Application of PS-affinity-based methods to extracellular vesicle research

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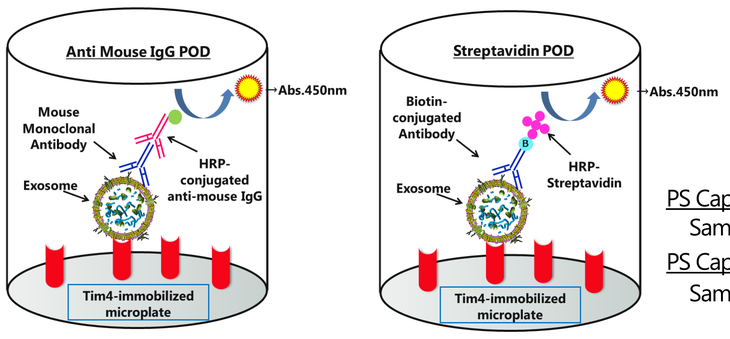
Abstract

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 for quantification of EVs in ELISA system, which shows higher sensitivity than western blot and conventional ELISA system. In addition, PS-affinity ELISA system can be applied for quality control of EV-depleted FBS by using suitable antibody. Furthermore, we could detect exosomal PD-L1 by using PS-affinity ELISA system, and that leads to the development of a useful tool for screening of disease markers on EVs. These findings suggest that PS-affinity ELISA system will find abundant applications in EV studies.

Characteristics of PS-affinity ELISA for detection of EV

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 HRP)	96 Reaction



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

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

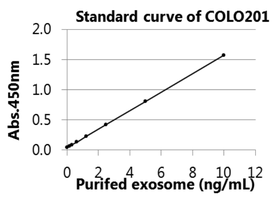
List of antibodies against EV marker

Name	Clone	Host	Code	Application	Animal Species			
					Human	Mouse	Rat	Bovine
Anti CD9, Monoclonal Antibody	1K	Mouse	014-27763	ELISA, WB, FCM, IP	○	×	△	○
	30B	Rat	019-29953 (Biotinylated)	ELISA, WB, FCM, IP	○	×	Weakly cross-reactive (≒1%)	×
	77B	Rat	-	ELISA, WB, FCM, IP	○	×	Weakly cross-reactive (≒1%)	×
Anti CD63, Monoclonal Antibody	3-13	Mouse	012-27063	ELISA, WB, FCM, IP	○	×	×	×
Anti CD81, Monoclonal Antibody	17B1	Mouse	011-27773	ELISA, WB, FCM, IP	○	×	×	○
	9B	Rat	-	ELISA, WB, FCM, IP	○	×	×	Weakly cross-reactive (≒1%)

Comparison of the sensitivity of EV detection between PS Capture™ ELISA with western blotting

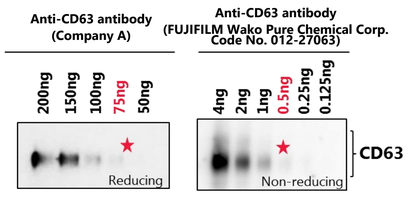
Samples : small EVs (sEVs) purified from COLO201 cell culture supernatant by MagCapture™ Exosome Isolation Kit PS

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)

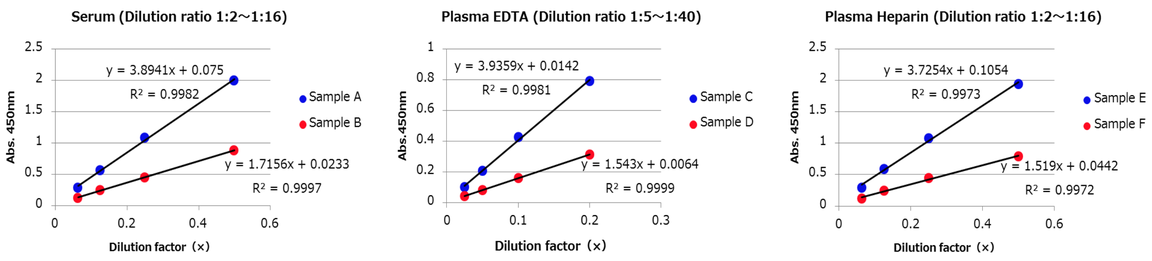
Western blotting



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 HRP)

