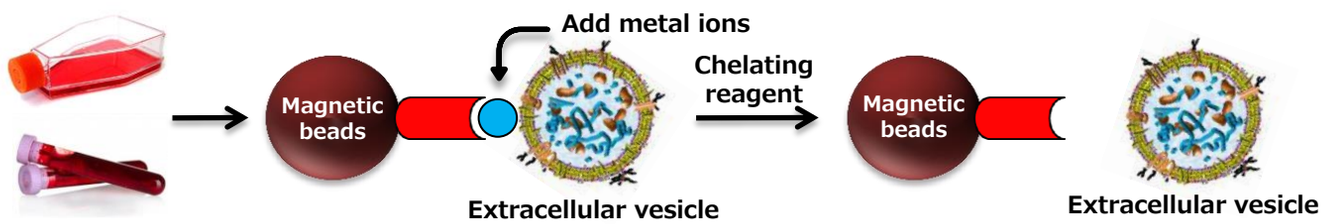
Wako Rafer INNOVACIÓN TECNOLÓGICA PARA LABORATORIO

Life Science

Isolation of high purity exosomes by a novel affinity molecule

MagCapture™ Exosome Isolation Kit PS

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



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

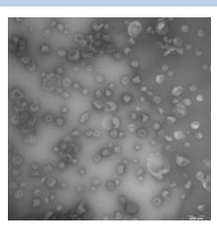
Feature ~MagCapture™ Exosome Isolation Kit PS~

Novel affinity method is adopted

- Recovering by PS-binding molecules
- Low background
- Mild elution with a chelating reagent under neutral pH condition

High purity and intact exosomes

High purity exosomes can be easily isolated by PS affinity method.

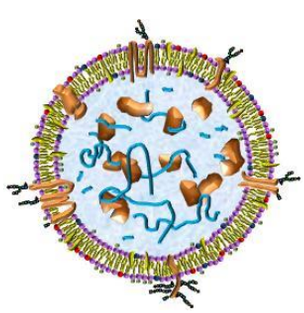


Not required ultracentrifugation

- Easy operation with magnetic beads
- Optimized protocol for isolation

High reproducibility

Comparison with other purification method			
Methods	Exosome purity	Exosome recovery	Intact vesicles recovery
MagCapture™ (PS affinity method)	■■■■■	■■■	Yes
Ultracentrifugation	■■	■■	Yes
Polymer-based precipitation	■	■■■■	Yes
Affinity method with antibody against surface antigen of exosome	■■■	■	No



Target samples: cell culture supernatant, serum, plasma, urine, *etc*.

Product Name	Package Size	Catalog No.	Storage
MagCapture™ Exosome Isolation Kit PS	2 purifications*1	299-77603	Keep at 2-10℃.
	10 purifications*1	293-77601	

*1 Used **Exosome Capture-immobilized beads** can be recycled up to 4 times and buffers of kit component also be contained enough for the case of recycling. When repeated isolations of extracellular vesicles from same sample are required, please try the recycling. However, when repeated isolations of extracellular vesicles from different kinds of samples are required, please don't try the recycling for preventing a contamination.

Comparing the yield of exosomes isolated from normal human serum

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

CD9, CD63 and CD81 are exosome markers.

Signals of each lanes are corresponding to 150 µL of serum sample. 15 µLof eluate and 5 µL of 4 × SDS sample buffer were mixed and applied all in each well.

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

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

Comparing the performance between conventional exosome isolation methods

The yield and purity were compared with exosomes isolated from a cell culture supernatant of K562 cells (human chronic mylogenous leukemia: CML) by using MagCapture™, ultracentrifugation and polymer-based precipitation method. The cell culture supernatant was prepared from serum-free medium, or 10% Exosome-depleted FBS included medium.

