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

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Abstract

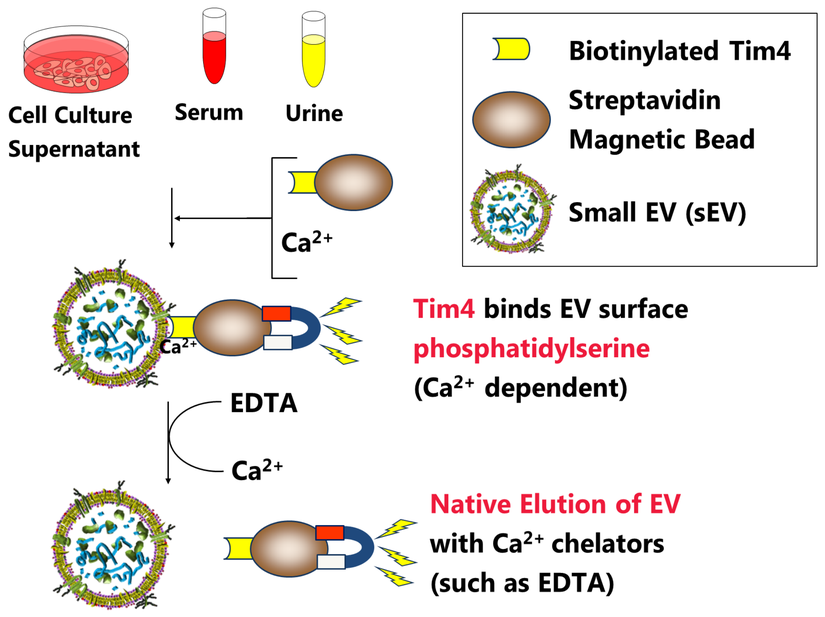
Extracellular vesicles (EVs) such as exosomes and microvesicles serve as messengers of intercellular network, allowing exchange of cellular components between cells. EVs carry lipids, proteins, and nucleic acids derived from their producing cells, and have potential as biomarkers specific to cell types 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. Isolation by PS-affinity method exhibited superiority to conventional method . 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.

PS-affinity method for isolation of EVs

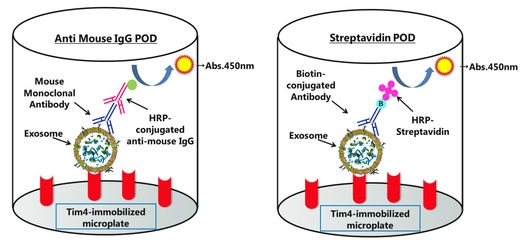
Code No.	Product Name	Package Size
299-77603	MagCapture™ Exosome Isolation Kit PS	2 test
293-77601	MagCapture™ Exosome Isolation Kit PS	10 test

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.



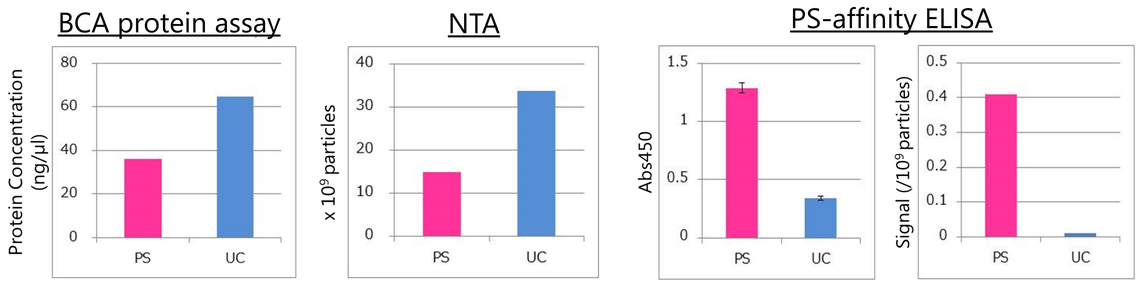
- ◆Can isolate **High Purity and Intact** Extracellular Vesicles
- ◆Can isolate EVs from **cell culture supernatant, serum, plasma, and urine**
- ◆**High Reproducibility** and stable recovery amount
- ◆**Easy Operation** (about 3.5hours)
- ◆Enable to use multiple samples (No need of ultracentrifugation)
- ◆Can apply to ELISA system : PS-affinity ELISA(4P-0639)



