

A novel affinity-based method for isolation and quantification of extracellular vesicles

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Abstract

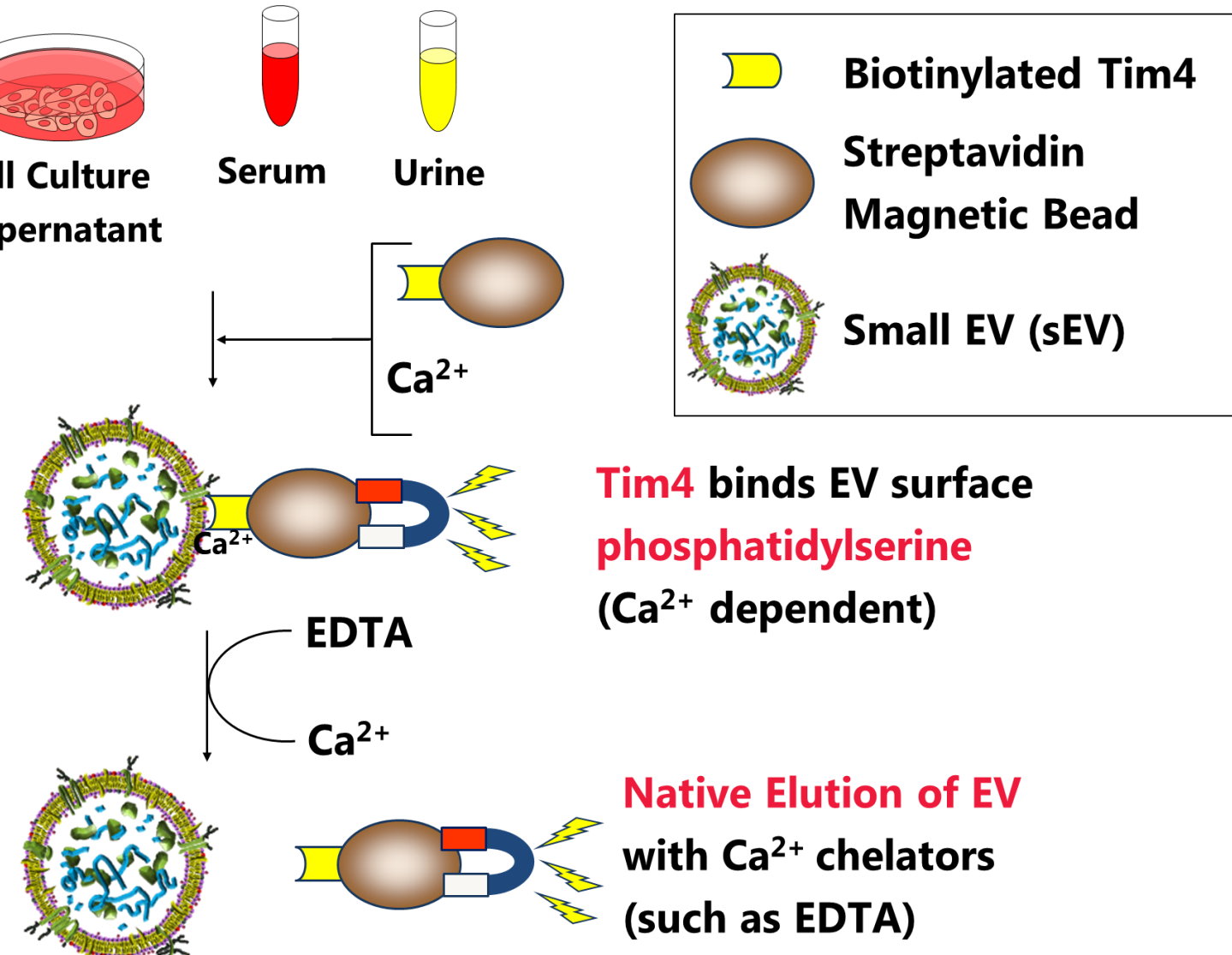
Extracellular vesicles (EVs) such as exosomes and microvesicles serve as messengers of intercellular network, allowing exchange of cellular components between cells. EVs carry lipids, proteins, and nucleic acids derived from their producing cells, and have potential as biomarkers specific to cell types 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 Ca²⁺-dependent, intact EVs can be easily released from Tim4 by adding Ca²⁺ chelators. Tim4 protein can also be used as a powerful tool for quantification of EVs in ELISA system. Particularly, we have used PS-affinity ELISA system for quality control of EV-depleted FBS. Furthermore, we found a polymer-based blocking reagent prevents absorbing EVs to labwares and reduced the loss of EVs. These findings suggest that the affinity of Tim4 for EVs will find 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 Tests
293-77601	MagCapture™ Exosome Isolation Kit PS	10 Tests



