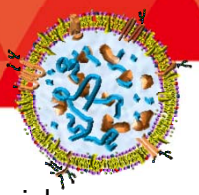
Wako

Life Science

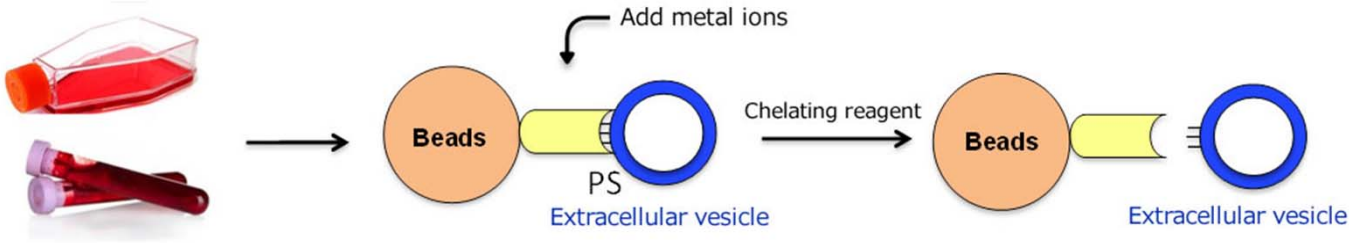
Exosome isolation by novel affinity molecule

MagCapture™

Exosome Isolation Kit PS



Affinity method for phosphatidylserine (PS) on membrane surface of extracellular microvesicles



Using Phosphatidylserine (PS)-binding protein, extracellular vesicles are captured in a metal ion-dependent manner, followed by eluting them with metal ion chelating reagent.

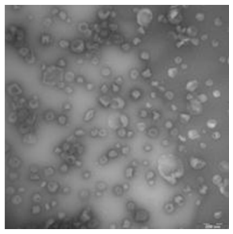
Features

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

Novel affinity method

- Recovery by a PS-binding molecule
- Low background
- Mild elution by a chelating reagent on a neutral pH condition

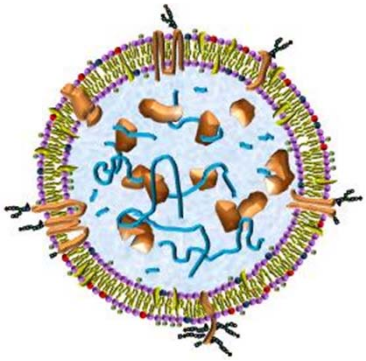
High purity of intact exosomes



Not required ultracentrifugation

Improved operation by using magnetic beads
Optimized protocol

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
Exosome surface antigen affinity method (using the antibody)	■■■	■	No

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

NOTE: This kit is NOT suitable for exosome isolation from plasma treated with chelating agents such as EDTA and citric acid.

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

The yield comparison of exosome isolated from human serum

Exosomes were isolated from human serum by using MagCapture™, ultracentrifugation and antibody affinity method, followed by western blot with the anti CD9, anti CD63 and CD81 antibodies.

CD9, CD63 and CD81 are exosome markers.

Lane 1: Ultracentrifugation
Lane 2: MagCapture™
Lane 3: Exosome Isolation kit (CD9) [Company A]
Lane 4: Exosome Isolation Kit (CD63)[Company A]
Lane 5: Exosome Isolation Kit (CD81)[Company A]
Lane 6: Exosome Isolation Kit (Antibody beads-mixture of CD9, CD63, CD81 & EpCAM)

The yield of exosomes by **MagCapture™** is higher than ultracentrifugation or antibody affinity method.

The performance comparison with conventional exosome isolation methods

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

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

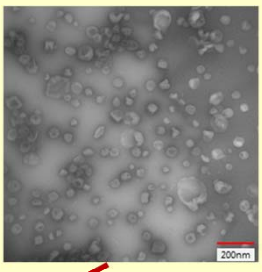
Ultracentrifugation
Exosome fractions were collected from 10 mL of pretreated (10,000 x g, 30 min.) K562 cell culture supernatant (serum-free medium or 10% exosome-depleted FBS medium) by ultracentrifugation (110,000 x g, 70 min.). The precipitates were suspended by TBS and then exosomes were recovered by ultracentrifugation (110,000 x g, 70 min.) as a pellet.

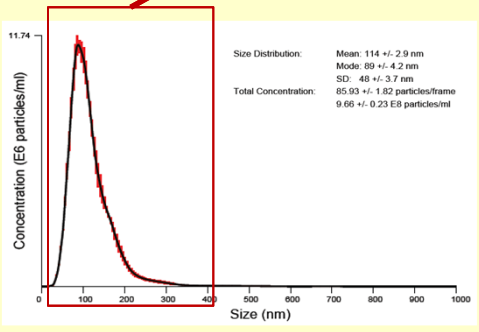
Polymer-based precipitation
Exosomes were collected from 1 mL of pretreated (10,000 x g, 30 min) K562 cell culture supernatant (serum-free medium or 10% exosome-depleted FBS medium) by using Company A's product protocol (Precipitation time: overnight).