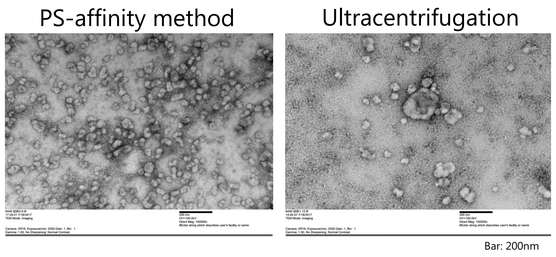
Performance comparison between PS-affinity method and conventional methods

◆Comparison with Ultracentrifugation(UC)

EVs in 10K supernatant of COLO201 cells were isolated by each method and examined by BCA assay, Nanoparticle Tracking Analysis (NTA), PS-affinity ELISA, and transmission electron microscope.



TEM

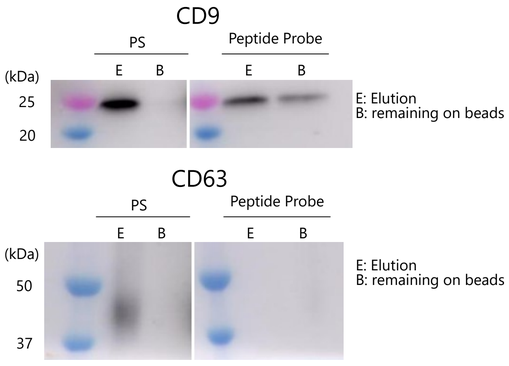


The PS-affinity method could isolate more than about three times as many sEVs as the ultracentrifugation method. However, twice of protein abundance and the number of particles were detected using BCA assay and NTA in sEV samples isolated by ultracentrifugation. Farthermore, sEVs isolated by PS-affinity method were not accompanied by huge EVs probably derived aggregated each other.

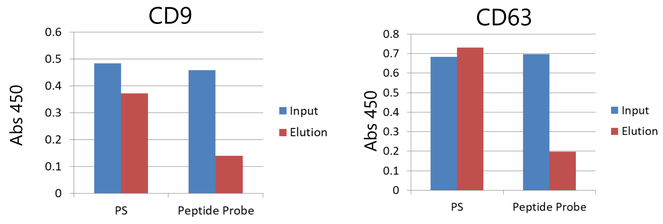
◆Comparison with Peptide Probe method

EVs in 10K supernatant of TIG3 cells were isolated by each method and examined by western blot and PS-affinity ELISA.

Western blot



PS-affinity ELISA



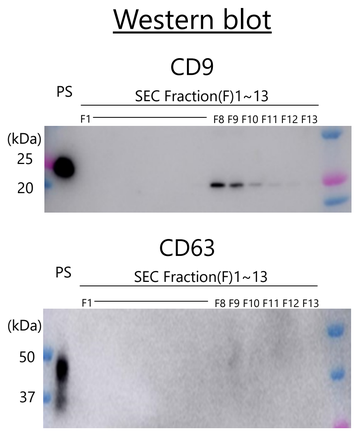
Recovery rate(%, sample signal/input signal)

	CD9	CD63
PS	76.74	107.00
Peptide Probe	30.54	28.21

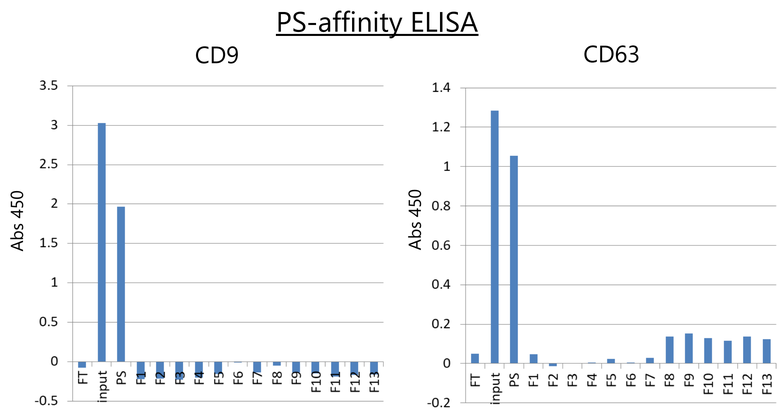
◆Comparison with Size exclusion chromatography(SEC)

EVs in 10K supernatant of COLO201 cells were isolated by each method and examined by western blot and PS-affinity ELISA.