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



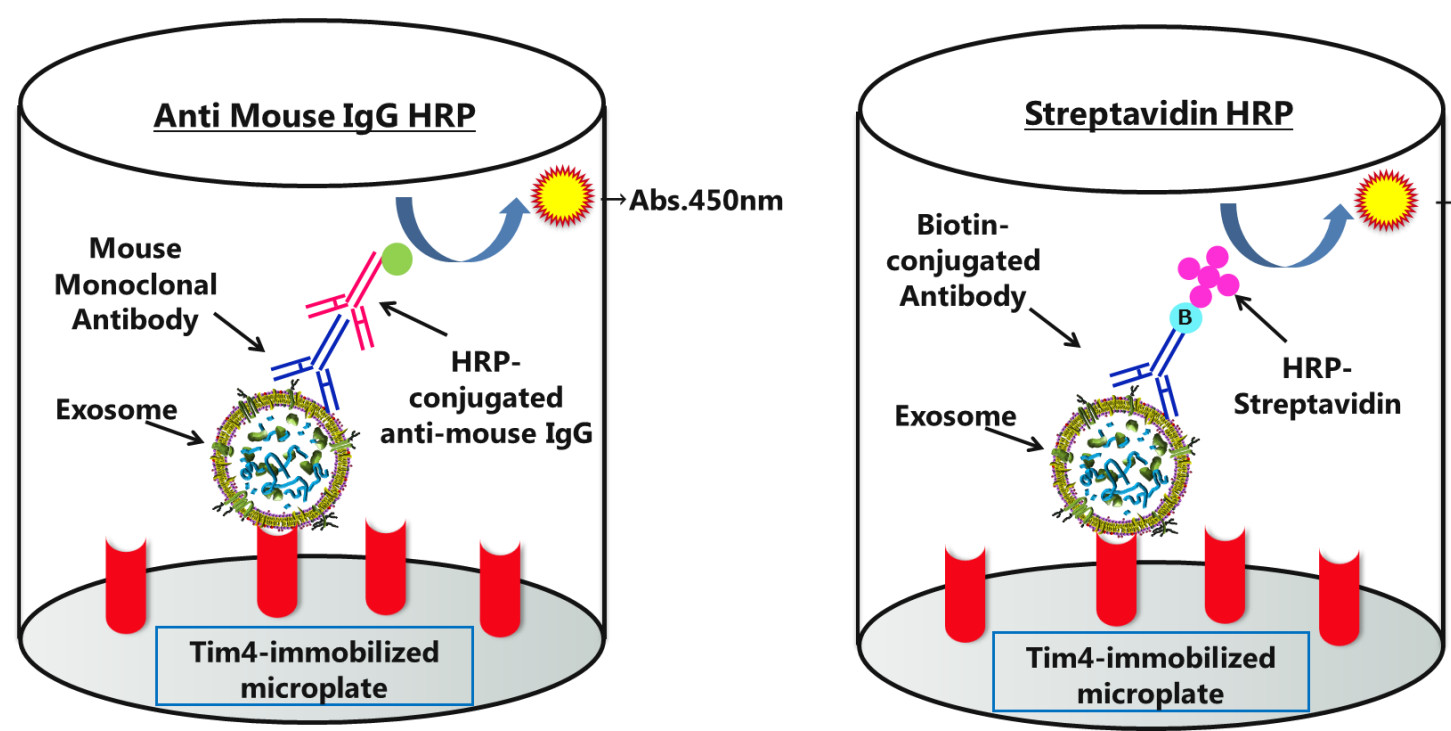
MagCapture™ Exosome Isolation Kit PS can purify EVs which expose phosphatidylserine on the outer surface of their lipid bilayer. It has been confirmed that this isolation kit can purify EVs from various animal species samples such as human, mouse, and bovine.

