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

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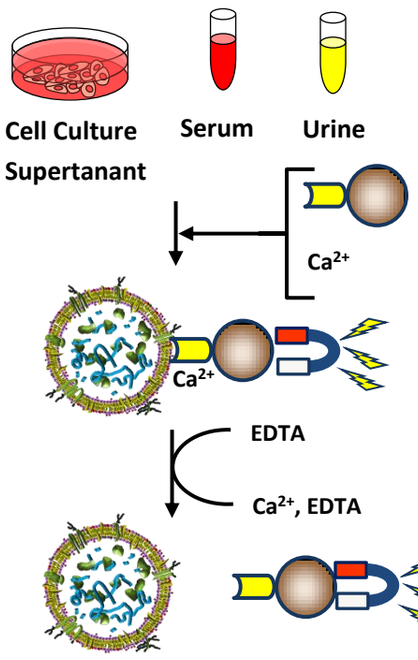
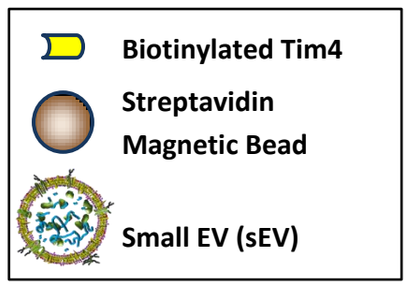
MagCapture™ Exosome Isolation Kit PS





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)	Ultracentrifu-gation	Polymeric precipitation	Antibody-based affinity purification
EV's Purity	■■■	■■	■	■■■
State of vesicles	Intact	Intact	Intact	Not Intact
Operability	Easy and Stable	Easy	Easy and Fast	Easy and Stable
Recovery amount	■■■	■■	■■■■	■■

