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Value from Innovation

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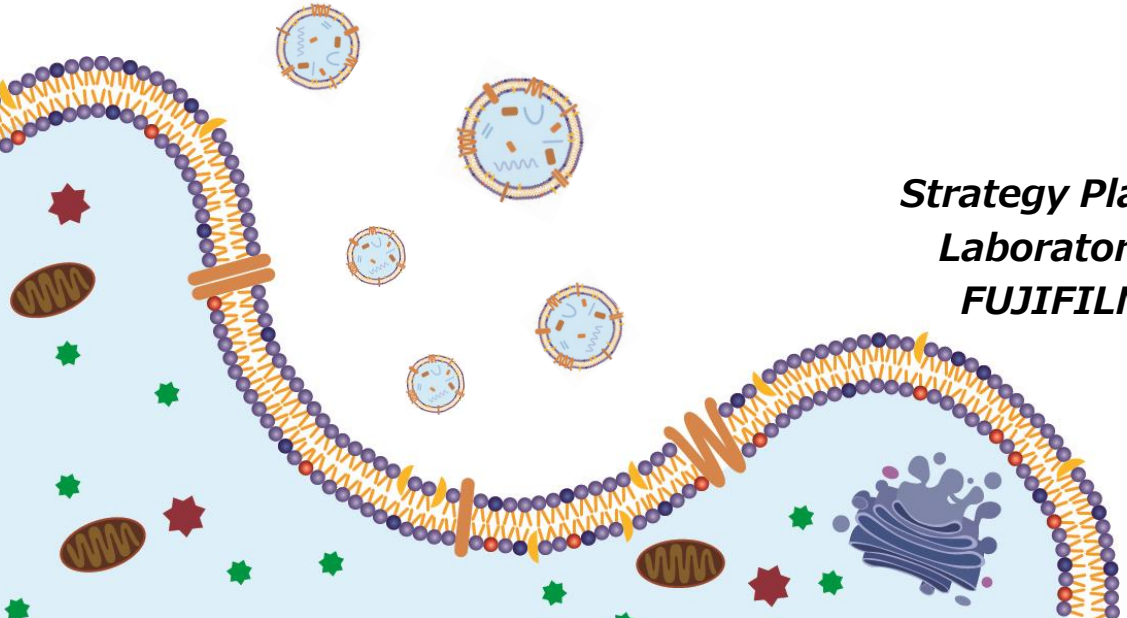
GSEV&ASEV meeting

Characteristics on PS Affinity for Isolation and Detection of EVs: Advantages Clarified from Comparison with Conventional Methods

Key word: extracellular vesicles, exosome, microvesicle, phosphatidylserine



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2022/10/19

Today's Outline

1. Introduction on PS Affinity

2. Comparing PS Affinity with Ultracentrifugation and SEC

3. Reference Data
   - -Proteomic Analysis
   - -Density Distribution

4. Conclusion

1. Introduction on PS Affinity

Introduction on PS Affinity Method

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NanoLSI
WPI KANAZAWA UNIVERSITY

- Collaborated with Prof. Rikinari Hanayama. (WPI Nano Life Science Institute, Kanazawa Uni.)
- Developed in 2015
- Isolating and Detecting EVs (e.g. Exosome etc.)
- Completely different from conventional methods
- Introducing highly-pure extracellular vesicles compared with conventional methods

MagCapture™
Exosome Isolation Kit PS
Launched on Dec in 2015

SCIENTIFIC REPORTS

OPEN

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

Nakai *et al., Sci Rep.* 2016 Sep 23;6

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EV Research Products using PS Affinity

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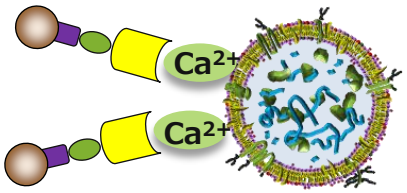
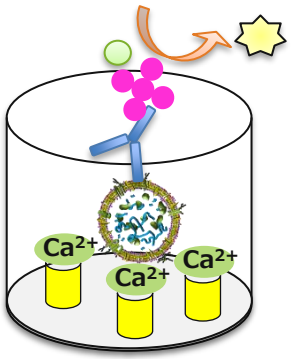
PS Affinity

MagCapture Exosome Isolation Kit PS



High purity & intact EVs (small EVs, large EVs, enveloped viruses)

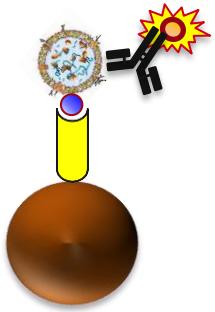
PS Capture Exosome ELISA Kit



High-sensitive qualitative and quantitative analyses

Direct detection without purification

PS Capture Exosome Flow Cytometry Kit



Simultaneous detection of multiple antigens

High-sensitive qualitative analysis

Direct detection without purification

Easy operation and high reproducibility

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Characteristics of PS Affinity Method

