Exorapid-qIC®
Immunochromatographic kit for extracellular vesicles (CD9)
[Cat.No. :DNT-EXO-K01]
Instruction Manual

Handling Precautions

- This product is for research use only.
  ※Since it is not subject to the Pharmaceuticals and Medical Devices Act, it cannot be used for diagnostic purposes.
- Store in a cool, dark place (4℃) away from heat and humidity.
- Please pay attention to the following points when using the Immunochromatographic Test Strip (Contents No.1).
  ①Do not use near open flames
  ②Moisten before disposal
  ③Avoid storing at room temperature
  ④Avoid direct sunlight
  ⑤Avoid impact and friction
- Dispose of waste liquid and other waste materials in accordance with local regulations.
- Wear recommended personal protective equipment (lab coat, safety glasses, mask, rubber gloves, etc.).
- Wash hands thoroughly after handling.
- Be careful not to inhale dust etc.
- If the solution gets into your eyes or mouth, immediately rinse thoroughly with water for several minutes. If irritation persists, seek medical advice.

➢ Introduction

Exorapid-qIC® is a rapid and simple kit for quantifying extracellular vesicles in samples.

➢ Kit Component

No.	Component	Quantitiy
1	CD9 antibody coated Immunochromatographic Test strip	40 strips※1
2	Gold nanoplate labeled antibody (CD9) [lyophilized product]	1 bottle (Equivalent to 1400 µL) ※2
3	Standard substance (Recombinant CD9 Protein :rCD9) [lyophilized product]	1 bottle (Equivalent to 105 ng) ※3
4	Dilution solution	1 bottle（1.2 mL）
5	Washing solution	1 bottle（10 mL）
6	Assay microplate 96 Well	1 plate
7	Instruction manual	1 copy

※1. The number of strips are contained about 10% (4 strips) more as a reserve.

※2. 40 analyses (1200 µL) + 200 µL reserve.

※3. Dissolve in 140 µL of purified water to prepare a solution with a concentration of 0.75 ng/µL.

➢ Materials Not Included in the kit

Micropipettes	Paper towels
Tabletop mixer (for tubes)	Microtube (recommended volume: 0.5-2.0 mL)
Tweezers	Purified water※4
Tapes	Tabletop Centrifuge

Immunochromatography measuring device or scanner

※4. Pure water, ultrapure water, distilled water, etc.

➢ Precautions for use

Please open the aluminum pouch and remove the freeze-dried product contents and Immunochromatographic Test Strips after returning them to room temperature.

Contents may be attached to the cap or inside wall of the tube.

Before opening, please remove any dirt by flushing with a tabletop centrifuge.

➢ Outline

Step0. Preparation

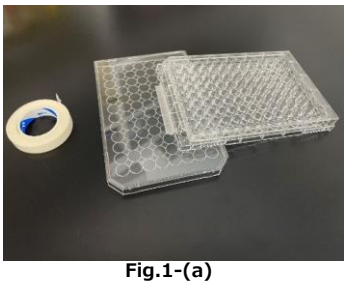


Fig.1-(a)

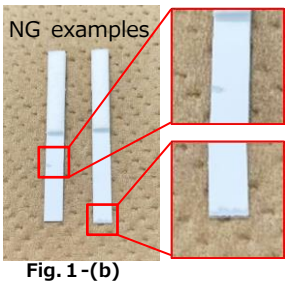


Fig.1-(b)

1. Setting up the microplate. [**Fig.1-(a)**]
   ☞Placing the lid underneath, can slope a plate to make it easier to spread samples.
2. Remove the required number of test strips. Do not use any that have large pieces of peeling off. [**Fig.1-(b)**]

Step1. Development of Samples

10 min.

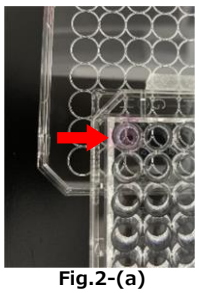


Fig.2-(a)

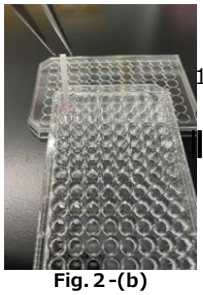


Fig.2-(b)

10 min. Later

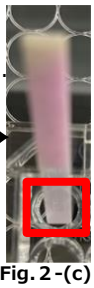


Fig.2-(c)

1. Inject 20 μL of the standard substance (rCD9 solution) and your sample solution into each well. [**Fig.2-(a)**]
2. Place the test strip in each well with the absorbent paper up.[**Fig.2-(b)**]
3. Check that the solution in the wells has disappeared. [**Fig.2-(c)**]
   ☞Once samples has been developed, proceed immediately to the next step to prevent the test strips from drying out.

