A novel affinity-based method for the isolation of highly purified extracellular vesicles

○Kodai Sasamoto[1], Naoko Imawaka[1], Takamasa Ishidome[1] Ken Naruse[1], Masayuki Yamane[1], Kazunari Hirayasu[1], Ryo Ukekawa[1], Takahiro Nishibu[1] & Yoshifusa Sadamura[1]

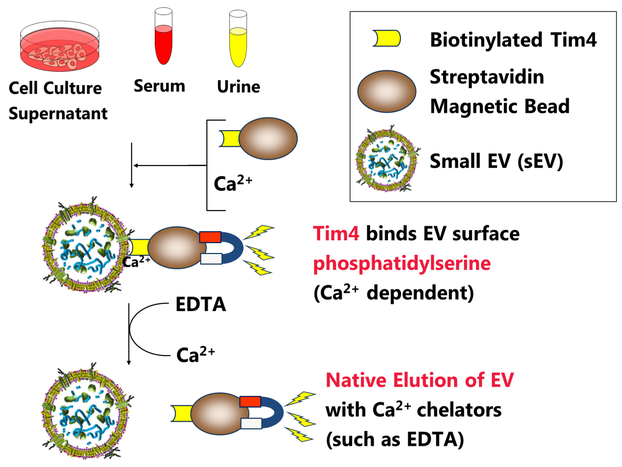
1 Life Science Research Laboratories, FUJIFILM Wako Pure Chemical Corporation, Japan

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 even cellular states. However, conventional methods, such as ultracentrifugation (UC) or polymeric precipitation for isolating EVs have disadvantages regarding purity and feasibility. Here, we have developed a novel method for EV purification, termed "PS-affinity method", 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. In addition, we have applied the PS-affinity method to ELISA system and it showed higher sensitivity than western blot and conventional ELISA system. Furthermore, we found a polymer-based blocking reagent prevents absorbing EVs to labware and reduces the loss of EVs, and we have used PS-affinity ELISA system for quality control of EV-depleted FBS. Therefore, the PS-affinity purification and detection system will be a powerful tool for EV researches.

PS-affinity method for isolation of EVs

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



	PS-affinity	Ultracentrifugation (UC)	Density (UC-Sucrose Cushion)	Size exclusion chromatography	Polymer
Method					
EVs Purity (Exosome protein coverage)	■■■■	■■	■■■	■■■	■
State of EVs	Intact	Intact	Intact	Intact	Intact
Operability	Easy and Stable	Easy	Complex	Easy	Easy
Total protein yield	■	■■■	■■	■■	■■■■

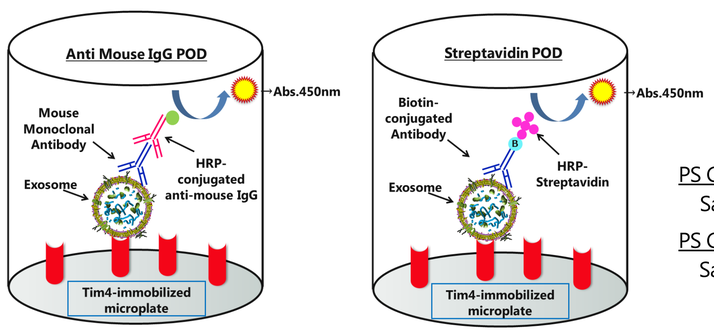
Sample Type: cell culture supernatant, serum, plasma, urine, etc.

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.

- ◆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 Reaction
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin POD)	96 Reaction

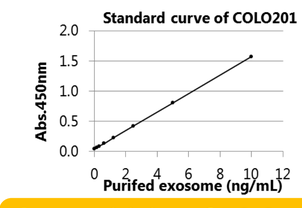


PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)
Sample Type : Purified EVs, Cell culture supernatant

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

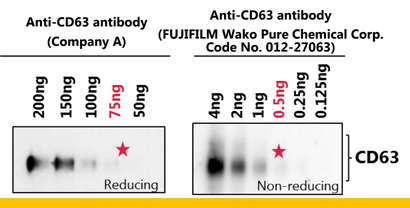
◆**Comparison of the sensitivity of EV detection between PS Capture™ ELISA with western blotting**
Samples : small EVs (sEVs) purified from COLO201 cell culture supernatant by MagCapture™ Exosome Isolation Kit PS

PS Capture Exosome ELISA Kit (Anti Mouse IgG POD)



