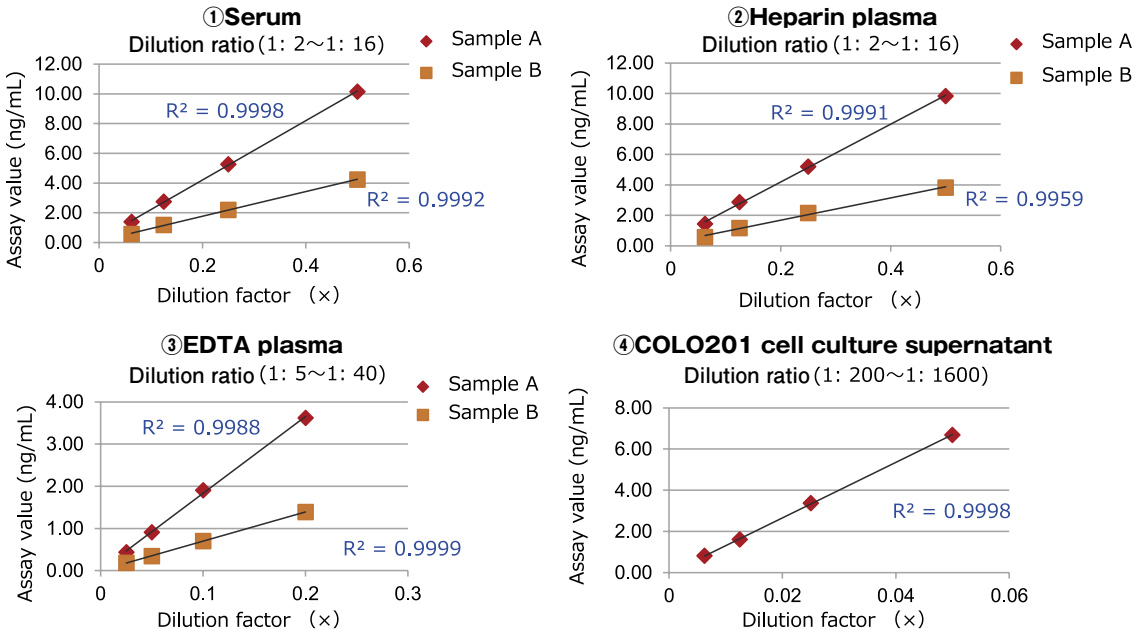
By preparing a standard curve using purified extracellular vesicles with an interested surface marker protein as a reference standard, permit the relative quantitation of extracellular vesicles which have the surface marker protein in cell culture supernatants and body fluids.

Note: Control Biotinylated Antibody Anti-CD63 in the kit is specific for human CD63. It does not react with mouse, rat, and bovine CD63. If any surface marker proteins except human CD63 are required to be detected, use appropriate biotinylated antibodies.

Application Data

Dilution Linearity of Cell Culture Supernatant and Blood Samples

Dilution linearity of four specimens, ① human serum diluent ② human heparin plasma diluent ③ human EDTA plasma diluent (2 specimens, 4-step serial dilution each) and ④ COLO201 cells cell culture supernatant diluent (4-step serial dilution), was assessed by measuring the concentration of exosomes (CD63 detection) with standard curve obtained using extracellular vesicles purified from cell culture supernatant of COLO201 cells.



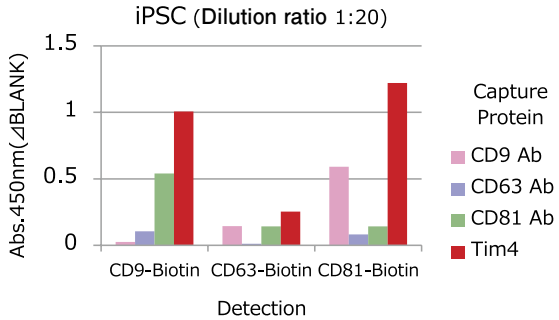
Samples in serum and heparinized plasma have given favorable linearity when diluted 2 folds or more.
Even samples in EDTA-treated plasma will give favorable linearity when diluted 5 folds or more.

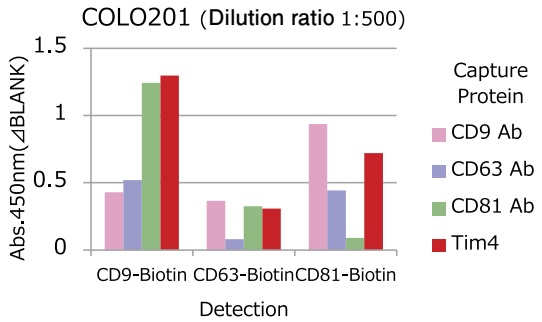
Comparison of Capture Ability between PS Affinity and Antibody

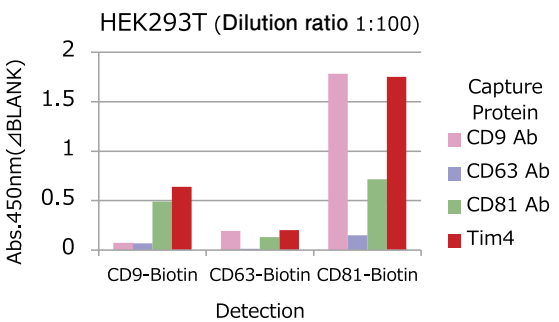
Microplate wells, pre-coated with anti-CD antibodies (CD9, CD63, CD81) and PS Affinity (Tim4), were incubated with pretreated※1 cell culture supernatants of iPSC, COLO201 and HEK293T cells. Captured exosomes on the plate were detected using biotinylated anti-CD antibodies (CD9, CD63, CD81).

※ 1 Pretreatment condition: 10,000×g, 30min.

Almost all of the exosomes derived from various cell lines were efficiently captured by Tim4 compared with anti-CD9, 63, 81 antibodies.



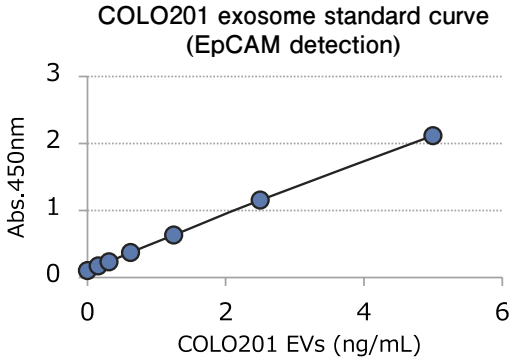




Application Data

Spike and Recovery Assay with Blood Samples【EpCAM】

Pretreated※2 pooled normal human serum, EDTA-treated plasma, and heparinized plasma were spiked with exosomes isolated and purified from COLO201 cell conditioned medium using MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601) at various concentrations. The spiked exosomes in a sample were detected using biotin-labeled anti-EpCAM※3 antibody (MBL), and the recovery rate of exosomes from the sample was calculated based on the standard curve prepared using exosomes purified from COLO201 cell conditioned medium.



Reaction Buffer (BLANK)	
Spiked value	Assay value
ng/mL	ng/mL
0	–
1.25	1.087
2.5	2.245
5	4.591

Actual conc.

Serum (Dilution ratio 1:2)				
Spiked value	Assay value	Recovery value	Recovery rate	
ng/mL	ng/mL	ng/mL	%	Mean
0	0.381	–	–	–
1.087	1.418	1.037	95	91
2.245	2.377	1.996	89	
4.591	4.429	4.048	88	

EDTA plasma (Dilution ratio 1:2)				
Spiked value	Assay value	Recovery value	Recovery rate	
ng/mL	ng/mL	ng/mL	%	Mean
0	1.861	–	–	–
1.087	2.945	1.084	100	94
2.245	3.898	2.037	91	
4.591	6.007	4.146	90	

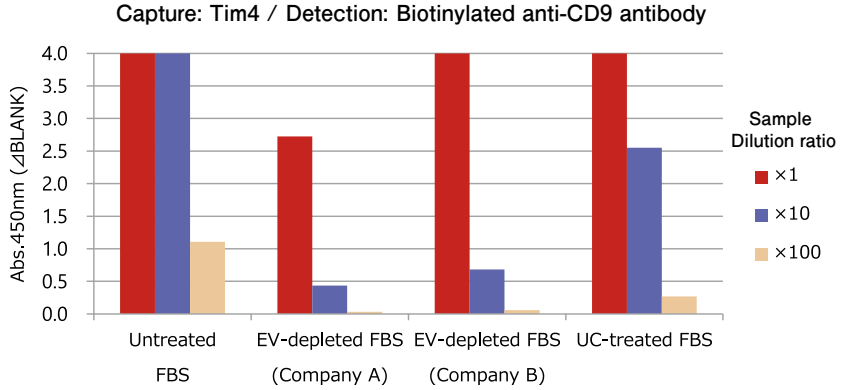
Heparin plasma (Dilution ratio 1:2)				
Spiked value	Assay value	Recovery value	Recovery rate	
ng/mL	ng/mL	ng/mL	%	Mean
0	0.433	–	–	–
1.087	1.524	1.091	100	96
2.245	2.566	2.133	95	
4.591	4.719	4.286	93	

The spiked recovery rates based on the detection of EpCAM fell within a range of 100%±10%, showing favorable recovery performance.

※ 2 Pretreatment condition: 10,000×g, 30min.　※ 3 EpCAM: Epithelial cell adhesion molecule

Test of Residual Extracellular Vesicles in EV-depleted FBS

Extracellular vesicles in untreated FBS, commercial EV-depleted FBS and ultracentrifugation (UC)-treated FBS※4 were detected using Biotinylated anti-CD9 antibody※5.



PS Affinity ELISA System enables test of residual extracellular vesicles in EV-depleted FBS.

※ 4 Ultracentrifugation condition: 160,000xg, 16h

※ 5 Anti-CD9 antibody (Code No. 014-27763) was biotinylated using Biotin Labeling Kit-SH (Code No. 348-90941).

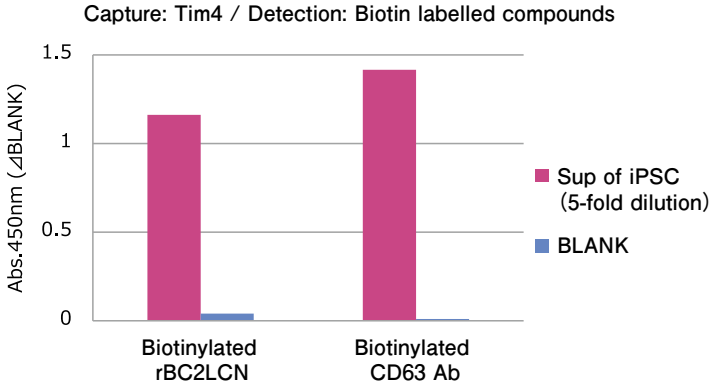
Application Data

Sugar Chain Analysis by Sandwich ELISA with rBC2LCN Lectin and Tim4

Exosomes in five-fold dilution of pretreated※6 cell culture supernatants of iPS cells were detected using Biotinylated anti-CD63 antibody (Code No. 019-27713) and Biotinylated rBC2LCN lectin※7.

※ 6 Pretreatment condition: 10,000×g, 30min.
※ 7 rBC2LCN binds specifically to Fucα1-2Galβ1-3GalNAc (GlcNAc) and is known as a undifferentiated marker of iPS and ES cells. rBC2LCN (Code No. 029-18061) was biotinylated using EZ-Link™ Sulfo-NHS-LC-LC-Biotin (ThermoFisher).

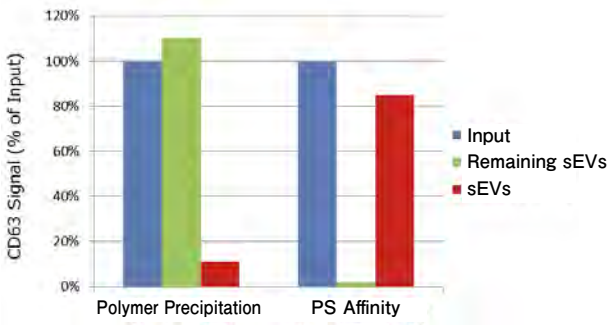
Reference: S. Saito, *et al*., Sci. Rep., **8**, 3997 (2018)



PS Affinity ELISA is applicable for sugar chain analysis on exosomes with a various combination of lectins.