MagCapture™ Exosome Isolation Kit PS

Exosomes were recovered from 1 mL of pretreated (10,000 x g, 30 min) cell culture supernatant of K562 cells (serum-free medium or 10% exosome-depleted FBS included medium) by using MagCapture™ standard protocol (reaction time: 3 hours)

Ultracentrifugation

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

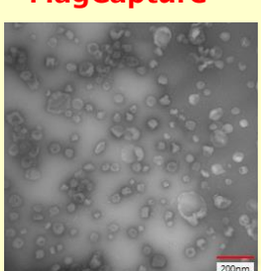
Polymer-based precipitation

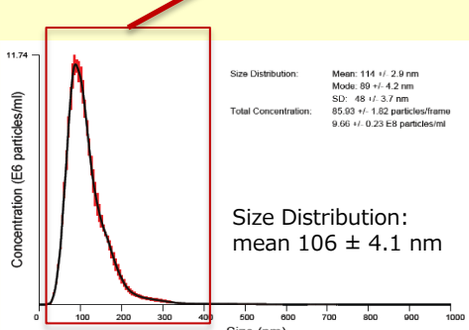
Exosomes were collected from 1 mL of pretreated (10,000 x g, 30 min) cell culture supernatant of K562 cells (serum-free medium or 10% exosome-depleted FBS included medium) by Company A's product in accordance with manual (Precipitation time: overnight).

Electron microscopic analysis and Nanoparticle tracking analysis of isolated exosomes

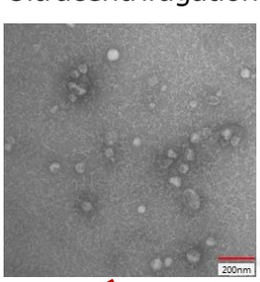
The particle size of exosomes isolated from cell culture supernatant of K562 cells (serum-free medium) using **MagCapture™**, ultracentrifugation and polymer-based precipitation, respectively was determined with NanoSight LM-10. In addition, the collected exosomes (2-4 x 10^{10} particles) were fixed by 2% paraformaldehyde and analyzed by electron microscopy.

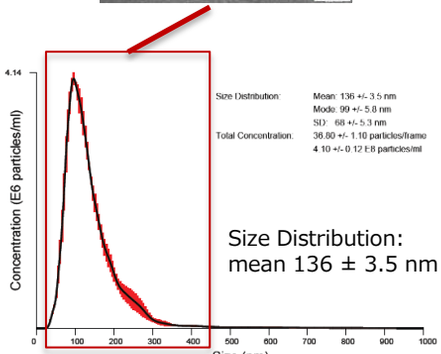
MagCapture™



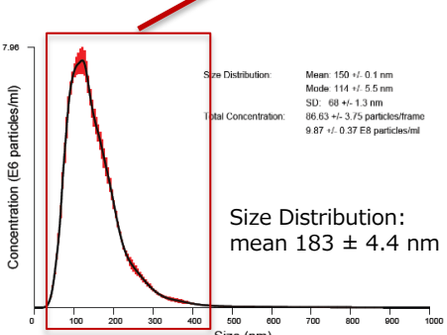


Ultracentrifugation





Polymer-based precipitation
(Company A's product)



Electron microscope images were provided by Dr. R. Hanayama at Graduate School of Medicine, Kanazawa University and Dr. W. Nakai at iFReC, Osaka University.

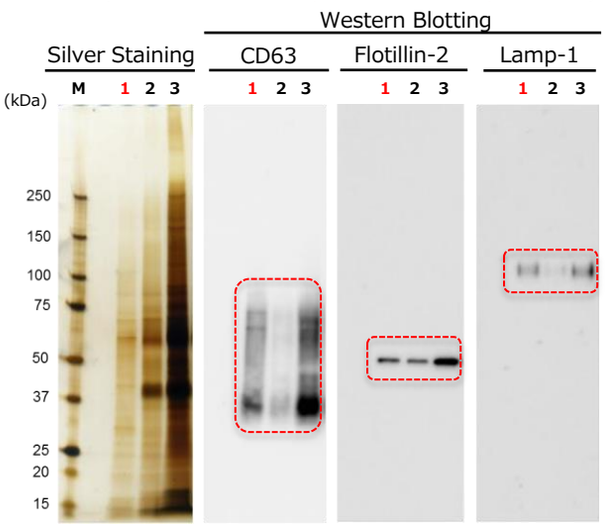
MagCapture™ collected many particles around 50-100 nm in size. With electron microscopy, numerous extracellular vesicles can be seen.