Step2. Development of Washing solution (1st time)

5 min.

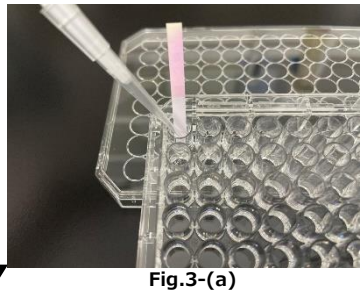


Fig.3-(a)

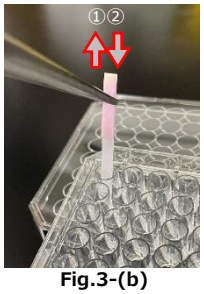


Fig.3-(b)

Inject 10 μL of Washing solution into the same wells as in **Step 1.** [**Fig.3-(a)**]

☞Make sure that the washing solution (droplets) falls to the bottom of the well.

☞If you use tweezers to lift the test strip and place it back into the same well, the water will be developed more quickly. [**Fig.3-(b)**]

Step3. Development of Gold nanoplate labeled antibodies

15 min.

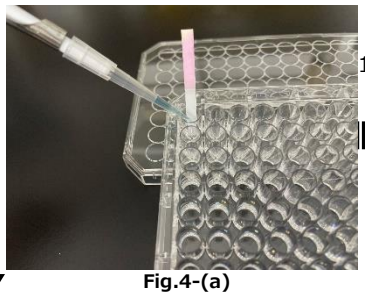


Fig.4-(a)

15 min. later

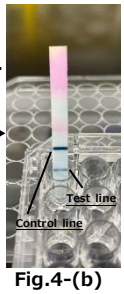


Fig.4-(b)

1. Inject 30 μL of Gold nanoplate labeled antibody solution into the same wells as in **Steps 1, 2**. [**Fig.4-(a)**]
   ☞ Please refer to the notes in **Step 2.**
2. When the Gold nanoplate labeled antibody solution is developed, the test line (lower side) and control line (upper side) gradually become colored.

Step4. Development of Washing solution (2nd time)

15 min.

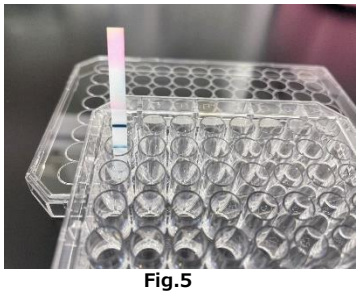


Fig.5

Add 50 μL of washing solution to each new well. [**Fig. 5**]

☞ Please refer to the notes in **Step 2.**

Analysis

➢ Protocol

<Detection Test>

1 **Preparation of standard substance (rCD9) solution for calibration curve**

Add 140 µL of purified water to a tube of rCD9 (lyophilized product) and dissolve by stirring using a tabletop mixer (rCD9 stock solution: concentration 15 ng/20µL).

Prepare three microtubes and prepare rCD9 diluents ① to ③ with different concentrations according to Table 1. Keep on ice until use. The data points for the calibration curve will be 0 (sample dilution solution), 2 (diluent ③), 4 (diluent ②), and 8 (diluent ①) [ng/20 µL].

Table1. rCD9 Diluent Solutions Preparation Table (n=3※5)

Abbreviation	rCD9 concentration (ng/20 µL)	Dilution solution preparation table (µL)				Preparation amount (µL)	Final Volume (µL)
		rCD9 stock solution (15 ng/20 µL)	Diluent ① (8.0 ng/20 µL)	Diluent ② (4.0 ng/20 µL)	Dilution solution		
Diluent ①	8.0	65.6	-	-	57.4	123	70.5
Diluent ②	4.0	-	52.5	-	52.5	105	70
Diluent ③	2.0	-	-	35	35	70	70

2 **Preparation of sample diluents**

Add Dilution solution to your sample solution containing extracellular vesicles so that the amount of diluents spread on each immunochromatographic test strip is 20 µL (e.g., 5 µL of your sample solution + 15 µL of Dilution solution).

After dilution, sample diluents are left on ice for 1 hour (to prevent clogging of the samples during immunochromatography).

3 **Preparation of Gold nanoplate labeled antibody solution**

Add 1400 µL of purified water to the Gold nanoplate labeled antibody (lyophilized product) and dissolve by stirring using a tabletop mixer.