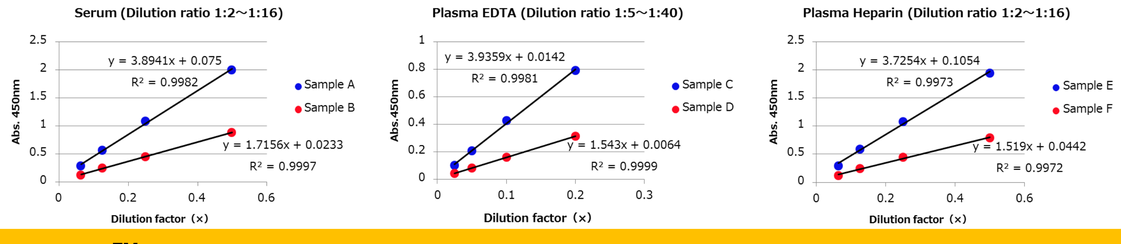
Limit of detection (Blank+3.3SD)	Limit of quantification (Blank+10SD)
0.11 ng/mL (Total 11 pg)	0.34 ng/mL (Total 34 pg)

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.

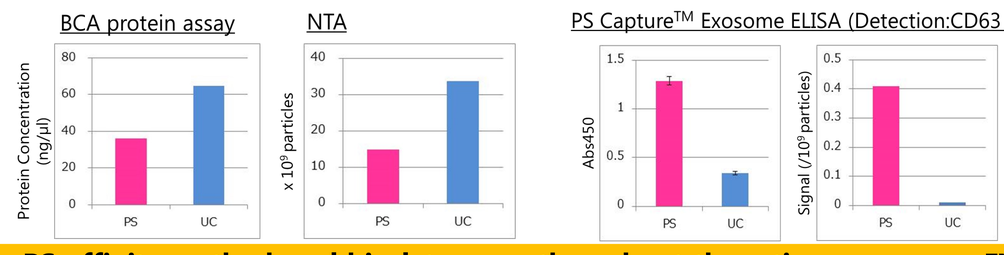
◆**Dilution linearity of serum and plasma by PS Capture™ ELISA (Streptavidin POD)**
Samples : normal human serum and plasma (Detection : Biotinylated CD63 Ab)



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



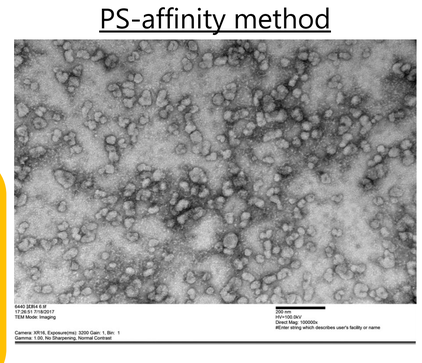
The PS-affinity method could isolate more than about three times as many sEVs as the ultracentrifugation method. However, twice of protein abundance and the number of particles were detected using BCA assay and NTA in sEV samples isolated by ultracentrifugation.

Particle analysis of sEVs isolated by PS-affinity method

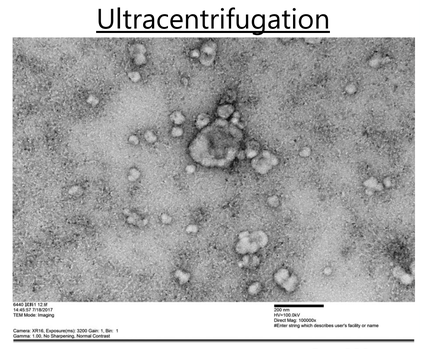
sEVs in 10K supernatant of COLO201 cells were isolated by each method and examined by transmission electron microscope (TEM).

sEVs isolated by PS-affinity method were not accompanied by huge EVs probably derived aggregated each other.

PS-affinity method



Ultracentrifugation



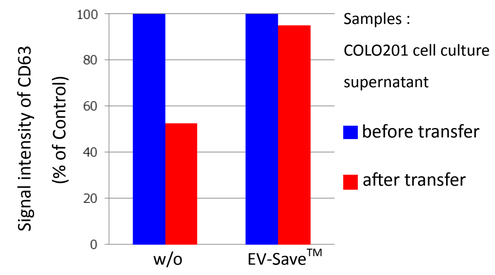
Bar: 200nm

Reducing the loss of EVs by polymer-based blocking reagent

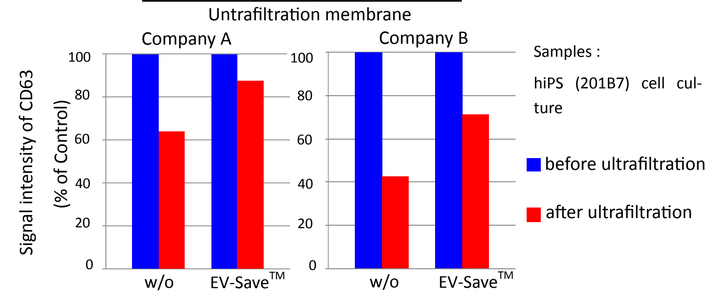
EV-Save™ Exracellular Vesicle Blocking Reagent is a polymer reagent to prevent absorbing EVs in cell culture supernatants to labware, which reduces the loss of EVs during experiments and storage.

