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What are exosomes?

Rikenari Hanayama
Professor, Department of Immunology,
Kanazawa University Graduate School of Medical Sciences

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.).

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.

In the nervous system, exosomes have been found to be involved not only in regulation of neural circuits 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. 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. 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. Recently, exosomes released from adipocytes have been reported to regulate gene expression in the liver. 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.

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. 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, miRNAs 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 miRNAs 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 classification 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. 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. 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. 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.

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. 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. Since Tim4 binds to phosphatidylinerse (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. 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. 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 or-

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. According to research, 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.

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. 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.

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. 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.

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.

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 CDB1 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.

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.

[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

[Product photo]

[Kit contents]
This kit includes 7 components.

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

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

*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 as a sample.

Additionally, when highly purified exosomes are needed, prepare 10,000 × g supernatant 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.

**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)

---

### Cell Culture Medium

<table>
<thead>
<tr>
<th>Sample (Cell Culture Medium)</th>
<th>Precipitation (ppt)</th>
<th>Supernatant (sup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cell)</td>
<td>360 × g, 5 min, 4°C</td>
<td>1,200 × g, 20 min, 4°C</td>
</tr>
</tbody>
</table>

- **Purification Step**
  - (1,200 × g sup. fraction) → 10,000 × g, 30 min, 4°C

### EDTA Plasma and Citrated Plasma

<table>
<thead>
<tr>
<th>Sample (EDTA/Citrated Plasma)</th>
<th>(1,200 × g EDTA plasma sup. fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Large EVs)</td>
<td>(10,000 × g sup. fraction)</td>
</tr>
</tbody>
</table>

- **Purification Step**
  - Add 1/100 volume of Exosome Binding Enhancer (×500)

**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. 

**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: UFC30Gv05) 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.

---

**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.

**Size Distribution :**

- **10,000 × g precipitate**
  - Mean : 219 ± 14.7 nm

- **10,000 × g supernatant**
  - Mean : 119 ± 0.7 nm
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.

[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.

Electron microscopic analysis of exosomes

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

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

Figure 2. Results of electron microscopic analysis

<Photographic data of exosomes>
Hanaichi Ultrastructure Research Institute
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.

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.

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

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

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

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

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.

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 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.

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.

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.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>295-71701</td>
<td>microRNA Extractor SP Kit</td>
<td>50 purifications</td>
<td>2 〜 10℃</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Rank</th>
<th>Protein Description</th>
<th>PS Affinity</th>
<th>Ultracentrifugation</th>
<th>Polymer Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heat shock cognate 71 kDa protein</td>
<td>DNA-dependent protein kinase catalytic subunit</td>
<td>Complement C3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Annexin A6</td>
<td>Transferrin receptor protein 1</td>
<td>Alpha-2-macroglobulin</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Transferrin receptor protein 1</td>
<td>Serum albumin</td>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>V-type proton ATPase catalytic subunit A</td>
<td>ATP-dependent RNA helicase A</td>
<td>Serum albumin</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Hmgb-1</td>
<td>Tubulin beta-5 chain</td>
<td>Thrombospondin-1</td>
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<td>7</td>
<td>4F2 cell-surface antigen heavy chain</td>
<td>Fatty acid synthase</td>
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<td>8</td>
<td>Annexin A1</td>
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<td>Annexin A2</td>
<td>Tubulin beta-48 chain</td>
<td>Tubulin beta-5 chain</td>
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</tbody>
</table>

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

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

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

(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 a purified exosome preparation and Elution Buffer (BLANK) on 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) Incubated the plate at 60℃ for 30 minutes.

(5) Allow the plate to cool at room temperature.

(6) Measure the absorbance at 560 nm.

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 200μL 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](image)

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
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</thead>
<tbody>
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<td>297-73101</td>
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<td>Room Temperature</td>
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<tr>
<td>015-25613</td>
<td>2 mg/mL Albumin Solution from Bovine Serum</td>
<td>1 mL × 10</td>
<td>Room Temperature</td>
</tr>
</tbody>
</table>

<Overview of BCA assay protocol>

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

(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 a purified exosome preparation and Elution Buffer (BLANK) on 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) Incubated the plate at 60℃ for 30 minutes.

