FUJIFILM
Value from Innovation

Code No. 294-85701

EV-Perm™ Permeabilization Pretreatment Kit for Exosome Membrane

【Product Information】

Code No.	Product Name	Volume	Storage
294-85701	EV-PermTM Permeabilization Pretreatment Kit for Exosome Membrane	1 kit	Frozen（-20℃）

【Kit Contents】

Reagent	Volume	Storage
Reagent A（20×）	500 µL×1	Frozen（-20℃）
Reagent B（10×）	5 mL×1	Before thawing : -20℃ After thawing : 2~10℃
Reagent C（100×）	500 µL×1	Before thawing : -20℃ After thawing : 2~10℃

【How to store each reagent】

Store each reagent acoording to the storage condition above.

Preparation of reagents

1. Reagent A（20×）

Following thaw, mix the vial by gently tapping, and prepare aliquots. Close the lid tightly and immediately store the remaining solution in the freezer (-20℃).

- If stored in a liquid state for a long time, components may precipitate.
- If precipitation is observed, heat it at 60℃ for about 30 minutes to dissolve it, then store it in the freezer.

2. Reagent B（10×）and Reagent C（100×）

Following thaw, mix the vial by gently tapping, and prepare aliquots. Close the lid tightly and immediately store the remaining solution in the refrigerator (2～10℃).

【About this product】
This product is a reagent that enables the detection of internal markers of extracellular vesicles. Until now, the detection targets of the PS Capture™ Exosome ELISA Kit Series and PS Capture™ Exosome Flow Cytometry Kit were limited to the detection of surface markers of extracellular vesicles. However, when used in combination with this product, it is possible to improve the permeability of the extracellular vesicle membrane surface and detect internal markers.

For PS Capture™ Exosome ELISA Kit

【Equipment and reagents to be supplied by user】

① ELISA Kit（□ Check）:

□ One of the following kits

Code No.	Product Name
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin HRP)

② Reagents（□ Check）:

□ Distilled Water

□ Specific antibody for Extracellular vesicle internal protein

If using the PS Capture™ Exosome ELISA Kit (anti-mouse IgG POD), please prepare a mouse monoclonal antibody, and if using the PS Capture™ Exosome ELISA Kit (Streptavidin HRP), prepare a biotin-labeled antibody.

③ PS Capture™ Exosome ELISA Kit included reagents

□ Washing Buffer（10×）

□ Exosome Binding Enhancer（100×）

□ Reaction Buffer

Please use the Exosome Binding Enhancer and Reaction Buffer in the PS Capture™ Exosome ELISA Kit. Even if you use Buffer with this kit, you will still have Buffer for the PS Capture™ Exosome ELISA Kit.

④ Equipments（□ Check）:

□ Microwell plate (PS Capture™ Exosome ELISA kit accessory)

□ Plate seal (PS Capture™ Exosome ELISA kit accessory)

□ Microplate shaker

□ Microplate reader

【Prepartaion of samples】

For PS Capture™ Exosome ELISA Kit (anti-mouse IgG POD)

It is necessary to optimize the concentration of extracellular vesicles used to detect the target marker using this product. The optimal concentration of extracellular vesicles was determined using the PS Capture™ Exosome ELISA kit (anti-mouse IgG POD) such that the resulting absorbance (450 nm minus 620 nm) was in the range of 0.2 to 2.5. To adjust the concentration of extracellular vesicles, dilute them with the Reaction/Wash Buffer (1x) included in the kit.

For PS Capture™ Exosome ELISA Kit (Streptavidin HRP)

It is necessary to optimize the concentration of extracellular vesicles used to detect the target marker using this product. The optimal concentration of extracellular vesicles was measured using the PS Capture™ Exosome ELISA Kit (Streptavidin HRP) and adjusted so that the resulting absorbance (450 nm minus 620 nm) was in the range of 0.2 to 2.5. To adjust the concentration of extracellular vesicles, dilute at least two times with the Reaction Buffer included in the kit.

【Prepartion of reagents】

1. Preparation of permeabilization solution

Dilute each reagent, referring to Table 1 below. After dilution, mix well.