Code No.	Product Name	Package Size
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL

Absorbing EVs to tube

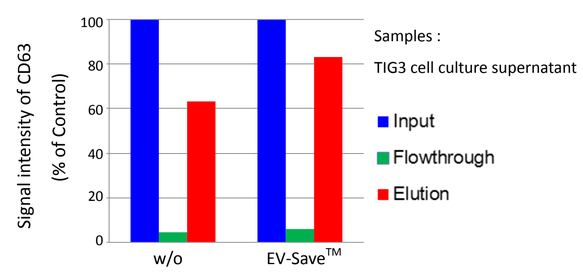


Loss of EVs during ultrafiltration

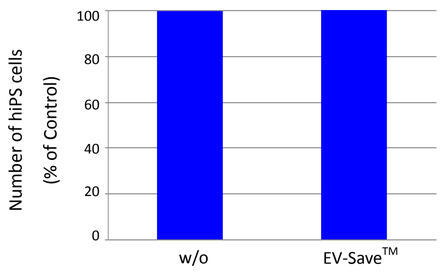


EV-Save™ improves the recovery rate of PS-affinity isolation method, and has no effect on cell growth of hiPS (201B7) cells.

PS-affinity isolation method



Cytotoxicity of blocking reagent

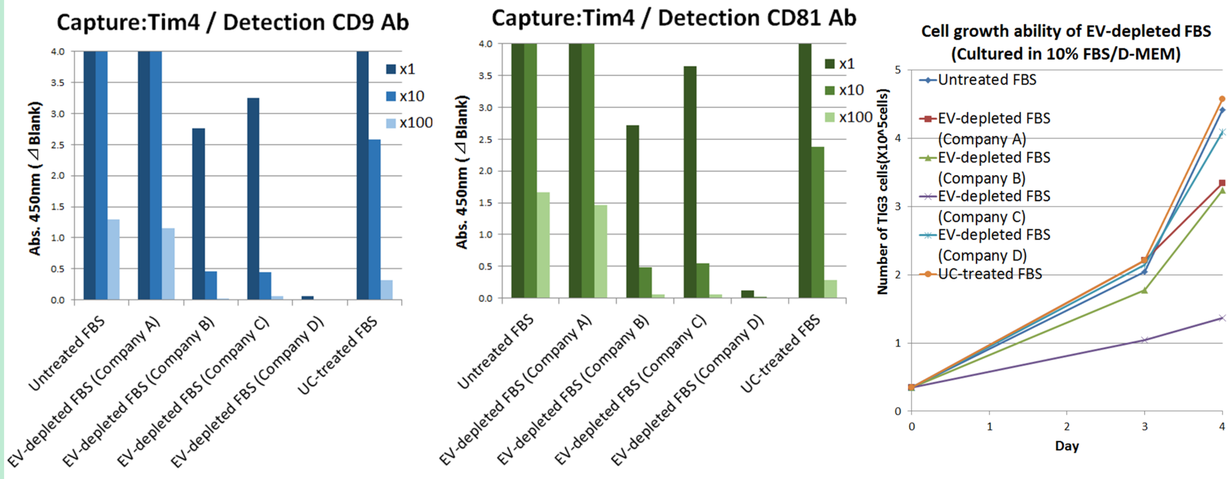


EV-Save™ Exracellular Vesicle Blocking Reagent reduces the loss of EVs effectively.

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

The residual EVs in untreated FBS, commercial products of EV-depleted FBS and ultracentrifugation-treated FBS were measured by PS-affinity ELISA system.

Code No.	Product Name	Package Size	Cross Reactibity
014-27763	Anti CD9, Monoclonal Antibody (1K)	100uL	Human, Bovine
011-27773	Anti CD81, Monoclonal Antibody (17B1)	100uL	Human, Bovine



PS-affinity ELISA system would be a useful tool for quality control of EV-depleted FBS.

Conclusion

- ◆**In EVs isolation step, ultracentrifugation has serious problems such as purity and recovery amount of 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 researches such as functional analysis of EVs and research of biomarkers.**