(5) Allow the plate to cool at room temperature.

(6) Measure the absorbance at 560 nm.
Exosomes purified by MagCapture™ Exosome Isolation Kit PS were labeled with PKH67 (Sigma) and confirmed their ability to be incorporated into HeLa cells.

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 3μg protein* to a 1.5 mL tube.
    * Prepare an appropriate amount as needed for the experiment.
4. Dissolve 2.0μL 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 100μL 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 100μL per column, multiple columns corresponding to the sample volume are recommended to be kept on hand where the sample amount is more than 100μL.
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 3μg, 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

![Assay principle diagram]

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)
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]

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

[Product photo]

[Features]
- 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.
- 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.
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.

**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.

- **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

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.
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.

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.

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

- Assay sample: 25-fold diluted COLO201 cell culture supernatant (4μL → diluted to 100μL)
- 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

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 10μM 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.
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]**

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<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
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<tr>
<td>× 1</td>
<td>1/16</td>
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<td>40 ng/mL</td>
<td>160 ng/mL</td>
<td>800 ng/mL</td>
<td>2000 μg/mL</td>
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<td>× 0.5</td>
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<td>1/2000</td>
<td>20 ng/mL</td>
<td>80 ng/mL</td>
<td>400 ng/mL</td>
<td>1000 μg/mL</td>
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<td>× 0.25</td>
<td>1/64</td>
<td>1/4000</td>
<td>10 ng/mL</td>
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<td>200 ng/mL</td>
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<td>5 ng/mL</td>
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*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).
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]**

<table>
<thead>
<tr>
<th>Kit composition (96 reactions)</th>
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<tbody>
<tr>
<td>Exosome Capture 96 Well Plate</td>
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<tr>
<td>Reaction Buffer</td>
<td>80 mL × 1 vial</td>
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<tr>
<td>Washing Buffer (10 ×)</td>
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<td>Exosome Binding Enhancer (100 ×)</td>
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**[Sample Type]**

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</tr>
<tr>
<td>Body fluid</td>
<td>Analyzable</td>
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</tbody>
</table>

**[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 Ca²⁺. 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.
Dilution Linearity of Four Specimens

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.

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).

Comparison of Capture Ability between PS Affinity and Antibody

Almost all of the exosomes derived from various cell lines were efficiently captured by Tim4 compared with anti-CD9, 63, 81 antibodies.
Spike and Recovery Assay with Blood Samples [EpCAM]

Pretreated 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 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.

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

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<th>Reaction Buffer (BLANK)</th>
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<th>Assay value</th>
<th>Recovery value</th>
<th>Recovery rate</th>
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<td>ng/mL</td>
<td>%</td>
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<td>2.245</td>
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<tr>
<td>5</td>
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</table>

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 were detected using Biotinylated anti-CD9 antibody.

<table>
<thead>
<tr>
<th>Serum (Dilution ratio 1:2)</th>
<th>Spiked value</th>
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<tr>
<th>EDTA plasma (Dilution ratio 1:2)</th>
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<th>Assay value</th>
<th>Recovery value</th>
<th>Recovery rate</th>
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<tbody>
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<td>ng/mL</td>
<td>ng/mL</td>
<td>ng/mL</td>
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<td>Mean</td>
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<td>4.591</td>
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<th>Assay value</th>
<th>Recovery value</th>
<th>Recovery rate</th>
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<tbody>
<tr>
<td>ng/mL</td>
<td>ng/mL</td>
<td>ng/mL</td>
<td>%</td>
<td>Mean</td>
</tr>
<tr>
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<td>4.591</td>
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※ 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).
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].

From 1 mL of pretreated[^8] COLO201 cell conditioned medium, exosomes were isolated and purified by PS affinity method or polymer precipitation method (competitor 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.

[^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).
[^8]: Pretreatment condition: 10,000×g, 30min.


