CD147/CD9 Exosome ELISA Kit, Human

【Ⅰ】About this kit

【Ⅰ－1】Background and Measurement Principal

Cluster of differentiation (CD) 147 is a transmembrane glycoprotein that is highly expressed at the tumor cell surface, which stimulates fibroblasts to produce a large number of matrix metalloproteinases and promotes tumor invasion and metastasis and tumor-induced angiogenesis.[1)]

It has been suggested that an increase in CD147-positive exosomes released into the bloodstream from cancer cells may be useful as a diagnostic and prognostic biomarker for colorectal cancer and other diseases.[2)]

This product is a two-step sandwich ELISA kit that uses high-performance antibodies against CD9 and CD147, which are exosome markers, to detect CD147 molecules expressed on the surface of exosomes secreted by cells in human serum or cell culture medium.

【Ⅰ－2】Features

- Directly quantitate CD147-positive 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 CD147/CD9 fusion protein (Standard Protein), instead of unstable/hard to store exosome itself, to implement stability and reproducibility.
- Normalization with a standard curve using CD147/CD9 fusion protein (Standard Protein) enable to relative quantitate each sample.
- Detect human CD147-positive exosomes by two-step sandwich method using immobilized anti-CD147 antibody and HRP conjugated anti-CD9 antibody.

For research use only, Not for diagnostic use.
Please read this manual thoroughly before use.

【Ⅰ－3】Kit Principle

This ELISA kit uses two-step sandwich ELISA principle. The ELISA plate provided in this kit has been pre-coated with an anti-CD147 antibody.

First, samples were added onto the plate to capture CD147 by the anti-CD147 antibody. Next, HRP conjugated anti-CD9 antibody will be added to react with CD9 on the surface of CD147-positive exosomes. Finally, substrate will be added, then measure the coloring by the plate reader to quantitate sample exosomes.

【Ⅰ－4】Kit Component

Storage temperature： 2～8℃

	Reagent	Volume	Quantity
1	Anti CD147 Antibody Immobilized Plate	96well (8well x 12 strips)	1 plate
2	Standard Protein (CD147/CD9 Fusion Protein) (20ng/mL)	200μL	1tube*1
3	Assay Buffer	25mL	1vial
4	Washing Buffer (10X)*2	25mL	1vial
5	HRP Conjugated Anti CD9 Antibody (500X)*3	20μL	1tube
6	Substrate Solution	12mL	1vial
7	Stop Solution (2N H_2SO_4)	6mL	1vial
8	Plate Seals		2sheets

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

*2 Crystals may precipitate in the Washing Buffer (10x) during refrigerated storage. Warm the buffer to dissolve it at 45℃ before use.

*3 If the kit is not going to be used immediately, remove the labeled antibody from the kit and store it at -20℃.

Required 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

【Ⅱ】Preparation of Reagents and Samples

【Ⅱ－1】Preparation of Washing Buffer

・Dilute Washing Buffer (10×) to 10 folds with purified water.
e.g. For 1 plate, add 225 mL of purified water to 25mL of Washing Buffer (10 x) and mix well.

【Ⅱ－2】Preparation of Standard Protein solution

	Concentration (pg/mL)	Standard Protein	Assay Buffer	Dilution factor
A	20000			
B	2000	50μL of A	450μL	10
C	1000	250μL of B	250μL	2
D	500	250μL of C	250μL	2
E	250	250μL of D	250μL	2
F	125	250μL of E	250μL	2
G	62.5	250μL of F	250μL	2
H	31.25	250μL of G	250μL	2

・To prepare Solution B, add 450μL of Assay Buffer into 50μL of Human 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 Human Standard Protein Solution(31.25～2000pg/mL) should be freshly prepared at each time before use.

【Ⅱ－3】Preparation of antibody solution

・Dilute HRP conjugated anti-Human CD9 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

Serum is measured as a sample diluted 20-fold with Assay Buffer.

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris.
Collect supernatants and assay.

Samples generating absorbance values greater than that of the highest standard should be further diluted using Assay Buffer and reanalyzed.

【Ⅱ－5】Sample Storage

After sample preparation, store at 2-8°C until measurement.

【Ⅲ】Sample measurement procedure

1. Bring anti CD147 antibody solid phased plate and the reagents to the room temperature.
2. Prepare Human Standard Protein solution by serial dilution. (from step【Ⅱ－2】)
3. Add 100μL each of serial diluted Standard Protein solution (31.25～2000pg/mL) or Sample solution into the well.
4. Seal the microplate with Plate Seals.
5. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
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 CD9 antibody (from step 【II-3】) to the well.

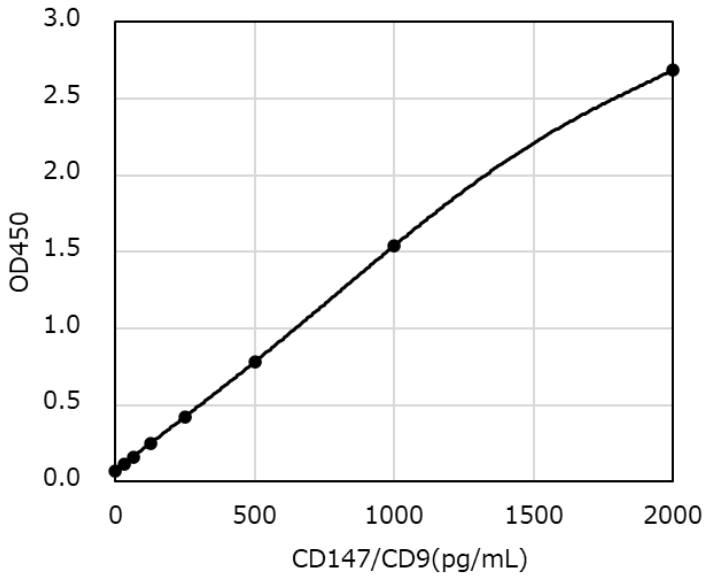
8. Seal the microplate with Plate Seals.
9. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
10. Discard the reaction solution, and then rinse each well with 300μL of Washing Buffer(from step 【II-1】). Repeat this step for 3 times.
11. Add 100μL of Substrate Solution into each well, and then incubate at room temperature protected from light 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 well on a spectrophotometer at the wavelength of 450nm.
14. Create a standard curve by plotting the absorbance value (y axis) against the Standard Protein concentration (x axis).
15. Calculate the concentration by comparing the absorbance obtained from the sample solution to the standard curve. Determine the CD147/CD9-positive exosome concentration (Unit/mL) in the sample solution as the CD147/CD9 fusion protein (pg/mL) equivalent. Multiply the resulting value by the appropriate sample dilution factor, to obtain the concentration of CD147/CD9-positive exosome in the sample.