Method	PS-affinity	Ultracentrifugation (UC)	Density (UC-Sucrose Cushion)	Size exclusion chromatography	Polymer
EVs Purity (Exosome protein coverage)	★★★★	★★	★★	★★	★
State of EVs	Intact	Intact	Intact	Intact	Intact
Operability	Easy and Stable	Easy	Complex	Easy	Easy
Total protein yield	★	★★	★★	★★	★★★★

- ◆ 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 3.5 hours)
- ◆ **Enable to use multiple samples** (No need of ultracentrifugation)

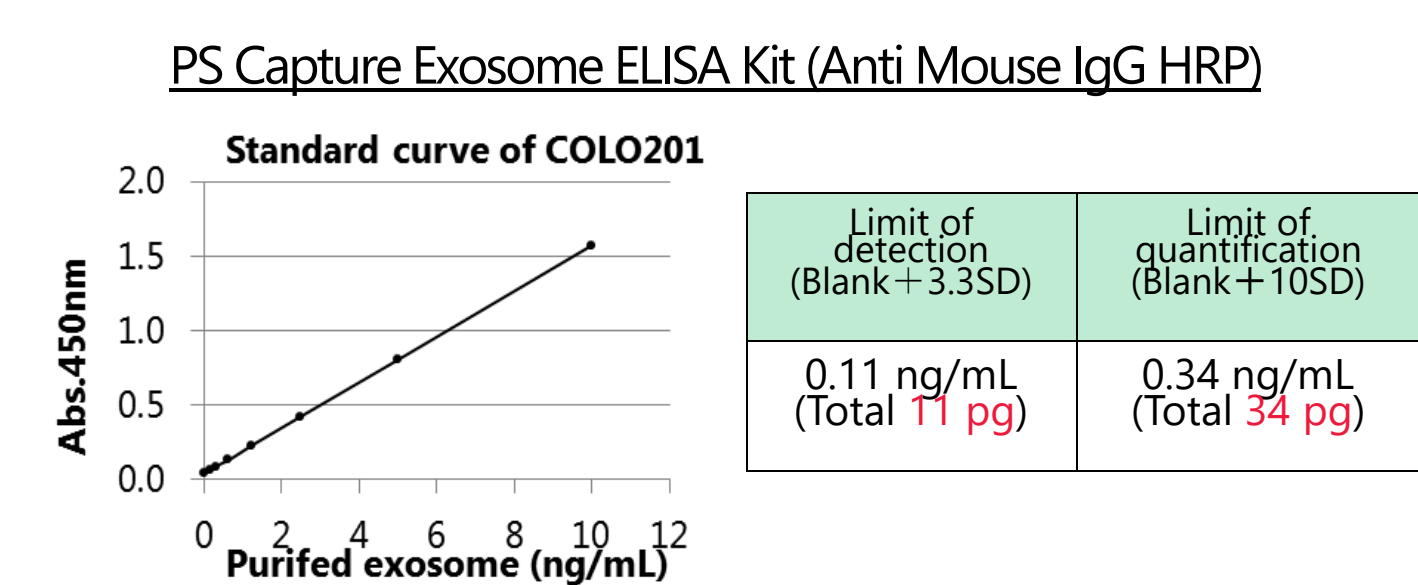
PS-affinity for detection of EVs

Code No.	Product Name	Package Size
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG HRP)	96 Reactions
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin HRP)	96 Reactions