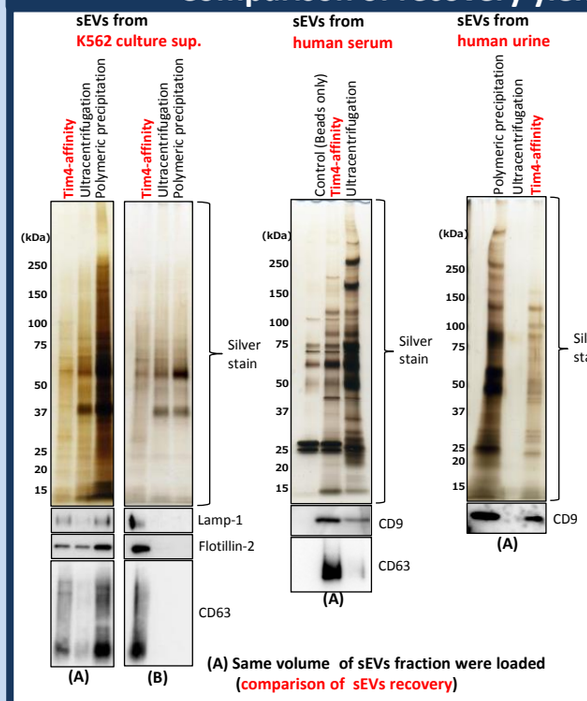
MagCpature™ Exosome Isolation Kit PS

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.**

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

Comparison of recovery yield and purity of sEVs

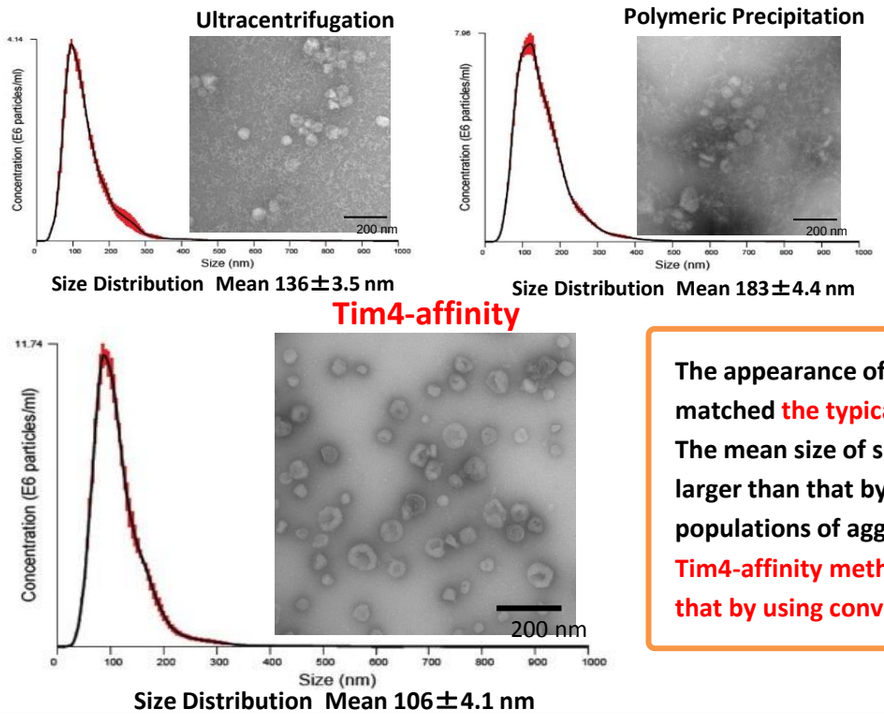


(A) Same volume of sEVs fraction were loaded (comparison of sEVs recovery)
(B) Equal amount of protein were loaded (comparison of sEVs purity)

sEVs from K562 culture supernatant, human serum, or human urine were purified by the Tim4-affinity method or conventional methods (Ultracentrifugation, Polymeric precipitation). Purified sEVs were analyzed by Silver stain or Western blotting.

Tim4-affinity is an ideal method to isolate sEVs containing exosome with much higher purity than that by using other conventional methods.
Tim4-affinity method is suitable for isolating sEVs from biofluids including serum and urine.

Particle analysis of sEVs purifeid by the Tim4-affinity methods



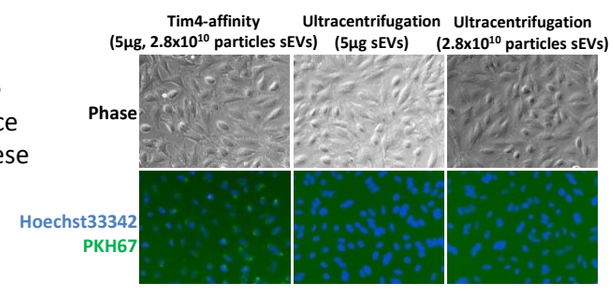
sEVs from K562 cells were examined by transmission electron microscope (TEM) and nanoparticle tracking analysis (NTA) using NanoSight

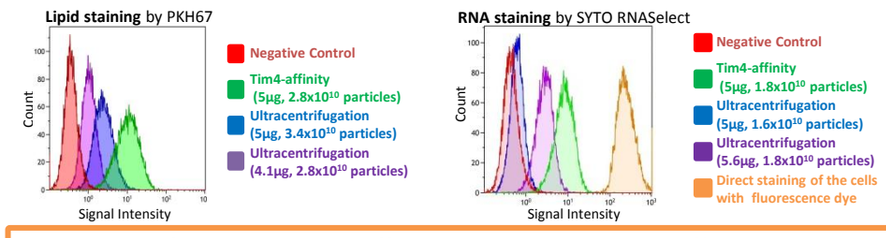
The appearance of sEVs isolated by the Tim4-affinity method matched the typical saucer-like shape as previous reported*. The mean size of sEVs purified by the conventional methods was larger than that by the Tim4-affinity method due to increased populations of aggregated or fused sEVs larger than 200nm. Tim4-affinity method could isolate sEVs with higher quality than that by using conventional methods.

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

The Uptake of sEV

sEVs from K562 cells were labeled by the fluorescence dye and the uptake of these sEVs by HeLa cells was examined.

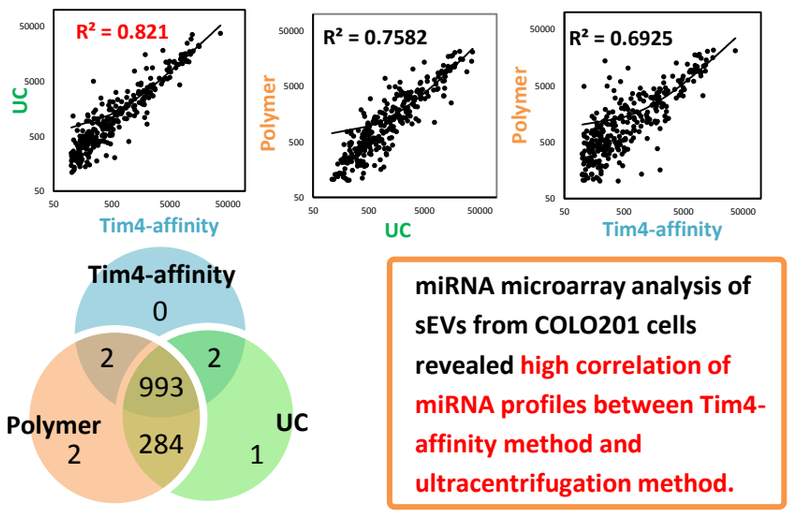




sEVs purified by the Tim4-affinity method were more efficiently incorporated into the HeLa cells than that by the ultracentrifugation.