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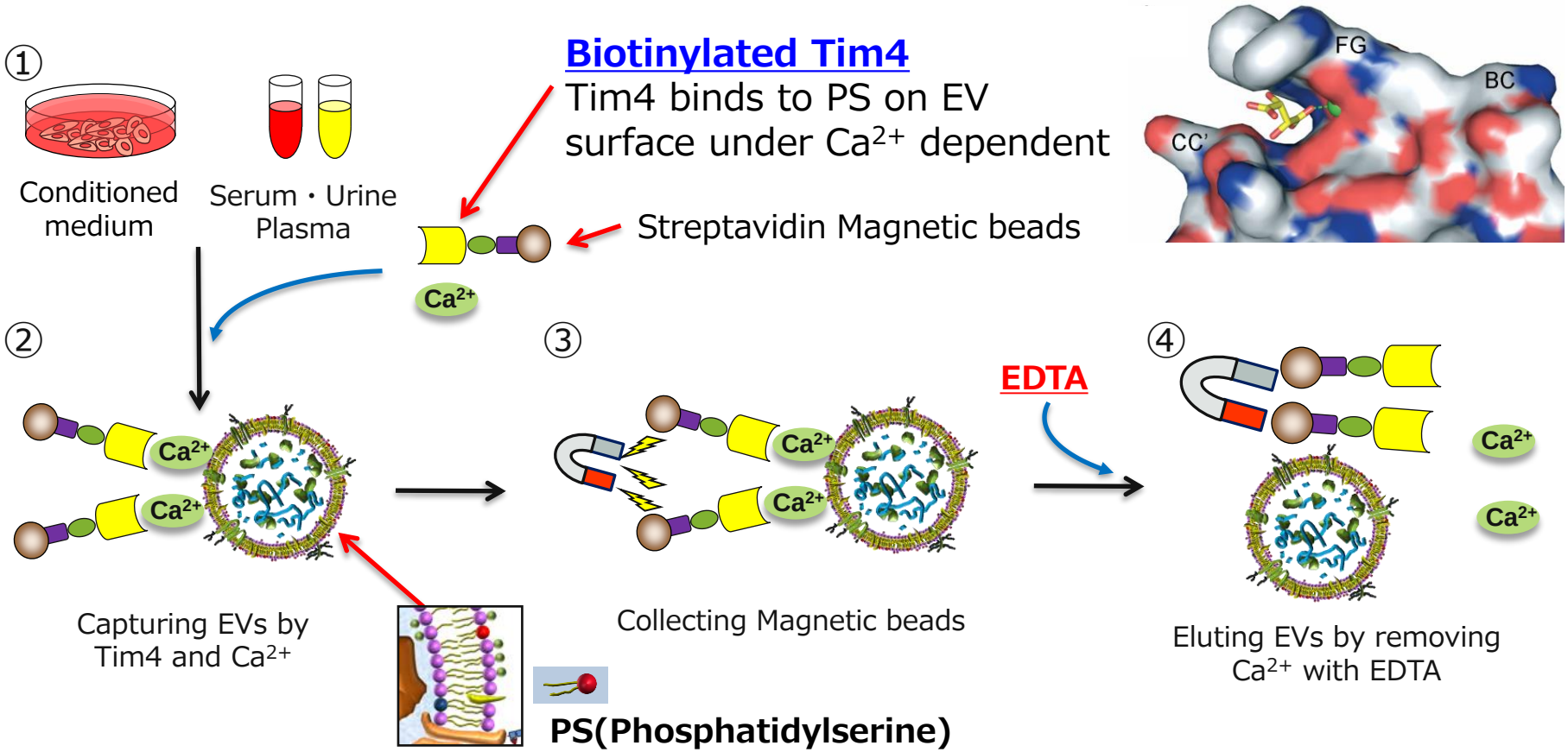
Scheme of phosphatidylserine (PS) affinity-based method



A novel affinity purification method is capable of purification of highly pure and intact extracellular vesicles

