Characteristics of PS-affinity method for isolation and detection of EVs

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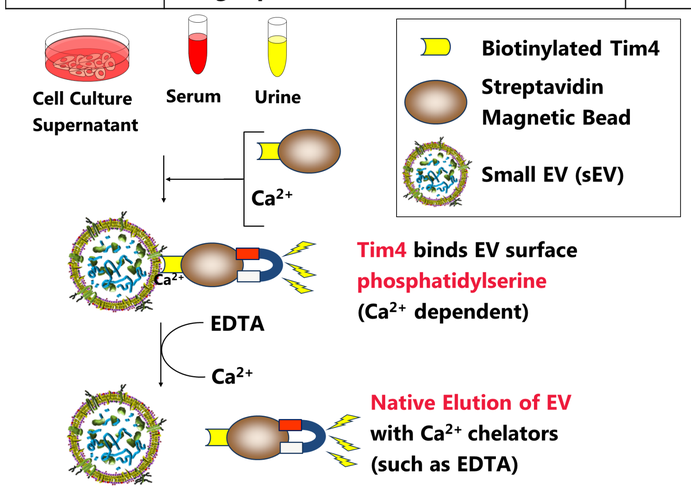
Abstract

Extracellular vesicles (EVs), such as exosomes and microvesicles serve as messengers of intercellular network, allowing exchange of cellular components between cells. EVs carry lipids, proteins, and nucleic acids derived from their producing cells, and have potential as biomarkers specific to cell types and cellular states. However, conventional methods, such as ultracentrifugation (UC) or polymeric precipitation (Polymer) for isolating EVs have disadvantages regarding purity and feasibility. Here, we have developed a novel method for EV purification by using Tim4 protein, which specifically binds the phosphatidylserine (PS) displayed on the surface of EVs. Because the binding is Ca^{2+}-dependent, intact EVs can be easily released from Tim4 by adding Ca^{2+} chelators. We termed this EVs purification system "PS-affinity method", and in which we have applied to cell conditioned media and biofluids, this is capable of yielding EVs of higher purity than those obtained using conventional methods. In addition, we have applied the PS-affinity method to ELISA system and it showed higher sensitivity than western blot and conventional ELISA system. Therefore, the PS-affinity purification and detection system will be a powerful tool for EV studies.

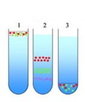
PS-affinity method for isolation of EVs

Code No.	Product Name	Package Size
299-77603	MagCapture™ Exosome Isolation Kit PS	2 tests
293-77601	MagCapture™ Exosome Isolation Kit PS	10 tests



Sample Type: cell culture supernatant, serum, plasma, urine, etc.

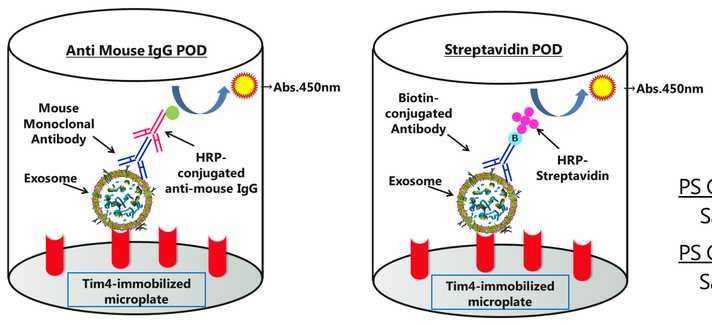
MagCapture™ Exosome Isolation Kit PS can purify EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that this isolation kit can be purified EVs from various animal species samples such as human, mouse, and bovine.

	PS-affinity	UC	Polymer	Density	Antibody
Method					
EV's Purity	■■■	■■	■	■■■■	■■■
State of vesicles	Intact	Intact	Intact	Intact	Not Intact
Operability	Easy and Stable	Easy	Easy and Fast	Complex	Easy and Stable
Recovery amount	■■■	■■	■■■■	■	■■

- Can isolate High Purity and Intact Extracellular Vesicles
- Can isolate EVs from cell culture supernatant, serum, plasma, and urine
- High Reproducibility and the recovery amount are stable
- Easy Operation (about 3.5hours)
- Enable to use multiple sample (No need of ultracentrifugation)

PS-affinity for detection of EVs

Code No.	Product Name	Package Size
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)	96 Reactions
Coming soon	PS Capture™ Exosome ELISA Kit (Streptavidin POD)	96 Reactions

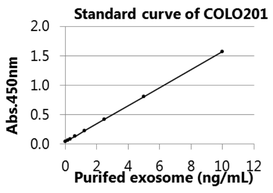


PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)
Sample Type : Purified EVs, Cell culture supernatant

PS Capture™ Exosome ELISA Kit (Streptavidin POD)
Sample type : Purified EVs, Cell culture supernatant, Serum, Plasma