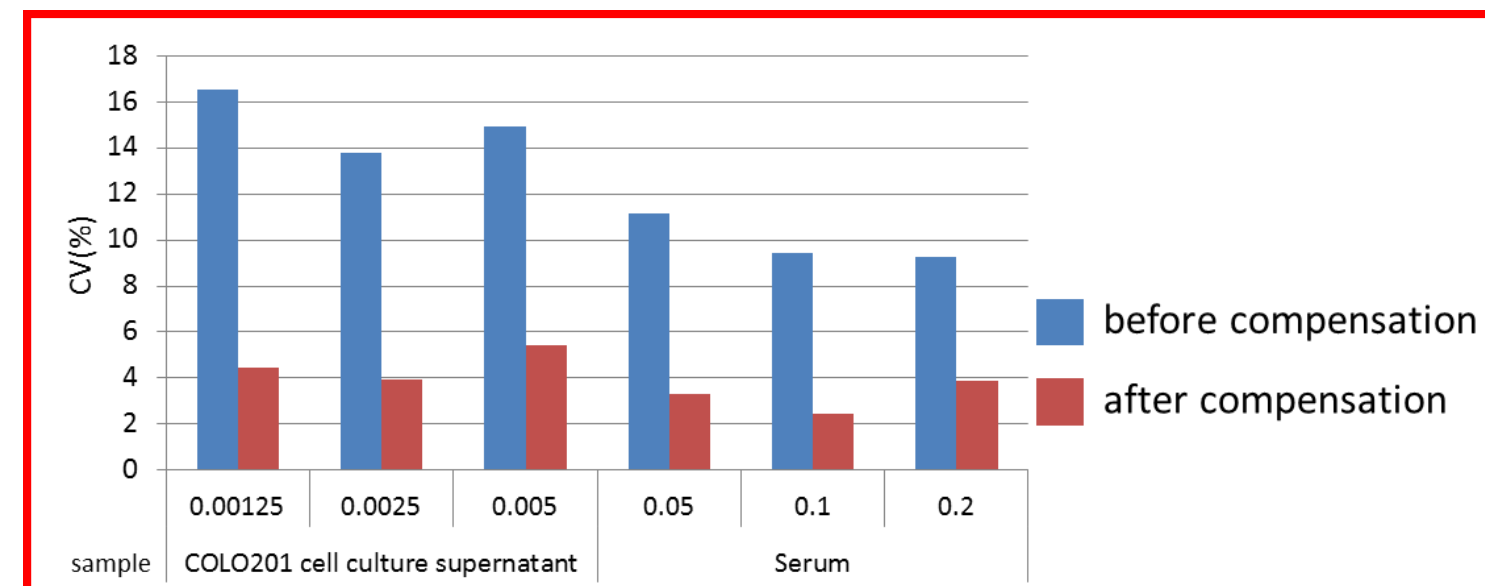


PS Capture™ Exosome ELISA Kit (Anti Mouse IgG HRP)
 Sample Type : Purified EVs, Cell culture supernatant
 PS Capture™ Exosome ELISA Kit (Streptavidin HRP)
 Sample type : Purified EVs, Cell culture supernatant, Serum, Plasma

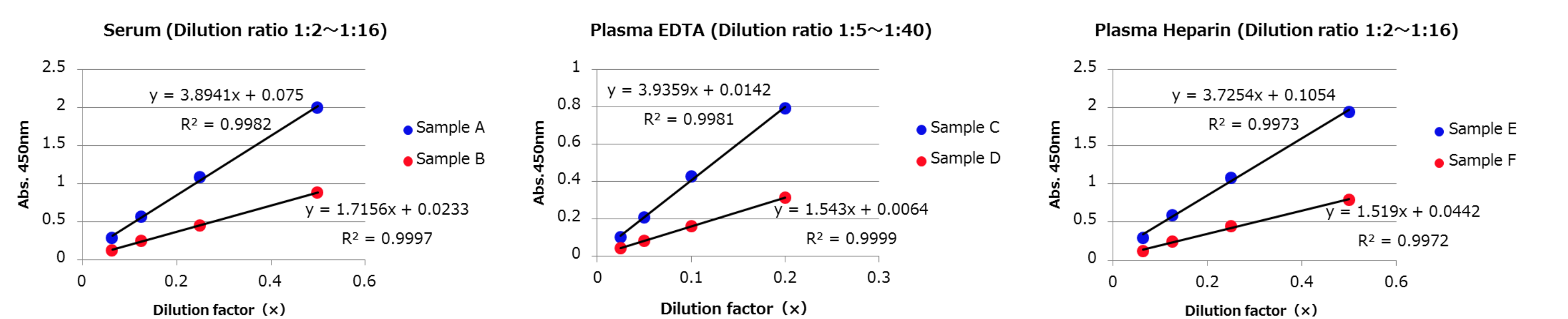
- ◆ **The sensitivity of EV detection by PS Capture™ ELISA**
- ◆ **Compensation of ELISA signals by standard curve of purified COLO201 sEVs**



The sensitivity of PS Capture™ Exosome ELISA Kit was able to detect ≥11 pg of sEVs