Microarray analysis of miRNA

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



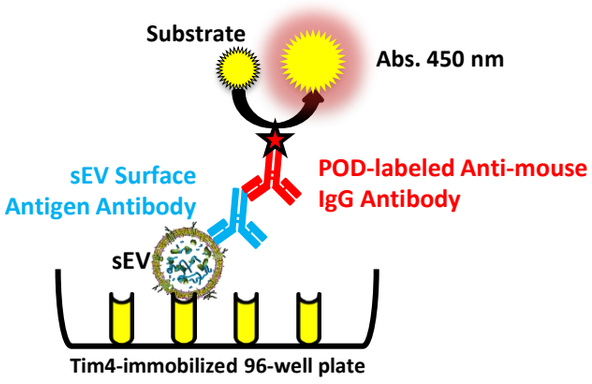
miRNA microarray analysis of sEVs from COLO201 cells revealed high correlation of miRNA profiles between Tim4-affinity method and ultracentrifugation method.

Conclusion

Tim4-affinity method

- **Tim4-affinity method can isolate high purity and quality extracellular vesicles from cell culture supernatant and biofluid.**
- **Tim4-affinity method can purify intact extracellular vesicles.**
- **sEVs purified by the Tim4-affinity method were efficiently taken up by the recipient cells.**
- **High correlation of microRNA profiles between Tim4-affinity method and ultracentrifugation was revealed.**

PS Capture™ Exosome ELISA Kit



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**

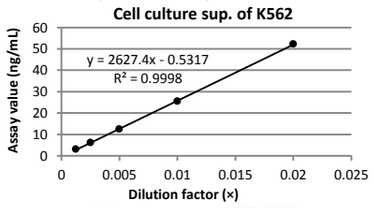
Code No.	Product Name	Package Size
297-79201	PS Capture™ Exosome ELISA Kit (Anti-mouse IgG POD)	96 Reactions

Linearity-of-Dilution Assessments

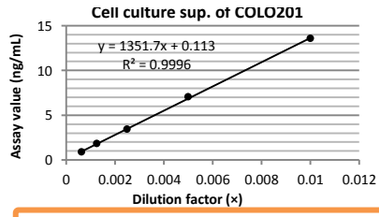
sEV standard : purified sEVs from K562 or COLO201 Culture supernatnat (sEVs were purified by the MagCapture™ Exosome Isolation Kit PS)

Sample : K562 or COLO201 cell culture supernatant.

Primary Antibody : Anti-human CD63 Antibody



Cell culture supernatant of K562					
CM volume (μL)	Dilution		Assay value	Expected value	% of expected
	Ratio	Factor (×)	ng/mL	ng/mL	
0.125	1:800	0.00125	3.02	3.07	98.2
0.25	1:400	0.0025	6.15	6.19	99.3
0.5	1:200	0.005	12.4	12.7	97.5
1	1:100	0.01	25.4	26.1	97.2
2	1:50	0.02	52.2	-	-



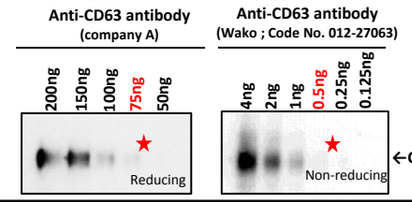
Cell culture supernatant of COLO201					
CM volume (μL)	Dilution		Assay value	Expected value	% of expected
	Ratio	Factor (×)	ng/mL	ng/mL	
0.0625	1:1600	0.000625	0.89	0.91	98.4
0.125	1:800	0.00125	1.82	1.72	105.6
0.25	1:400	0.0025	3.44	3.52	97.8
0.5	1:200	0.005	7.04	6.78	103.9
1	1:100	0.01	13.6	-	-

The linearity-of-dilution was good over the wide range of dilution of cell culture supernatant.
Tim4-based ELISA Kit could detect sEVs included in the 0.1μL Culture Supernatant.

Western blotting vs Tim4-based ELISA

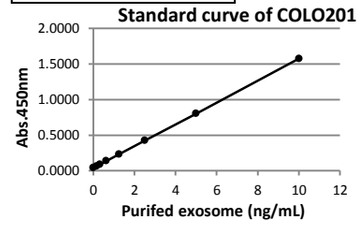
Samples : Purified sEVs from COLO201 cell culture supernatant (sEVs were purified by the MagCapture™ Exosome Isolation Kit PS)

Western blotting



Limit of Detection was about **75 ng or 0.5ng**

Tim4-based ELISA



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)

Limit of Detection (COLO201) was **11pg**

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