Table 1. Dilution ratio and amount of reagent added for each reagent used in permeabilization solution

Reagent	Dilution	12 samples※ (24 wells)	24 samples※ (48 wells)	48 samples※ (96 wells)
Distilled Water	-	2.1 mL	4.2 mL	8.4 mL
Reagent A（20×）	1:20	125 µL	250 µL	500 µL
Reagent B（10×）	1:10	250 µL	500 µL	1.0 mL
Reagent C（100×）	1:100	25 µL	50 µL	100 µL
Total	**-**	**2.5 mL**	**5.0 mL**	**10.0 mL**

※ For performing in duplicate.

2. Preparation of negative control solution

Dilute each reagent referring to Table 2 below. After diluting, mix well

(Do not add Reagent A when preparing the negative control solution.)

Table 2. Dilution ratio and amount of reagent added for each reagent used for negative control

Reagent	Dilution	12 samples※ (24 wells)	24 samples※ (48 wells)	48 samples※ (96 wells)
Distilled Water	-	2.2 mL	4.5 mL	8.9 mL
Reagent B（10×）	1:10	250 µL	500 µL	1.0 mL
Reagent C（100×）	1:100	25 µL	50 µL	100 µL
Total	-	**2.5 mL**	**5.0 mL**	**10.0 mL**

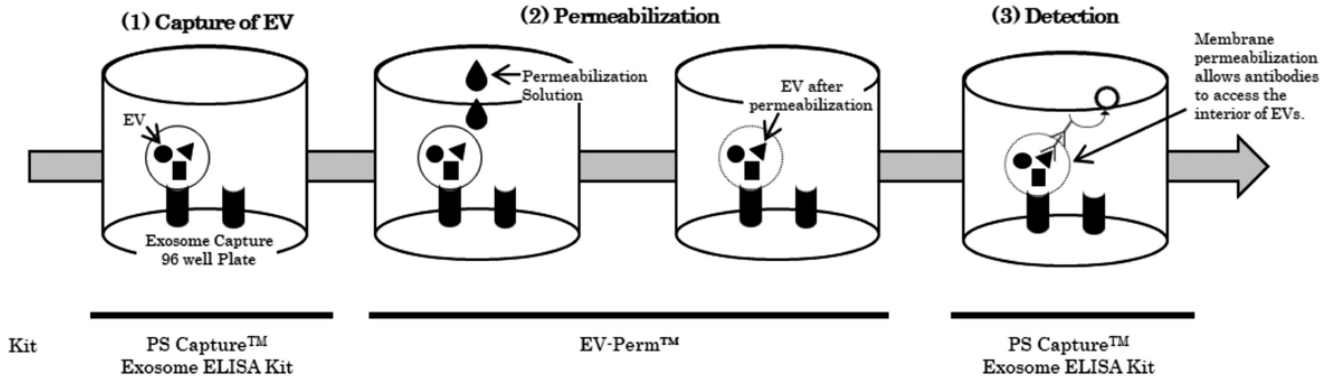
※ For performing in duplicate.

3. Preparation of washing solution (1x)

Washing Buffer (10x) included with the PS Capture™ Exosome ELISA Kit was diluted 10 times with purified water. After that, add 1/100 amount of Exosome Binding Enhancer (100x) to the diluted solution and mix well. 10

【Procedure for detection using EV-Perm™】

■ If you use EV-Perm™ in conjunction with the PS Capture™ Exosome ELISA Kit series



【Procedure】

1. Process Flow

Process			Reagent・Equipment	Time
1.	Capture Exosome	Washing the plate	Washing buffer（1×）	>2 hrs
		Capture Exosome on the plate	Sample Capture （1× Reaction Buffer）	
		Washing the plate	Washing buffer（1×）	
2.	Permeabilization	Permeabilization※	① **permeabilization solution** ② **negative control solution**	>1 hr
		Washing the plate	Washing buffer（1×）	

※ The permeabilization solution and negative control solution are separate for each sample and detection condition in the permeabilization process.

The following steps are performed according to the PS Capture™ Exosome ELISA kit.

Process			Reagent・Equipment	Time
3.	1st antibody reaction	1st antibody reaction	Add detection antibody	1 hr
		Washing the plate	Washing buffer（1×）	
4.	2nd antibody / streptavidin-HRP reaction	2nd antibody / streptavidin-HRP reaction	2nd antibody / streptavidin-HRP reaction	>1 hr
		Washing the plate	Washing buffer（1×）	
5.	Substrate	Substrate addition	TMB Solution Stop Solution	>30 min
		ELISA plate reading	Microplate reader	

2. The procedure of #2 permeabilization in the process flow above.

(1) Follow the instructions for the PS Capture™ Exosome ELISA kit and proceed to the washing step Procedure# 4 before adding the 1st antibody reaction solution.