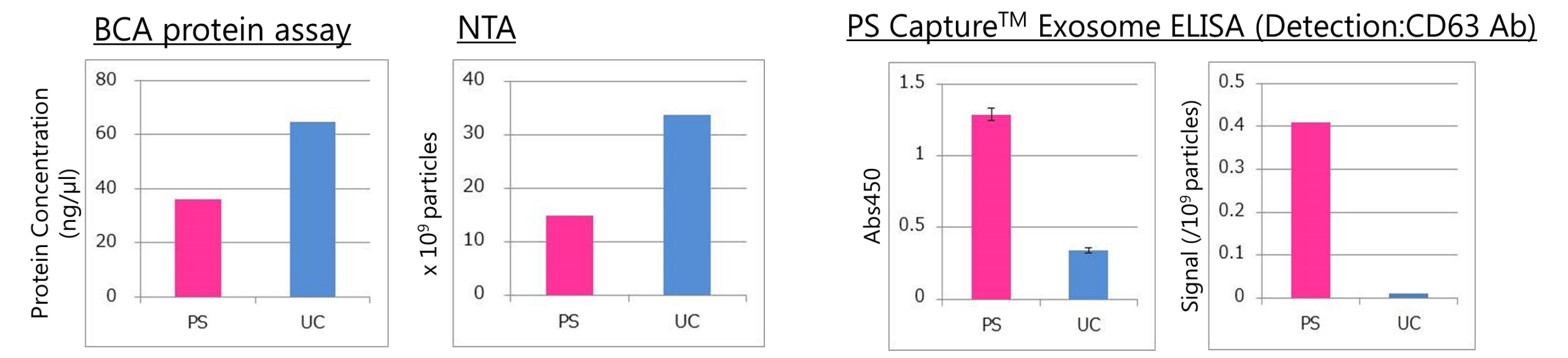


- ◆ **Dilution linearity of serum and plasma by PS Capture™ ELISA (Streptavidin HRP)**



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

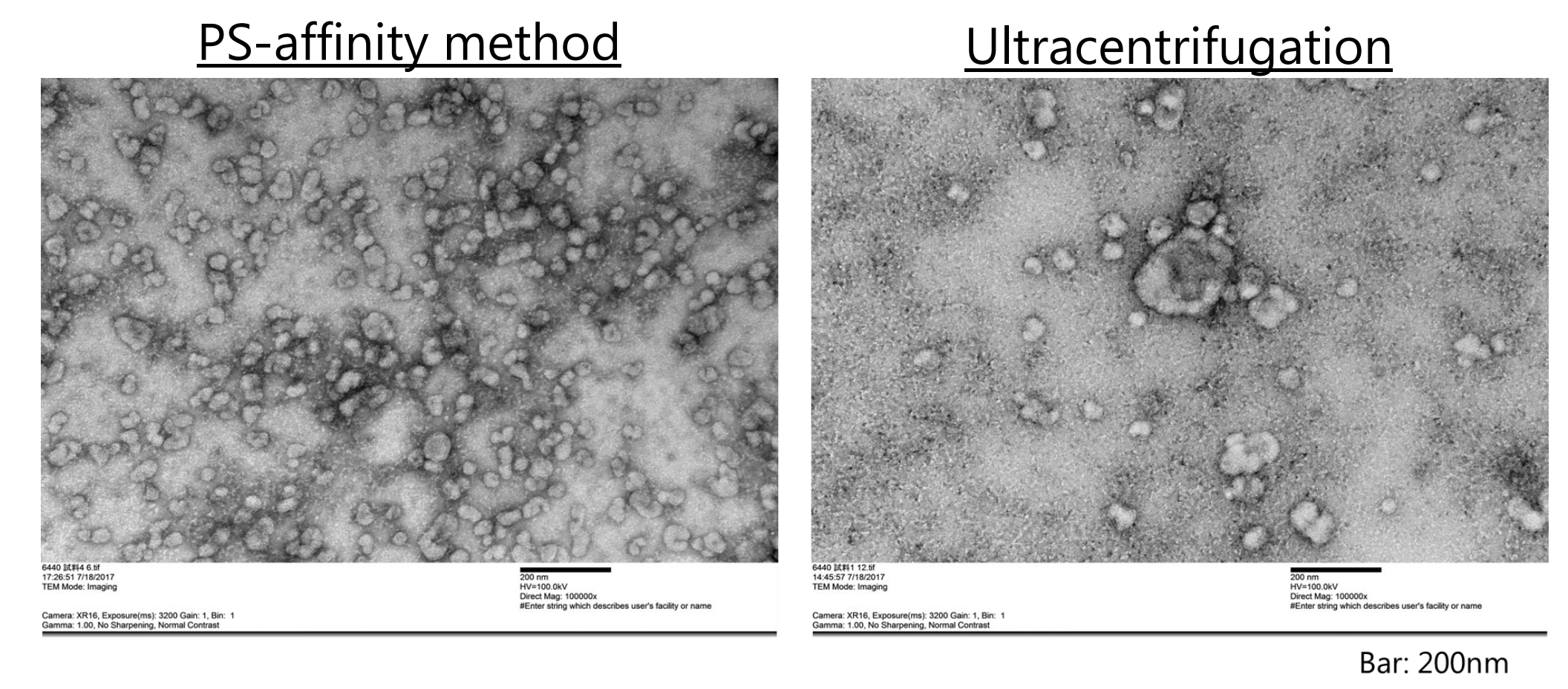
sEVs in 10K supernatant of COLO201 cells were isolated by each method and examined by BCA assay, Nanoparticle Tracking Analysis (NTA) and PS Capture™ Exosome ELISA Kit.