Nakai *et al., Sci Rep.* 2016 Sep 23;6

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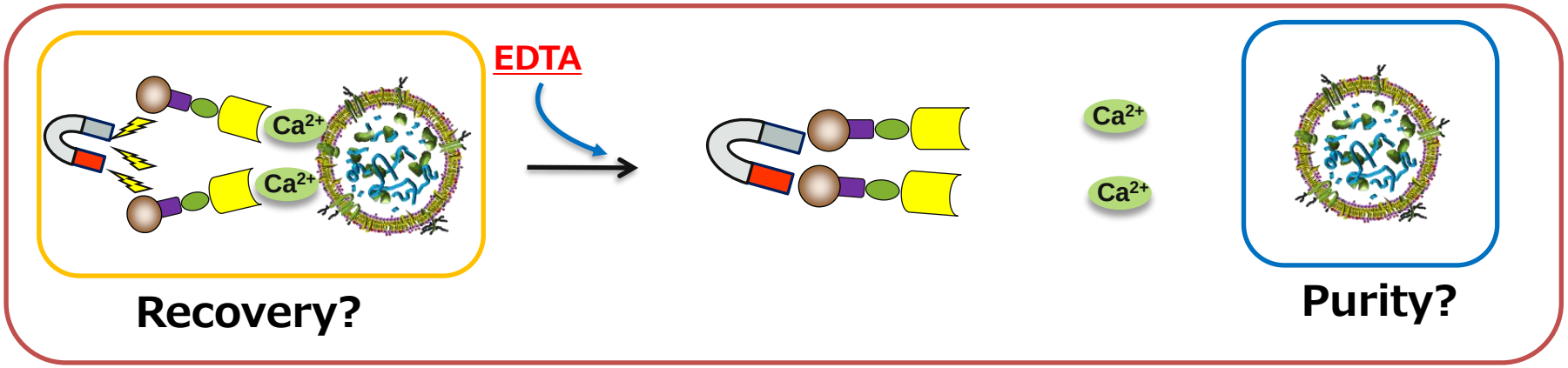
Investigating Superiority of PS Affinity



Validity?

Evaluation Points of PS affinity method

1. Recovery amount
2. Purity
3. Validity as method

For proving superiority in terms of the above, we carried out comparison between conventional methods and commercial kit.

2. Comparing PS Affinity with Ultracentrifugation

1. Appearance
2. Purity
3. Recovery Amount and Recovery Efficiency

Comparing PS Affinity with UC

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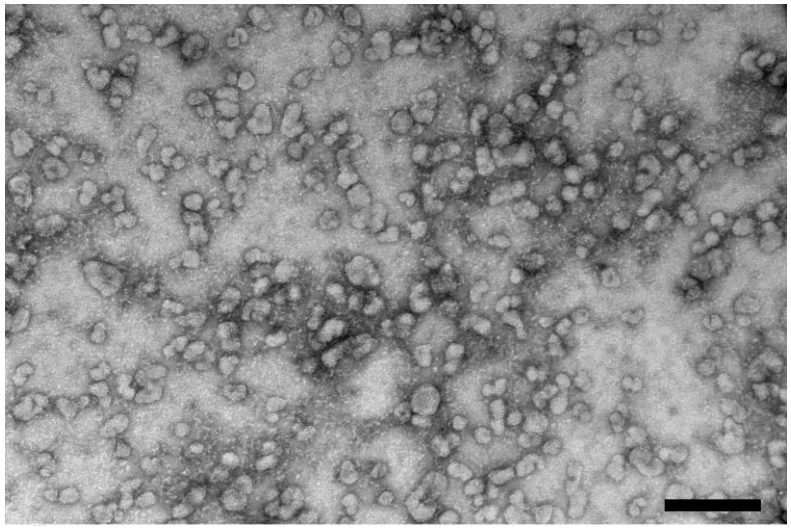
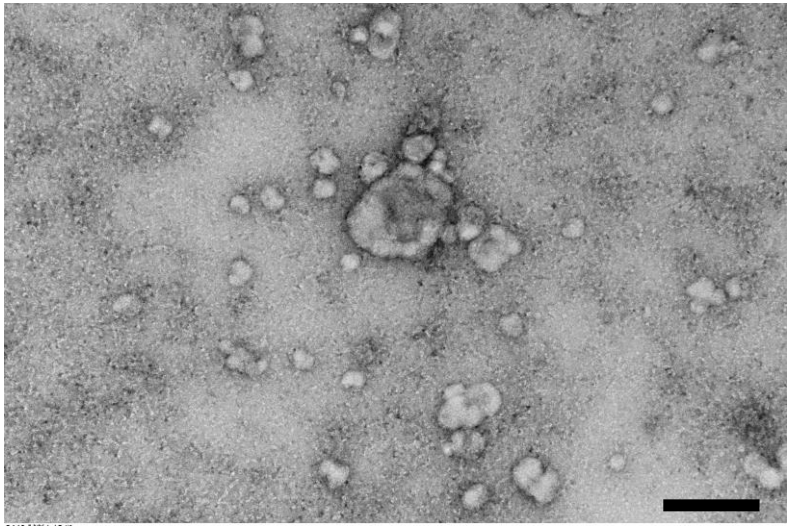
- in terms of appearance by TEM analysis -

■ sEVs in 10K sup of COLO201 cells were isolated by each method, followed by transmission electron microscope (TEM)

PS Affinity



Ultracentrifugation



x 100,000 Bar : 200nm

PS Affinity isolated many particles of around 50-100 nm in size. Numerous small EVs can be seen.