Comparison of recovery efficiency with Polymer Precipitation

From 1 mL of pretreated※8 COLO201 cell conditioned medium, exosomes were isolated and purified by PS affinity method or polymer precipitation method (competiter A) and then measured for signals of CD63, an exosome marker, using PS Capture™ Exosome ELISA Kit (streptavidin HRP). From absorbance of each sample, the amount of residual exosomes in a post-purification sample and the recovery rate of exosomes were determined for comparison.



※ 8 Pretreatment condition: 10,000×g, 30min.

< Calculation of recovery efficiency >
Input: Measured value of 100-fold diluted conditioned medium※Ⅰ
Remaining sEVs: Measured value of post-purification 100-fold diluted conditioned medium※Ⅰ
Recovered sEVs: Measured value of 1000-fold diluted purified sample※Ⅱ

※Ⅰ Measured value of 100-fold diluted sample has been confirmed to fall within the measurement range. In the preliminary investigation, a dilution ratio appropriate for ELISA is recommended to be calculated for each sample
※Ⅱ Exosome samples purified with this kit and polymer reagents are theoretically concentrated 10 folds.
(e.g. 1 mL of input → 100μL of purified exosomes)
Value obtained by multiplying the concentration ratio by the dilution ratio for ELISA corresponds to integrated dilution ratio of the purified exosome for ELISA

The ELISA system allowing highly sensitive detection of exosomes will ease efficiency comparison among exosome recovery methods and validation of the method.

PS Capture™ Exosome Flow Cytometry Kit

Introduction and Features

This product is capable of capturing extracellular vesicles on magnetic beads by a new affinity method (PS affinity method) using magnetic beads and Tim4 protein specifically binding phosphatidylserine (PS) and then detecting surface marker proteins at a high sensitivity by flow cytometry. It realizes direct qualitative analysis of surface marker proteins without purification of extracellular vesicles from cell culture supernatant and body fluid specimens (*e.g.*, serum, plasma).

This product requires a primary antibody against the surface marker protein of interest and a fluorescence-labeled secondary antibody, or a fluorescence-labeled primary antibody against the surface marker protein of interest.

[Features]
- High-Sensitive Qualitative Analysis
- Easy Operation by Magnetic Beads
- Direct Detection without Purification
- Total 3 hours
  ～ from Isolation to Staining ～

[Kit contents]

Kit composition (300 assays)	Quantity
Exosome Capture Beads	3 mL × 1 bottle
Washing Buffer（10 ×）	45 mL × 2 bottles
Exosome Binding Enhancer (100 ×)	15 mL × 1 bottle

【Sample Type】
- Cell Culture Supernatant
- Serum
- Plasma（Heparin and EDTA）

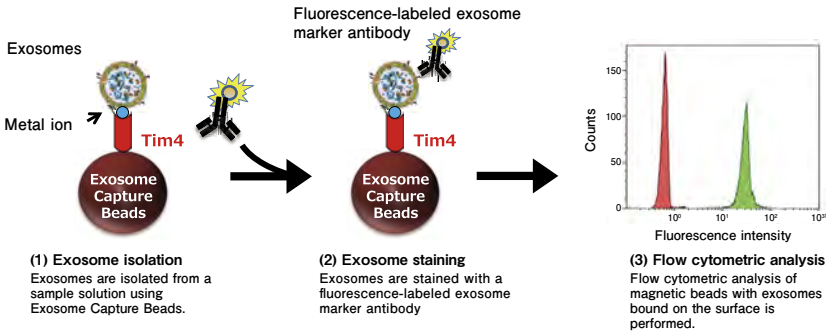
[Recommended Reaction Scale]

Basic protocol is set as 2 reactions using a 1.5 mL microcentrifuge tube to isolate EVs from samples with Exosome Capture Beads. For scale-up, increase the amount of Exosome Capture Beads and samples.

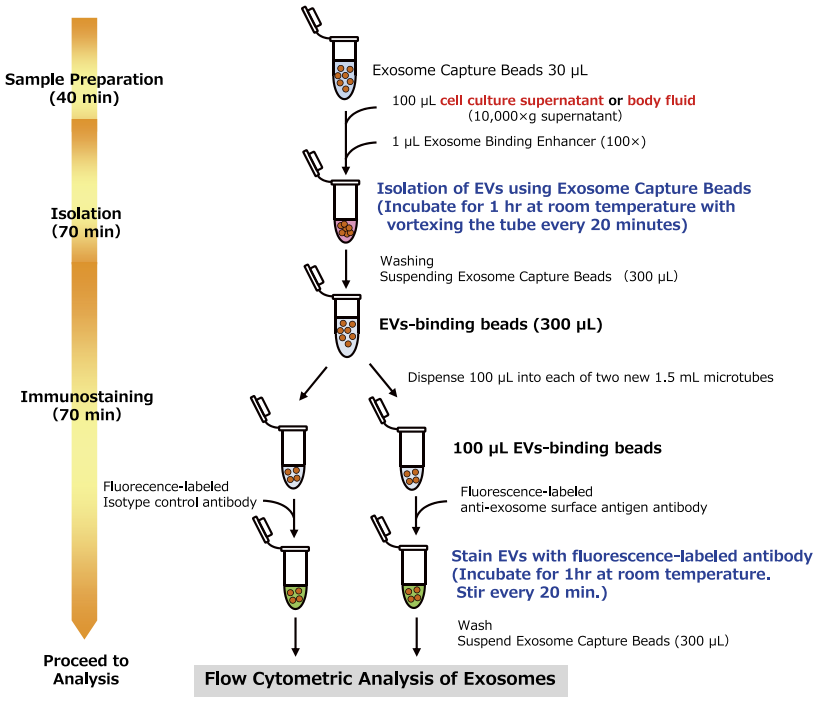
※The maximum reactions per 1.5 mL microcentrifuge tube is 10.

Qty of Reaction	Exosome Capture Beads (μL)	Sample volume (μL)
2 reactions (basic)	30	100
3 reactions	40	133
4 reactions	50	167
5 reactions	60	200
6 reactions	70	233
7 reactions	80	267
8 reactions	90	300
9 reactions	100	333
10 reactions	110	367

[Assay method]

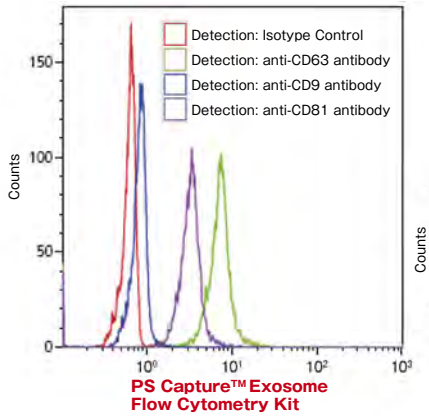


Outline of Procedure ~ Basic Protocol for 2 reactions ~

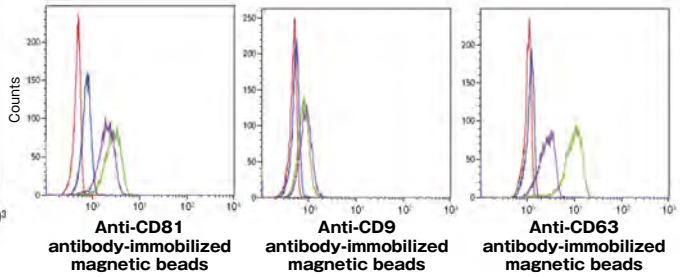


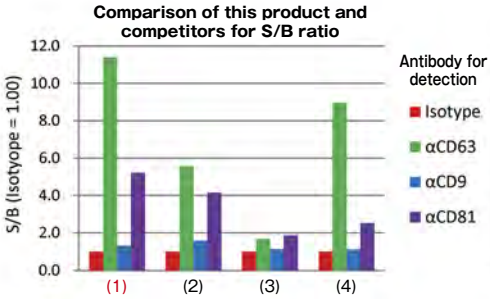
Application Data

Surface antigen analysis of exosomes contained in K562 cell culture supernatant



Exosomes in cell culture supernatant of K562 cells were isolated using PS Capture™ Exosome Flow Cytometry Kit or each of anti-CD81-, CD9-, and CD63-antibody-immobilized magnetic beads (supplier A), followed by flow cytometric analysis of exosome surface antigens after immunostaining with fluorescence- labeled antibodies.





(1) PS Capture™ Exosome Flow Cytometry Kit
(2) Competitor: Anti-CD81 antibody-immobilized magnetic beads
(3) Competitor: Anti-CD9 antibody-immobilized magnetic beads
(4) Competitor: Anti-CD63 antibody-immobilized magnetic beads

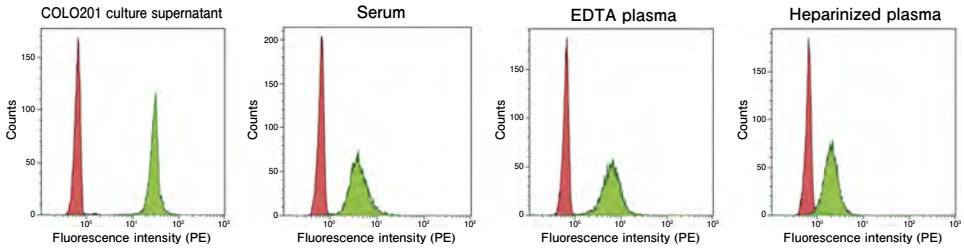
Whichever antibody was used for detection, this product detected exosome surface antigens at a sensitivity higher than those of competitors.

■ Sample
Cell culture supernatant of K562 cells: 33 μL/Assay
■ Detection antibody
PE-anti-CD63 (BD Biosciences)
PE-anti-CD9 (Novus Biologicals)
PE-anti-CD81 (Novus Biologicals)

Surface antigen analysis of exosomes contained in COLO201 cell culture supernatant, human serum, and human plasma (EDTA plasma, heparinized plasma)

Exosomes contained in COLO201 cell culture supernatant, human serum, and human plasma (EDTA plasma, heparinized plasma) were isolated by this product. Exosomes were detected using PE-labeled mouse IgG isotype control or PE-labeled anti-human CD9 antibody.

・Samples: 33 μL/assay each　　・Detection: PE-labeled anti-CD9 antibody, Novus Biologicals



Whichever sample was analyzed, the shift of fluorescence intensity peak was observed. This demonstrated that this product was capable of detecting exosomes in cell culture supernatant, serum, and plasma.

Code No.	Description	Package Size	Storage
297-79701	PS Capture™ Exosome Flow Cytometry Kit	300 reactions	-20℃

Exosomal Marker Antibody

Introduction and Features

The tetraspanin family such as CD9, CD63 and CD81 are used as marker proteins for exosomes. We offer highly sensitive monoclonal antibodies established by the DNA immunization method and have confirmed that it can be used for Western blot, flow cytometry, ELISA and immunoprecipitation.