The PS-affinity method isolated more than about three times as many CD63+ 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.

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).

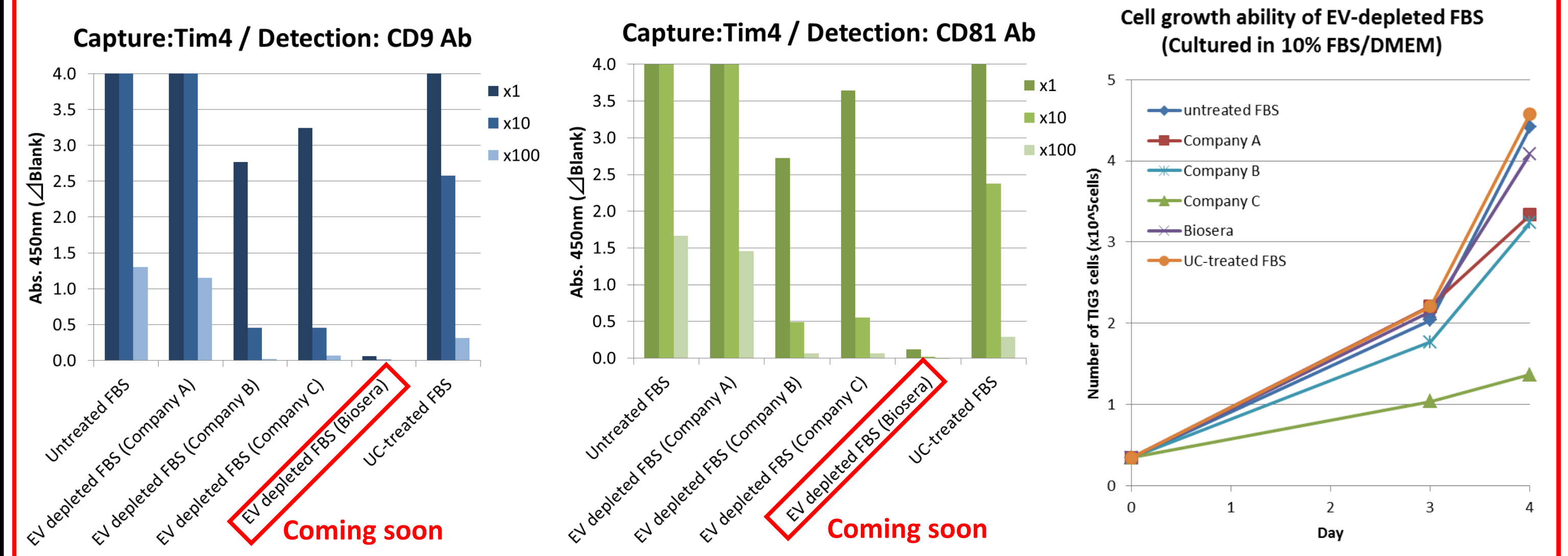


sEVs isolated by PS-affinity method were not accompanied by huge EVs probably derived aggregated each other.

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.

Code No.	Product Name	Package Size	Cross Reactivity
014-27763	Anti CD9, Monoclonal Antibody (1K)	100uL	Human, Bovine
011-27773	Anti CD81, Monoclonal Antibody (17B1)	100uL	Human, Bovine