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Comparing PS Affinity with UC

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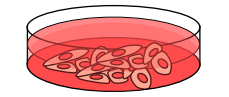
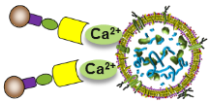
- in terms of purity of small EVs by biochemical analysis -



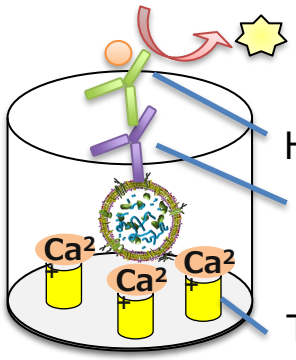
Conditioned medium of COLO201



or

Analyses

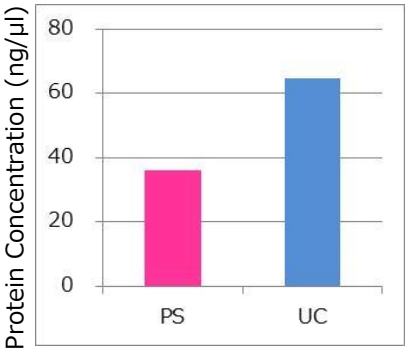
- BCA assay
- NTA
- PS ELISA



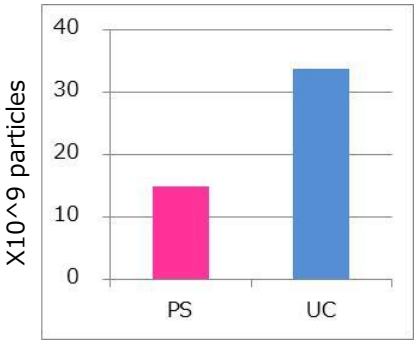
PS ELISA

PS ELISA (amount of purified exosome)

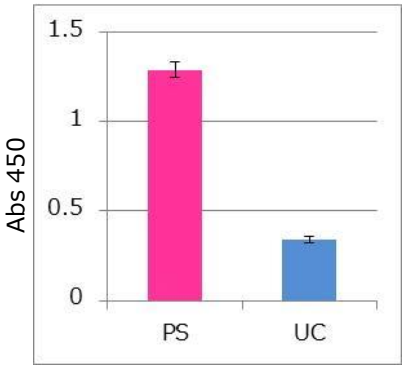
BCA Assay (Protein conc.)



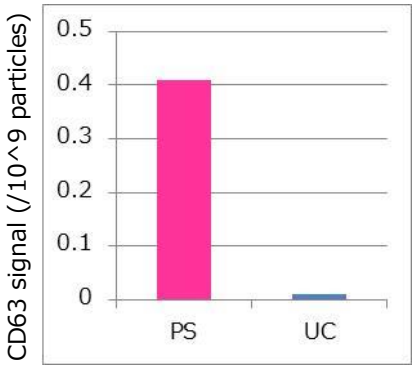
NTA (Number of Counted Particles)



Comparison of CD63 signals



CD63 signal per Counted particles



PS: PS affinity
UC: Ultracentrifugation

PS affinity method enables the isolation of highly pure small EVs.

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Comparing PS Affinity with UC

