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

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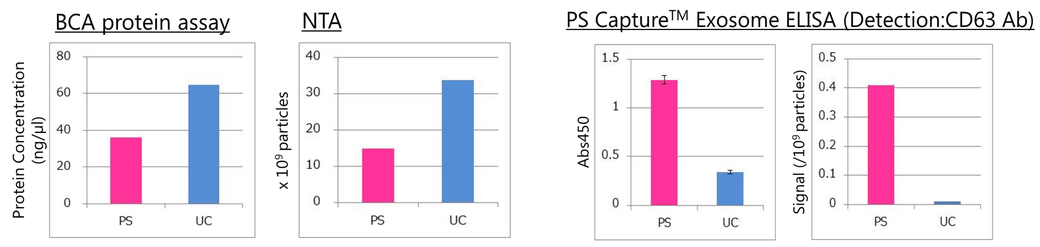
Abstract

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 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. Tim4 protein can also be used as a powerful tool to quantification of EVs in ELISA system. Furthermore, we found a polymer-based blocking reagent prevents absorbing EVs to labwares and reduced the recovery loss of EVs. These findings suggest that the affinity of Tim4 for EVs will contribute to abundant applications in EV studies.

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

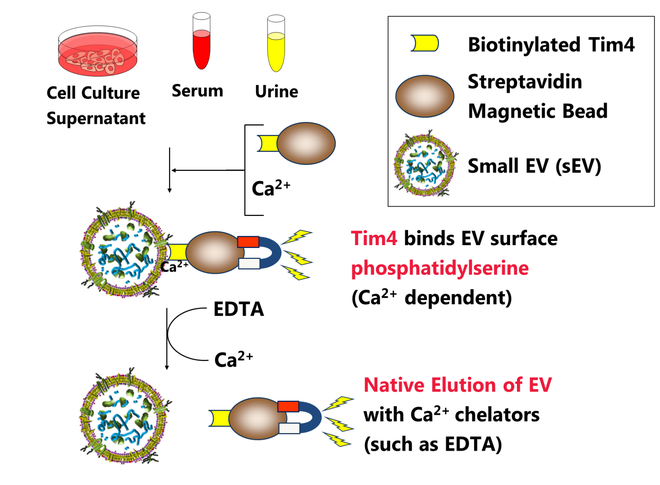
sEVs in 10K supernatant of COLO201 cells were isolated by each method and examined by BCA assay, Nanoparticle Tracking Analysis (NTA) and PS Capture™ Exosome ELISA Kit.

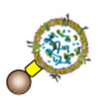
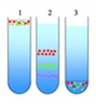
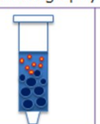


The PS-affinity method could isolate more than about three times as many sEVs as the ultracentrifugation method. However, twice of protein abundance and the number of particles were detected using BCA assay and NTA in sEV samples isolated by ultracentrifugation.

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.

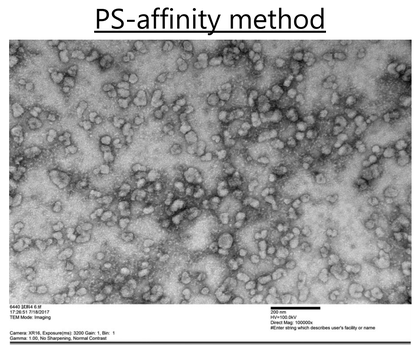
- **Can isolate High Purity and Intact Extracellular Vesicles**
- **Can isolate EVs from cell culture supernatant, serum, plasma, and urine**
- **High Reproducibility and the recovery amount are stable**
- **Easy Operation (about 3.5hours)**
- **Enable to use multiple samples (No need of ultracentrifugation)**

Particle analysis of sEVs isolated by PS-affinity method

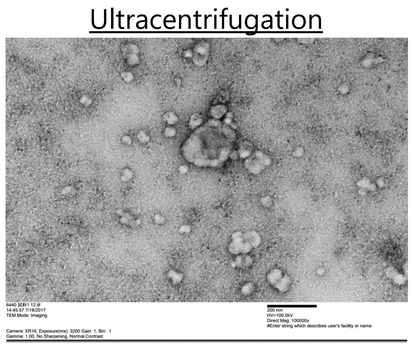
sEVs in 10K supernatant of COLO201 cells were isolated by each method and examined by transmission electron microscope (TEM).