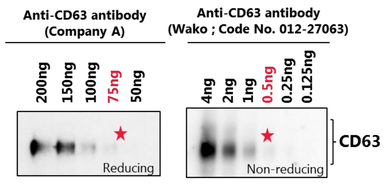
◆**Comparison of the sensitivity of EV detection between PS Capture™ ELISA with western blotting**
Samples : small EVs (sEVs) purified from COLO201 cell culture supernatant by MagCapture™ Exosome Isolation Kit PS

PS Capture Exosome ELISA Kit (Anti Mouse IgG POD)



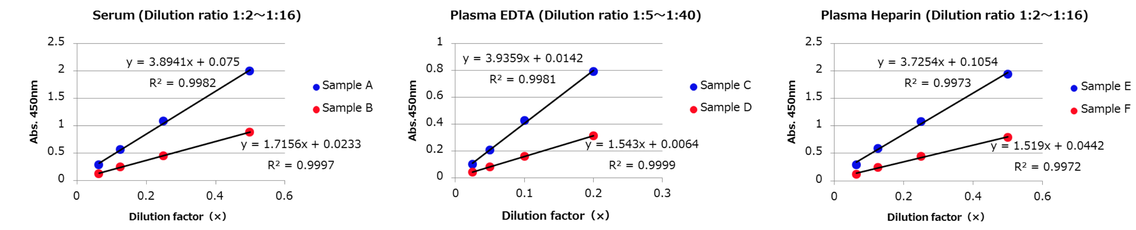
Limit of detection (Blank+3.3SD)	Limit of quantification (Blank+10SD)
0.11 ng/mL (Total 11 pg)	0.34 ng/mL (Total 34 pg)

Western blotting



The sensitivity of PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) was 50 to 1,000 times higher than that of western blotting.

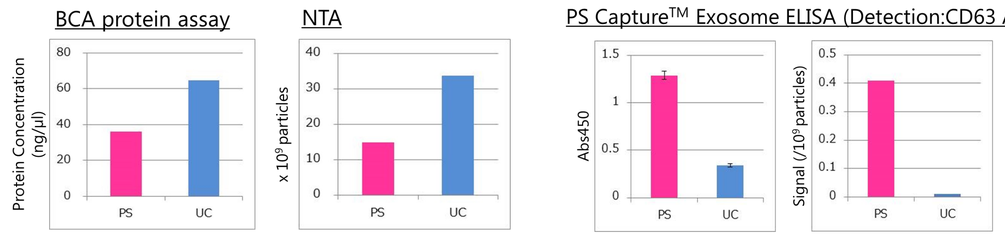
◆**Dilutiom linearity of serum and plasma by PS Capture™ ELISA (Streptavidin POD)**
Samples : normal human serum and plasma (Detection : Biotinylated CD63 Ab)



PS Capture™ Exosome ELISA Kit (Streptavidin POD) showed good dilution linearity in the assay using serum and plasma samples. Therefore, the ELISA kit can measure EVs quantitatively.

The yield and purity of sEVs isolated by PS-affinity method

sEVs in 10K sup of COLO201 cells were isolated by each method and examined by BCA assay, Nanoparticle Tracking Analysis (NTA) and PS Capture™ Exosome ELISA Kit.

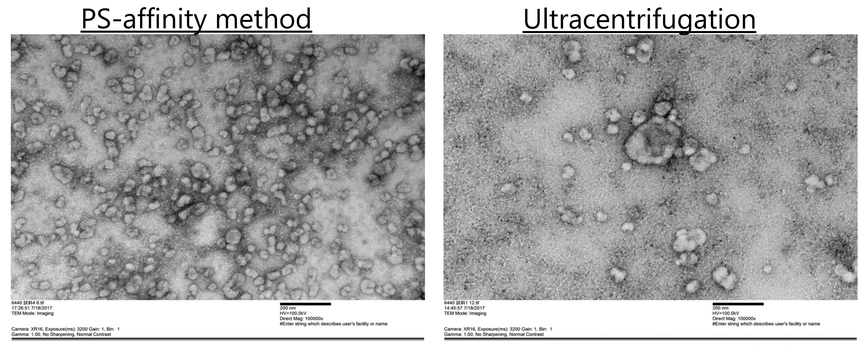


The PS-affinity method could isolate more than about three times as many sEVs as the ultracentrifugation method. However, twice of protein abundance and the number of particles were detected using BCA assay and NTA in sEV samples isolated by ultracentrifugation.

Particle analysis of sEVs isolated by PS-affinity method

sEVs in 10K sup of COLO201 cells were isolated by each method and examined by transmission electron microscope (TEM).