Electron microscopic analysis and Nano analysis of isolated exosomes

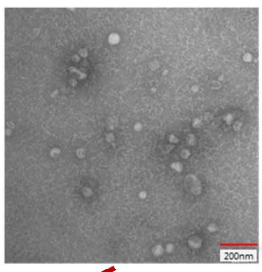
The particle size of exosomes from K562 cell culture supernatant (serum-free medium) using **MagCapture™**, ultracentrifugation and polymer-based precipitation, respectively was determined by using NanoSight LM-10. The collected exosomes (2-4 x 10^{10} particles) were fixed by 2% paraformaldehyde and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

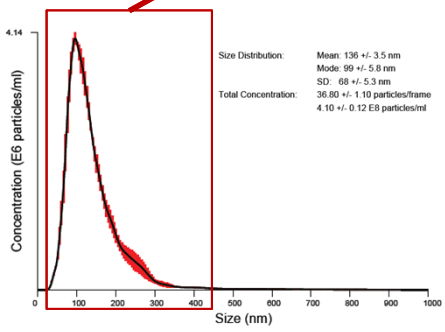
MagCapture™



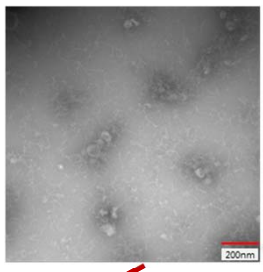


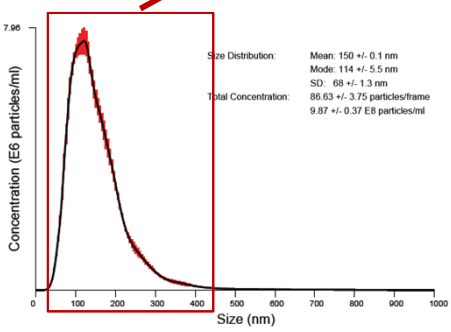
Ultracentrifugation





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.

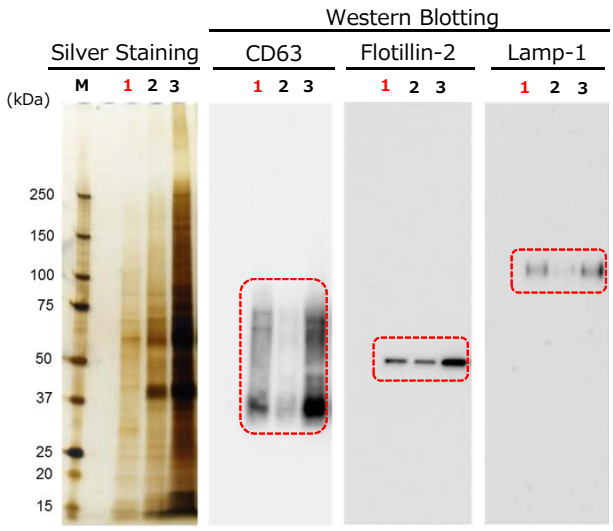
MagCapture™ could enrich exosomes which were uniformed particles (~100 nm)!

The comparison of recovery amount and purity of exosomes (1)

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

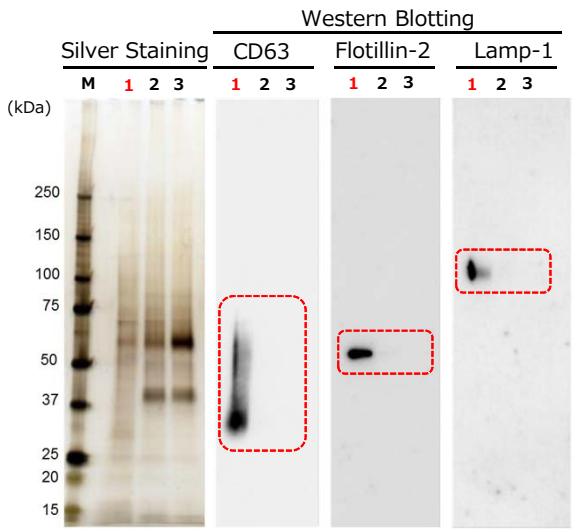
Comparison of recovery amount

(Recovery amount from 150μL cell culture supernatant)



