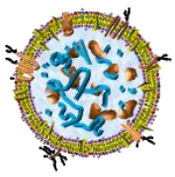
A novel tool for qualitative and quantitative analysis of extracellular vesicles in sample of cell culture supernatant

PS Capture™

Exosome ELISA Kit (Anti Mouse IgG POD)

Introduction



The kit includes reagents for enzyme-linked immunosorbent assay (ELISA) available for a **Qualitative Analysis** of extracellular vesicles purified from **cell culture supernatant** or **samples from body fluid** as well as a **Quantitative Analysis** of extracellular vesicles **in samples of cell culture supernatant**.

It can detect extracellular vesicles, which have any surface marker protein, with high sensitivity by using a mouse monoclonal antibody against any surface marker proteins of extracellular vesicles as a primary detection antibody and HRP-conjugated anti mouse IgG antibody of the kit as a secondary detection antibody after extracellular vesicles are captured by a plate on which proteins that specifically bind with phosphatidylserine (PS) on the surface of extracellular vesicles are immobilized.

*Reference : "A novel affinity-based method for the isolation of highly purified extracellular vesicles", W. Nakai, T. Yoshida, D. Diez, Y. Miyatake, T. Nishibu, N. Imawaka, K. Naruse, Y. Sadamura & R. Hanayama, Sci Rep **6**, 33935 (**2016**).*

Feature ~PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)~

PS-binding molecule, Tim4, immobilized plate is adopted

- **Capturing by PS-binding molecule※1**
- **Can detect extracellular vesicles in samples of cell culture supernatant corresponding to 0.1μL**
- **Quantitative analysis with reference standard of purified extracellular vesicles※2**
- **Can detect with 50-1,000 times higher sensitivity than WB※3**

High-sensitive qualitative and quantitative analysis

※1 Available for high-sensitive qualitative and quantitative analysis than ELISA kit based on antibody-immobilized plate.
※2 Use reference standard of purified extracellular vesicles from a sample with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601).
※3 WB: western blot.

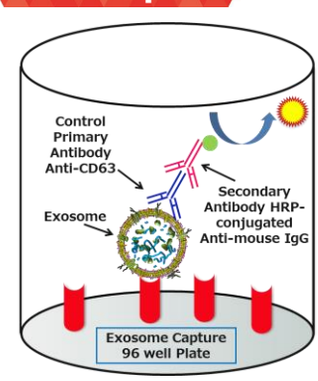
Optimized reagent is kitted

- **Optimized control primary antibody※4**
- **Optimized secondary antibody, HRP-conjugated※5**
- **Chromogenic method is adopted**

Easy operation High reproducibility

※4 Anti-human CD63 mouse antibody is contained in the kit. When intending to detect other surface marker proteins, use an optional mouse monoclonal antibody.
※5 Anti-mouse IgG antibody, HRP-conjugated is contained in the kit.

Principle



Component

Components (for 96 reactions)	Amount
Exosome Capture 96 Well Plate	1 plate (8 well×12 strips)
Reaction / Washing Buffer (10×)	50 mL×2 vials
Exosome Binding Enhancer (100×)	10 mL×1 vial
Control Primary Antibody Anti-CD63 (100×)	120 μL×1 vial
Secondary Antibody HRP-conjugated Anti-mouse IgG (100×)	120 μL×1 vial
TMB Solution	12 mL×1 vial
Stop Solution	12 mL×1 vial
Plate Seal	4 sheets
Instruction Manual	1 copy

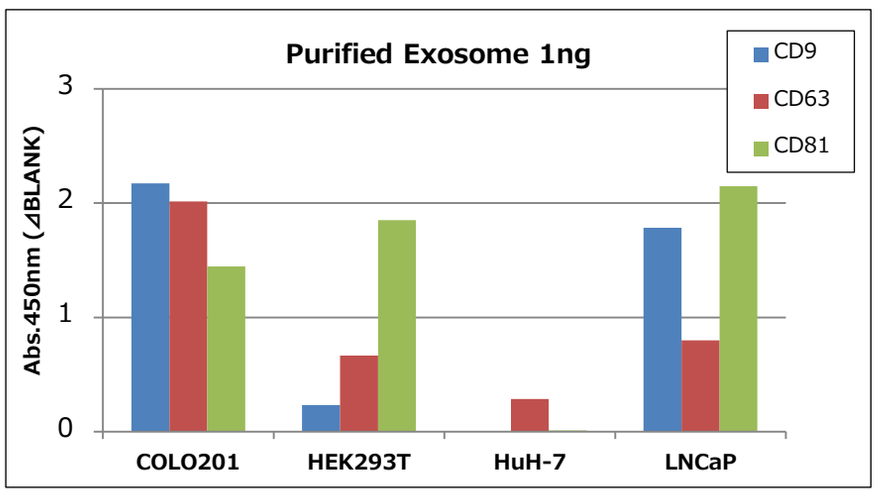
Product Name	Package Size	Catalog No.	Storage
PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)	96 reactions	297-79201	Keep at 2-10℃