[Features]
- High Sensitivity
- Cost-Effective Price
- High Specifity
- Recognize Nonreducing Samples

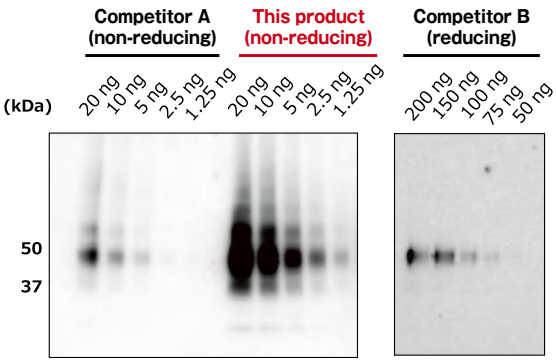
Marker		CD63	CD9	CD81
Clone No.		3-13	1K	17B1
Immunized Animal		Mouse	Mouse	Mouse
Subclass		IgG1	IgG1	IgG2b
Reactivity	Human	++	++	++
	Bovine	−	++	++
	Mouse	−	−	−
	Rat	−	+	−
Applications		Western Blot Flow Cytometry ELISA Immunoprecipitation	Western Blot Flow Cytometry ELISA Immunoprecipitation	Western Blot Flow Cytometry ELISA Immunoprecipitation
Product Lineup		Unconjugated Fluorescein conjugated Red Fluorochrome (635) conjugated Biotin conjugated	Unconjugated	Unconjugated

[Reactivity information] ++ : Reactive. + : Weak Reactive. − : Non-reactive.

DNA immunization: It is a technique to establish antibodies against the target protein, which is expressed in an animal body transfected with an expression vector including a gene of the target protein. Because this technique allows preparation of antibodies recognizing the target protein in a native form, it would have advantages in preparation of antibodies intended for therapeutic/diagnostic use, ones recognizing membrane proteins, and neutralizing/functional ones.

Anti CD63, Monoclonal Antibody (3-13)

Exosomes were purified from COLO201 cell culture supernatant and used for comparison of detection sensitivity of Western Blotting.



This product was capable of detecting CD63 protein on exosome surface at a sensitivity higher than those of Competitors A and B.

Exosome isolation: MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
Gel: SuperSep™ Ace 5-20%, 17 wells (Code No. 194-15021)
Running buffer: SDS-PAGE Buffer, pH8.5 (Code No. 192-16801)
Blocking: 3% Skim milk/PBS-T
Chemiluminescent reagent: ImmunoStar® Zeta (Code No. 295-72404)
Primary antibody: diluted 1,000-fold
Secondary antibody: anti-mouse IgG (H+L, peroxidase-conjugated, diluted 10,000-fold) (for Competitor B, the secondary antibody included in the product was used)

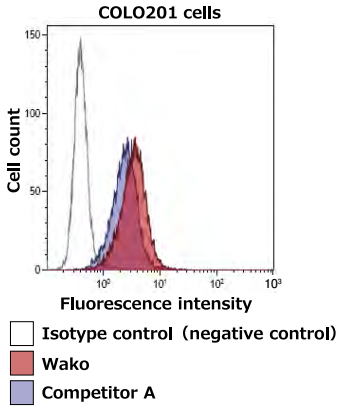
Anti CD63, Monoclonal Antibody (3-13), Fluorescein conjugated

Ex / Em = 494 nm / 521 nm

■ FCM analysis of COLO201 cells

Flow cytometric analysis of COLO201 cells was performed using Wako (3-13) and competitor A.

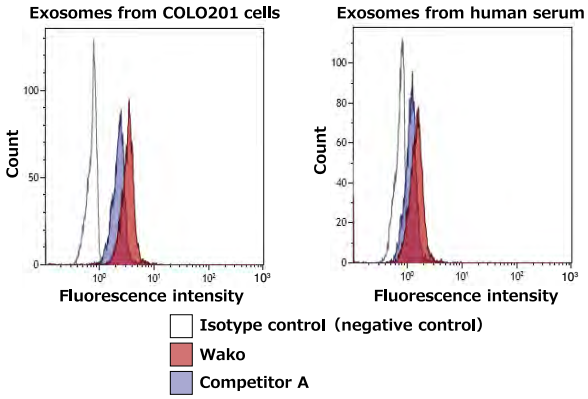
Sample amount: 1 × 10^6 cells
Antibody amount: 10 μL (1 test)



■ Detection of CD63 on the surface of exosome in COLO201 cell culture supernatant and human serum

Exosomes in pretreated※ COLO201 cell culture supernatant and human serum were isolated with PS Capture™ Exosome Flow Cytometry Kit (Code No. 297-79701) and detected using Wako (3-13) and competitor A.

※ Pretreatment condition: 10,000 × g, 30min.
Sample amount (supernatant / serum): 33 μL



This product achieved more sensitive detection of CD63 on the COLO201 cell surface and exosome surface than the other company product.

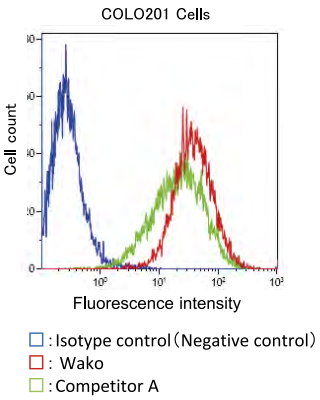
Anti CD63, Monoclonal Antibody (3-13), Red Fluorochrome (635) conjugated

Ex / Em = 634 nm / 654 nm

■ FCM analysis of COLO201 cells

Flow cytometric analysis of COLO201 cells was performed using Wako (3-13) and competitor A.

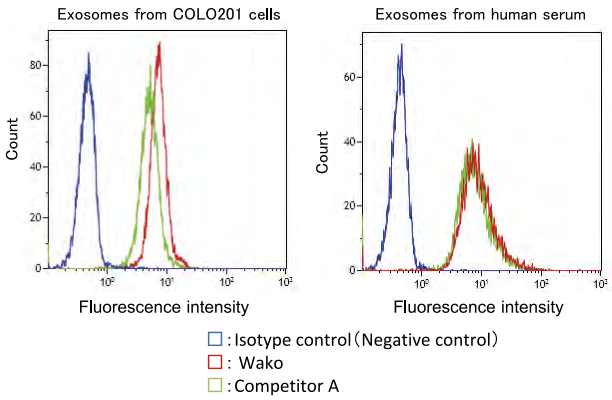
Sample amount: 1 × 10^6 cells
Antibody amount: 10 μL (1 test)



■ Detection of CD63 on the surface of exosome in COLO201 cell culture supernatant and human serum

From pretreated※ COLO201 cell conditioned medium (8-fold dilution) and human serum, exosomes were isolated using PS Capture™ Exosome Flow Cytometry Kit (Code No. 297-79701) and analyzed by flow cytometry using this antibody or the other company product.

※ Pretreatment condition: 10,000 × g, 30min.
Sample amount (supernatant / serum): 33 μL

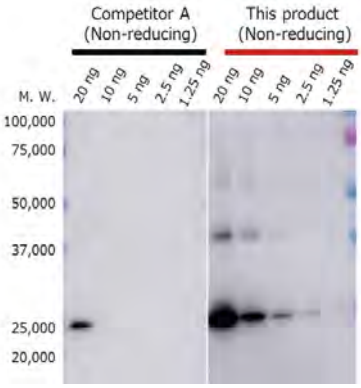


This product achieved detection of CD63 on the COLO201 cell surface and exosome surface comparable to or more sensitive than the other company product.

Anti CD9, Monoclonal Antibody (1K)

■ Western Blot

Exosomes (1.25 to 20 ng) isolated and purified from COLO201 cell conditioned medium using MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601) were detected with this antibody or competitor A.

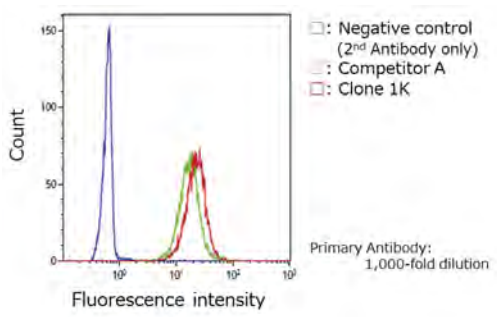


Isolation of Exosomes: MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
Gel: SuperSep™ ACE 10-20%, 17well (Code No. 198-15041)
Running Buffer: SDS-PAGE Buffer, pH8.5 (Code No. 192-16801)
Blocking: 3% Skim milk/PBS-T
Chemiluminescent reagent: ImmunoStar® Zeta (Code No. 295-72404)
Primary Antibody: 1,000-fold dilution
Secondary Antibody: Anti Mouse IgG (H+L), Peroxidase Conjugated, 10,000-fold dilution.

■ Flow Cytometry

From pretreated※ 8-fold diluted COLO201 cell conditioned medium, exosomes were isolated using PS Capture™ Exosome Flow Cytometry Kit (Code No. 297-79701) and subjected to detection of the exosome surface antigen by flow cytometry using this antibody or competitor A and fluorescence-labeled anti-mouse IgG antibody (Code No. 569-79631).

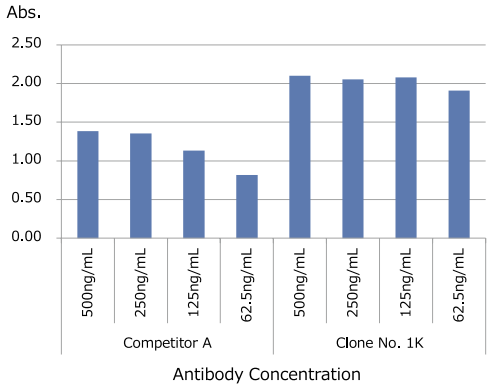
※ Pretreatment condition: 10,000 × g, 30min.



■ ELISA

Exosomes in pretreated※ 500-fold diluted COLO201 cell conditioned medium were measured using this antibody or competitor A as the primary antibody. As reagents other than the primary antibody, ones included in PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (Code No. 297-79201) were used.

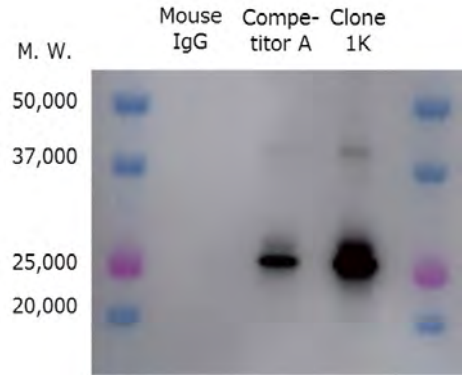
※ Pretreatment condition: 10,000 × g, 30min.



■ Immunoprecipitation

Exosomes in pretreated※ COLO201 cell conditioned medium were captured by immunoprecipitation using this antibody or competitor A and detected using HRP-labeled anti-CD9 antibody (competitor B).

※ Pretreatment condition: 10,000 × g, 30min.



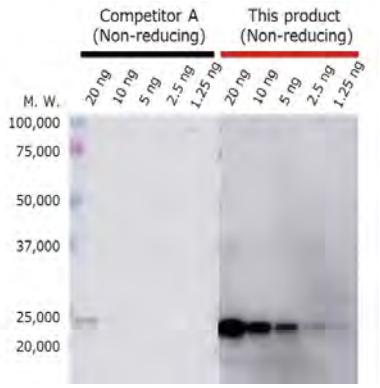
Gel: SuperSep™ ACE 10-20%, 17well (Code No. 198-15041)
Running Buffer: SDS-PAGE Buffer, pH8.5 (Code No. 192-16801)
Blocking: 3% Skim milk/PBS-T
Chemiluminescent reagent: ImmunoStar® Zeta (Code No. 295-72404)
HRP conjugated Anti-CD9 antibody: 1,000-fold dilution
COLO201 cell culture supernatant: 1 mL
Antibody amount: 5 μg

This product successfully detected CD9 on the exosome surface at a high sensitivity. Also, CD9 positive exosomes were efficiently collected.

Anti CD81, Monoclonal Antibody (17B1)

■ Western Blot

Exosomes (1.25 to 20 ng) isolated and purified from COLO201 cell conditioned medium using MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601) were detected with this antibody or competitor A.

