FUJIFILM

Wako

Code No. 294-84101 (2 purifications)
290-84103 (10 purifications)

For Genetic Research

MagCapture™ Exosome Isolation Kit PS Ver.2

【Introduction】

Extracellular Vesicles (EVs) represented by exosomes contain a protein, mRNA, microRNA, DNA, and lipids on the surface or inside. After being secreted from various cells, EVs stably exist in body fluids such as blood, urine, saliva, cerebrospinal fluid, and breast milk. Thus, it is attracting attention as a messenger for cell-to-cell communication and as a biomarker for diseases. As a method for isolating exosomes, an ultracentrifugation method, an affinity method using an antibody against a surface antigen, a precipitation method using a polymer reagent etc. are generally used. However, in all respects such as recovery efficiency, purity, and operability, there was no satisfying way.
MagCapture™ Exosome Isolation Kit PS Ver.2 adopts a novel affinity purification method using magnetic beads and phosphatidylserine (PS)-binding protein (PS affinity method). The PS affinity method can easily isolate exosomes and other EVs with PS on the membrane surface from the cell culture medium and body fluids with high purity and high efficiency. Using the 10,000×g supernatant as a sample, PS affinity can get more highly purified exosomes. Besides, PS affinity enables the elution of intact EVs from magnetic beads with a chelating agent because PS affinity captures EVs in a metal ion-dependent manner. Therefore, purified EVs can be used for various applications such as electron microscopic analysis, nanoparticle tracking analysis, uptake assay, and analysis of constituent molecules (proteins, lipids, nucleic acids, etc.).

【Feature】

- Adopted new affinity method (PS affinity method).
- Can obtain EVs with higher yield and higher purity than ultracentrifugation.
- Enables the elution of intact EVs, and can be used for various applications.
- Easy operation with magnetic beads.
- No ultracentrifugation is required.

【Kit contents】

This kit includes 6 components.

・2 purifications

(1) Biotin Capture Magnetic Beads	120 μL×1 tube
(2) Biotin-labeled Exosome Capture	20 μL×1 tube
(3) Exosome Immobilizing/Washing Buffer (10×)	5 mL×1 bottle
(4) Exosome Binding Enhancer (500×)	300 μL×1 tube
(5) Exosome Elution Buffer (10×)	300 μL×1 tube
(6) Reaction Tubes	4 tubes

・10 purifications

(1) Biotin Capture Magnetic Beads	600 μL×1 tube
(2) Biotin-labeled Exosome Capture	100 μL×1 tube
(3) Exosome Immobilizing/Washing Buffer (10×)	25 mL×1 bottle
(4) Exosome Binding Enhancer (500×)	1500 μL×1 tube
(5) Exosome Elution Buffer (10×)	1500 μL×1 tube
(6) Reaction Tubes	22 tubes

【Storage】

Store at 2-10℃

【Additional required materials】

Reagents :

1) Distilled Water
2) TBS (as necessary)

Equipment :

1) Microcentrifuge (Max > 10,000×g)
2) Vortex mixer
3) Tabletop centrifuge
4) Magnetic stand
5) Rotator or tube mixer
6) Micro pipette
7) Pipette tip
8) Microcentrifuge tube (1.5 mL)
9) Centrifuge tube (15 mL) (as necessary)
10) Centrifuge tube (50 mL) (as necessary)
11) Ultrafiltration unit (Vivaspin6, M.W. cut off : 100 K, Code No. VS0641 or Vivaspin20, M.W. cut off : 100 K, Code No. VS2041) (as necessary)

【Precaution for use】

1. Equipment

Please use sterile or DNase and RNase free microcentrifuge tubes and pipette tips. We recommend the use of gloves and a mask to avoid contamination of DNase and RNase.