Note: when intending to analyze extracellular vesicles of body fluids, qualitative analysis is available by using purified extracellular vesicles from body fluids with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601).

Qualitative analysis of extracellular vesicles purified from various cell culture supernatants

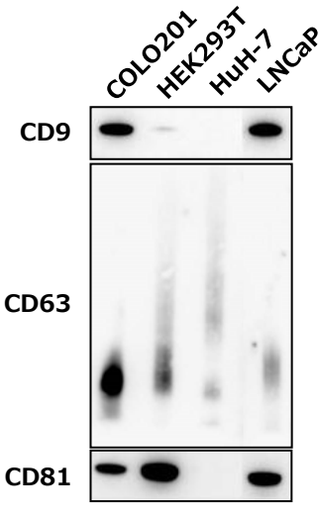
Add 1 ng of extracellular vesicles purified from various cell culture supernatants to a well and surf-ace marker levels were compared by a qualitative analysis with the kit using a primary detection antibody, which recognize cell surface markers, CD9, CD63, or CD81 each. In addition, as reference comparative data, 150 ng of extracellular vesicles purified from various cell culture supernatants were electrophoresed and protein levels of each surface markers were detected by Western blot using a primary detection antibody, which recognize cell surface markers, CD9, CD63, or CD81 each. Then, qualitative analysis was conducted.

・Comparison of qualitative data per 1 ng of purified exosomes



<Reference comparative data>

Purified exosome: 150ng

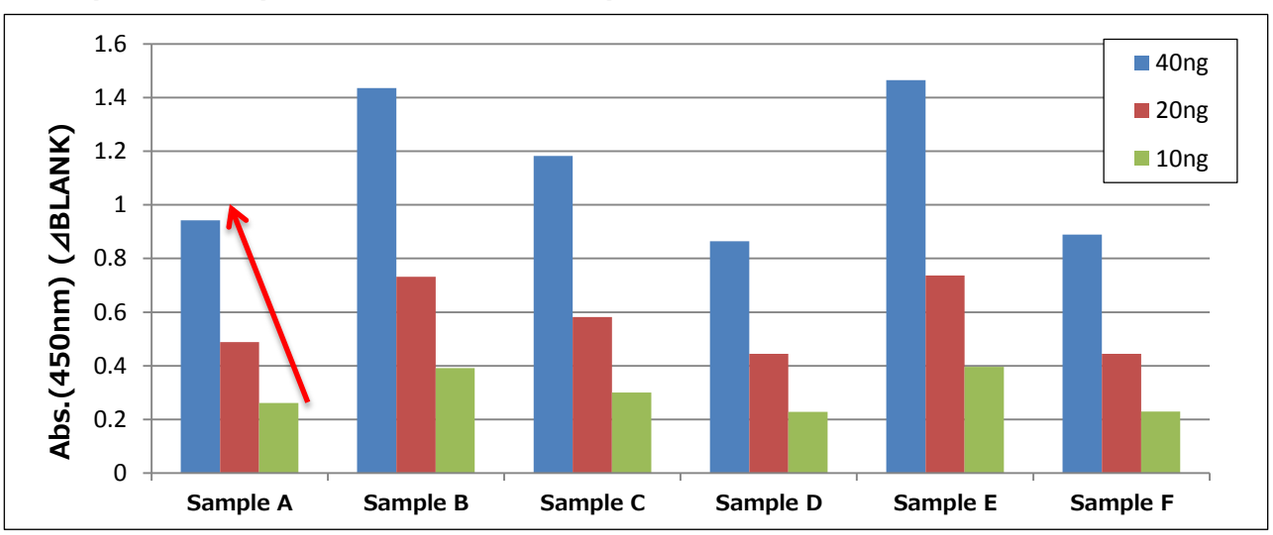


Expression pattern of marker proteins in data of ELISA have a correlation with that of WB!