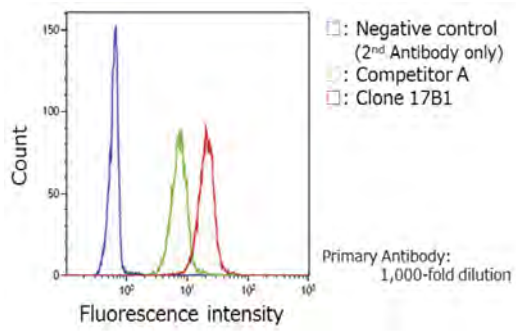


Isolation of Exosomes: MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
Gel: SuperSep™ ACE 10-20%, 17well (Code No. 198-15041)
Running Buffer: SDS-PAGE Buffer, pH8.5 (Code No. 192-16801)
Blocking: 3% Skim milk/PBS-T
Chemiluminescent reagent: ImmunoStar® Zeta (Code No. 295-72404)
Primary Antibody: 2,000-fold dilution
Secondary Antibody: Anti Mouse IgG (H+L), Peroxidase Conjugated, 10,000-fold dilution.

■ Flow Cytometry

From pretreated※ 8-fold diluted COLO201 cell conditioned medium, exosomes were isolated using PS Capture™ Exosome Flow Cytometry Kit (Code No. 297-79701) and subjected to detection of the exosome surface antigen by flow cytometry using this antibody or competitor A and fluorescence-labeled anti-mouse IgG antibody (Code No. 569-79631).

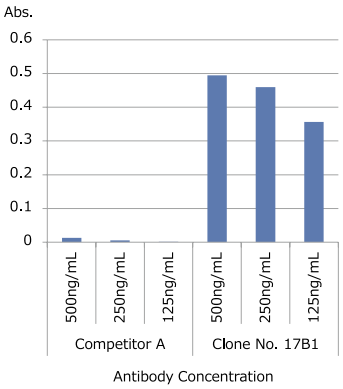
※ Pretreatment condition: 10,000 × g, 30min.



■ ELISA

Exosomes in pretreated※ 500-fold diluted COLO201 cell conditioned medium were measured using this antibody or competitor A as the primary antibody. As reagents other than the primary antibody, ones included in PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (Code No. 297-79201) were used.

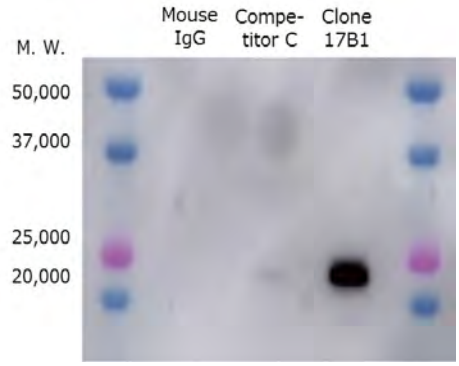
※ Pretreatment condition: 10,000 × g, 30min.



■ Immunoprecipitation

Exosomes in pretreated※ COLO201 cell conditioned medium were captured by immunoprecipitation using this antibody or competitor A and detected using HRP-labeled anti-CD9 antibody (competitor B).

※ Pretreatment condition: 10,000 × g, 30min.



Gel: SuperSep™ ACE 10-20%, 17well (Code No. 198-15041)
Running Buffer: SDS-PAGE Buffer, pH8.5 (Code No. 192-16801)
Blocking: 3% Skim milk/PBS-T
Chemiluminescent reagent: ImmunoStar® Zeta (Code No. 295-72404)
HRP conjugated Anti-CD81 antibody: 1,000-fold dilution
COLO201 cell culture supernatant: 1 mL
Antibody amount : 5μg

This product successfully detected CD81 on the exosome surface at a high sensitivity. Also, CD81 positive exosomes were efficiently collected.

EV-Save™ Extracellular Vesicle Blocking Reagent

Introduction and Features

EV-Save™ Extracellular Vesicle Blocking Reagent is a polymer reagent to prevent adsorbing extracellular vesicles in cell culture supernatants to laboratory tools such as tubes and pipet tips, which reduces the loss of extracellular vesicles during experiments and storage. Add into samples before ultrafiltration of cell culture supernatants, isolation and storage of extracellular vesicles.

【Features】

- Strong suppression of adsorption of extracellular vesicles in culture supernatant and after purification to laboratory tools
- Simple operation just to add to the sample

【Precautions for use】

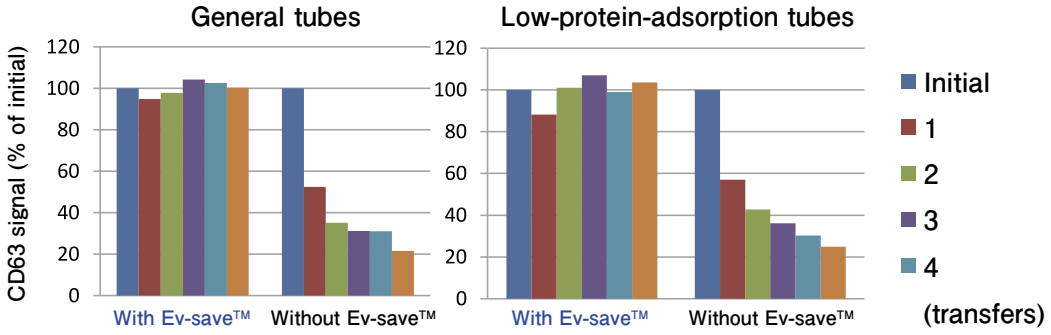
- The effect of EV-Save™ cannot be obtained when it is used for serum, plasma, or samples containing a lot of impurities.
- The product contains a polymer. Do not use EV-Save™ if the polymer may affect the experimental results in the post process.
  It have been confirmed that there are no effect by EV-Save™ in the following analyses using extracellular vesicles.
  1. Nanoparticle Tracking Analysis
  2. Western blot
  3. ELISA
  4. Microarray analysis
  5. Cell culture

Anti-adsorption effect of EV-Save™ — Tubes —

Whether EV-Save™ could suppress adsorption of purified exosomes to tubes or not was investigated.

【Results】

Loss of exosomes occurred associated with transfers using either general tubes or low-protein-adsorption tubes. Addition of EV-Save™ almost completely suppressed loss of exosomes associated with tube transfers.



【Experiment conditions】

Using MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601), exosomes were isolated from COLO201 cell conditioned medium. A suspension of exosomes at 3 ng/μL was placed in a tube and allowed to stand for 3 minutes. Then, the suspension was transferred to another tube. Such standing-transfer operation was repeated 4 times. In the "With EV-Save™" condition, EV-Save™ was initially added to the suspension to achieve 100-fold dilution in volume before the standing-transfer operations.

Using PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (Code No. 297-79201), CD63 signals of the exosomes were measured to determine the reduction rate of exosomes with the number of transfers. The results were reflected in a graph setting the initial CD63 signal as 100% relatively.

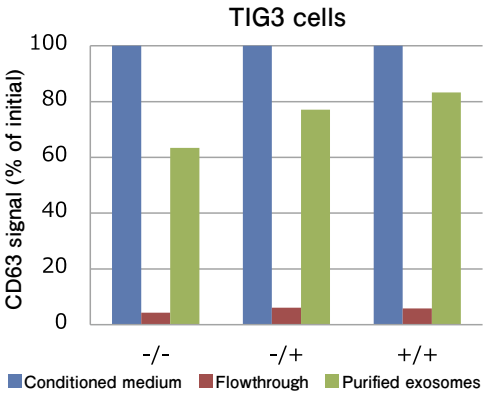
Anti-adsorption effect of EV-Save™
— Combination with MagCapture™ Exosome Isolation Kit PS —

【Outline of experiment】

Whether EV-Save™ would suppress loss of exosomes in an exosome purification process from TIG3 cell conditioned medium using MagCapture™ Exosome Isolation Kit PS or not was investigated.

【Results】

The yield of exosomes from the process with EV-Save™ added to both TIG3 cell conditioned medium and "Exosome Elution Buffer" included in MagCapture™ Exosome Isolation Kit PS (+/+) was 20% higher than that from the process without EV-Save™ added to either (−/−).



− − Without EV-Save™ added to conditioned medium/ without EV-Save™ added to "Exosome Elution Buffer"
− + Without EV-Save™ added to conditioned medium/ with EV-Save™ added to "Exosome Elution Buffer"
+ + With EV-Save™ added to conditioned medium/ with EV-Save™ added to "Exosome Elution Buffer"

【Experiment conditions】

Using MagCapture™ Exosome Isolation Kit PS, exosomes were purified from 1 mL of the conditioned medium each of COLO201, TIG3, and human iPS cells. The conditioned medium was not subjected to concentration by ultrafiltration. Exosomes were purified from the "conditioned medium with and without EV-Save™ added" using "'Exosome Elution Buffer' included in MagCapture™ Exosome Isolation Kit PS with and without EV-Save™ added."

Using PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (Code No. 297-79201), CD63 signals of exosomes were measured as an indicator of the exosome amount. The results were reflected in a graph setting the CD63 signal in the conditioned medium as 100% relatively.

Cryoprotective effect of EV- Save™

【Experimental overview】

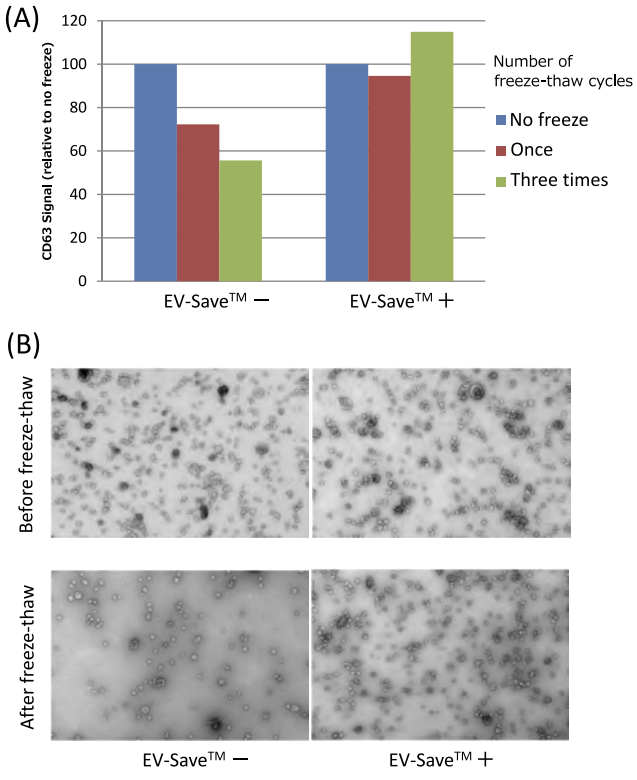
It is known that exosomes are damaged by repeated freeze-thaw (Witwer, K. W. *et al*., 2013). It was examined whether such damage for exosomes could be suppressed by the addition of EV-Save™.

【Experimental conditions】

The COLO201 cell-derived exosomes purified by MagCapture™ Exosome Isolation Kit PS were freeze-thawed. Thereafter, measurement with a PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (Code No. 297-79201) (A) and analysis with a transmission electron microscope (TEM) were performed (B).

【Result】

Although freeze-thaw reduced CD63 signal, such reduction was suppressed by the addition of EV-Save™ (A). Furthermore, from the result of electron microscopic analysis (B), it was confirmed that freezing and thawing caused a marked decrease in the number of particles, but the suppression effect by EV-Save™ was also recognized for such a phenomenon.



Q&As and Troubleshooting

• MagCapture™ Exosome Isolation Kit PS

■ Kit specifications and performance

Q1: What is the principle of this purification method?
This method for purification of extracellular vesicles (EVs) including exosomes utilizes a protein called Tim4 that binds phosphatidylserine (PS), a phospholipid located on the surface of EVs including exosomes, in a manner dependent on metal ion.
This is an affinity purification technique not using an antibody.

Q2: What kind of EVs are purified using this kit?
Exosomes and microvesicles with PS exposed on the surface of lipid membrane are purified using this kit.