Purity comparison

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



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

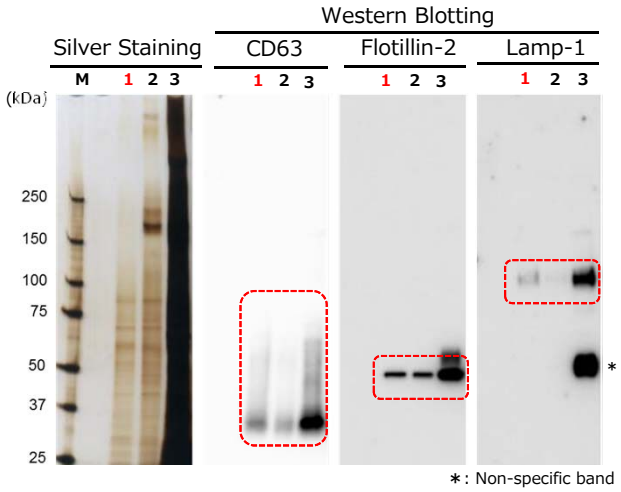
With **MagCapture™**, the recovery performance of exosomes is excellent and the amount of contaminant proteins is very low, so the balance of purity and recovery efficiency is the best!!

The comparison of recovery amount and purity of exosomes (2)

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

Comparison of recovery amount

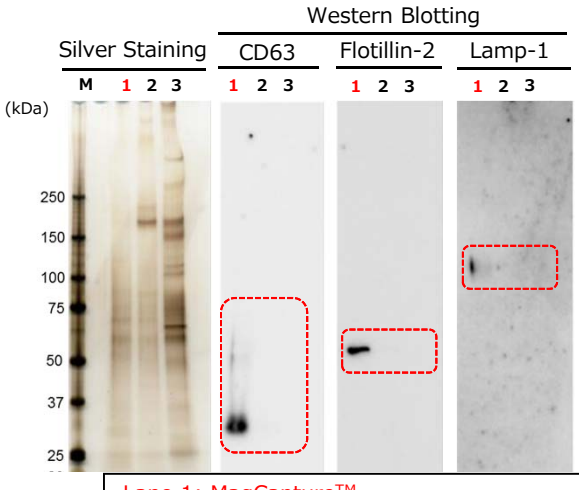
(Recovery amount from 150μL cell culture supernatant)



*: Non-specific band

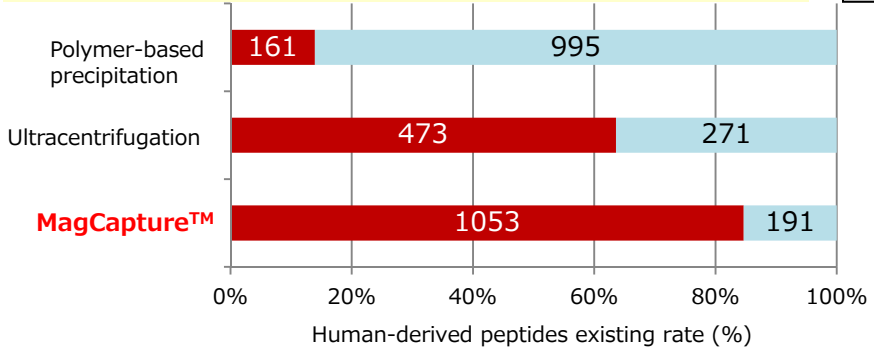
Purity comparison

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



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

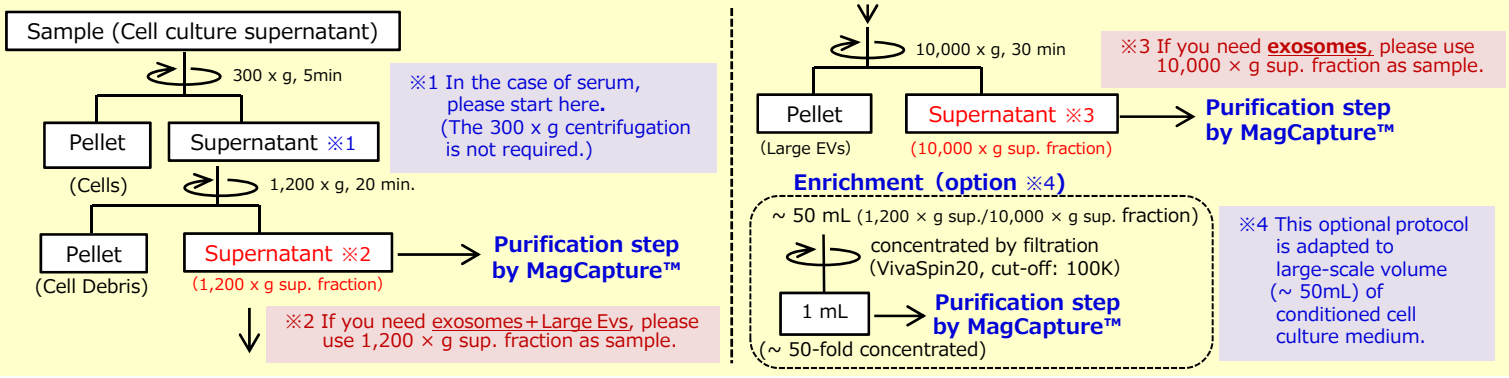
Comparison of human-derived peptides identified by MASS analysis