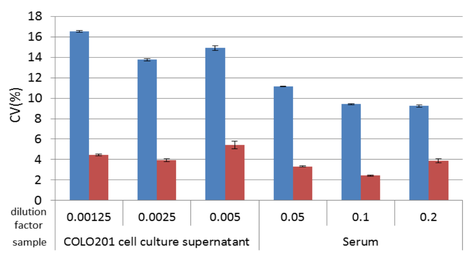
Samples : normal human serum and plasma(detection:CD63 Ab)



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

Compensation of ELISA signals by standard curve of purified COLO201 sEVs

Standard curve : Exosomes, from COLO201 cells, purified
Samples : COLO201 cell culture supernatant, normal human serum (detection:CD63 Ab)

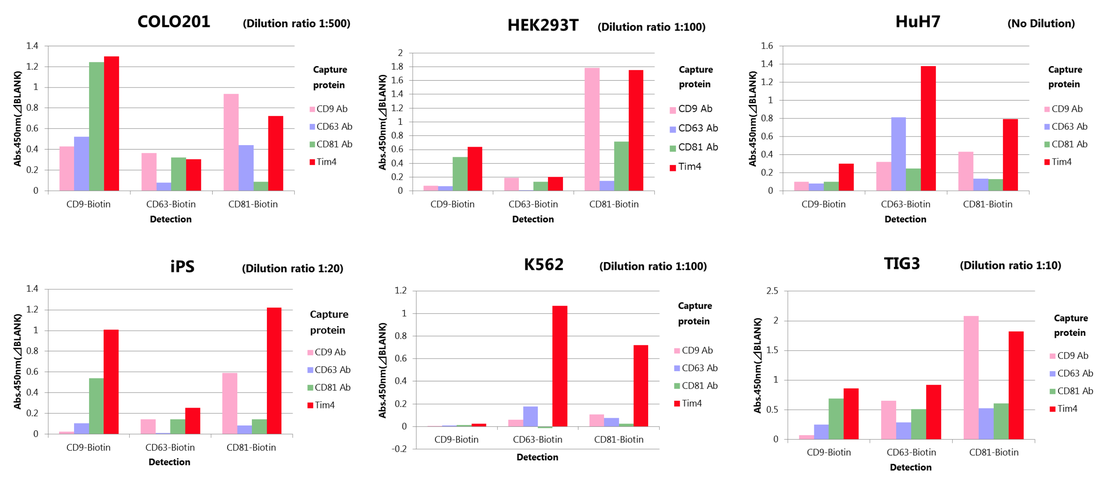


Code No.	Product Name	Package Size
052-09301	Exosomes, from COLO201 cells, purified	50µL

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

Comparison of capturing ability of sEVs

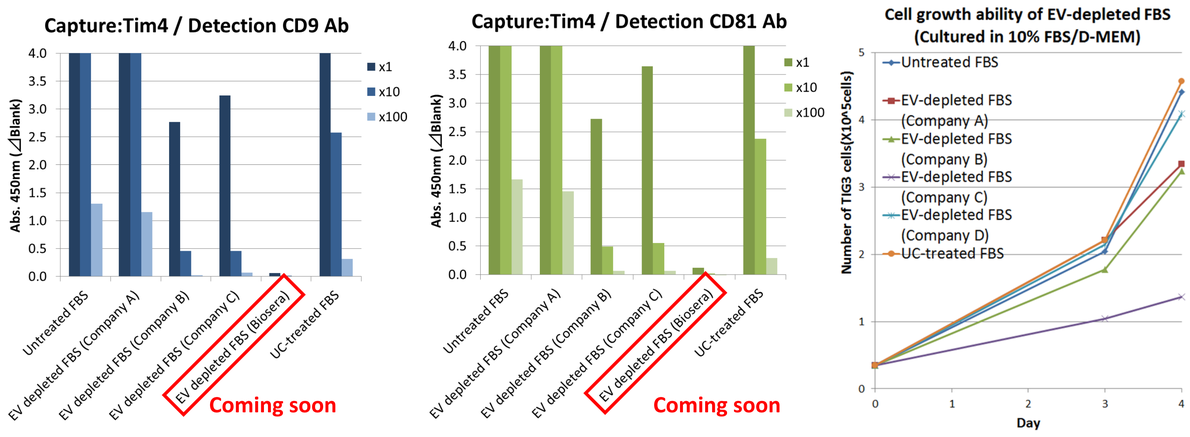
sEVs in 10K sup of various cells were diluted and incubated in each well of microplate immobilized anti-CD9, anti-CD63, anti-CD81 antibodies or Tim4. And then, bound sEVs were detected with biotinylated antibodies against EV surface marker such as CD9, CD63 or CD81.