Q3: What is the difference between exosomes and microvesicles?
Exosomes and microvesicles are distinguished from each other by difference in generation pathway. Exosomes are defined as extracellular vesicles secreted from late endosomes, while microvesicles as extracellular vesicles directly budding from cell membrane. Exosomes and microvesicles differ in size distri-bution: exosomes are considered to have a particle size of approximately 40-100 nm, while microvesicles have a particle size of approximately 100-1,000 nm. However, much smaller microvesicles have been reported. Thus, exosomes and microvesicles may not be clearly distinguished by size.

Q4: Can exosomes and microvesicles be purified separately?
As described above, exosomes and microvesicles cannot be clearly distinguished by size and their complete separation by size is impossible. We recommend the following method for preliminary separation of exosomes and microvesicles to obtain major fractions of individual vesicles using this kit. To purify extracellular vesicles with small particle size (Small EVs) including exosomes, use the supernatant obtained after centrifugation at 10,000 × g as a sample.
To purify extracellular vesicles with large particle size (large EVs) including microvesicles, then, collect the supernatant obtained after centrifugation at 1,200 × g, centrifuge this supernatant at 10,000 × g to isolate the precipitate, suspend the precipitate with TBS, and use this suspension as a sample.
To purify both vesicles together, use the supernatant obtained after centrifugation at 1,200 × g as sample.
See instruction manual for details of sample pretreatment conditions.

Q5: Is there anything else co-purified with exosomes and microvesicles?
Enveloped viruses, if present in sample, are known to be recovered together with exosomes and microvesicles. This is because enveloped viruses have PS exposed on the viral membrane surface. Utilizing this property, this kit may potentially be applied to recovery of enveloped viruses.
Separation of enveloped viruses from exosomes and microvesicles recovered using this kit requires affinity purification with a virus-specific antibody. This applies also to purification methods using antibodies against exosome markers located on the envelope (e.g., CD63).

Q6: Is there citation for virus purification?
In the following paper, virus purification using PS affinity method is reported.
"Vesicle-Cloaked Virus Clusters Are Optimal Units for Inter-organismal Viral Transmission", M. Santiana, *et al., Cell Host & Microbe*, **24**, 208-220 (2018)

Q7: Do all exosomes have PS exposed on the membrane surface?
Although no finding has been obtained that indicates that all exosomes have exposed PS, we have not confirmed samples that could not be detected in our experience (see Purification results and Detection results on page 36 Q7) so far.
In the following paper, cryo-electron microscopic analysis and phenotypic analysis of activated platelet-derived extracellular vesicles using immune-gold colloidal particles are reported. In this paper, it is reported that about 75% of extracellular vesicles from activated platelets have PS exposed on the surface.
"Extracellular vesicles from activated platelets: a semiquantitative cryo-electron microscopy and immuno-gold labeling study", B. Alain R, *et al.*, *Platelets*, **28** (3), 263-271 (2017)

Q8: From what samples are exosomes purified using this kit?
We have experiences of successful exosome recovery from cell culture supernatant, serum, heparinized plasma, EDTA-plasma, urine, and feces. User-reported applications of this kit include exosome purification from cerebrospinal fluid and saliva.

Q9: How much is the number of exosomes recovered per purification?
Although it greatly varies depending on the type and amount of sample, we experienced recovery of approximately 30 μg/mL protein (as measured by BCA method) and 1-2 × 10^{10} particles/mL (as measured using NanoSight LM10) per purification (by single purification from K562 cell culture supernatant collected after enhancement of exosome secretion with monensin sodium salt and subsequently concentrated from 5 mL to 1 mL). We also experienced recovery of approximately 34 μg/mL protein (as measured by BCA method) and 5 × 10^{9} particles/mL (as measured using NanoSight LM10) per purification from 1 mL of pooled human normal serum. This kit yields an eluate in a final volume of 100 μL.

■ Comparison with conventional methods

Q10: What are advantages of this kit over ultrafiltration?
This kit is capable of easier and more reproducible exosome recovery at a higher purity and efficiency than those of ultrafiltration. This kit is also confirmed to be capable of recovering exosomes from samples difficult to precipitate by ultra-centrifugation.
The purity of exosomes recovered using this kit is high: an exosome fraction at a high purity comparable to that of exosome isolated by a combination of ultrafiltration and density gradient centrifugation.

Q11: What are advantages of this kit over the affinity method using antibodies?
While the affinity method with antibodies uses antibodies against exosome surface antigens and therefore requires exosome recovery by dissociation of bound exosomes by elution with a denaturant or under an acidic condition, this kit allows elution of bound exosomes under a neutral condition with a chelating agent and recovery of almost intact exosomes. Since no denaturant is required for elution, the resulting exosome contains less amount of contaminating proteins non-specifically adsorbed on magnetic beads and is recovered at a higher purity. Another confirmed advantage of this method is a high recovery efficiency. Because the expression level of the marker protein differs depending on the exosome-derived cell compared with the conventional antibody affinity method targeting one exosome surface marker protein, this kit targeting a membrane lipid component is expected to capture a wider range of exosomes. In addition, although antibodies recognizing surface marker proteins may fail to recognize homologous antigens from different animal species, this kit is applicable to a wide range of animal species (we have experiences of its application to human, mice, cattle, and monkeys).

Q12: What are advantages of this kit over polymer precipitation?
Compared with polymer precipitation, this kit yields exosomes at a higher purity and higher recovery efficiency.

■ Operational procedures and composition of the kit

Q13: How long is the operation time for exosome purification using this kit?
Sample pretreatment requires approximately 1 hour, while the entire kit process takes 3 and a half hours including immobilization of Exosome Capture onto magnetic beads for approximately 15 minutes, incubation with sample for 3 hours, and washing plus elution of exosomes for approximately 35 minutes. Although the time for incubation with sample may be reduced to 1 hour, make sure in advance to confirm that this change does not affect experimental results.

Q14: What are the steps for operation of this kit requiring particularly careful manipulation?
(1) At the final step of washing after incubation of Exosome Capture-immobilized magnetic beads with sample, thoroughly remove the washing buffer. Do not proceed to elution step until complete removal of the washing buffer is confirmed.
(2) At the elution step, after addition of the elution buffer, thoroughly suspend the beads to ensure that no beads remain aggregated.

Q15: What is the composition of Elution Buffer?
It is a Tris-based buffer solution containing 1 mM chelating agent, salt, and a preservative agent. If any of these components may interfere with subsequent analysis, change to an appropriate buffer by ultrafiltration (Sartorius Vivaspin500, molecular weight cutoff 100K, Product No.: VS0141) or gel filtration.

Q16: Are magnetic beads with immobilized Exosome Capture compatible with recycling?
Yes. Used magnetic beads are reusable up to 4 times after regeneration to ensure recovery of exosomes remaining in sample. The kit includes all necessary buffers in sufficient amounts, and the kit specifications allow reuse of magnetic beads up to 50 times (in case of 10 test kit) in cases of repeated extraction from an identical sample or no concern of contamination. Reuse is recommended for recovery from a sample with a volume of 1 mL or larger or a concentrated sample. See instruction manual for details.

Q17: Are Exosome Capture-immobilized magnetic beads compatible with storage?
Yes. If Exosome Capture-immobilized magnetic beads after elution of exosomes are to be reused, store them refrigerated in Washing Buffer included in the kit or TBS prepared separately (Our experience: usable after 2 years of storage).

Q18: Is there any step that can be carried over to next day?
Incubation of the Exosome Capture-immobilized magnetic beads with sample (for 3 hours in the standard protocol) may be prolonged up to overnight without any problems.

■ Sample volume

Q19: How much is the minimum sample volume for a single purification?
To assure consistent mixing of magnetic beads and the sample solution, the sample volume should be 500 μL or larger for mixing with a rotator and 100 μL or larger for mixing with a tube mixer. When the sample volume is smaller than the relevant lower limit, add TBS to make a sample volume exceeding it before incubation with the Exosome Capture-immobilized magnetic beads. In addition, it is recommended to add EV-Save™ Extracellular Vehicle Blocking Reagent (Code: 058-09261) to the TBS for fill-up.

Q20: Is this kit compatible with recovery from large-volume samples?
Yes, this kit is compatible with large-volume samples after concentration. For cell culture supernatant, it is compatible with a sample volume up to 50 mL. Concentrate 50 mL of supernatant to 1 mL by ultrafiltration (filter recommended: Sartorius VivaSpin20, molecular weight cutoff 100K, Product No.: VS2041). It is compatible not only with serum-free medium but also with 10% FBS-supplemented medium. Since serum samples cannot be concentrated, this kit is compatible with a serum sample volume only up to 1 mL. See instruction manual for details.
In addition, it has been confirmed that concentration by ultrafiltration decrease exosomes due to absorption to containers and filters. When performing concentration by ultrafiltration, add EV-Save™ Extracellular Vehicle Blocking Reagent Kit (Code: 058-09261) to prevent adsorption loss. However, since this product contains a polymer, using as samples for proteomics analysis is not recommended.

Q21: Why is a molecular weight cut off of 100K recommended for ultrafiltration concentration?
We compare 100K, 300K and 1000K ultrafiltration filters and recommend 100K based on the concentration time and results of concentrated exosomes. 10K and 30K can be used, concentration time will be longer. In addition, in the case of a medium containing albumin, the recovery efficiency may decrease because the albumin is concentrated.

■ Analysis after exosome extraction

Q22: What are the components of exosomes?
Exosomes are reported to contain proteins, lipids, and nucleic acids (DNA, microRNA, mRNA) and others.

Q23: What kind of downstream application do I use the extracellular vesicles purified using this kit?
Since intact extracellular vesicles are obtained using this kit, you can use them for any analysis.
(Examples)
- Protein analysis: protein electrophoresis, western blotting, proteomics analysis, flow cytometry, ELISA, etc.
- Nucleic acid analysis: qPCR, microarray, next-generation sequencing, etc.
- Particle analysis: electron microscopy, NanoSight (NTA), etc.
- Functional analysis: *in vitro*/*in vivo* administration experiments, etc.

Q24: Can I use the extracellular vesicles purified with this kit for uptake assay to cells without any pre-treatment?
Because cytotoxic effects of preservatives have been confirmed, prepare PBS buffer containing 2 mM EDTA separately and use it instead of the elution buffer supplied with the kit. For the purified extracellular vesicles sample obtained, sterilize it using a centrifugal filter unit (Millipore Ultrafree - MC, GV 0.22 μm, sterile, Catalog No.: UFC30GV0S). After sterilization treatment, use it for uptake assay etc. Even if EV-Save™ is added to prevent adsorption, it can be used as it is.

Q25: How much is the exosome amount required for electron microscopic analysis?
We have an experience of performing electron microscopic analysis using 2-4×10^{10} exosome particles/mL particle concentration (measured by NanoSight LM10).

Q26: How much exosome is required for microarray analysis?
In our experiment, we have been able to perform microarray analysis by extracting RNA from the number of particles below (measured by NanoSight LM10);
COLO201: 4.6 × 10^{10} particles
TIG3: 1.7 × 10^{10} particles
iPS: 1.9 × 10^{9} particles

Q27: How much exosome is required for proteomics analysis?
For P. 12 proteomics analysis (W. Nakai, *et al*., *Sci. Rep*., **6**, 33935, 2016), approximately 1 μg of purified exosomes were used. (It is derived from approximately 1.5 mL of K562 culture supernatant sample. Since this sample promotes exosome secretion with monensin, the amount of exosome is large.)

Q28: How should I store the extracellular vesicles purified with this kit?
Add EV-Save™ and store at 2 ~ 10 ℃ or -20 ℃. Store at -80 ℃ for long-term storage. Due to cryoprotective effect of EV-Save™ ensures that the purified extracellular vesicles are not broken even when they are stored frozen. However, since it contains a polymer, using this sample for proteomics analysis is not recommended.

Q29: How do I perform western blotting analysis using the recoverd exosome?
At our laboratory, 15 μL of eluate and 5 μL of 4 × SDS sample buffer are mixed and applied for SDS-PAGE. All western blotting experiments described in this guidebook are applied under this condition.