Qualitative analysis of extracellular vesicles purified from healthy human serum

40, 20, or 10 ng of extracellular vesicles purified from 6 healthy human serum samples were added to each well and qualitative analysis was conducted using a control primary detection antibody which recognize the cell surface marker CD63.

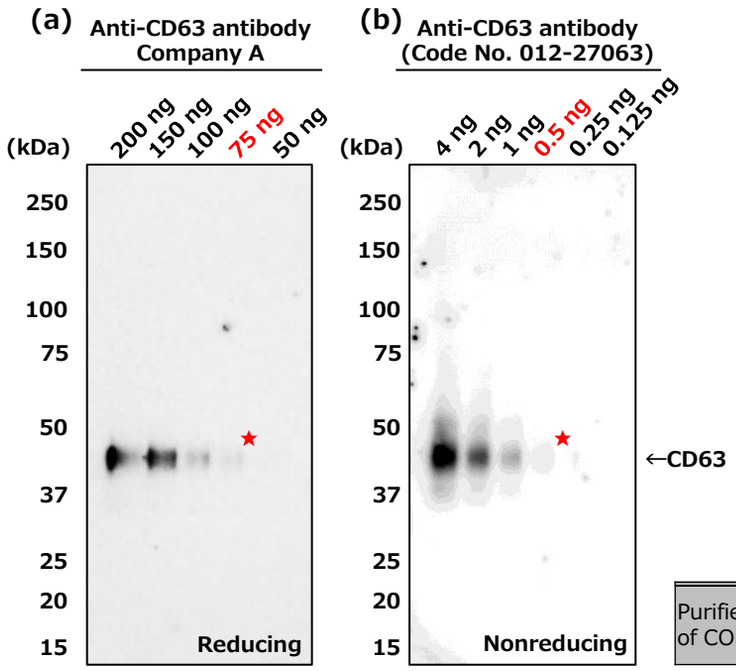
<Comparison of qualitative data of each purified extracellular vesicles>



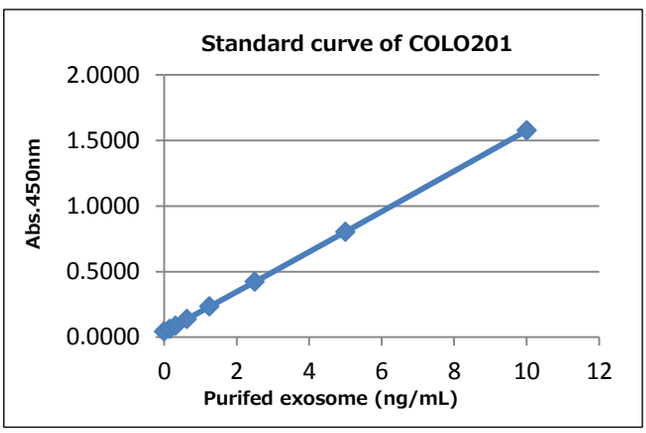
Linearity was confirmed properly when extracellular vesicles purified from body fluids are used!

Reference data: detection sensitivity of extracellular vesicles purified from cell culture supernatant

Extracellular vesicles were purified from cell culture supernatant of COLO201, then detection sensitivity of the kit and western blot was compared.



(c)



	Detection limit (pg) (Blank + 3.3SD)	Determination limit (pg) (Blank + 10SD)
Purified exosomes of COLO201	0.11 ng/mL (11 pg)	0.34 ng/mL (34 pg)

Comparing a detection sensitivity of western blot and ELISA

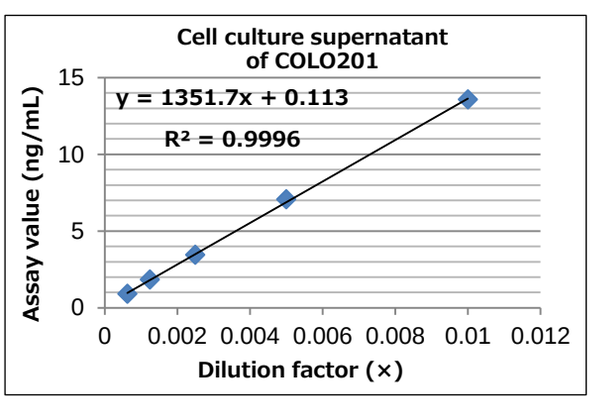
(a), (b) Data of a detection sensitivity by western blot with each anti-CD63 antibody (company A and Wako: Code No. 012-27063).
Sample: purified extracellular vesicles from cell culture supernatant of COLO201 with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601)
★ : detection limit by western blot