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

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

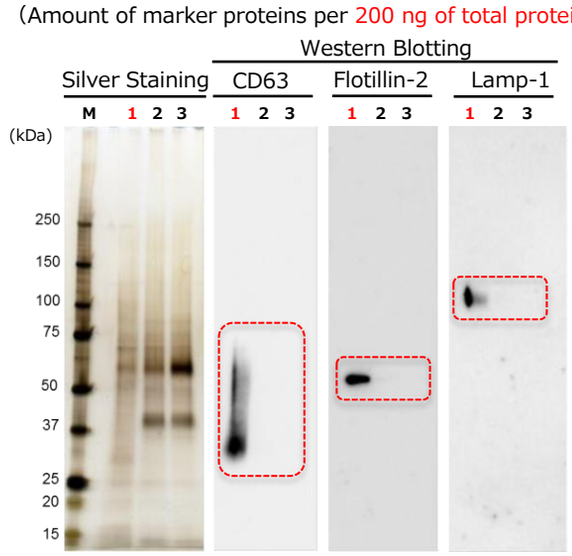
Comparison of recovery amount

(Recovery amount from 150 μL cell culture supernatant)



Comparison of purity

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



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

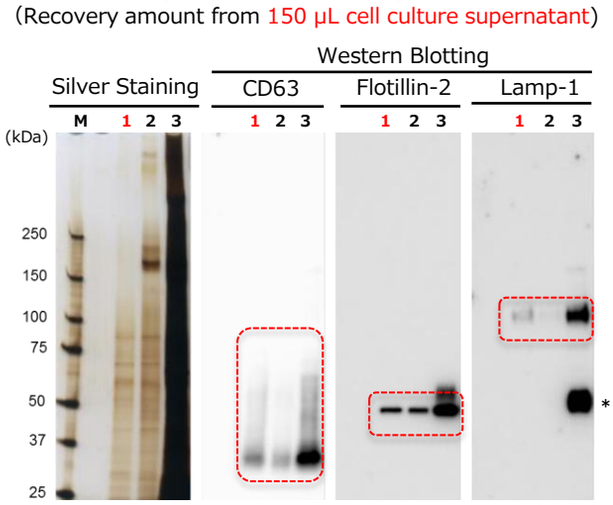
With **MagCapture™**, the recovery performance of exosomes is excellent and the amount of contaminant of proteins is very low, so the balance of purity and recovery amount is the best!!

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

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

Comparison of recovery amount

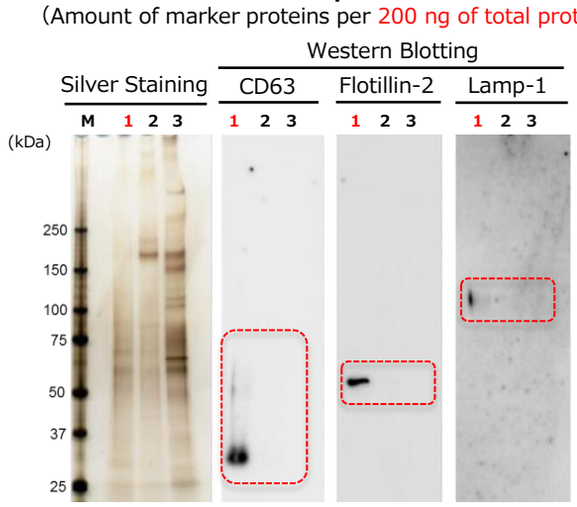
(Recovery amount from 150 μL cell culture supernatant)