■ Exosome markers

Q30: How are the exosomes purified using this kit identified?
They have been identified by western blotting and ELISA using antibodies against exosome surface antigens, electron microscopy, density gradient centrifugation, and particle size measurement (NanoSight LM10), etc.

Q31: What are the marker proteins identified in exosomes by western blotting?
CD9, CD63, CD81, Tsg101, Alix, Flotillin-2, and Lamp-1, etc.

■ Related products

Q32: Are exosome marker antibodies for western blotting available from Wako?
We distribute the following antibodies successfully used for this purpose at our laboratory.

Antigen	Reactivity	Antibody	Manufacturer	Application
CD63	Human	Anti CD63, Monoclonal Antibody (3-13)	Wako, Code: 012-27063	WB、ELISA、FCM、IP
CD81	Human, Bovine	Anti CD81, Monoclonal Antibody (17B1)	Wako, Code: 011-27773	WB、ELISA、FCM、IP
CD9	Human, Bovine	Anti CD9, Monoclonal Antibody (1K)	Wako, Code: 014-27763	WB、ELISA、FCM、IP
TSG101	Human	Tsg101 Antibody (4A10)	Novus, Code:NB200-112 (Wako 553-30151)	WB
Alix	Human	Alix Antibody (3A9)	Novus, Code:NB100-65678 (Wako 552-30121)	WB

Q33: Is a kit for purification of RNA from the purified extracellular vesicles available from Wako?
Yes. Our microRNA Extractor SP Kit (Code: 295-71701) are capable of purify-ing microRNA and mRNA more efficiently than AGPC method.

Q34: Is a magnetic stand available from Wako?
We distribute Magnetic stand (Code: 290-35591).

■ Experimental conditions

Q35: What is the conditions for exosome secretion enhancement with monensin sodium salt.
The final concentration of monensin sodium salt used for culture of K562 cells is 10 μM. Monensin sodium salt is dissolved in ethanol to make a concentration of 10 mM and 1/1000 volume of this solution is added to the culture medium. Exosome Release Is Regulated by a Calcium-dependent Mechanism in K562 Cells., *J Biol Chem*., 2003 May 30, **278** (22), 20083-90.

Q36: How should I prepare a positive control sample?
Culture any control cells such as HEK293, prepare the required amount of culture supernatant, and purify exosomes using this kit. The purified exosomes show exosome markers such as CD9, CD63, and CD81 in western blotting and ELISA.
At our laboratory, control cells are cultured in serum-supplemented medium for one day, change to a serum-free medium or exosome-depleted serum-containing medium for about 3 days. Then collect the culture supernatant.

Q37: What is the protocol for BCA assay?
A calibration curve for standards is prepared according to the following protocol.

Since protein concentrations of exosomes isolated and purified with this kit is low, it is recommended to measure without diluted.
(1) Pipette 25μL per well of standard BSA solutions (250, 125, 62.5, 31.25, 15.625μg/mL) and a standard BLANK into a 96-well plate.
(2) Pipette 25μL per well of purified exosomes and Elution Buffer (BLANK) to a 96-well plate.
(3) Add 200μL per well of a mixture of Reagent A and Reagent B (A:B=50:1) of Protein Assay BCA Kit (Code: 297-73101) to each sample-containing well.
(4) Incubate the plate for 30 minutes at 60℃ .
(5) Allow the plate to cool at room temperature.
(6) Measure the absorbance at 560 nm.

■ Troubleshooting

Q38: My purification results are not satisfactory. How should I prepare for a successful purification?

Prepare a positive control in reference to Q36. Enlarge the culture scale as the amount of extracellular vesicles in the culture medium may be small.

Q39: The total protein amount of the exosome sample purified by this kit is smaller than that of exosome sample collected by other methods. What causes it?

Exosome samples collected by other methods contaminated with many impurities, resulting in a large amount of total protein. On the other hand, although the total protein amount of the exosome sample purified with this kit is small, the amount of exosome actually obtained may be larger than that of the others due to the high purity of the sample.

・PS Capture™ Exosome Flow Cytometry Kit

■ Kit specifications and performance

Q1: Does this kit include antibodies for detection of exosomes?

This kit does not include fluorescent labeled antibodies for detection of exosomes. Additional purchase of any fluorescein-labeled anti-CD63 antibody (Code: 018-27641), red fluorescent-labeled anti-CD63 antibody (Code: 011-27751), or other appropriate fluorescent-labeled antibody is required.

Q2: Is it able to use a fluorescent labeled secondary antibody for detection?

Yes. detection with a fluorescent labeled antibody is possible. After isolation of exosomes, please proceed with the primary antibody reaction, secondary antibody reaction, and flow cytometry analysis in accordance with the following protocol.
(1) Mix an unlabeled primary antibody and Exosome Capture Beads, and allow to stand at room temperature for 1 hour. During the incubation, vortex the mixture for about 5 seconds at 20 minutes, 40 minutes, and 1 hour to stir the magnetic beads.
(2) Wash the beads 2 times with 300μL of WB (+ Enhancer).
(3) Dilute a PE-labeled secondary antibody (Code: 115-115-164) (Jackson Immuno Research Laboratories) 100-fold with WB (+Enhancer), and add the secondary antibody solution to the magnetic beads in (2).
(4) Allow to stand at room temperature for 1 hour. During the incubation vortex the mixture for about 5 seconds at 20 minutes, 40 minutes, and 1 hour to stir the magnetic beads.
(5) Wash the beads 3 times with 300μL of WB (+ Enhancer).
(6) Suspend the magnetic beads in 300μL of WB (+ Enhancer).
(7) Perform flow cytometry analysis.

Q3: Can this kit be used for non-human biological species?

Yes. Exosomes from humans, mice, cattle, and monkeys have been successfully detected.

Q4: Is it possible to use MagCapture™ Exosome Isolation Kit PS (Code: 293-77601) for flow cytometry analysis?

MagCapture™ Exosome Isolation Kit PS is a kit for purification of exosomes but not for flow cytometry analysis. For PS Capture™ Exosome Flow Cytometry Kit, magnetic beads and washing buffer are optimized for flow cytometry analysis.

■ Operational procedures and composition of the kit

Q5: How long does it take to run an experiment with this kit?

Pretreatment of the samples takes about 1 hour, and the process of this kit takes about 2 hours and 20 minutes. The process consists of a reaction with the sample for 1 hour, staining of exosomes with fluorescent labeled antibody for 1 hour, and washing for 20 minutes.

Q6: Multiple Exosome Capture Beads may bind to an exosome, forming aggregates of magnetic beads. In such case, is an accurate analysis possible?

It is recommended to adjust gating parameters based on a plot of the forward and lateral scattered lights for gating of a singlet bead fraction only and then detect fluorescent signals of the Exosome Capture Beads in the gated fraction. Generally, the singlet bead fraction accounts for 50% to 70% of the sample overall.

Q7: Is there any method to measure purified exosome samples?

It is recommended to dilute the purified exosomes to an appropriate concentration and proceed to 2. Isolation of extracellular vesicles in instruction manual. For dilution of the purified exosomes, it is recommended to use the washing solution after diluting the washing solution (10 ×) 10 fold with ultrapure water. Purified exosomes can be detectable in the concentration range from 125 to 1000 ng/mL. For a detection antibody, Anti CD63, Monoclonal Antibody (3-13), Red Fluorochrome (635) Conjugated (Code: 011-27751) is recommended.

Q8: Detection using about 10 types of antibodies is planned. What amounts of samples and magnetic beads are necessary for such experiment?

Please refer to Table 2 Recommended reaction scale in instruction manual. In the bottom row of Table 2, amounts for 10 reactions are described. Mix 367μL of the sample with 110μL of Exosome Capture Beads to isolate exosomes, wash these beads with WB (+Enhancer), suspend these beads in 1100μL of WB (+Enhancer); and then proceed to 3. Immunostaining of extracellular vesicles in which the suspended beads are dispensed into 100-μL portions.

■ Sample volume

Q9: How much is the minimum sample volume for detection?

About 33μL of a sample is required. If an amount of extracellular vesicles in a sample is small, concentration by ultrafiltration of a conditioned medium pretreated with centrifugation is recommended to prepare the sample for detection. (recommended filter; Sartorius Vivaspin 20, cut-off molecular weight 100 K, Product code: VS2041)

■ Related products

Q10: Is a magnetic stand available from Wako?

We distribute Magnetic stand (Code: 290-35591).

■ Troubleshooting

Q11: Magnetic beads are not gathered to the magnetic stand.

The Exosome Capture Beads of this kit are optimized for flow cytometry detection, and thus the concentration of magnetic beads is low. Therefore, magnetic beads gathered by the magnet may be hardly visible. Before the washing operation, it is recommended to keep the tube still on the magnetic stand for at least 1 minute. Discard the washing buffer gently not to suction the magentic beads.

• PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)
• PS Capture™ Exosome ELISA Kit (Streptavidin HRP)

■ Kit specifications and performance

Q1: Do I have to prepare a standard for every assay? And do I have to use MagCapture™ Exosome Isolation Kit PS for preparation of the standard?

When you perform a quantitative assay, prepare an extracellular vesicle sample as standard. Although an extracellular vesicle sample purified by ultracentrifugation or polymer precipitation may also be used as standard, an extracellular vesicle sample purified by the PS affinity method based on the principle identical with that of the assay is recommended (see instruction manual included in the kit for details of the preparation method).

Q2: Why does this kit include no standard?

The standard and assay samples must be derived from identical cell species, because the type and amounts of surface marker proteins on extracellular vesicles may vary depending on the cell type. Therefore, this kit does not include a standard. Prepare a standard purified from culture super-natant of cells identical with the source cells of assay samples.

Q3: Can this kit measure extracellular vesicles in serum and plasma directly?

PS Capture™ Exosome ELISA Kit (Streptavidin HRP) is available. PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) is not recommended for direct assay of serum and plasma samples from human, mouse, and rat, because the secondary antibody for detection included in the kit reacts with human, mouse, and rat IgG non-specifically.

Q4: Can this kit measure extracellular vesicles in cell culture supernatant directly?

Both kits are available. PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) is compatible with direct assay of cell culture supernatant from both serum-free and FBS-supplemented media because primary antibody (Anti-CD63 antibody) and secondary antibody included in the kit don't react with bovine IgG non-specifically. In addition, biotin-labeled antibody (anti-CD63 antibody-biotin) and Streptavidin HRP in PS Capture™ Exosome ELISA Kit (Streptavidin HRP) do not react in the same manner. Utilize this kit for both quantitative and qualitative analyses of extracellular vesicles in cell culture supernatant samples.

Q5: Can I replace the primary antibody with another antibody?

Yes. When using PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD), choose a mouse antibody against the surface marker of interest and investigate the optimal concentration according to instruction manual.
When using PS Capture™ Exosome ELISA Kit (Streptavidin HRP), select a biotin-labeled antibody for detection of any surface marker, and optimize concentration according to instruction manual.
If a biotin-labeled antibody is not available, label the antibody with Biotin Labeling Kit-SH (Code: 348-90941) or Biotin Labeling Kit-NH2 (Code: 347-90891).

Q6: How can I store the remaining reagents?

See [6. The storage method of each reagent when the kit is separately used] in the instruction manual included in the kit.

Q7: What kinds of cell lines can I use for this kit?

The following is a list of cell lines successfully isolated and detected by Wako.