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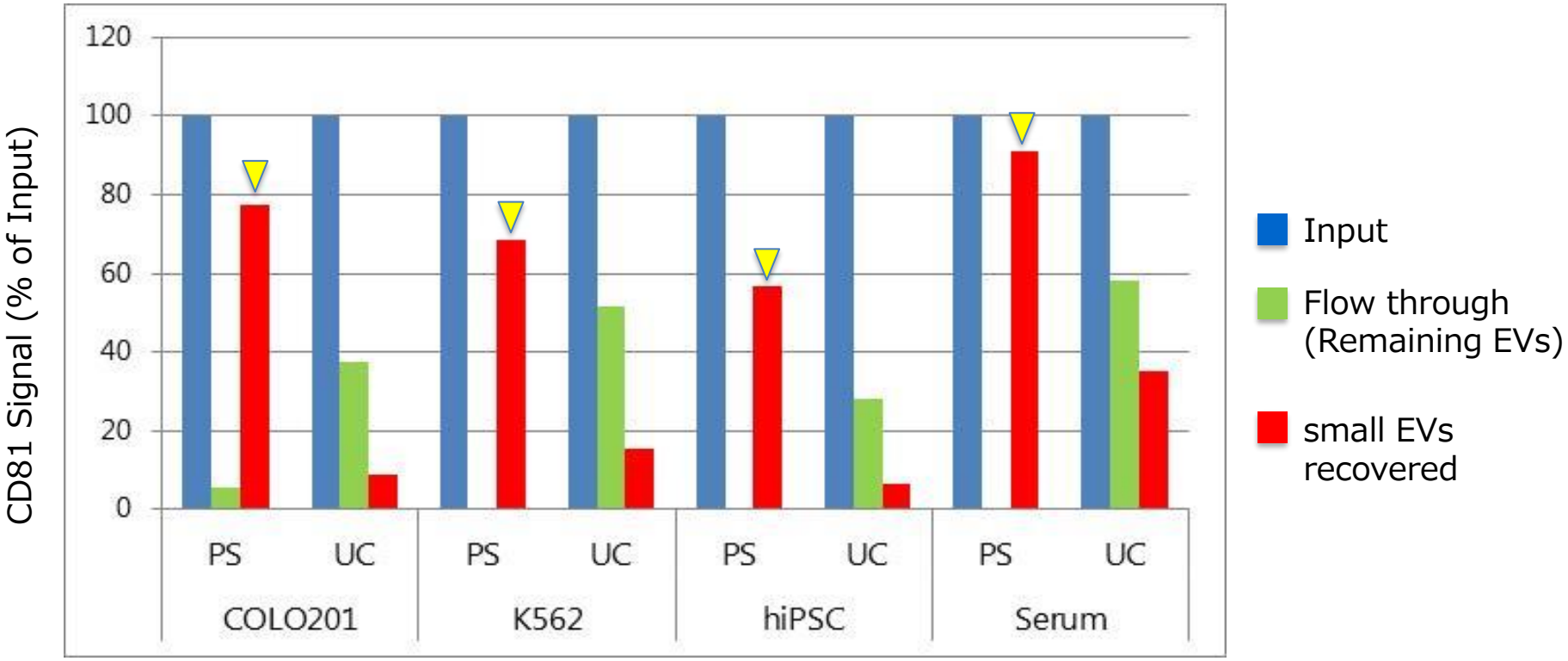
- in terms of recovery efficiency by PS ELISA -



<u>These results indicates that PS affinity can recover sEVs derived from various cell lines and serum more efficiently than ultracentrifugation.</u>

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3. Reference Data

1. Proteomic Analysis
2. Density Distribution

Proteomic Analysis

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Top 15 proteins identified in the isolated sEVs

	Tim4 method	Ultracentrifugation	PEG precipitation
1	Heat shock cognate 71 kDa protein	**DNA-PK catalytic subunit**	Complement C3
2	**Annexin A6**	Transferrin receptor protein 1	Alpha-2-macroglobulin
3	Transferrin receptor protein 1	Serum albumin	Fibronectin
4	V-type proton ATPase subunit A	**ATP-dependent RNA helicase A**	Serum albumin
5	**Flotillin-2**	Tubulin beta-5 chain	Thrombospondin-1
6	Programmed cell death 6	Heat shock cognate 71 kDa protein	Complement C4
7	4F2 cell-surface antigen heavy chain	Fatty acid synthase	Alpha-1-antiproteinase
8	**Annexin A1**	4F2 cell-surface antigen heavy chain	Apolipoprotein B-100
9	Kinase D-interacting substrate	**U5 small nuclear RNP helicase**	Hemoglobin fetal subunit beta
10	**Annexin A2**	Tubulin beta-4B chain	Tubulin beta-5 chain
11	**Flotillin-1**	**Ribonucleoprotein M**	Fatty acid synthase
12	V-type proton ATPase subunit B	Hemoglobin fetal subunit beta	Adiponectin
13	**Annexin A11**	Clathrin heavy chain 1	Fibulin-1
14	**Annexin A7**	Fibronectin	Complement C4A
15	**Syntenin-1**	Tubulin alpha-1B chain	Complement C7

