COSMO BIO CO., LTD.

CD9/CD63 Exosome ELISA Kit, Human

For Research Use Only

Cat. No. EXH0102EL

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www.cosmobio.com

Ⅰ. About this kit

【Ⅰ－1】Background and Measurement Principal

Exosomes are membrane vesicles, which are secreted from many cells and have a diameter of about 30 nm ～ 200 nm. They are present in many bodily fluids, including saliva and blood[(1) ～ (5)].It is also known that exosomes are secreted into the mammalian cell culture medium in vitro. Exosomes are enveloped with a lipid bilayer membrane, so like cells, membrane proteins exist at their surface, and they encase various molecular constituents, including proteins and microRNAs. When the target cells receive the exosomes, the proteins or microRNAs inside will fulfill their function, and in this way exosomes are thought to play a role of cell-to-cell communication [(6)]. One of the exosomes' structural characteristics is the tetraspanin family which is located on their surface. CD9 and CD63 are member molecules of this family, and known to be exosome surface markers [(7)].

The conventional way to quantify exosomes is indirect. For example, it measures encapsulated protein abundance or performs Nanoparticle Tracking Analysis to determine the size distribution profile of small particles[(8)]. The drawback for these methods is that they require exosome purification utilizing ultracentrifugation, for instance. The direct methods to quantitate exosomes in body fluids or culture supernatant is extremely limited, and there were no common methods available until now.

The CD9/CD63 Exosome ELISA Kit is a Sandwich ELISA kit, which utilizes high-performance anti-CD9 and anti-CD63 antibodies. This product detects exosome markers, CD9, and CD63 molecules that are located on the exosome surface in the body fluids or cell culture supernatant.

【Ⅰ－2】Features

- Directly quantitate exosomes in human blood samples or cell culture supernatant.
- No special equipment is required. Standard microplate reader capable of reading at 450nm will do the job.
- Utilize CD9/CD63 fusion protein (Standard Protein), instead of unstable/hard to store exosome itself, to implement stability and reproducibility.
- Normalization with CD9/CD63 fusion protein (Standard Protein) enable to relative quantitate each samples.
- Capture exosomes with solid phase anti-CD9 antibody (12A12), then detect using HRP conjugated anti-CD63 antibody.

【Ⅰ－3】Kit Principle

An anti-CD9 antibody was immobilized and placed on the plate. First, samples were added onto the plate to capture exosomes by the anti-CD9 antibody. Next, HRP conjugated anti-CD63 antibody will be added to react exosome surface antigen, CD63. Finally, substrate will be added, then measure the coloring by the plate reader to quantitate sample exosomes.

【Ⅰ－4】Kit Components

Storage temperature : 4℃

	Component	Volume	Quantity
1	anti-CD9 antibody immobilized 96-well plate	8-well x 12 strips	1 plate
2	Standard Protein (2000 pg/mL) (Red cap)	200 µL	1 vial*
3	Assay Buffer	25 mL	1 tube
4	Washing Buffer (10 X)	25 mL	1 tube
5	HRP conjugated anti-CD63 antibody (500 X) (Green cap)	20 µL	1 vial
6	Substrate Solution	12 mL	1 tube
7	Stop Solution (2N H_2SO_4)	6 mL	1 tube
8	Plate Seals		2 sheets

* Sufficient to create 4 standard curves with n=2.

【Ⅰ－5】Materials Not Included in the Kit

- Micropipettes (10 ~ 1000 µL)
- Multichannel micropipette
- Multichannel micropipette Reservoir
- Plate shaker
- Microplate reader (enable to measure at wavelength 450nm)
- Plate washer

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【II】 Preparation of Reagents and Samples

【II－1】Preparation of Washing Buffer

・Dilute Washing Buffer (×10) to x1 with purified water.

e.g. For 1 plate, add 225 mL of purified water to 25mL of Washing Buffer (x10) and mix well.

【II－2】Preparation of Standard Protein solution

	Concentration(pg/mL)	Standard Protein	Assay Buffer	Dilution factor
A	2000	n/a	n/a	1
B	200	50 µL of A	450 µL	10
C	100	250 µL of B	250 µL	2
D	50	250 µL of C	250 µL	2
E	25	250 µL of D	250 µL	2
F	12.5	250 µL of E	250 µL	2
G	6.25	250 µL of F	250 µL	2
H	3.125	250 µL of G	250 µL	2

・To prepare Solution B, add 450µL of Assay Buffer into 50µL of Standard Protein (Solution A), and then mix well (10 times dilution). To prepare Solution C, add 250µL of Assay Buffer into 250µL of Solution B, and then mix well (2 times dilution). Similarly, 2 times dilution series for Solution D through H should be prepared.
・Use 100µL for measurement, using 2 wells for each solution (n=2).
・Diluted Standard Protein Solution should be freshly prepared at each time before use.