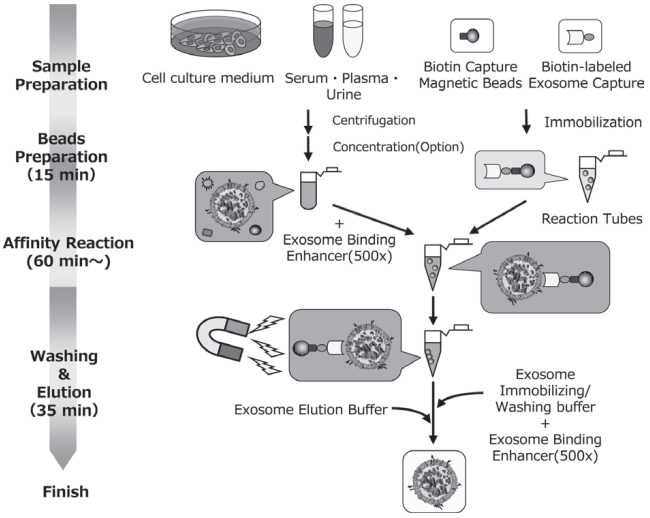
2. Reagents

If you analyze the nucleic acid (RNA, DNA) after isolation of exosomes and other EVs, please handle reagents carefully and keep them as sterile as possible. Additionally, if you analyze the mass spectrometry of proteins after isolation of exosomes and other EVs, please be careful of contamination from other proteins.

3. Handling of biohazardous waste

Please treat human serum, human plasma, and human tissue samples as infectious samples. Biohazardous wastes, which include waste liquid and equipment such as microcentrifuge tubes, pipette tips, and gloves, must be disposed of according to the guidelines of the institution.

【Outline of procedure】



【Procedure】

1. Preparation of Sample

This is the section to prepare samples. When exosomes and other large EVs are needed, prepare **1,200×g supernatant** as a sample. Additionally, when highly purified exosomes are needed, prepare **10,000×g supernatant** as a sample※1. This sample preparation protocol describes the protocol when using cell culture supernatant and serum/plasma sample. When using other body fluid samples, refer to the serum/plasma sample protocol and consider the appropriate pretreatment protocol.

※1 To remove Large EVs such as apoptotic bodies and microvesicles from a sample, use a centrifugal filter unit (Millipore, Ultrafree-MC, GV 0.22μm sterile, Code No : UFC30GV0S) after centrifuging the supernatant at 10,000×g for 30 minutes. The filtrate can be used as a test sample.

In the case of cell culture medium

1) Culture the cells under the appropriate condition※2.
2) Harvest cell culture medium.
3) Centrifuge the cell culture medium for 5 minutes at 300×g at 4℃ to remove cells and debris.
4) Transfer the supernatant from step 3) into a new tube.
5) Centrifuge 4) at 1,200×g for 20 minutes at 4℃ to remove the cell debris.
6) Transfer the supernatant from step 5) into a new tube.… **1,200×g supernatant**
7) Centrifuge 6) at 10,000×g for 30 minutes at 4℃ to remove the large EVs.
8) Transfer the supernatant from step 7) into a new tube.… **10,000×g supernatant**

〔**Optional : Concentration of cell culture supernatant by ultrafiltration**〕

If the volume of **1,200×g supernatant** or **10,000×g supernatant** is over 1 mL, concentrating the sample to 1 mL or less is available with ultrafiltration unit. The maximum sample volume as ultrafiltration is 50 mL (50-fold concentration). However,

although EVs can be obtained efficiently by concentrating the sample, it may cause physical damage or inactivation of biological activity. Please consider the appropriate processing method according to the purpose of use.

Precaution for concentration by ultrafiltration

Since EVs may be adsorbed to the centrifugal ultrafiltration unit, and the recovery amount may be reduced, we recommend adding EV-Save™ Extracellular Vesicle Blocking Reagent (Code No : 058-09261) (sold separately) to the sample for preventing loss of EVs. However, this product contains a polymer component, please be careful when using it as a sample for proteomics analysis※3.

※2 Please examine appropriate culture conditions depending on cell lines. Since the amount of EVs in the medium is small, please increase the culture scale as much as possible. (Example : 20 mL or more is better) Besides, to ensure that isolated EVs originate from your cells of interest, please use EV-depleted FBS prepared by ultracentrifugation (Example : at 110,000×g for 18 hours) or commercialized products.