(2) Add 100 µL of permeabilization solution or 100 µL of negative control solution to the extracellular vesicle sample (well).

(3) Apply a plate seal and incubate at room temperature for 1 hour while stirring at 500 rpm using a microplate shaker.

(4) Decant the reaction solution and wash each well three times with 300-350 µL of washing solution (1x).

(5) From the process flow "3. 1st antibody reaction", proceed to step "[Procedure] 5)" according to the PS Capture™ Exosome ELISA Kit instruction manual.

Please refer to the PS Capture™ Exosome ELISA Kit Instruction Manual for post-measurement calculations and analysis methods.

For PS Capture™ Exosome Flow Cytometry Kit

【Equipment and reagents to be supplied by user】

① PS CaptureTM Exosome Flow Cytometry Kit（□ Check）:

□ The Kit

Code No.	Product Name
297-79701	PS Capture™ Exosome Flow Cytometry Kit

② Reagents（□ Check）:

□ Distilled Water

□ Specific antibody for Extracellular vesicle internal protein

Please prepare fluorescent labeled antibodies.

③ PS Capture™ Exosome Flow Cytometry Kit included reagents

□ Washing Buffer（10×）

□ Exosome Binding Enhancer（100×）

□ Exosome Magnetic Beads

Please use the Exosome Binding Enhancer and Reaction Buffer in the PS CaptureT™ Exosome Flow Cytometry Kit. Even if you use Buffer with this kit, you will still have Buffer for the PS Capture™ Exosome Flow Cytometry Kit.

④ Equipments（□ Check）:

□ Centrifuge tubes（15 mL）

□ Centrifuge tubes（1.5 mL）

□ Vortex Mixer

□ Desktop Centrifuge

□ Magnetic stand

□ Flow Cytometry

【Prepartion of reagents】

1. Preparation of permeabilization solution

Dilute each reagent, referring to Table 1 below. After dilution, mix well.

Table 1. Dilution ratio and amount of reagent added for each reagent used in permeabilization solution

Reagent	Dilution	2 Reactions (100 µL) ※	4 Reactions (167 µL) ※	8 Reactions (300µL) ※
Distilled Water	-	126 µL	184.8 µL	294 µL
Reagent A（20×）	1:20	7.5 µL	11 µL	17.5 µL
Reagent B（10×）	1:10	15 µL	22 µL	35 µL
Reagent C（100×）	1:100	1.5 µL	2.2 µL	3.5 µL
Total	**-**	**150 µL**	**220 µL**	**350 µL**

※ For the amount to add, please refer to Table 2, "Sample (µL)" in the instruction manual of the PS Capture™ Exosome Flow Cytometry Kit.

2. Preparation of negative control solution

Dilute each reagent referring to Table 2 below. After diluting, mix well （Do not add Reagent A when preparing the negative control solution.)

Table 2. Dilution ratio and amount of reagent added for each reagent used for negative control※

Reagent	Dilution	2 Reactions (100 µL) ※	4 Reactions (167 µL) ※	8 Reactions (300µL) ※
Distilled Water	-	133.5 µL	195.8 µL	311.5 µL
Reagent B（10×）	1:10	15 µL	22 µL	35 µL
Reagent C（100×）	1:100	1.5 µL	2.2 µL	3.5 µL
Total	**-**	**150 µL**	**220 µL**	**350 µL**

※ For the amount to add, please refer to Table 2, "Sample (µL)" in the instruction manual of the PS CaptureTM Exosome Flow Cytometry Kit.