【II－3】Preparation of antibody solution

・Dilute HRP conjugated anti-CD63 antibody (500x) to 500 folds using Assay Buffer.
e.g. For 1 plate, add 20µL of antibody (500x) into 10mL of Assay Buffer. Mix by inverting the tube.
* Diluted antibody solution should be freshly prepared at each time before use.

【II－4】Preparation of Samples

Note: Each sample should be assayed in duplicate.

① For cell culture medium supernatant

The culture medium containing 10% fatal bovine serum (FBS) can be directly used to quantitate exosomes. Grow cells until it reach to the confluent state. Collect the medium supernatant, centrifuge at 2000 xg for 10min, and then used as a sample. If necessary, dilute with Assay Buffer.

② Serum

Dilute the serum sample at least four times with Assay Buffer.
* Out of 100µL reaction solution, serum should not be exceeded more than 25µL.

【II－5】Sample Storage

Samples should be stored at 4℃ after preparation. If the samples are stored for more than one month after preparation, it should be stored at -80℃ immediately. Samples should be used right away after thawing. Never re-freeze the samples.

【III】Sample measurement procedure

1	Bring anti-CD9 antibody solid phased plate and the reagents to the room temperature.
2	Prepare Standard Protein solution by serial dilution (from step 【II － 2】)
3	Add 100µL each of Serial diluted Standard Protein solution (3.125 ~ 200 pg/mL) or Sample solution into the well.
4	Seal the microplate with Plate Seals, place into plate shaker, and then shake it at 800 rpm for 30 sec.
5	Incubate at room temperature for 2hrs for static reaction.
6	Discard all the reaction solution, and then rinse each well with 300µL of Washing Buffer (from step【II-1】). Repeat this step for 3 times.
7	Add 100µL each of diluted HRP conjugated anti-CD63-antibody to the well.
8	Seal the plate, and then shake in the plate shaker.
9	Incubate at room temperature for 2hrs for static reaction.
10	Discard the antibody solution, and then rinse each well with 300µL of Washing Buffer. Repeat this step for 3 times.
11	Add 100µL of Substrate Solution into each well, and then incubate at room temperature for 20min for static reaction.
12	Visually confirm the coloring, and then add 50µL each of Stop Solution.
13	Place into the plate reader, and read the absorbance of each microwell on a spectrophotometer at the wavelength of 450nm.
14	Plot absorbance on the X-axis, and the measurement of Standard Protein on the Y-axis, and then draw a Standard curve (Fig. 1).

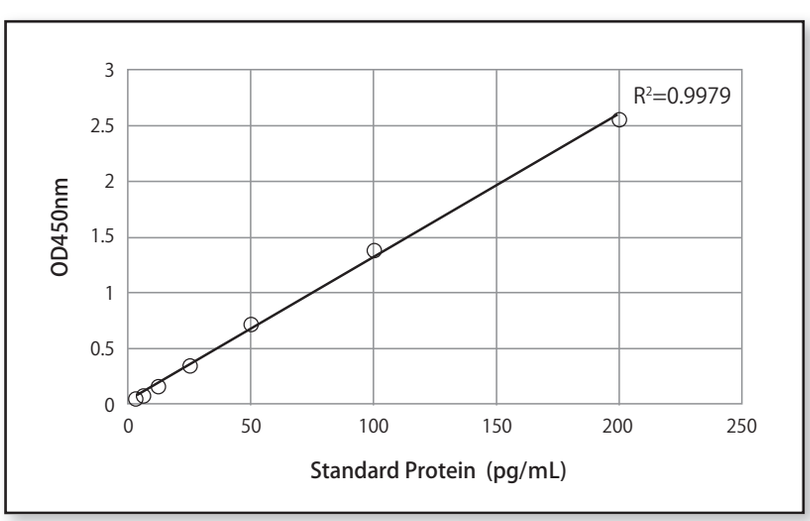

Fig. 1 Example of Standard curve for Standard Protein.

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【IV】Example of Results

【IV -1】Cell culture medium supernatant

The culture medium containing 10% fatal bovine serum (FBS) was used to culture various cell lines (HCT116, HT29, AsPC-1, MDA-MB-231, and PC3). After 8 days of cultivation, supernatants were collected, centrifuged, and then used as samples. Samples were diluted appropriately so that the measurements will be inside the Standard curve window. With this kit, relative quantification of exosomes in the sample will be performed using Standard Protein as a reference. Based on the measurements for the Standard Protein, plot the absorbance on the X-axis, and the measurements of Standard Protein on the Y-axis (Fig.2A). Based on this Standard curve and the sample absorbance, exosome concentration in the samples will be calculated as equivalent to the Standard Protein amount (Fig. 2B). Since Standard curve will be created for each experiment, one could compare exosome amount directly between the experiments.

