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

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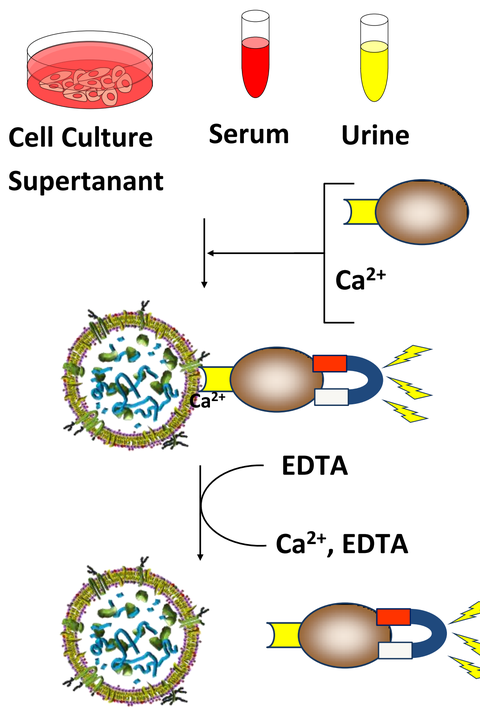
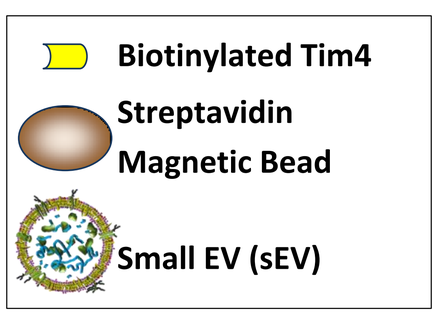
Abstract

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 or polymeric precipitation) 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 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 purification, which we have applied to cell conditioned media and biofluids, is capable of yielding EVs of a higher purity than those obtained using conventional methods. Tim4 protein can also be used as a powerful tool for quantification of EVs in both ELISA and flow cytometry formats. Therefore, the affinity of Tim4 for EVs will find abundant applications in EV studies.

MagCapture™ Exosome Isolation Kit PS

Tim4-affiity purification method





Tim4 binds EV surface phosphatidylserine (PS) (Ca^{2+} dependent)

Native Elution of EV with Ca^{2+} chelators (such as EDTA)

Method	Tim4-affinity method (MagCapture™ Exosome Isolation Kit PS)	Ultracentri-fugation	Antibody-based affinity purification
EV's Purity	■■■	■■	■■■
State of vesicles	Intact	Intact	Not Intact
Operability	Easy and Stable	Easy	Easy and Stable
Recovery amount	■■■	■■	■■

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

MagCpature™ Exosome Isolation Kit PS

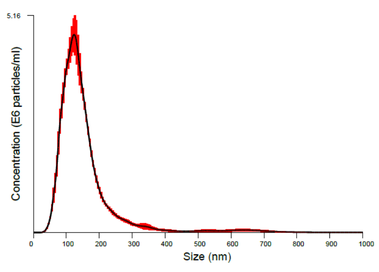
Sample Type: cell culture supernatant, serum, plasma, urine, *etc.*

◆ MagCpature™ Exosome Isolation Kit PS can purify any EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that human, mouse, and bovine EVs can be purified by this isolation kit.

Particle analysis of sEVs purifeid by the Tim4-affinity methods

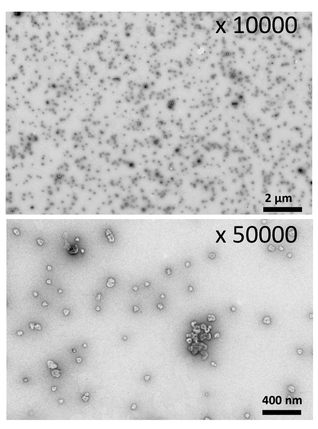
Tim4-affiity purification

NTA



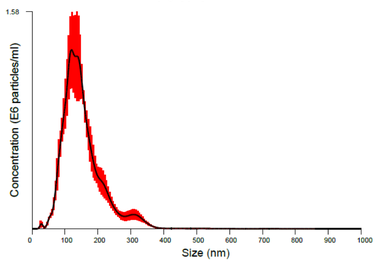
Size Distribution
Mean : 159 +/- 3.9 nm
Mode : 116 +/- 8.7 nm