The results indicated that PS-affinity can detect sEVs derived from various cell lines more efficiently and universally than EV surface markers.

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.



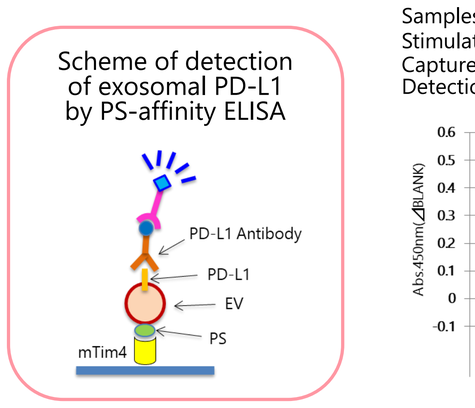
PS-affinity ELISA system would be a useful tool for quality control of EV-depleted FBS.

Detection of disease marker on sEVs

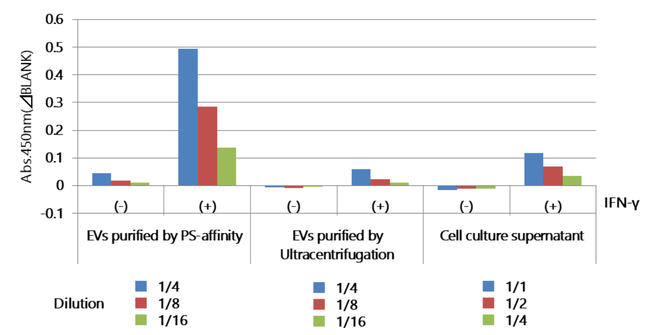
Detection of PD-L1 on sEVs derived from melanoma cells stimulated with IFN-γ

Based on the report that stimulation with IFN-γ increases the amount of exosomal PD-L1 derived from melanoma cells※, we tested the detection of PD-L1 on sEVs derived from IFN-γ -stimulated A375 cells by PS-affinity ELISA.

※Nature. 2018 Aug;560(7718):382-386



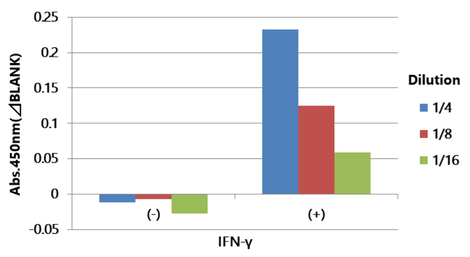
Samples : sEVs derived from melanoma A375 cell culture supernatant
Stimulation : ±IFN-γ
Capture : mTim4-Fc
Detection : Biotinylated PD-L1 Ab (clone:MIH1)



PS-affinity ELISA can detect exosomal PD-L1 derived from IFN-γ-stimulated A375 cells. Especially, PD-L1 on sEVs purified by PS-affinity method are detected intensely.

Application of PS-affinity ELISA to drug evaluation

We detected PD-L1 on sEVs purified from A375 cells by PS-affinity ELISA using antibody used for drug evaluation of Nivolumab/Opdivo®.



Samples : sEVs purified from melanoma A375 cell culture supernatant by MagCapture™ Exosome Isolation Kit PS
Stimulation : ±IFN-γ
Capture : mTim4-Fc
Detection : Biotinylated PD-L1 Ab (clone:28-8)
※FDA approved (It is used for drug evaluation of Nivolumab/Opdivo® by IHC)

PS-affinity ELISA system would be a useful tool for drug evaluation of antibodies against therapeutic target.

Conclusion

- **PS-affinity ELISA system can detect EVs high sensitively and quantitatively.**
- **PS-affinity ELISA system can detect EVs derived from various cell lines more efficiently and universally than conventional ELISA.**
- **PS-affinity ELISA system will be a useful tool for drug evaluation of antibodies against therapeutic target.**