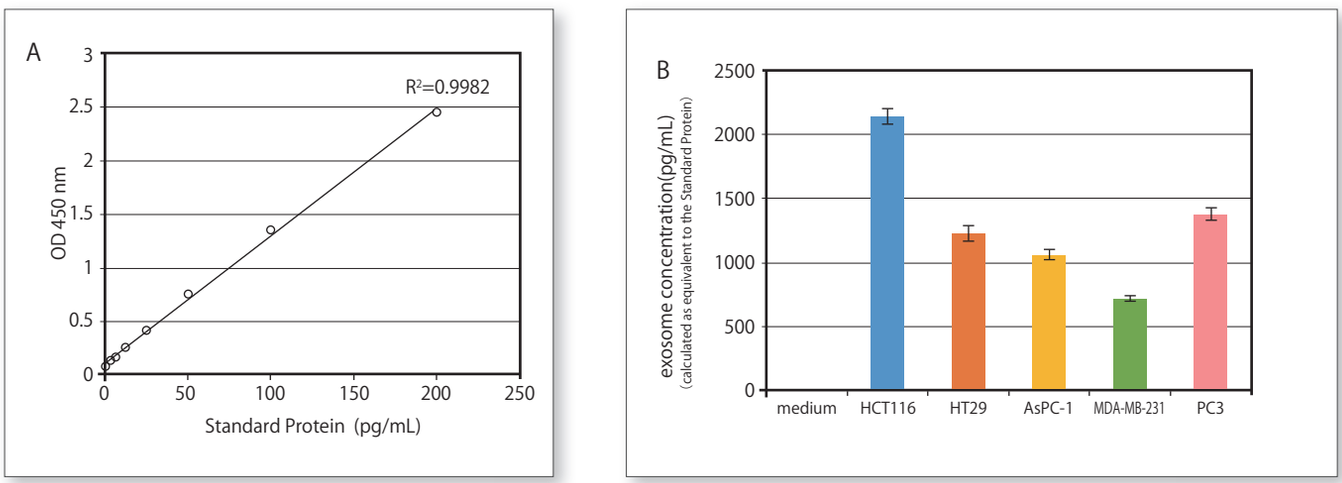

Fig. 2 Example of measurements for cell culture supernatant.

【IV -2】Purified exosomes

The culture medium of breast cancer cell line, MDA-MB-231, was collected, and exosomes were purified by ultracentrifugation. The purified exosomes, 0.781, 1.56, 3.13, 6.25, 12.5, 25, and 50 ng each, were added to the well, and measured using this kit (Fig.3A).

The Standard curve was created using the measurement of Standard Protein (CD9/CD63 fusion protein) (Fig. 3A). From here (Fig.3B), assuming 5 pg of CD9/CD63 fusion protein as 1 unit, the OD450 for 1unit will be 0.7. For the exosomes purified from MDM-MB-231, the protein concentration for 1 unit of OD450, which will correspond to 0.7, is about 50ng (Fig.3C). Therefore, 50 ng of MDM-MB-231 derived exosomes can be considered as 1unit of CD9 and CD63 positive exosomes. Presenting the exosome measurement by the units, we could standardize it and/or normalize the measurements, and then will be able to compare exosome measurements directly between different samples or different experiments.