Cell Line	Origin	Isolation	ELISA
A549	Human Caucasian lung carcinoma	–	○
BxPC-3	Human primary pancreatic adenocarcinoma	–	○
COLO201	Human Caucasian colon adenocarcinoma	○	○
COS7	Monkey African green kidney	○	○
FM3A	Mouse C3H mammary carcinoma	○	○
HCT116	Human colon carcinoma	○	○
HEK293	Human Embryo Kidney	○	○
HEK293T	Human Embryo Kidney	○	○
HeLa	Human cervix carcinoma	○	○
HPAF Ⅱ	Human pancreatic tumor	–	○
HuH-7	Human hepatocellular carcinoma	○	○
HUVEC	Human umbilical vein endothelial cell	○	○
iPS	induced pluripotent stem cell	○	○
K562	Human Caucasian chronic myelogenous leukaemia	○	○
LNCaP	Human Caucasian prostate carcinoma	○	○
P388D1	Mouse leukemia	○	○
Panc-1	Human Caucasian pancreas	–	○
RAJI	Human Burkitt's lymphoma	○	○
SH-SY5Y	Human neuroblastoma	–	○
TIG-3	Normal human diploid cells.	○	○
THP-1	Human monocytic leukaemia	○	○
U2OS	Human Osteosarcoma	○	○
BM-MSC	Human Mesenchymal Stem Cells from Bone Marrow	○	○
iCell-MSC	Mesenchymal Stem Cells from iPS-01279	○	○

– : No test
○ : Succeeded

Q8: Can the recovery rate of extracellular vesicles be measured with this kit?

Yes, it can. Please refer to P. 24; Comparison of recovery efficiency with polymer precipitation.

■ Comparison with conventional methods

Q9: Is the detection sensitivity of this kit higher than other methods?

This kit has been confirmed to detect extracellular vesicles at sensitivity higher than those of ELISA methods using immobilized antibodies or direct immobilization of purified samples on the plate. In addition, it has been confirmed that there is a correlation with the Western blot method.

■ Operational procedures and composition of the kit

Q10: How long is the operation time of this kit?

The entire process of PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) takes approximately 5 hours, including immobilization of extracellular vesicle samples onto a 96-well plate for 2 hours, reaction with the primary antibody for 1 hour, reaction with the secondary antibody for 1 hour, and reaction with tetramethylbenzidine (TMB) for 30 minutes. With washing and other operations included, the assay is completed in approximately 5 hours.
The entire process for PS Capture™ Exosome ELISA Kit (Streptavidin HRP) is approximately 6 hours. The breakdown of the process is 2 hours for immobilizing extracellular vesicle samples on the plate, 1 hour for the primary antibody reaction, 2 hours for the streptavidin-HRP reaction, and 30 minutes for the TMB reaction. It can be measured in about 6 hours including other washing steps.
After addition of Stop Solution, measure the absorbance at the main wavelength 450 nm and the complementary wavelength 620 nm (600 - 650 nm).

Q11: Can I reuse an Exosome Capture 96 Well Plate?

No. An Exosome Capture 96 Well Plate cannot be reused because Stop Solution denatures proteins on the plate.

Q12: Is there any step that can be carried over to next day?

Immobilization of individual samples onto the plate may be prolonged up to overnight at 4℃.

■ Sample volume

Q13: How much is the minimum sample amount required for detection using this kit?

Extracellular vesicles corresponding to 1 ng protein are detectable using this kit. The detection limit of extracellular vesicles purified from COLO201 cell culture supernatant was 11 pg (The detection limit varies depending on the cell lines).

Q14: How much sample volume is required for direct assay of culture supernatant and body fluid sample?

Culture supernatant or body fluid sample of a few μL in volume (1-5μL) is sufficient for assay. This is recommended for monitoring changes in number of extracellular vesicles in culture medium over time and assay of new cell culture supernatant. However, depending on the cell type (iPS cells, etc.), the amount of extracellular vesicles in the medium may be small. If the amount of extracellular vesicles in the sample is unknown, preliminary experiments for an appropriate amount of sample is recommended.

■ Related products

Q15: Is there a recommended primary antibody for detection?

We have confirmed that the following antibodies can be used for ELISA.

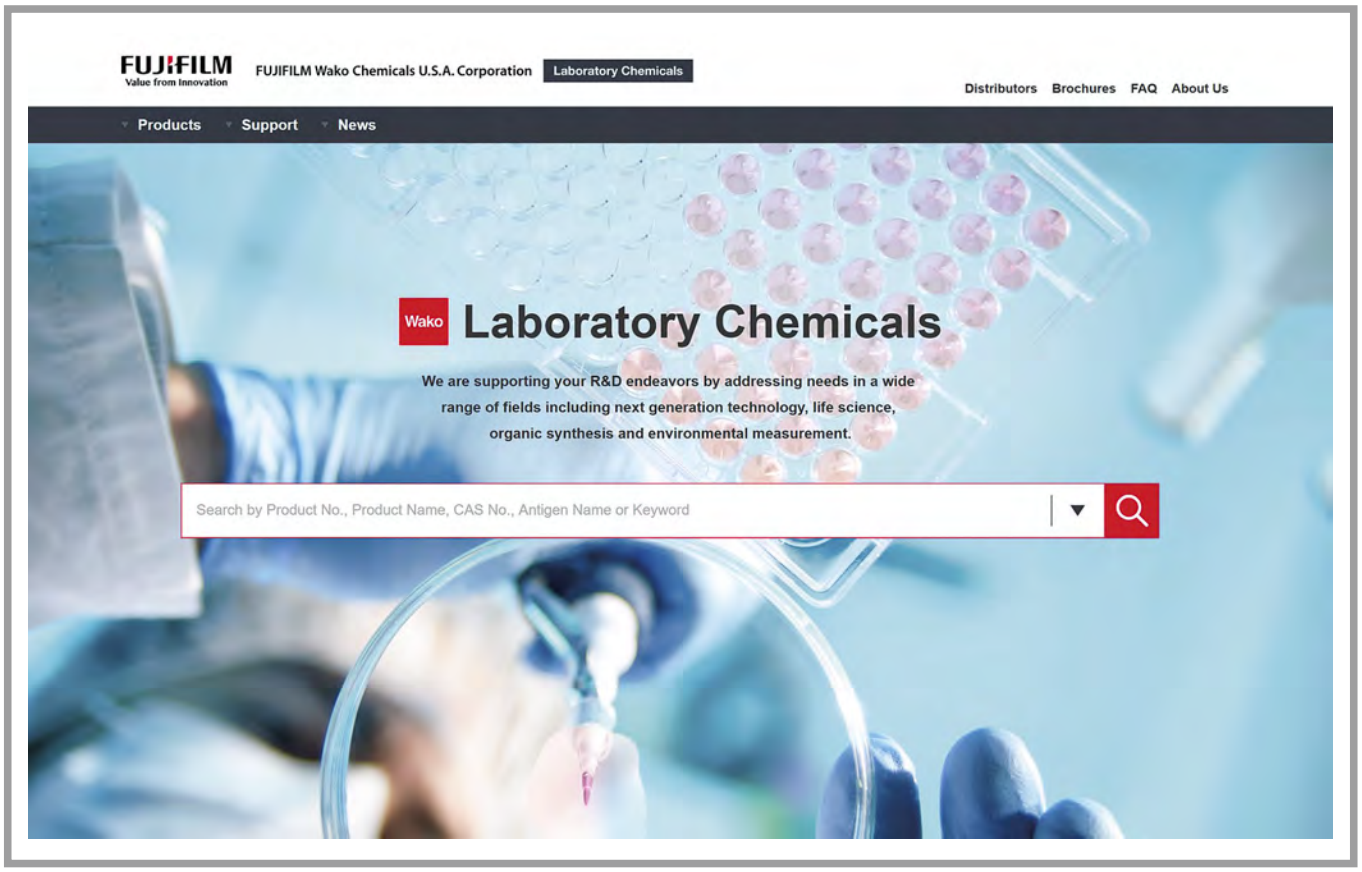
Antigen	Reactivity	Antibody	Manufacturer	Application
CD9	Human, Bovine	Anti CD9, Monoclonal Antibody (1K)	Wako, Code: 014-27763	WB、ELISA、FCM、IP
CD63	Human	Anti CD63, Monoclonal Antibody (3-13)	Wako, Code: 012-27063	WB、ELISA、FCM、IP
CD81	Human, Bovine	Anti CD81, Monoclonal Antibody (17B1)	Wako, Code: 011-27773	WB、ELISA、FCM、IP
CD9	Mouse	Rat anti-CD9 monoclonal antibody (MZ3)	Bio Legend, Code:124802	WB、ELISA
CD63	Mouse	Rat anti-CD63 monoclonal antibody (NVG-2)	Bio Legend, Code:143902	WB、ELISA
CD81	Mouse	Armenian hamster anti-CD81 monoclonal antibody (Eat-2)	Bio Legend, Code:104902	WB、ELISA

■ Troubleshooting

Q16: My detection results are not satisfactory. What should I check?

Check if any of the reagents has been expired. Be sure to add Exosome Binding Enhancer (100 ×) to the washing solution. When you fail to detect a positive signal even with Control Primary Antibody Anti CD63 (100 ×) or Control Biotinylated Antibody Anti-CD63 (100 ×) (included in this kit), it may be ascribable to an expression level of CD63 under the detection limit or some other cause. Please inquire to us in such a case.

Please visit FUJIFILM Wako Laboratory Chemicals site.
It's now quicker and simpler to get everything you need on desktop or mobiles.



https://labchem-wako.fujifilm.com/

50,000 products for Life Science, Green Chemistry, Analytical Chemistry will be able to search on this web site.

- ◆ Flexible search function
- ◆ Chemical Name
- ◆ CAS Number
- ◆ Wako Catalogue No.
- ◆ Molecular Formula
- ◆ Brochure…

Related Products

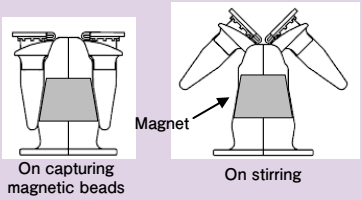
■ Magnetic stand

This product is a magnetic stand for capture of magnetic beads. It is intended for use in purification of particular components contained in cell culture supernatant, serum, urine, and other specimens by magnetic beads products represented by MagCapture™ series. It holds 16 × 1.5 mL (2.0 mL) microtubes simultaneously. The strong magnet embedded inside the stand base allows for quick capture of magnetic microbeads and avoids waiting for separation.

[Features]

■ Microtube holders are movable (flipped upward and downward).
Flipping the tube holders upward isolates tubes from the magnet to allow for re-suspension of magnetic beads in individual tubes by stirring with a vortex mixer.

■ Flipping the tube holders downward allows for capture of magnetic beads in all 16 tubes simultaneously and subsequent discarding of the supernatant or wash buffer by pipetting.



■ Simply changing the angle of the movable tube holders enables switching between the tube positions optimal for stirring and capture, thereby allowing efficient manipulation.

■ Use of a neodymium magnet and its configuration to allow direct contact with the side wall of each tube minimizes the waiting time for capture of magnetic beads

■ Use of a synthetic resin body assures visibility of samples and realizes reduction in size and weight


Photograph of the product

■ Specifications and performance

- Size: W198.8 × D49 × H49 (mm)
- Weight: 235g
- Time for magnetic beads capture 1.0 μm beads, 1 mL: approximately 25 secs
  2.7 μm beads, 1 mL: approximately 10 secs
  4.5 μm beads, 1 mL: approximately 2 secs
- Working volume 20 μL-1,500 μL (2,000 μL)

* The time for magnetic beads capture may vary depending on the properties and volume of liquid.

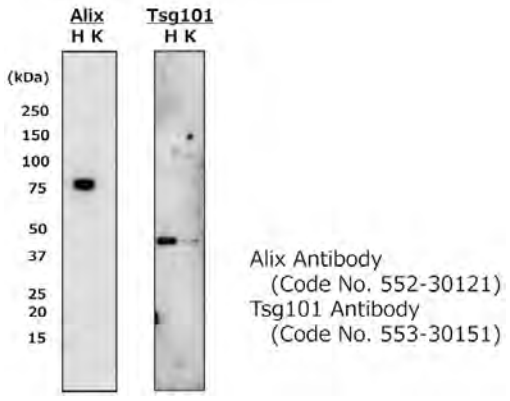
Code No.	Description	Package Size	Storage
290-35591	Magnet stand	1 unit	60,000

■ Alix Antibody (3A9)
■ TSG101 Antibody (4A10)

Comparison of detection sensitivity of Western Blotting using various exosome marker antibodies

Samples: Extracellular vesicles purified from HEK293T and K562 cell culture supernatants (using MagCapture™ Exosome Isolation Kit PS)

Extracellular vesicles purified from individual cell culture supernatants and assayed by BCA method were supplemented with Sample Buffer (containing 3-mercapto-1,2-propanediol) (×4) (Code No. 196-16142) and heated at 98℃ for 5 minutes. Then the samples (200 ng each) were separated by electrophoresis and subjected to detection by Western Blotting using various exosome marker antibodies each diluted to 1 μg/mL.