**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].

![Graph: Capture: Tim4 / Detection: Biotin labelled compounds](image)

**Calculation of recovery efficiency**

1. **Input**: Measured value of 100-fold diluted conditioned medium[^1].
2. **Remaining sEVs**: Measured value of post-purification 100-fold diluted conditioned medium[^1].
3. **Recovered sEVs**: Measured value of 1000-fold diluted purified sample[^2].

[^1]: 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.

[^2]: Exosome samples purified with this kit and polymer reagents are theoretically concentrated 10 folds. (e.g. 1 mL of input → 100 μL 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.
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.

[Assay method]

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

Outline of Procedure ~ Basic Protocol for 2 reactions ~

[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.

<table>
<thead>
<tr>
<th>Qty of Reaction</th>
<th>Exosome Capture Beads (μL)</th>
<th>Sample volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>3 reactions</td>
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<td>333</td>
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<tr>
<td>10 reactions</td>
<td>110</td>
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</tbody>
</table>
**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.

*Sample*
- Cell culture supernatant of K562 cells: 33μL/Assay

*Detection antibody*
- PE-anti-CD63 (BD Biosciences)
- PE-anti-CD9 (Novus Biologicals)
- PE-anti-CD81 (Novus Biologicals)

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

### 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.*

---

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
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</thead>
<tbody>
<tr>
<td>297-79701</td>
<td>PS Capture™ Exosome Flow Cytometry Kit</td>
<td>300 reactions</td>
<td>-20°C</td>
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</table>
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 Specificity
- Recognize Nonreducing Samples

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD63</th>
<th>CD9</th>
<th>CD81</th>
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<td>Clone No.</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>Rat</td>
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</table>

**Applications**
- Western Blot
- Flow Cytometry
- ELISA
- Immunoprecipitation

**Product Lineup**
- Unconjugated
- Fluorescein conjugated
- Red Fluorochrome (635) conjugated
- Biotin conjugated


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

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

Sample amount: $1 \times 10^6$ cells
Antibody amount: 10μL (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): 33μL

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

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

Sample amount: $1 \times 10^6$ cells
Antibody amount: 10μL (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): 33μL

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.

**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.

**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.

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.

### 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.

### 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.

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

![Graph showing CD63 signal reduction with and without EV-Save™](image)

**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.
【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 (−/−).

【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.

【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**
  - **Comparison with conventional methods**
  - **Operational procedures and composition of the kit**
  - **Sample volume**
  - **Analysis after exosome extraction**

- **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: From what samples are exosomes 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 distribution: 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 is by size 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 a 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.


Q7: Do all exosomes have PS exposed on the membrane surface?

Although no finding has been obtained that all exosomes have exposed PS, we have identified 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.


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 × 10^13 particles/mL (as measured using NanoSight LM10) per purification (by single purification from K562 cell culture supernatant spiked with approximately 10^11 exosomes/mL). Although no finding has been obtained that all exosomes have PS exposed on the viral membrane surface. Utilizing this property, this kit may potentially be applied to recovery of enveloped viruses. 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.

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 with sample, thoroughly remove the washing buffer, and add elution buffer to elute exosomes. 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 a buffer 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 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 ultracentrifugation (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 ultracentrifugation decrease exosomes due to absorption to containers and filters. When performing concentration by ultracentrifugation, 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 ultracentrifugation concentration?
We compare 100K, 300K and 1000K ultracentrifugation filters and recommend 100K based on the concentration time and results of concentrated extracellular vesicles. 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 cytotoxicity 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 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 x 10^6 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):
- COL0201: 4.6 x 10^5 particles
- TIG3: 1.7 x 10^9 particles
- PJS: 1.9 x 10^7 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°C or -20°C. Store at -80°C 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 recovered exosome?
At our laboratory, 15 μL of eluate and 5 μL of 4 x SDS sample buffer are mixed and applied for SDS-PAGE. All western blotting experiments described in this guidebook are applied under this condition.