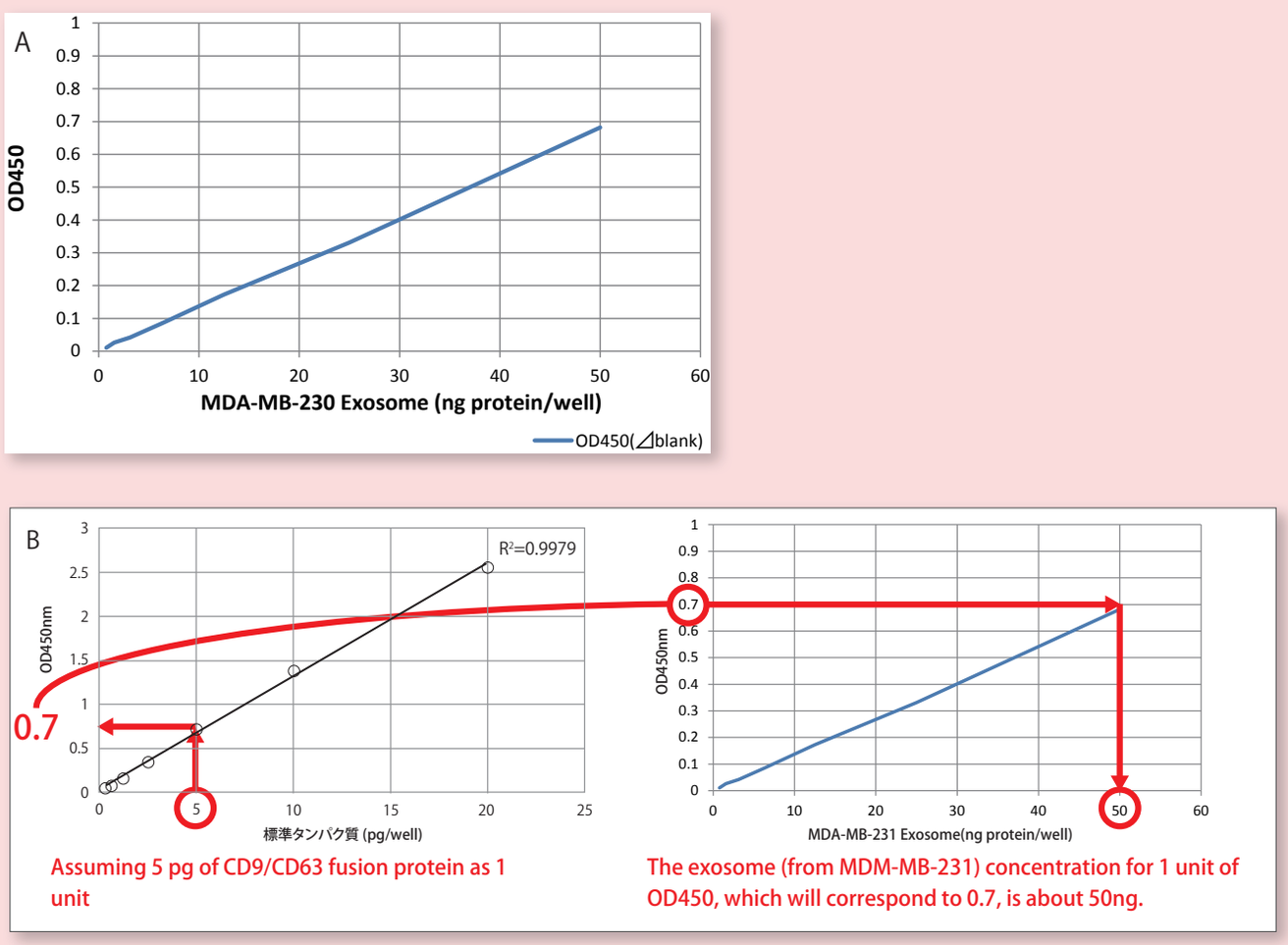

Fig. 3 Overview of standardization using CD9/CD63 fusion protein measurement and relative quantification.

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【V】Reference

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【VI】Related products

COSMO BIO CO., LTD (CAC)

Product name	Host	Label	Catalog number	Size	Price
Anti CD9, Human (Mouse) Unlabeled, 12A12	Mouse	Non-labeled	SHI-EXO-M01	100 µL (1 mg / mL)	¥65,000
Anti CD63, Human (Mouse) Unlabeled, 8A12			SHI-EXO-M02	100 µL (1 mg / mL)	¥65,000
Anti CD81, Human (Mouse) Unlabeled, 12C4			SHI-EXO-M03	100 µL (1 mg / mL)	¥65,000
Anti CD9, Human (Mouse) Biotin, 12A12	Mouse	Biotin	SHI-EXO-M01-B	100 µL (1 mg / mL)	¥85,000
Anti CD63, Human (Mouse) Biotin, 8A12			SHI-EXO-M02-B	100 µL (1 mg / mL)	¥85,000
Anti CD81, Human (Mouse) Biotin, 12C4			SHI-EXO-M03-B	100 µL (1 mg / mL)	¥85,000
Anti CD9, Human (Mouse) Label(Tide Fluor™5), 12A12	Mouse	Tide Fluor™5	SHI-EXO-M01-TF5	100 µL (1 mg / mL)	¥85,000
Anti CD63, Human (Mouse) Label(Tide Fluor™5), 8A12			SHI-EXO-M02-TF5	100 µL (1 mg / mL)	¥85,000
Anti CD81, Human (Mouse) Label(Tide Fluor™5), 12C4			SHI-EXO-M03-TF5	100 µL (1 mg / mL)	¥85,000

Storage：−70℃

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