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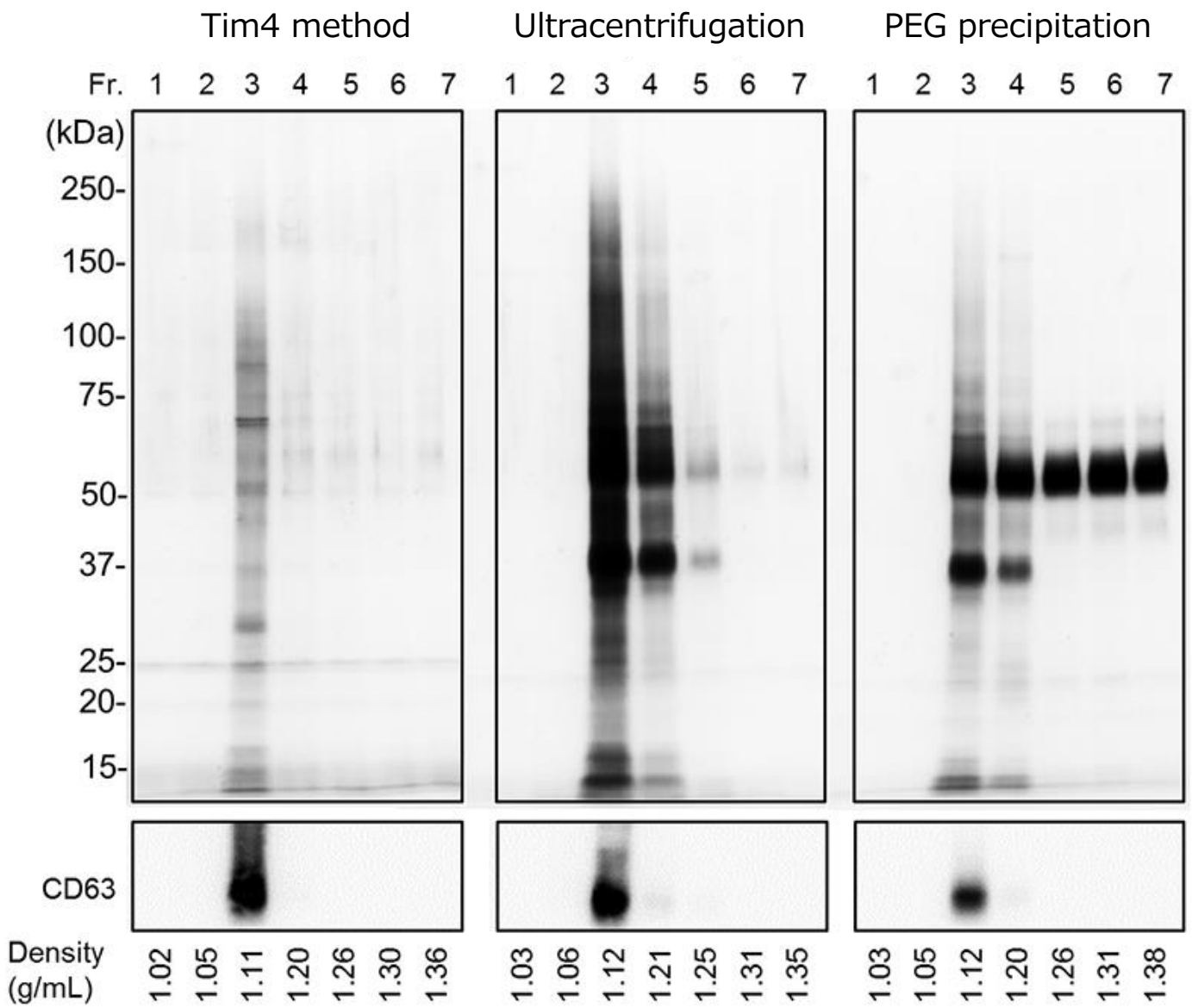
EV markers

White columns: human proteins from sEVs

Gray columns: bovine proteins from FBS

Density distribution of isolated sEVs



4. Conclusion

Conclusion

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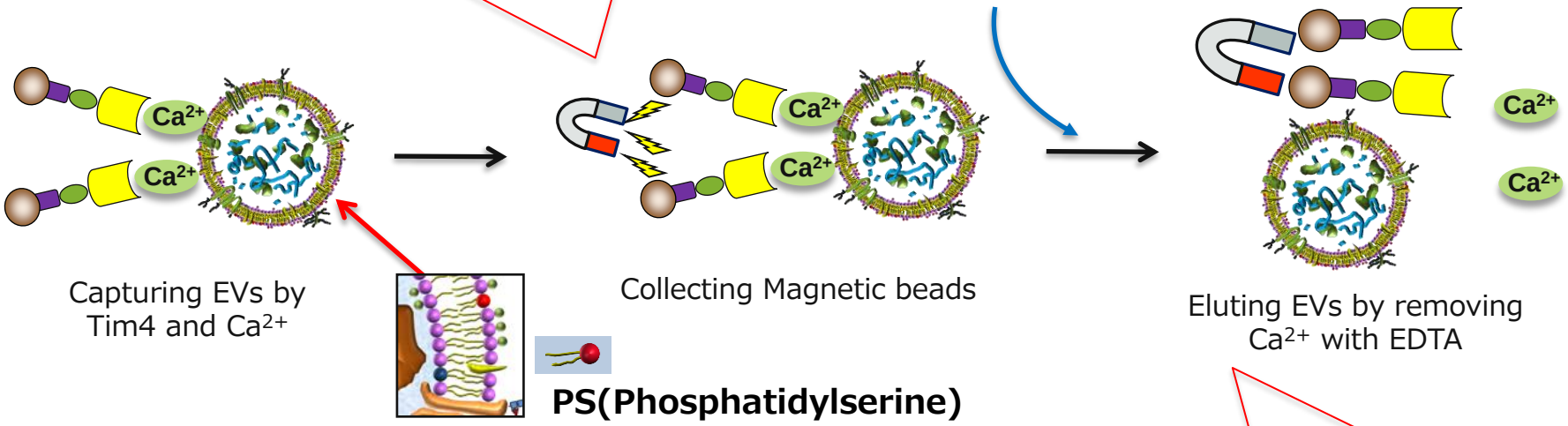
PS affinity introduced:

High purity isolation!