Q30: 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.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reactivity</th>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD6 Human</td>
<td>Anti CD63, Monoclonal Antibody (3-13) Wako, Code: 012-27063</td>
<td>WB</td>
<td>ELISA, FCM, IP</td>
<td></td>
</tr>
<tr>
<td>CD81 Human</td>
<td>Anti CD61, Monoclonal Antibody (17B8) Wako, Code: 011-27773</td>
<td>WB</td>
<td>ELISA, FCM, IP</td>
<td></td>
</tr>
<tr>
<td>CD9 Human</td>
<td>Anti CD9, Monoclonal Antibody (1K) Wako, Code: 014-27763</td>
<td>WB</td>
<td>ELISA, FCM, IP</td>
<td></td>
</tr>
<tr>
<td>Tsg101 Human</td>
<td>Tsg101 Antibody (4410) Novus, Code: N8600-112 (Wako 553-32151)</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alix Human</td>
<td>Alix Antibody (3A9) Novus, Code: N8610-66578 (Wako 553-32151)</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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., 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 purified 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.

Q38: What are the CD markers of exosome identified in exosomes by western blotting?
CD9, CD63, CD81, Tsg101, Alix, Flotillin-2, and Lamp-1, etc.

Q39: What are the exosome marker antibodies for western blotting available from Wako?
We distribute the following antibodies successfully used for this purpose at our laboratory.
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 Eutlon 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.

Q6: 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)

Q10: Is a magnetic stand available from Wako?

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

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

The Exosome Capture Beads of this kit are optimized for flow cytometry detection. For PS Capture™ Exosome Flow Cytometry Kit, magnetic beads and washing buffer are optimized for flow cytometry analysis.

Q5: How long does it take to do 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.

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 100μL portions.

Q9: How is the absorbance measured?

Wash the beads 3 times with 300μL of WB (+ Enhancer).

Q10: Is a magnetic stand available from Wako?

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

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 magnetic beads.
Q1: Do I have to prepare a standard for every assay? And do I have to use MagCaptureTM 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 not include 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 supernatant of cells identical with the source cells of assay samples.

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

PS CaptureTM Exosome ELISA Kit (Streptavidin HRP) is available. PS CaptureTM 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 CaptureTM 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 CaptureTM Exosome ELISA Kit (Streptavidin HRP) do not react in the same manner. Utilize this kit for both qualitative and quantitative analyses of extracellular vesicles in cell culture supernatant samples.

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

Yes. When using PS CaptureTM Exosome ELISA Kit (Anti Mouse IgG POD), choose a mouse antibody against the surface marker of interest and investi- gate the optimal concentration according to instruction manual. When using PS CaptureTM 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 is included 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.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Origin</th>
<th>Isolation</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Human Caucasian lung carcinoma</td>
<td>−</td>
<td>〇</td>
</tr>
<tr>
<td>BxPC-3</td>
<td>Human primary pancreatic adenocarcinoma</td>
<td>−</td>
<td>〇</td>
</tr>
<tr>
<td>COLO205</td>
<td>Human Caucasian colon adenocarcinoma</td>
<td>−</td>
<td>〇</td>
</tr>
<tr>
<td>CO97</td>
<td>Monkey African green kidney</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>FMSA</td>
<td>Mouse C3H mammary carcinoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HT-116</td>
<td>Human immortalized intestinal epithelial cell</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HEK-293</td>
<td>Human Embryo Kidney</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HEK293T</td>
<td>Human Embryo Kidney</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HU-1</td>
<td>Human cancer cell</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HP-1</td>
<td>Human pancreatic tumor</td>
<td>−</td>
<td>〇</td>
</tr>
<tr>
<td>HuH-7</td>
<td>Human hepatocellular carcinoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human umbilical vein endothelial cell</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>IPS</td>
<td>Induced pluripotent stem cell</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>K562</td>
<td>Human Caucasian chronic myelogenous leukemia</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>LNCaP</td>
<td>Human Caucasian prostate carcinoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>Mott</td>
<td>Mouse tumor</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>Panc-1</td>
<td>Human Caucasian pancreas</td>
<td>−</td>
<td>〇</td>
</tr>
<tr>
<td>RHL</td>
<td>Human Burkitt’s lymphoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>SH-SV57</td>
<td>Human neuroblastoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>HT-29</td>
<td>Normal human diploid cells</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>THP-1</td>
<td>Human monocytic leukemia</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>U-2666</td>
<td>Human breast carcinoma</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>BM MSC</td>
<td>Human Mesenchymal Stem Cells from Bone Marrow</td>
<td>〇</td>
<td>−</td>
</tr>
<tr>
<td>Cell-MSC</td>
<td>Mesenchymal Stem Cells from IPS-01279</td>
<td>〇</td>
<td>−</td>
</tr>
</tbody>
</table>

− No test 〇 Successed

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.