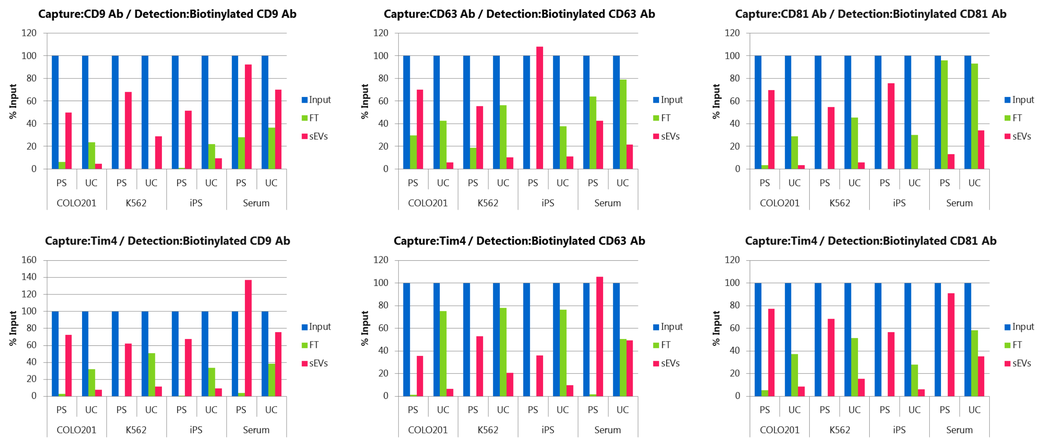
sEVs isolated by ultracentrifugation were accompanied by huge EVs probably derived aggregated each other.



Bar: 200nm

Comparison of sEV recovery between ultracentrifugation and PS-affinity method

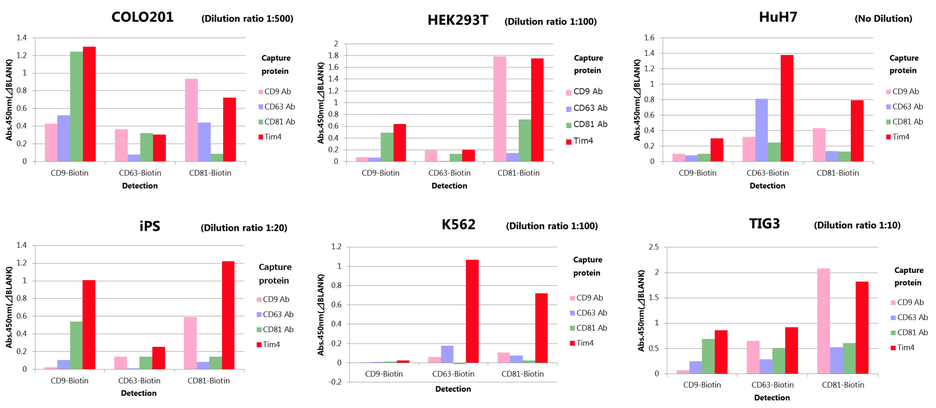
sEVs in 10K sup of various cells were isolated by each methods and recovery rate of sEVs were examined by anti-CD9, anti-CD63, anti-CD81 antibodies or Tim4-immobilized ELISA.



These results indicated that PS-affinity method can recover sEVs derived from various cell lines more efficiently than ultracentrifugation.

Comparison of capturing ability of sEVs

sEVs in 10K sup of various cells were diluted and incubated in each well of microplate immobilized anti-CD9, anti-CD63, anti-CD81 antibodies or Tim4. And then, bound sEVs were detected with biotinylated antibodies against EV surface marker such as CD9, CD63 or CD81.

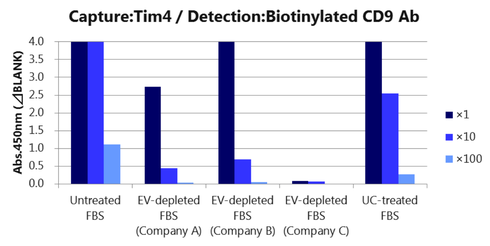


The results indicated that PS-affinity ELISA can detect sEVs derived from various cell lines more efficiently and universally than EV surface marker antibody-immobilized ELISA.

Quality control of EV-depleted FBS by using PS-affinity ELISA

The residual EVs in untreated FBS, three products of EV-depleted FBS and ultracentrifugation-treated FBS were measured by PS Capture™ Exosome ELISA Kit.

The results indicated that PS Capture™ Exosome ELISA Kit would be a useful tool for quality control of EV-depleted FBS.



Conclusion

- In EV isolation step, ultracentrifugation has serious problems such as purity and recovery amount of isolated EVs.
- PS-affinity can isolate and detect EVs derived from various cell lines more efficiently and universally than conventional methods such as ultracentrifugation.
- PS-affinity isolation and detection system will be a powerful tool in EV studies such as functional analysis of EVs and research of biomarkers.