3. Preparation of WB（+Enhancer）

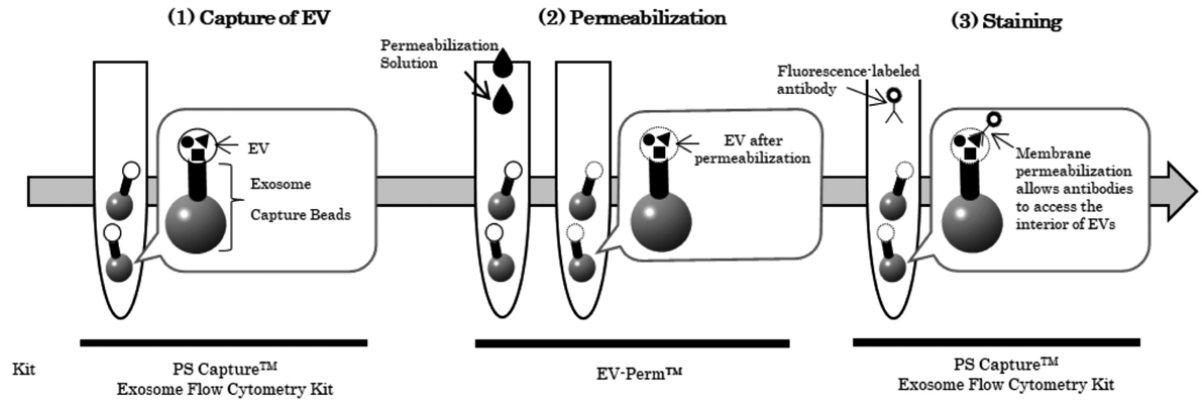
Add 0.5 mL of Washing Buffer (10X) and 4.5 mL of purified water to a 15 mL centrifuge tube and mix by vortexing. Add 50 µL (1/100 volume) of Exosome Binding Enhancer (100x) and mix on a vortex mixer.

This is the liquid volume for a reaction system (2 reactions) using a 1.5 mL microtube. If you wish to increase the reaction scale, please scale up according to Table 2 in the instruction manual included with the PS Capture™ Exosome Flow Cytometry Kit.

4. Preparation of extracellular vesicle-bound magnetic beads

Prepare according to "2. Isolation of extracellular vesicles" in the instruction manual included with the PS Capture™ Exosome Flow Cytometry Kit.

■ If you use EV-Perm™ in conjunction with the PS Capture™ Flow Cytometry Kit



【Procedure】

1. Process Flow

Process			Reagent・Equipment	Time
1.	Exosome Isolation	Exosome Isolation	WB（+Enhancer） Exosome Capture Beads	>70 mins
		Capture Exosome	WB（+Enhancer）	
2.	Permeabilization	Permeabilization※	① **Permeabilization solution** ② **Negative control solution**	>1 hr
		Washing	WB（+Enhancer）	

The following steps are performed according to the PS Capture™ Flow Cytometry kit.

Process			Reagent · Equipment	Time
3.	Antibody incubation	Antibody incubation	fluorophore-conjugated antibody or fluorophore-conjugated isotype control	>1 hr
		Washing	WB（+Enhancer）	
4.	Detection	Detection	Flow Cytometry	

※ The permeabilization solution and negative control solution are separate for each sample and detection condition in the permeabilization process.

2. Protocol (process flow “2. Permeabilization” when analyzing one type of extracellular vesicle marker)

(1) Follow the instructions for the PS Capture™ Exosome Flow Cytometry Kit and proceed to “2. Isolation of extracellular vesicles” to prepare extracellular vesicle magnetic beads (700 µL) for 6 reactions.

(2) Dispense 300 µL of extracellular vesicle-bound magnetic beads into two microtubes.

(3) Centrifuge the microtube and spin down the magnetic beads.

(4) Leave the microtube on the magnetic stand for 1 minute, and remove the supernatant with a pipette.

(5) Add 300 µL of WB (+Enhancer) to the microtube and mix by vortexing.

(6) Repeat steps (3) to (5) twice.

(7) Add 100 µL of permeabilization solution or 100 µL of negative control solution to each tube and mix by vortexing.

(8) Leave the microtube from (7) at room temperature, mix by vortexing after 20 and 40 minutes, and permeabilize the exosomes for 1 hour.

(9) Leave the microtube on the magnetic stand for 1 minute, and remove the supernatant with a pipette.

(10) Add 300 µL of WB (+Enhancer) to the microtube and wash by vortexing.

(11) Repeat washing steps (10) to (11) twice.

(12) Add 300 µL of WB (+Enhancer) to the microtube and mix by vortexing.

(13) From the process flow “3. Staining of extracellular vesicles”, proceed to step “3. Staining of extracellular vesicles” according to the PS Capture™ Exosome Flow Cytometry Kit instruction manual.

Please refer to the PS Capture™ Flow Cytometry Kit Instruction Manual for post-measurement calculations and analysis methods.

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