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

PS-affinity method

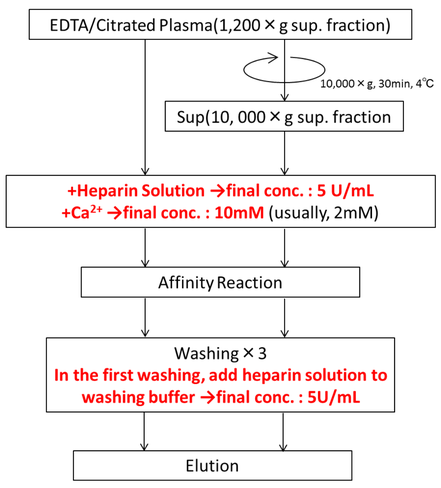


Ultracentrifugation



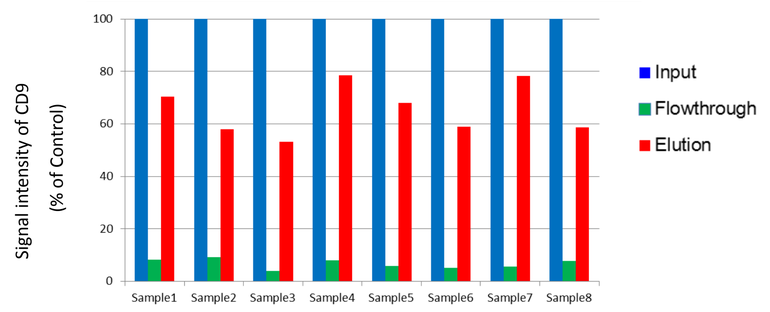
Bar: 200nm

Isolation of EVs from EDTA/Citrated plasma by PS-affinity method



In the case of EDTA and citrated plasma, the anticoagulant contained in the sample inhibits the binding of extracellular vesicles to Tim4-binding magnet beads. But, by adding heparin sodium solution to the centrifuged sample, EVs from them could be isolated by PS-affinity method.

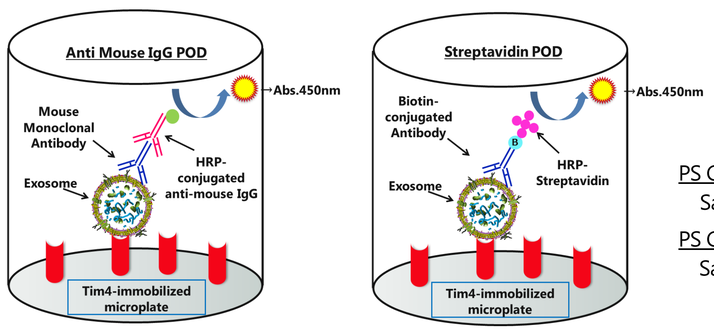
Isolation of EVs from EDTA plasma by PS-affinity method



PS-affinity method is available to isolation of EVs from EDTA/Citrated plasma.

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



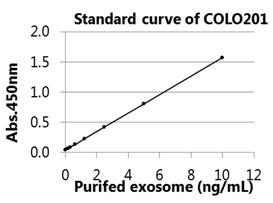
PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)
Sample Type : Purified EVs, Cell culture supernatant

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

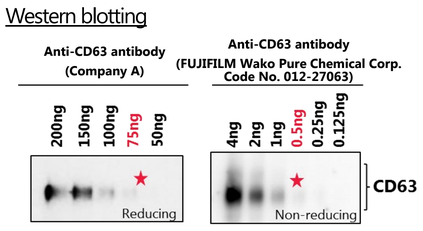
Samples : small EVs (sEVs) purified from COLO201 cell culture supernatant by MagCapture™ Exosome Isolation Kit PS

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)

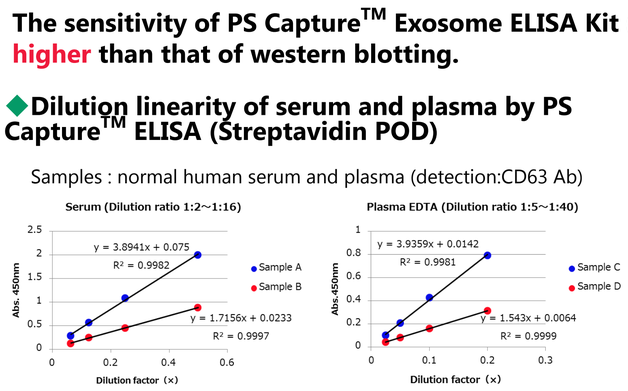
Western blotting



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

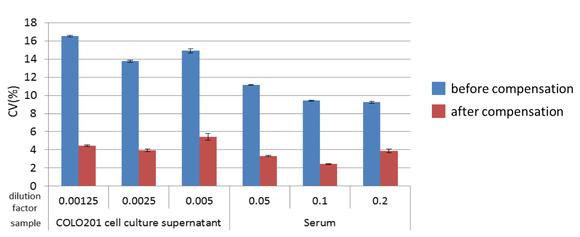
◆Dilution linearity of serum and plasma by PS Capture™ ELISA (Streptavidin POD)