TEM



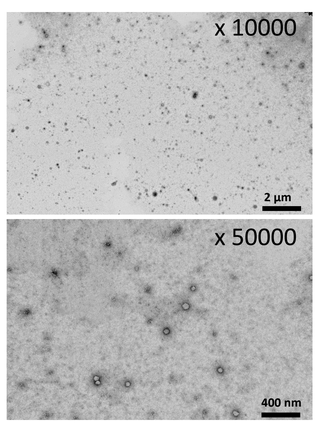
Ultracentrifugation

NTA



Size Distribution
Mean : 153 +/- 2.3 nm
Mode : 137 +/- 11.3 nm

TEM



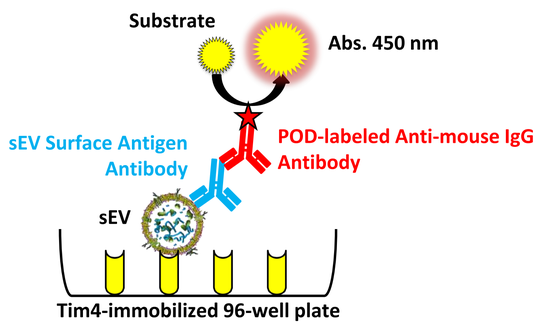
sEVs from 10K sup of COLO201 cells isolated by each method were examined by nanoparticle tracking analysis (NTA) using NanoSight and transmission electron microscope (TEM)

The appearance of sEVs isolated by the Tim4-affinity purification method matched the typical saucer-like shape as previous reported*, and almost no contaminants could be observed. In contrast, sEVs isolated by UC were accompanied by a large number of small precipitates probably derived from supplements added in advance into the medium.

*Raposp, G. *et al.* (1996)

PS Capture™ Exosome ELISA Kit

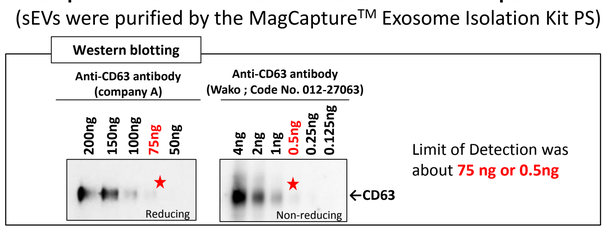
Tim4-based ELISA method

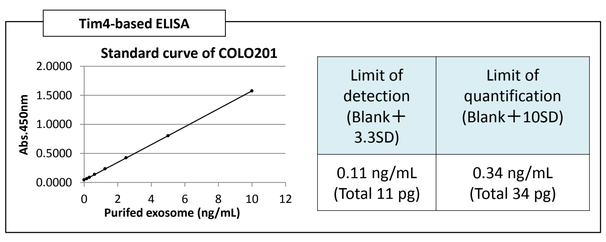


1. sEV binds to the Tim4 immobilized on the 96-well plate
2. Detect the sEV with sEV surface antigen antibody and POD-labeled Anti-Mouse Antibody

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

Samples : Purified sEVs from COLO201 cell culture supernatant
(sEVs were purified by the MagCapture™ Exosome Isolation Kit PS)

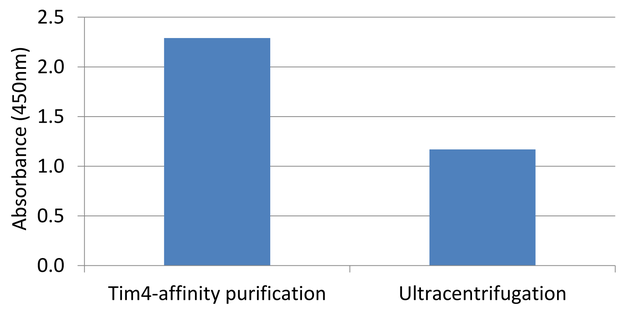




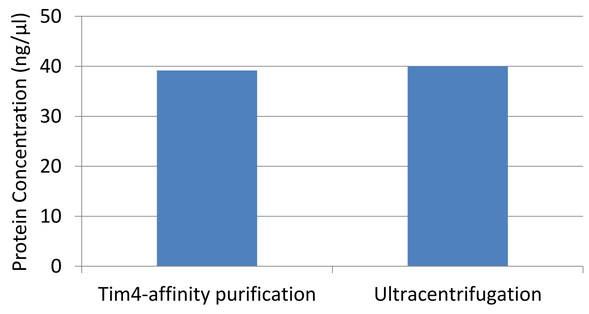
Limit of detection (Blank＋3.3SD)	Limit of quantification (Blank＋10SD)
0.11 ng/mL (Total 11 pg)	0.34 ng/mL (Total 34 pg)

The sensitivity of the Tim4-based ELISA was 50 to 1,000 times higher than that of western blotting.