(C) Data of a detection sensitivity by Exosome ELISA Kit
A standard curve was prepared using blank value of buffer and absorbance value of 2-fold serial dilution samples of extracellular vesicles purified from cell culture supernatant of COLO201 cells with MagCapture™ Exosome Isolation Kit PS. Then, the detection limit of extracellular vesicles purified from cell culture supernatant of COLO201 was calculated using its standard curve. (each dilution point: n=6, blank: n=12)

Exosome ELISA Kit detected the marker proteins with higher sensitivity than WB!

Reference data: dilution linearity of cell culture supernatant

A standard curve was prepared using a reference standard of extracellular vesicles purified from cell culture supernatant of COLO201, then dilution linearity of 5-step dilution samples of cell culture supernatant of COLO201 cells (1:100 to 1:1600) was evaluated.



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	-	-

Reference standard: extracellular vesicles purified from cell culture supernatant of COLO201 with MagCapture™ Exosome Isolation Kit PS
Measured sample: cell culture supernatant of COLO201
Primary antibody: anti-CD63 antibody in the kit

Good dilution linearity and detecting extracellular vesicles in cell culture supernatant corresponding to 0.1 μL were confirmed!

Q&A

Q.1 Do I have to prepare a reference standard certainly? In addition, do I have to use MagCapture™ Exosome Isolation Kit PS absolutely in the case?

A.1 When doing a quantitative measurement, prepare extracellular vesicles as a reference standard. It is also available to use extracellular vesicles purified by ultracentrifugation and polymer-based method as reference standard, however we recommend using extracellular vesicles purified by PS affinity method because its principle is same to that of measurement in PS Capture™ Exosome ELISA Kit. (Refer to instruction manual.)

Q.2 Why doesn't this kit contain a reference standard?

A.2 It is necessary to make the derived cell of a reference standard and a measured sample identical because there is a heterogeneity in the kind and the amount of surface marker proteins of extracellular vesicles secreted from every cells. This kit therefore doesn't contain a reference standard. Please purify extracellular vesicles from the cell culture supernatant of the identical derived cell line as a reference standard.

Q.3 Is it possible to directly measure extracellular vesicles in serum and plasma?

A.3 No, it isn't recommended because Secondary Antibody HRP-conjugated Anti-mouse IgG (100×) in the kit react to human, mouse, and rat IgG nonspecifically. However, it can qualitatively analyze extracellular vesicles purified from body fluids with MagCapture™ Exosome Isolation Kit PS (Code No. 293-77601). Additionally, it can also qualitatively analyze extracellular vesicles purified by ultracentrifugation and polymer-based method.

Q.4 Is it possible to directly measure extracellular vesicles in cell culture medium?

A.4 Yes it is. Furthermore, This kit can also directly measure extracellular vesicles in FBS included medium as well as serum-free medium because Secondary Antibody HRP-conjugated Anti-mouse IgG (100×) in the kit doesn't react to bovine IgG nonspecifically. Please use a qualitative analysis and a quantitative analysis of extracellular vesicles in cell culture supernatant.

Q.5 Can I change a primary antibody?

A.5 Yes, you can change. Please use an optional mouse monoclonal antibody against surface marker proteins of interest and examine an optimized concentration of antibody in accordance with instruction manual.

Q.6 Are there primary antibodies you would recommend?

A.6 The following antibodies were used for ELISA application.

- Anti-CD63 antibody (3-13) (Code No. 012-27063)
- Anti-CD9 antibody (HI9a) (Novus Biologicals, LLC:NB100-77915)
- Anti-CD81 antibody (M38) (Novus Biologicals, LLC:NBP1-44861)

Isolation of high purity exosomes by a novel affinity molecule

Product Name	Package Size	Catalog No.	Storage
MagCapture™ Exosome Isolation Kit PS	2 purifications*1	299-77603	Keep at 2-10℃.
	10 purifications*1	293-77601	

*1 Used **Exosome Capture-immobilized beads** can be recycled up to 4 times and buffers of kit component also be contained enough for the case of recycling. When repeated isolations of extracellular vesicles from same sample are required, please try the recycling. However, when repeated isolations of extracellular vesicles from different kinds of samples are required, please don't try the recycling for preventing a contamination.

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