【Ⅳ】Measurement example

【Ⅳ－１】Standard curve

As an example, the graph of absorbance (OD450) against Human Standard Protein concentration is drawn as shown in Figure 1.

However, draw a new standard curve for each assay to calculate the concentration in the sample.



Standard Protein (CD147/CD9 Fusion Protein) (pg/mL)	Absorbance (450nm)		mean
	1	2	
0	0.074	0.071	0.073
31.25	0.117	0.116	0.117
62.5	0.160	0.160	0.160
125	0.252	0.253	0.253
250	0.421	0.428	0.425
500	0.782	0.784	0.783
1000	1.491	1.589	1.540
2000	2.667	2.707	2.687

Fig.1　Standard curve and measured values

【Ⅳ−2】Example of sample measurement

1．Purified exosomes

Exosomes（15.6, 31.3, 62.5, 125, 250, 500, 1000ng/mL）purified by ultracentrifugation from the culture supernatant of HCT116 were measured using this kit. Figure 2 shows the exosome concentration (ng/mL) and absorbance (OD450) graphically.

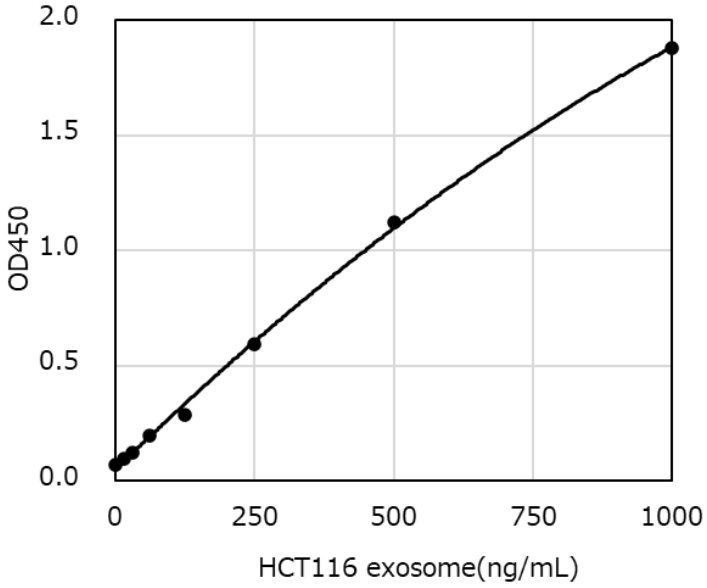


Fig.2 Measurement of exosomes

2. Cell culture supernatant

Culture supernatant of colon cancer cell line HCT116 was collected every two days and measured with this kit. Figure 3 shows the graph of the number of days of culture and the amount of CD147/CD9 positive exosomes (absorbance: OD450) in the culture supernatant.

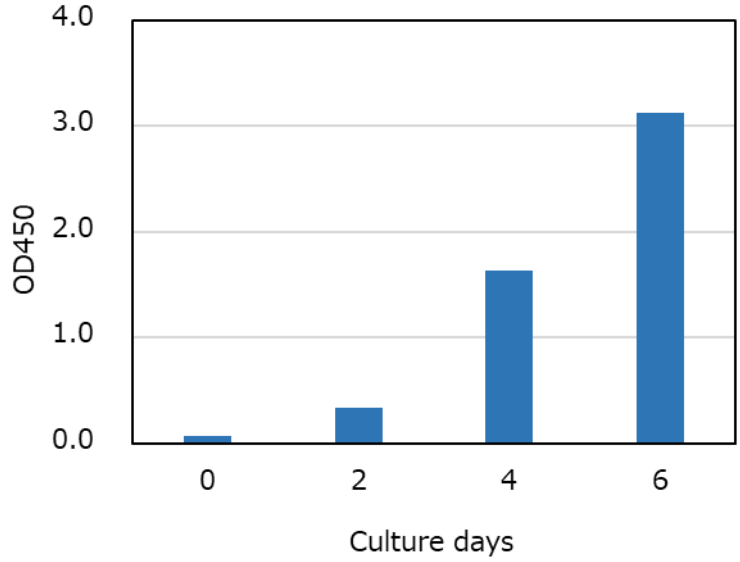


Fig.3 Time course of CD147/CD9 positive exosomes in HCT116 culture supernatant

【V】Kit expiry date and storage

Expiry date : 6 months after the manufacturing date.

(The manufacturing date is indicated on the kit box label)

Storage : Refrigeration (2-8℃)

【Reference】

1) S. Yang, et al.: *Oncol Lett*., **13**, 898 (2017).

2) Y. Yoshioka, et al.: *Nat Commun*., **5**, 3591 (2014).