Western blot



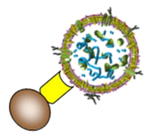
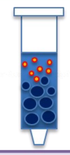
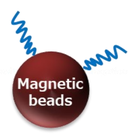
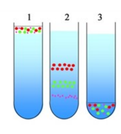
PS-affinity ELISA



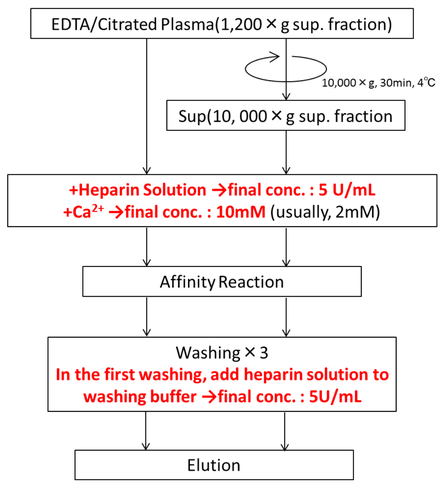
Recovery rate(%, sample signal/input signal)

	CD9	CD63
PS	64.93	82.04
SEC (sum of F7~F10)	Not calculated	34.63

PS-affinity method exhibited the superiority to conventional method

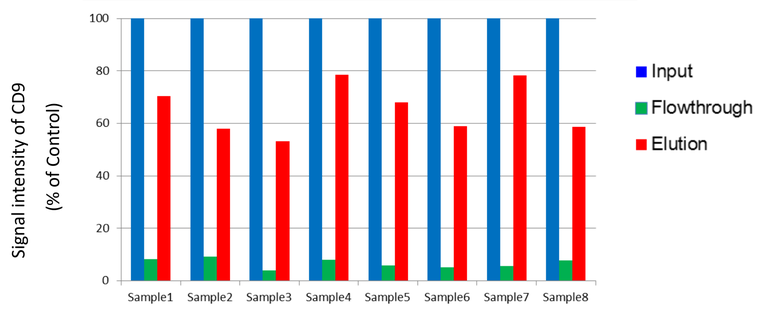
	PS-affinity	Ultracentrifugation (UC)	Size exclusion chromatography (SEC)	Peptide Probe	Density	Polymer
Method						
EVs Purity	■■■	■■	■■■	Not checked	■■■	■
Operability	Easy and Stable	Easy	Easy	Easy and Stable	complex	Easy and Fast
Recovery amount	■■■	■■	■	■	■	■

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. However, 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



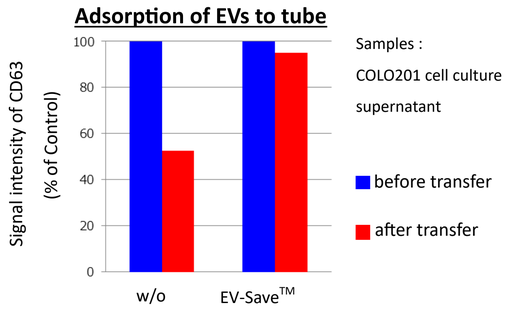
PS-affinity method is available to isolation of EVs from EDTA/Citrated plasma.

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 labwares.

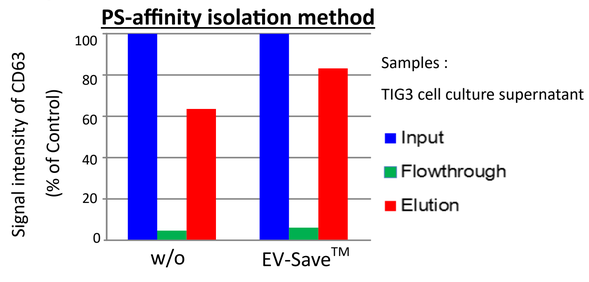
Code No.	Product Name	Package Size
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL

Adsorption of EVs to tube



EV-Save™ improves the recovery rate of PS-affinity isolation method.

PS-affinity isolation method



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

Conclusion

- ◆**In EVs isolation step, conventional methods such as ultracentrifugation have serious problems such as purity , laboriousness and low recovery amount of EVs.**
- ◆**PS-affinity system can isolate EVs derived from various samples more efficiently and universally than conventional methods such as ultracentrifugation.**
- ◆**PS-affinity system will be a powerful tool to EV researches.**