High recovery efficiency!

EDTA+EV-Save™
(Adsorption preventing reagent)



Capturing EVs by Tim4 and Ca^{2+}

Collecting Magnetic beads

Eluting EVs by removing Ca^{2+} with EDTA

PS(Phosphatidylserine)

Continuous research on function of EVs, PS positive and negative!

Validity as purification method of EVs is better!

PS Affinity exhibited the superiority to conventional method such as ultracentrifugation and also presented new challenge!

~Additional Information~

Do you know the loss and adsorption of EVs to labwares?

EV-Save™ Extracellular Vesicle Blocking Reagent

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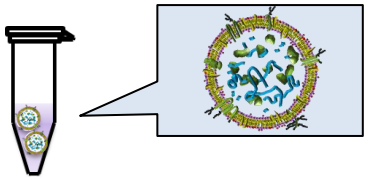
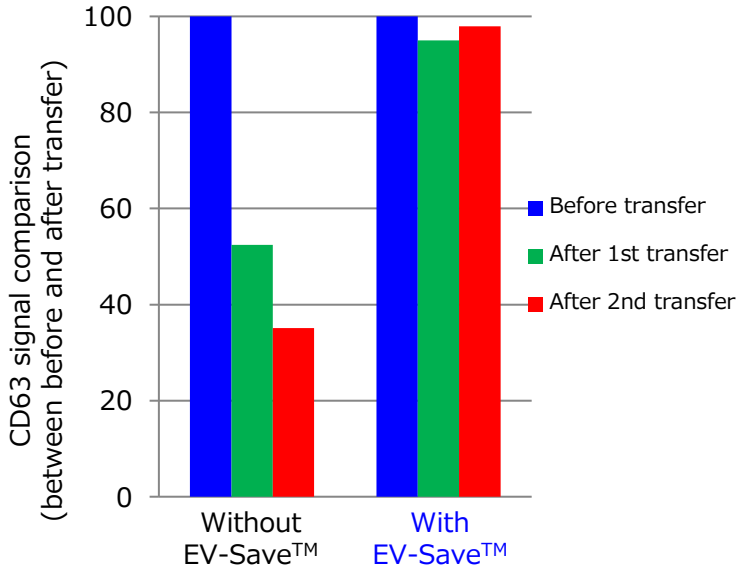
Features

- Strongly suppressing adsorption of EVs to laboratory tools
- Simple operation just to add to the sample
- Cryoprotective effect to EVs