4 **Injection of each developing solution and development of the solutions onto Immunochromatographic Test Strips**

Inject 20 µL of ① Dilution solution (blank solution), ② rCD9 solution of each concentration prepared in "1 Preparation of standard substance (rCD9) solution for calibration curve", and ③ sample diluents prepared in "2 Preparation of sample diluents" into separate wells.

Remove the Immunochromatographic Test Strip that has been brought to room temperature from the aluminum pouch and place it on the paper towels without touching the bottom of the test paper. Discard any that have significant peeling.

Using tweezers, soak the strip vertically into the wells into which the above solutions have been poured, allowing each solution to developed into the Immunochromatographic Test Strip (time required: about 10 minutes).

5 **Washing of Immunochromatographic Test Strips**

After confirming that each solution has been fully developed, inject 10 µL of Washing solution into the same well and re-soak the Immunochromatographic Test Strip (time required: about 5 minutes).

6 **Development of Gold nanoplate labeled antibodies**

Add 30 μL of Gold nanoplate labeled antibody solution to the same well and re-soak the Immunochromatographic Test Strip.

At this stage, the test line and control line will turn blue (time required: about 15 minutes).

7 **Washing of Immunochromatographic Test Strips**

Inject 50 μL of Washing solution into an unused well and soak the Immunochromatographic Test strip in the Washing solution for 15 minutes.

8 **Quantification of extracellular vesicles in samples**

The intensity of the test line is measured using ①CMS※6, ②ImageJ※7, ③an immunochromatographic measuring device, etc.

A caliburation curve is formed from the rCD9 detection results, and the amount of extracellular vesicles (equivalent to CD9) in samples are calculated.

※5. Recommend testing with n=3

※6. Abbreviation for Color Management System released by TOPPAN Corporation. **[Currently under consideration for implementation]**

(Release article : https://www.holdings.toppan.com/ja/news/2024/02/newsrelease240227_1.html)

※7. See the next page for the analysis method using ImageJ.

<Analysis method　Example: Staining intensity measurement using ImageJ>

1 Photographing the test strip

After samples are spread, the test paper is photographed with a digital camera or scanner, and the image data is saved in JPEG format.

☞ It will be easier to photograph it if you attach it to a mount or similar before photographing it.

2 Preparation of ImageJ

① Download

Download the public domain image processing software "Image J" from the following URL.

URL : https://imagej.net/ij/ (As of July 22, 2024)

Select "ImageJ" from the downloaded file set and launch it.

For detailed basic operations, please refer to the following URL.

URL : https://imagej. nih. gov/ij/docs/guide/user-guide. pdf(As of July 22, 2024)

② Setup

Select the “Analyze” tab → “Set Measurements” and check “Area” and “Mean grey value”.

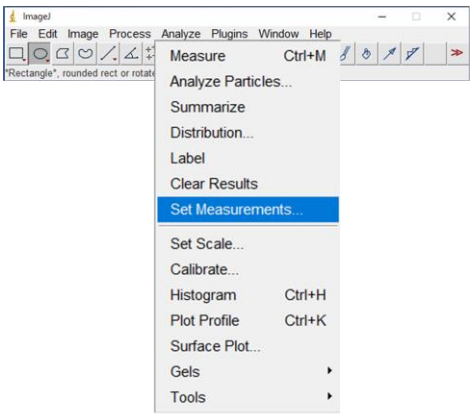


Fig.6-(a)

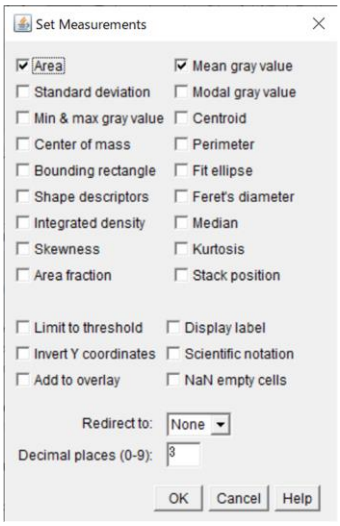


Fig.6-(b)

3 Loading test strips image

Select the “File” tab → “Open” to load the image file.

Images in formats other than JPEG may not be analyzed correctly, so convert them to JPEG before use.