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.

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

The entire process for PS CaptureTM 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.

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°C.

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 COLO205 cell culture supernatant was 11 pg. (The detection limit varies depending on the cell line).

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.

Q15: Is there a recommended primary antibody for detection?

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

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 x) to the washing solution. When you fail to detect a positive signal even with Control Primary Antibody Anti CD63 (100 x) or Control Biotinylated Antibody Anti CD63 (100 x) (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.

**Specifications and performance**
- Size: W198.8 × D49 × H49 (mm)
- 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
- Weight: 235g
- 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.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>290-35591</td>
<td>Magnet stand</td>
<td>1 unit</td>
<td>60,000</td>
</tr>
</tbody>
</table>

**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°C 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.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>552-30121</td>
<td>Alix Antibody (3A9)</td>
<td>0.1 mg</td>
<td>-20°C</td>
</tr>
<tr>
<td>553-30151</td>
<td>TSG101 Antibody (4A10)</td>
<td>0.1 mL</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
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)

**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.

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™**

\[
A:B = 1:1
\]

Medium volume are the same between A and B

**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.

![Image of cell cultures and exosome uptake](image)

Cell A
Red: Cell nuclei

Cell B
Green: CD63-GFP on exosomes

Exosomes from cell B were uptaked 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.

![Comparison diagram](image)

Exosome Density (mean)

- UniWells™
  - Cell Side: 178.0 x10^7
  - Medium Side: 218.0 x10^7
- Conventional co-culture plate
  - Cell Side: 67.2 x10^7
  - Medium Side: 32.7 x10^7

More exosomes migrated to medium side well in UniWells™
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.

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

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

Product listing

Horizontal Co-Culture Plat

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

RNA extraction Kit from Exosome

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>295-71701</td>
<td>microRNA Extractor SP Kit</td>
<td>50 reactions</td>
<td>2 ~ 10℃</td>
</tr>
</tbody>
</table>

Quantitative Kit for Protein Concentration

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>297-73101</td>
<td>Protein Assay BCA Kit</td>
<td>250 assays</td>
<td>Room temperature</td>
</tr>
<tr>
<td>015-25613</td>
<td>2 mg/mL Albumin Solution from Bovine Serum</td>
<td>1 mL × 10</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>
### Product listing

#### Isolation Kit for High Purity Extracellular Vesicles -No ultracentrifugation-

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>299-77603</td>
<td>MagCapture™ Exosome Isolation Kit PS</td>
<td>2 purifications</td>
<td>2 ~ 10°C</td>
</tr>
<tr>
<td>293-77601</td>
<td></td>
<td>10 purifications</td>
<td></td>
</tr>
</tbody>
</table>

#### -High Sensitive Detection- Exosome ELISA Kit

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>297-79201</td>
<td>PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)</td>
<td>96 tests</td>
<td>2 ~ 10°C</td>
</tr>
<tr>
<td>298-80601</td>
<td>PS Capture™ Exosome ELISA Kit (Streptavidin HRP)</td>
<td>96 tests</td>
<td>2 ~ 10°C</td>
</tr>
</tbody>
</table>

#### -High Sensitive Qualitative Analysis for Flow Cytometry-

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>297-79701</td>
<td>PS Capture™ Exosome Flow Cytometry Kit</td>
<td>300 reactions</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

#### -Suppressing Adsorption of Extracellular Vesicles-

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>058-09261</td>
<td>EV-Save™ Extracellular Vesicle Blocking Reagent</td>
<td>1 mL</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

#### -High Sensitive Detection Antibody Series-

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

#### -Magnetic Stand for Collecting Magnetic Beads-

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Description</th>
<th>Package Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>290-35591</td>
<td>Magnet Stand</td>
<td>1 piece</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>