*: Non-specific band

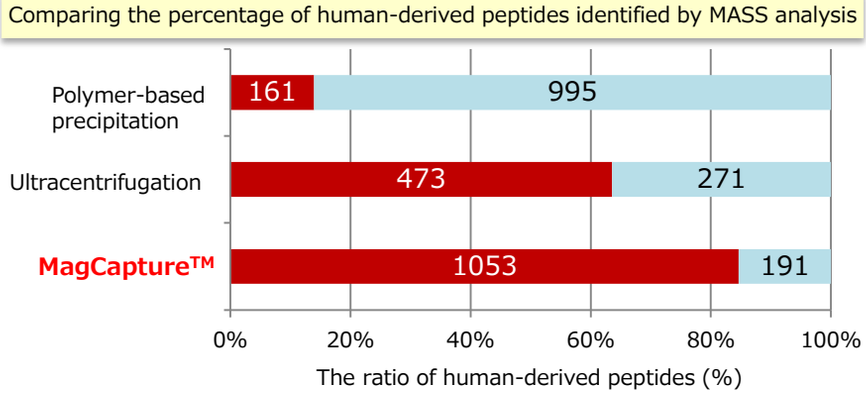
Comparison

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



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

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



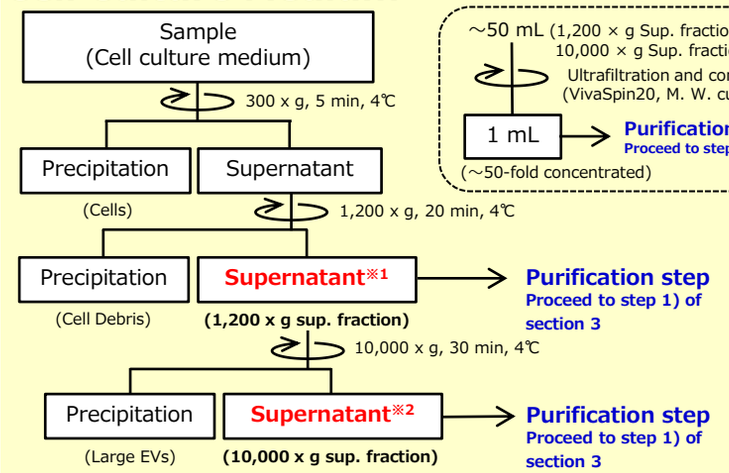
■ Number of human-derived peptides
■ Number of FBS-derived peptides

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

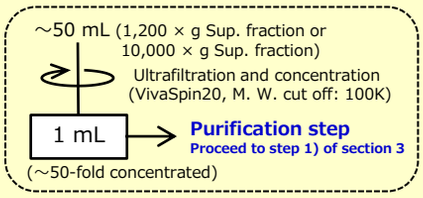
MASS analysis data was provided by Dr. R. Hanayama at Graduate School of Medicine, Kanazawa University and Dr. W. Nakai at iFReC Osaka University.

Pretreatment protocol for cell culture supernatant, serum, and plasma

Cell Culture Medium



Concentration (Optional※3)

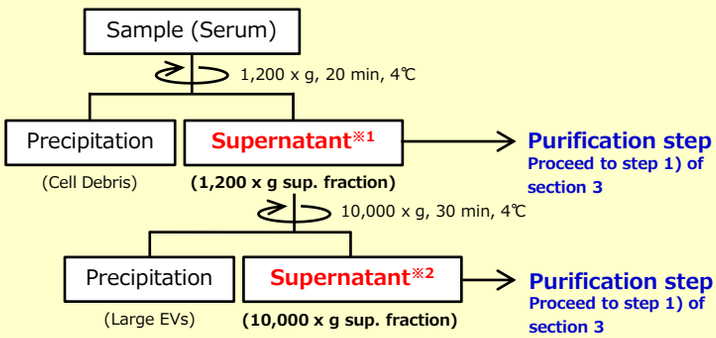


※1 When **exosomes and large EVs** are needed, use 1,200 × g sup. fraction as sample.

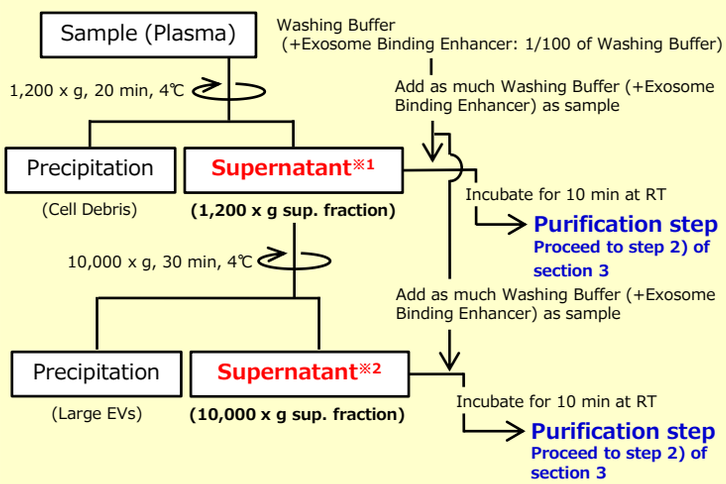
※2 When **exosomes** are needed, use 10,000 × g sup. fraction as sample.