Samples : normal human serum and plasma (detection:CD63 Ab)



PS Capture™ Exosome ELISA Kit (Streptavidin POD) showed good dilution linearity, indicating that EVs can be measured quantitatively.

◆Compensation of ELISA signals by standard curve of purified COLO201 sEVs



As compensation by standard curve of purified COLO201 sEVs reduced CV(%), more accurate comparison is possible by using the purified sEVs.

Anti-adsorption and Cytoprotective effect of EV-Save™

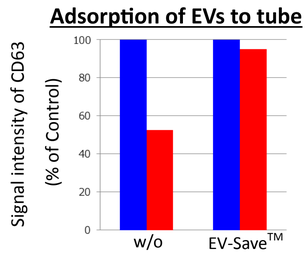
◆Anti-adsorption effect

EV-Save™ Exracellular Vesicle Blocking Reagent is a polymer reagent to prevent absorbing EVs in cell culture supernatants to labwares.

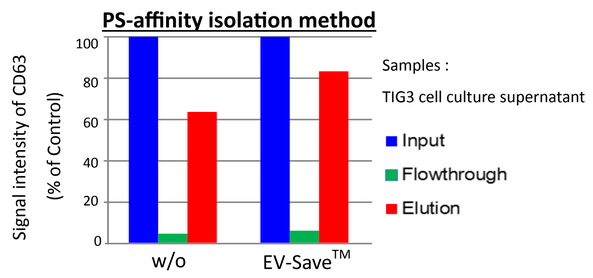
Code No.	Product Name	Package Size
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL

EV-Save™ improves the recovery rate of PS-affinity isolation method, and has no effect on microarray analysis of sEVs.

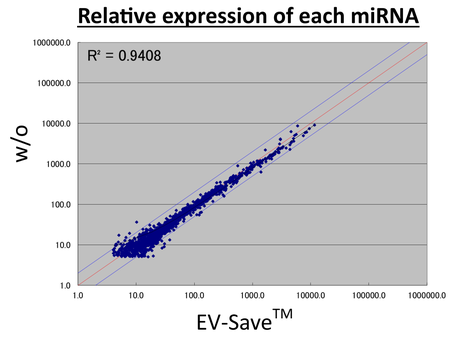
Adsorption of EVs to tube



PS-affinity isolation method



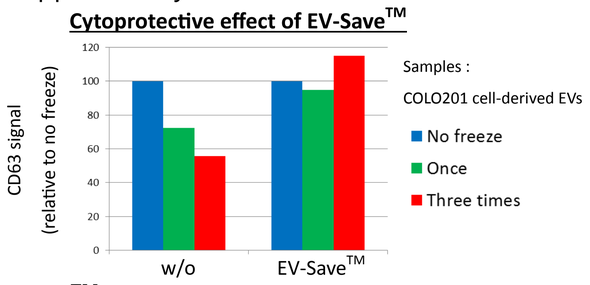
Relative expression of each miRNA



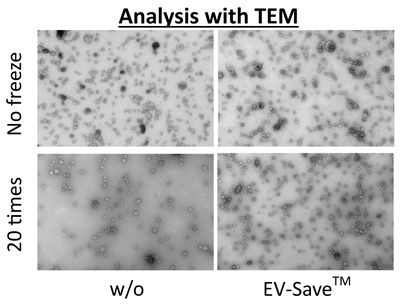
◆Cytoprotective effect

It is known that exosomes are damaged by repeated freeze-thaw. Such damage for EVs could be suppressed by the addition of EV-Save™.

Cytoprotective effect of EV-Save™



Analysis with TEM



EV-Save™ Exracellular Vesicle Blocking Reagent reduces the loss of EVs during experiments and storage.

Conclusion

- **In EVs isolation step, ultracentrifugation has serious problems such as purity , laboriousness and low recovery amount of EVs.**
- **PS-affinity system 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 to EV researches such as functional analysis of EVs and research of biomarkers.**