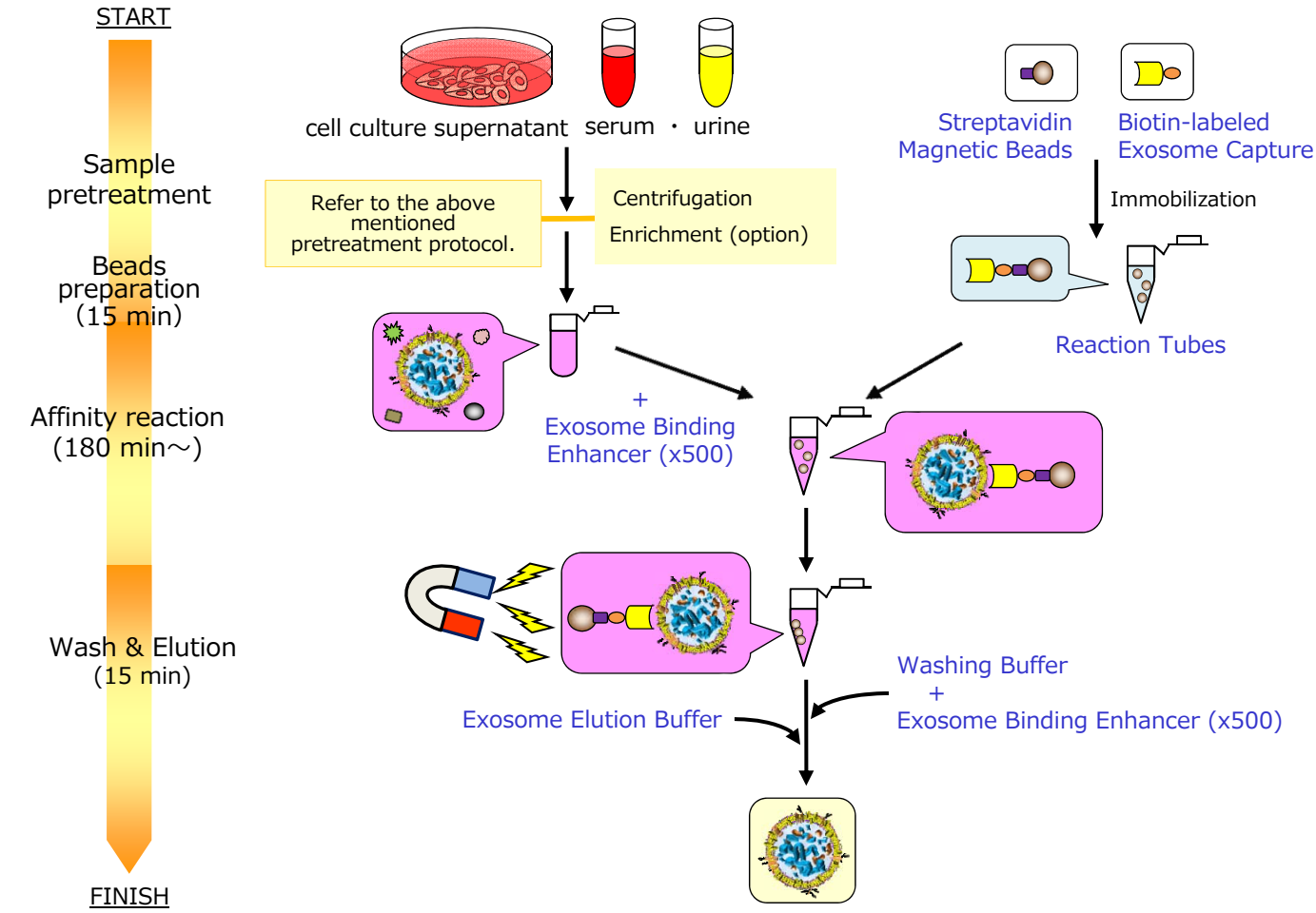
With **MagCapture™**, high purity exosomes are recovered even from culture medium with FBS, so MASS analysis with low background can be done!

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 from cell culture supernatant or serum



Exosome Isolation Flow Chart



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