H: Exosomes purified from HEK293T cells, 200 ng
K: Exosomes purified from K562 cells, 200 ng

Code No.	Description	Package Size	Storage
552-30121	Alix Antibody (3A9)	0.1 mg	-20℃
553-30151	TSG101 Antibody (4A10)	0.1 mL	-20℃

Related Products

UniWells™ Horizontal Co-Culture Plate

This plate is an ideal co-culture device that connects two wells laterally. Horizontal connection provides easy viewing of cells in both wells simultaneously using various type of microscopes.

It is a versatile tool for studying cell-cell interactions such as transport, migration and invasion.

【Features】

- Simultaneous observation using a time-lapse microscope
- No more filter clogging
- Cells are cultured in each well under the same condition (same material on the bottom and same volume)
- Filters of any membrane types and pore sizes are usable
- Both wells are independent (connect in a free combination)

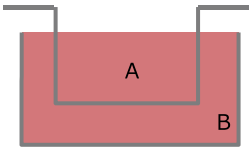
【Cpmponents in a set】

Filters for UniWells™ are not included and sold separately.

Excellent point

1) Both cells can be cultured under the same medium volume

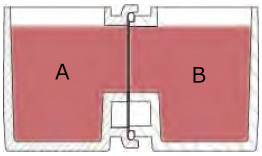
Conventional co-culture plate



A:B = 1:3

Medium volume in A is more than in B and cell secreted factors from A are diluted in B.

UniWells™

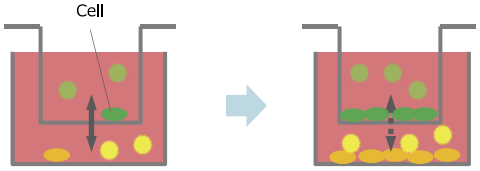


A:B = 1:1

Medium volume are the same between A and B

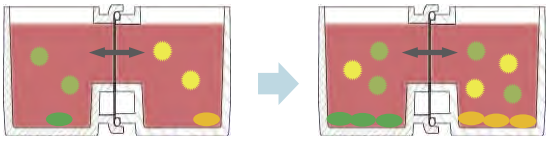
2) Filter is prevented from being clogged by cells

Conventional co-culture plate



The filter is clogged by cells cultured in the upper well, which leads to interfere with migration of cell secreted factors between the upper and lower wells.

UniWells™



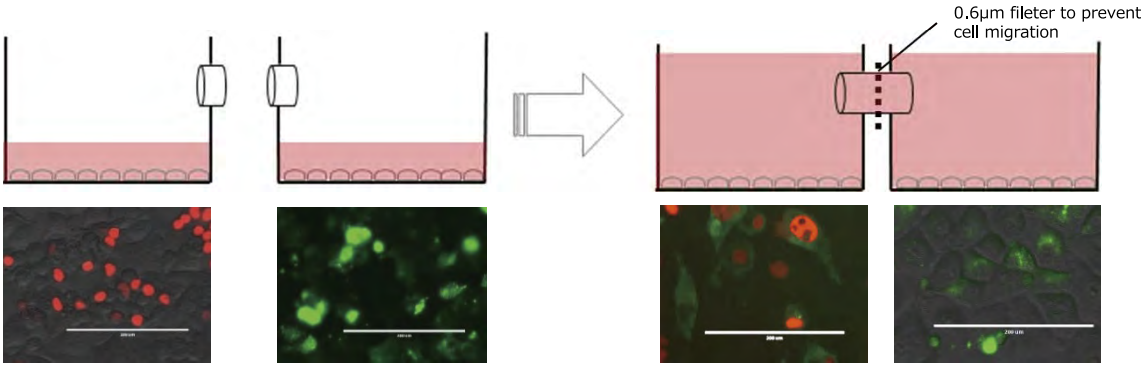
The filter is NOT clogged by cells.

Related Products

Example of use

- Uptake of exosomes -

UniWells™ Horizontal Co-Culture Plate enables the observation that exosomes derived from cell B permeate the filter and are uptaked by cell A.



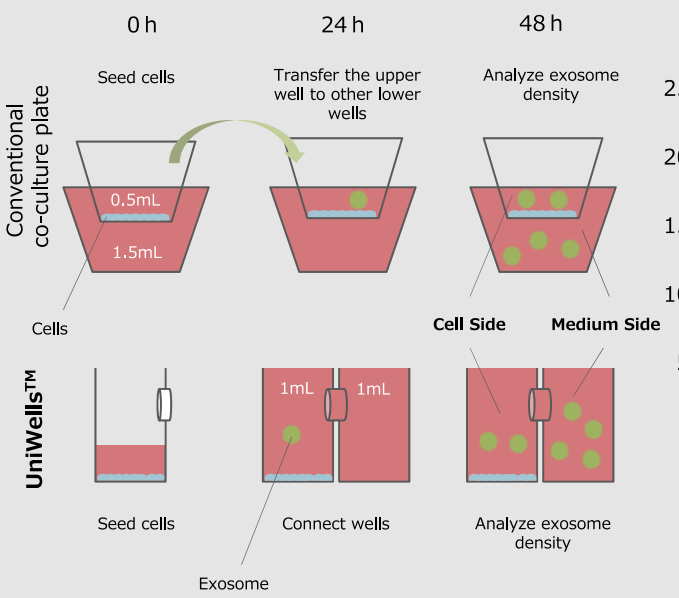
Cell A
Red : Cell nuclei

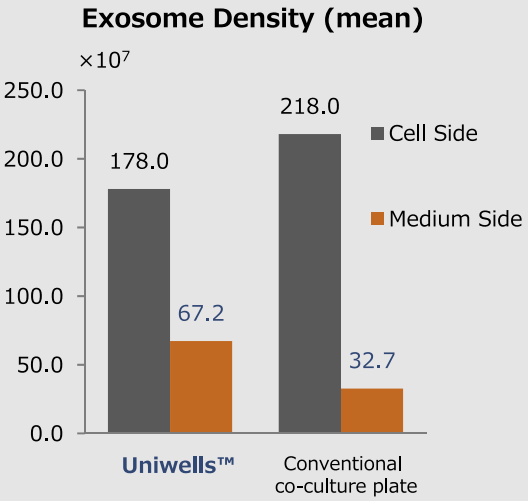
Cell B
Green : CD63-GFP on exosomes

Exosomes from cell B were upateked by cell A without cells being mixed.

Comparison of permeability testing of exosome between conventional co-culture plate and UniWells™

1. Cells were seeded to be the same cell numbers into the upper well in conventional co-culture plate and one side well in UniWells™.
2. Co-culture started after 24 hours from cell seeding.
3. Exosome densities of cell side and medium side were analyzed.



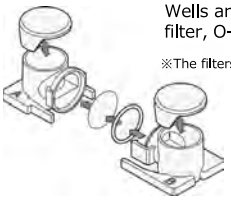


More exosomes migrated to medium side well in UniWells™

Related Products

Instructions for use

For connected use



Wells are connected by fitting a filter, O-ring and cover.

※The filters are sold separately.

For single use

Fit a common cover and cover to a well.

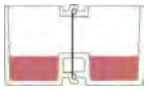
Use in a microscope

Set wells to the adapter provided.

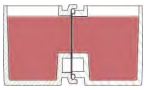
※The adapter is attached to the main unit.

Connection methods are as below;

A) Connect the wells cultured independently by aspirating the culture solution once.
B) Connect first and increase the volume of the culture solution to achieve co-culture



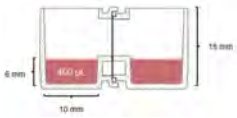
Non-co-culture condition



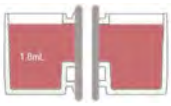
Co-culture condition

Size

Medium volume less than 400 µL is not shared between the wells.



The maximum medium volume on each well is 1.8 mL.



FAQ

What is UniWells™ made of ?

Main body and common cover are made of polystyrene and low density polyethylene, respectively. UniWells™ filter, which is sold separately, is made of polycarbonate.

Is Uniwells™ Sterilized ?

Uniwells™ is sterilized by electron beam sterilization. Do not sterilize it by autoclaving.

Are the main body surfaces coated with anything ?

No, they are not coated. Coat the main body surfaces with attachment matrixes as needed.

Product listing

Horizontal Co-Culture Plat

Code No.	Description	Intended use	Package Size	Storage
384-14421	UniWells™ Horizontal Co-Culture Plate	Culture vessel body (material, polystyrene)	10 packs	Room temperature
381-14431	UniWells™ Filter 0.03 µm	Specialized filter (pore size, 0.03 µm)	50 filters	Room temperature
388-14441	UniWells™ Filter 0.6 µm	Specialized filter (pore size, 0.6 µm)	50 filters	Room temperature
385-14451	UniWells™ Adapter 96	Holder for 96-well plate	1 piece	Room temperature

RNA extraction Kit from Exosome

Code No.	Description	Package Size	Storage
295-71701	microRNA Extractor SP Kit	50 reactions	2 ~ 10℃

Quantitative Kit for Protein Concentration

Code No.	Description	Package Size	Storage
297-73101	Protein Assay BCA Kit	250 assays	Room temperature
015-25613	2 mg/mL Albumin Solution from Bovine Serum	1 mL × 10	Room temperature

Product listing

Isolation Kit for High Purity Extracellular Vesicles -No ultracentrifugation-

Code No.	Description	Package Size	Storage
299-77603	MagCapture™ Exosome Isolation Kit PS	2 purifications	2 ~ 10℃
293-77601		10 purifications	

-High Sensitive Detection- Exosome ELISA Kit

Code No.	Description	Package Size	Storage
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)	96 tests	2 ~ 10℃
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin HRP)	96 tests	2 ~ 10℃

-High Sensitive Qualitative Analysis for Flow Cytometry-

Code No.	Description	Package Size	Storage
297-79701	PS Capture™ Exosome Flow Cytometry Kit	300 reactions	-20℃

-Suppressing Adsorption of Extracellular Vesicles-

Code No.	Description	Package Size	Storage
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL	-20℃

-High Sensitive Detection Antibody Series-

Code No.	Description	Package Size	Storage
016-27061	Anti CD63, Monoclonal Antibody (3-13)	20μL	-20℃
012-27063		100μL	
018-27641	Anti CD63, Monoclonal Antibody (3-13), Fluorescein Conjugated	25 tests	2 ~ 10℃
014-27643		100 tests	
013-27711	Anti CD63, Monoclonal Antibody (3-13), Red Fluorochrome(635) Conjugated	25 tests	2 ~ 10℃
019-27713		100 tests	
011-27751	Anti CD63, Monoclonal Antibody (3-13), Biotin Conjugated	20μL	-20℃
017-27753		100μL	
018-27761	Anti CD9, Monoclonal Antibody (1K)	20μL	-20℃
014-27763		100μL	
015-27771	Anti CD81, Monoclonal Antibody (17B1)	20μL	-20℃
011-27773		100μL	
552-30121	Alix Antibody (3A9)	0.1 mg	-20℃
553-30151	TSG101 Antibody (4A10)	0.1 mL	-20℃

-Magnetic Stand for Collecting Magnetic Beads-

Code No.	Description	Package Size	Storage
290-35591	Magnet Stand	1 piece	Room temperature

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; https://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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