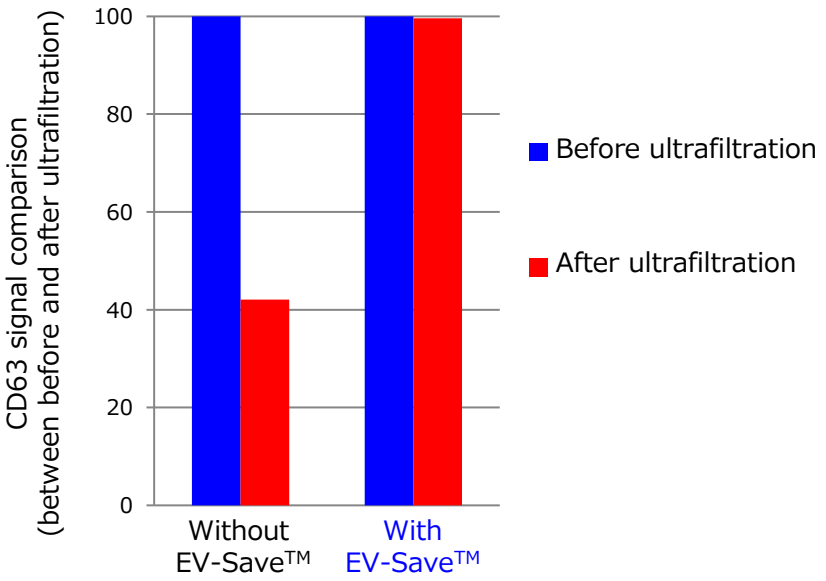
Save EVs



Tube Transfer



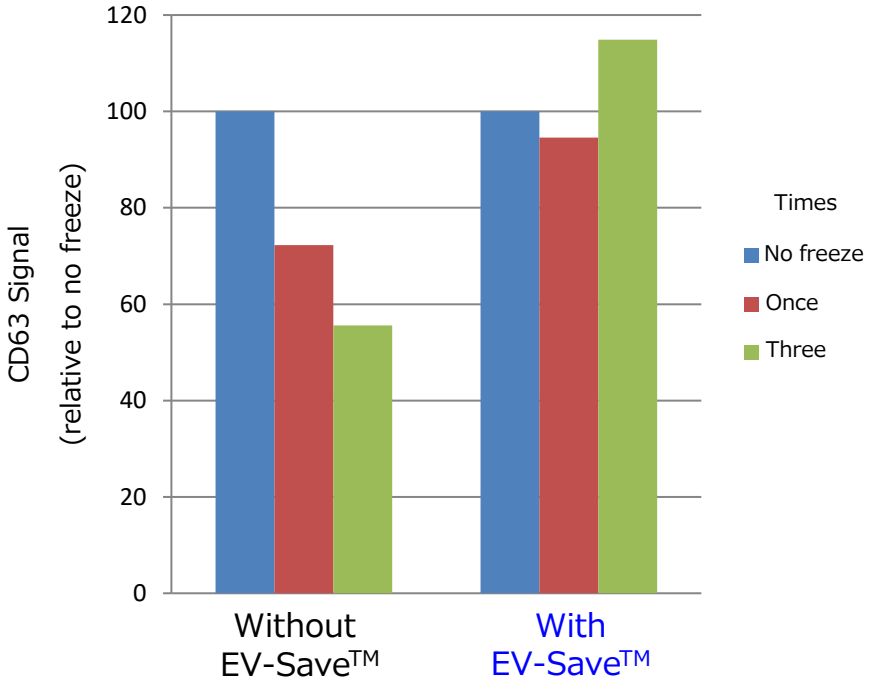
Ultrafiltration

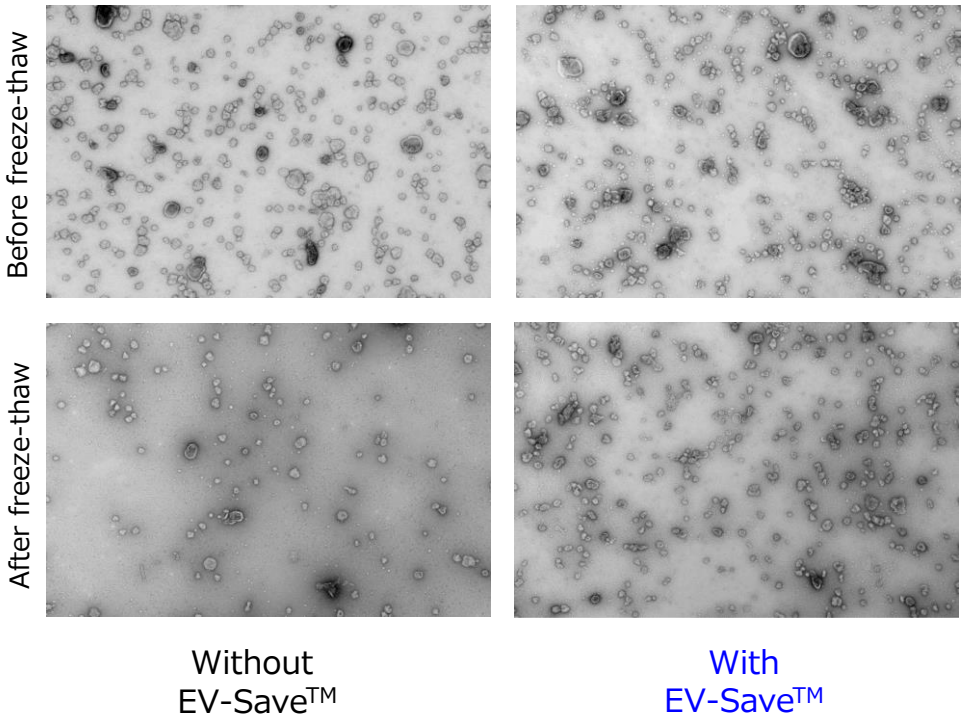


Description	Package Size	Catalog No.	Storage
EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL	058-09261	Keep at -20℃.

Cryoprotective effect by EV-Save™

Repeating freeze & thaw





【Result】

Although freeze-thaw reduced CD63 signal, such reduction was suppressed by EV-Save™ (A). Furthermore, TEM results (B) indicated that freezing and thawing caused a marked decrease in the number of particles, but the EV-Save™ suppress it.

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