Tim4-based ELISA



BCA protein assay

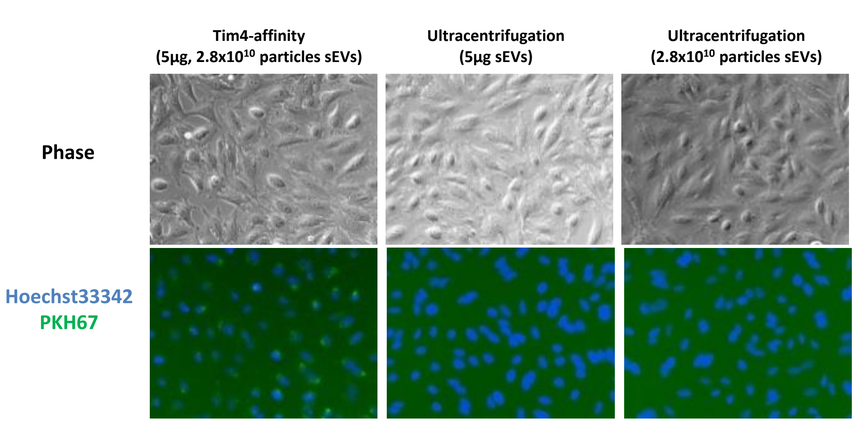


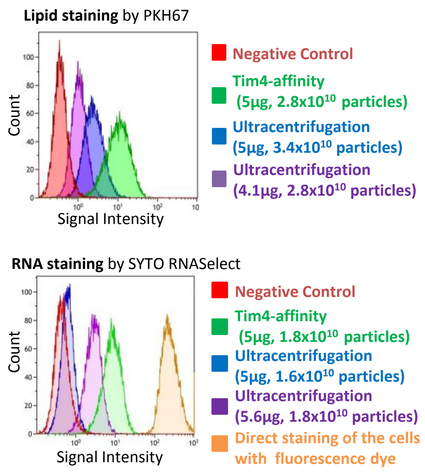
sEVs from 10K sup of COLO201 cells isolated by each method were examined by Tim4-based ELISA analysis and BCA protein assay

The Tim4-affinity purification method could isolate about twice as many sEVs as the Ultracentrifugation method. In contrast, equal amount of proteins were detected in sEV fractions using BCA protein assay.

The Uptake of sEV

Lipid staining by PKH67



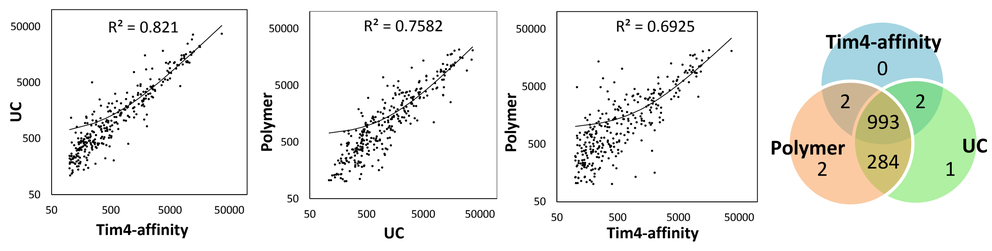


RNA staining by SYTO RNASelect

sEVs from COLO201 cells were labeled by the fluorescence dye and the uptake of these sEVs by HeLa cells was examined.

sEVs purified by the Tim4-affinity purification method were more efficiently incorporated into the HeLa cells than that by the ultracentrifugation.

Microarray analysis of miRNA



miRNA microarray analysis of sEVs from COLO201 cells was performed by the 3D-Gene (TORAY)

miRNA microarray analysis of sEVs from COLO201 cells revealed high correlation of miRNA profiles between Tim4-affinity purification method and ultracentrifugation method.

Conclusions

- Tim4-affinity purification method could be used for the efficient isolation of sEVs
- Tim4-affinity purification method can purify intact extracellular vesicles.
- sEVs purified by the Tim4-affinity purification method were efficiently taken up by the recipient cells.
- High correlation of microRNA profiles between Tim4-affinity purification method and ultracentrifugation was revealed.