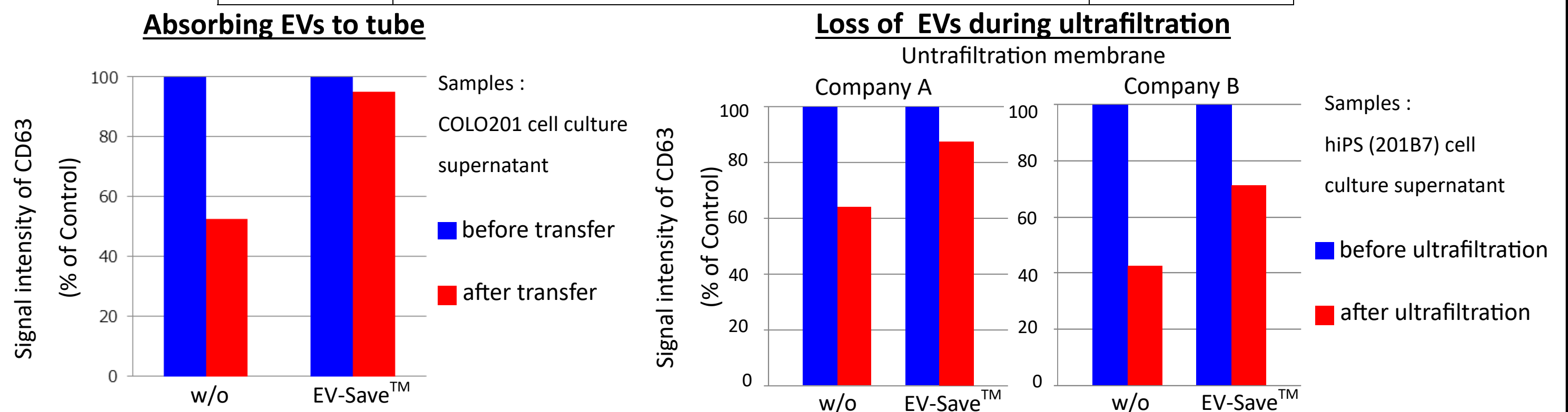


PS-affinity ELISA system would be a useful tool for quality control of EV-depleted FBS.