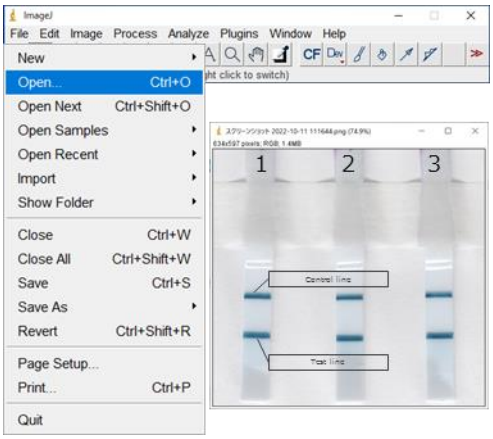
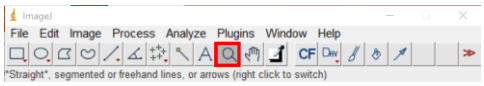


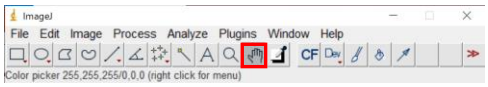
Fig. 7

☞ Magnifier tool



Click on the tool to zoom the image.

☞ Scroll tool



To move the image in the window, select this tool and move it.

※If you want to continue the analysis after using the above tools, please select a new rectangular ROI first.

4 ROI Managerを開く

Select the Rectangle ROI (rectangle area selection tool) from the toolbar.

Open “Analyze” tab → “Tools” → “ROI Manager”.

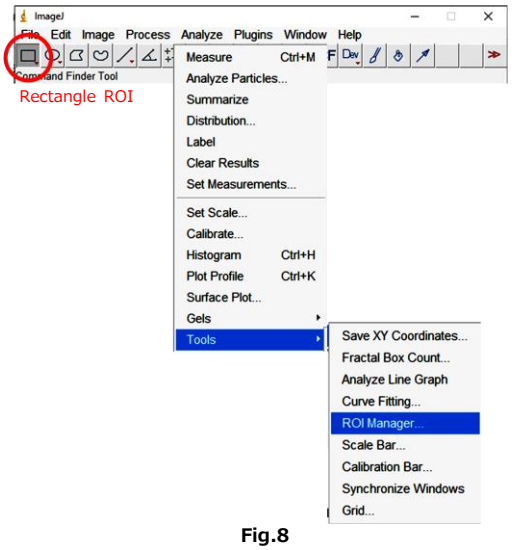


Fig.8

5 Test line, background staining intensity measurement

① Enter test line information

Draw a rectangle around the test line area[※7] in the image and type "T" on the keyboard to enter the information about the area into the ROI Manager.

② Enter background information

Drag and drop a rectangle of the same area as the test line area to select the uncolored part of the test paper, and enter the background area information into the ROI Manager using the same procedure as in ①.

Repeat steps ① and ② above for all test strips to be analyzed.

Check "Show All" and "Labels" to display the selection area and numbers on the image, and check that the selection area and order are correct.

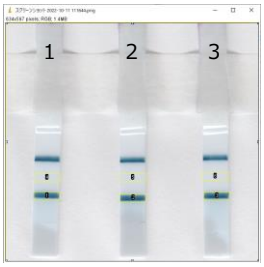


Fig.9-(a)

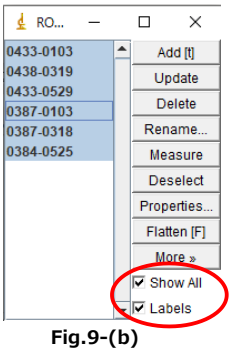


Fig.9-(b)

※7. If the amount detected is small, the center of the test line may be faint, but this does not affect the quality.

6 Detection strength calculation

Select the information entered in the ROI Manager in the previous section, and click "Measure" in the ROI Manager window. The Results window will open and the average luminance (Mean) of the area enclosed in a rectangle will be displayed. Select all of these, paste them into Excel, and subtract the "Mean" of the test line from the "Mean" of the background to calculate the staining intensity (detection intensity).

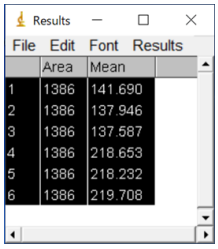


Fig.10-(a)

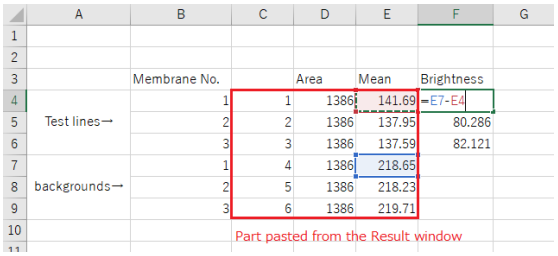


Fig.10-(b)

7 Quantification of extracellular vesicles in samples

The amount of extracellular vesicles (equivalent to CD9) in samples are calculated using a calibration curve formed from the results of rCD9 luminance analysis.[※9,※10]

※9. Detection lines may appear in blank tests. This does not affect the quality of the product.

※10. We recommend using a polynomial (quadratic) calibration curve.

➢ Contact

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