※3 This is optional protocol for large-scale volume (~50 mL) of cell culture medium.

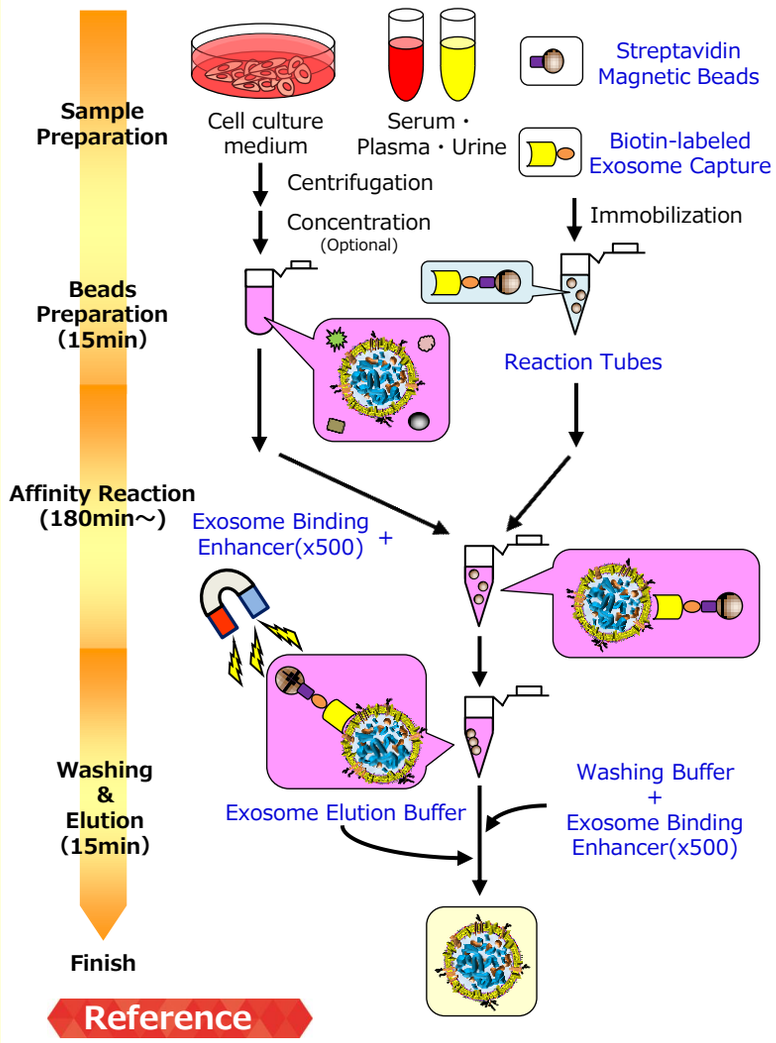
Serum



Plasma



Outline of procedure



Reference

"A novel affinity-based method for the isolation of highly purified extracellular vesicles", W. Nakai, T. Yoshida, D. Diez, Y. Miyatake, T. Nishibu, N. Imawaka, K. Naruse, Y. Sadamura & R. Hanayama, *Sci Rep* **6**, 33935 (**2016**).

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www.rafer.es

Barcelona 93 645 50 28 barcelona@rafer.es
Bilbao 94 499 85 80 bilbao@rafer.es
La Coruña 981 93 89 26 galicia@rafer.es
Madrid 91 365 15 70 madrid@rafer.es
Málaga 639 359 792 malaga@rafer.es
Sevilla 954 369 334 sevilla@rafer.es
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Lisboa 21 154 19 98 lisboa@rafer.es