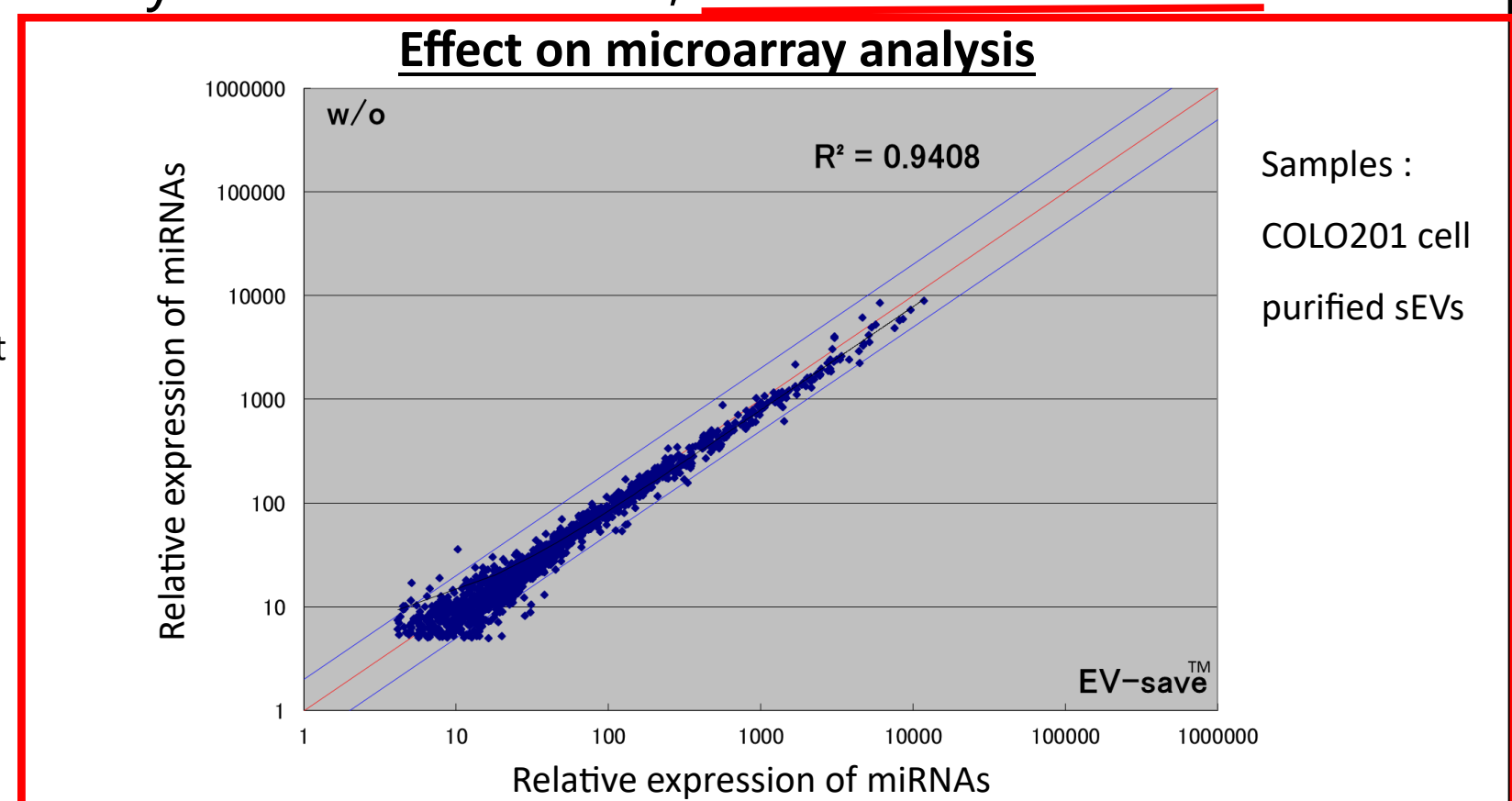
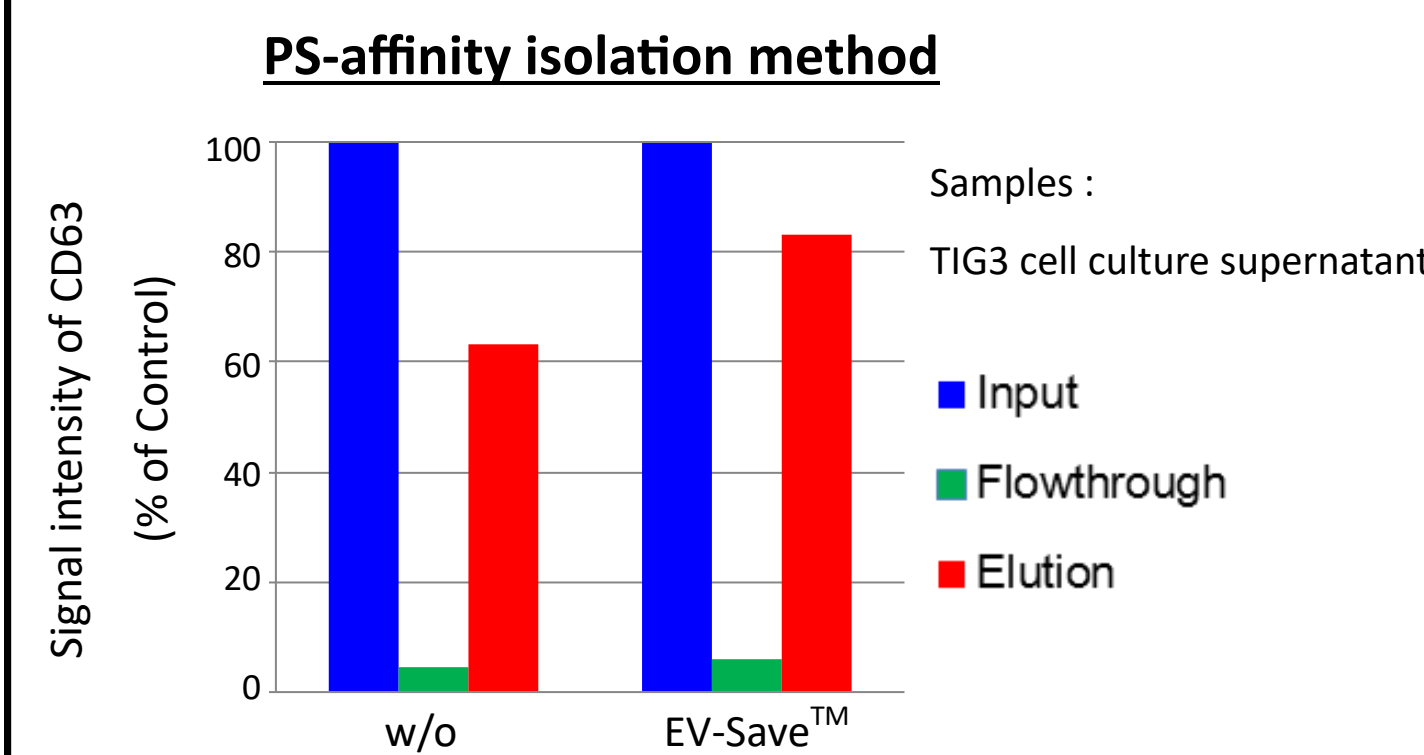
Reducing the loss of EVs by polymeric blocking reagent

EV-Save™ Extracellular Vesicle Blocking Reagent is a polymer reagent to prevent absorbing EVs in cell culture supernatants to labwares, which reduces the loss of EVs during experiments and storage.

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.



EV-Save™ Extracellular Vesicle Blocking Reagent reduces the loss of EVs effectively.

Conclusion

- ◆ In EVs isolation step, ultracentrifugation has serious problems such as purity, and recovery amount of EVs.
- ◆ PS-affinity system can isolate and detect EVs derived from various samples more efficiently and universally than conventional methods such as ultracentrifugation.
- ◆ PS-affinity isolation and detection system will be a powerful tool in EV researches such as functional analysis of EVs and research of biomarkers.