※3 When performing proteomics analysis, remove the polymer by appropriate preprocessing such as acetone precipitation.

In the case of Serum and Heparin Plasma

Please centrifuge serum and heparin plasma used in this section at 1,200×g beforehand or prepare the processed sample.

When exosomes and Large EVs are needed, use 1,200×g sup. fraction as a sample for purification. If a floating matter is observed in 1,200×g centrifuged samples, centrifuge again at 1,200×g and use as a sample for exosomes and Large EVs purification.

1) Centrifuge a sample at 10,000×g for 30 minutes at 4℃ to remove the large EVs※4.
2) Transfer the supernatant from step 1) into a new tube.… (**10,000×g supernatant**)

※4 When Large EVs are needed, use ppt of Large EVs obtained by centrifugation at 10,000×g as a sample after suspending it with 500μL to 1 mL of TBS.

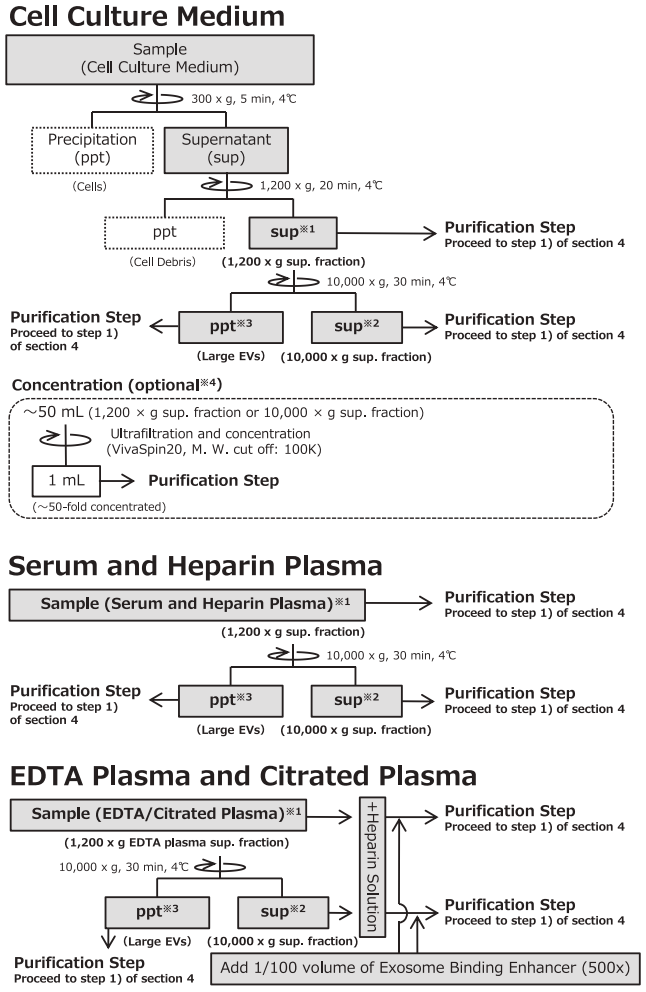
In the case of EDTA Plasma and Citrated Plasma

Please centrifuge EDTA plasma and citrated plasma used in this section at 1,200×g beforehand or prepare the processed sample.

In the case of EDTA and citrated plasma, the anticoagulant contained in the sample inhibits the binding of EVs to Exosome Capture Beads. Therefore, adjust the heparin sodium solution just before the affinity reaction, add it to the centrifuged sample, and proceed to the purification step. Details are described in the section on **EDTA Plasma and Citrated Plasma** in 4. Affinity Reaction.

1) Centrifuge a sample at 10,000×g for 30 minutes at 4℃ to remove the large EVs※4.
2) Transfer the supernatant from step 1) into a new tube.… (**10,000×g supernatant**)

〔Flowchart of Sample Preparation (Section 1)〕



※1 When **exosomes and large EVs** are needed, use 1,200 × g sup. fraction as sample.
※2 When **exosomes** are needed, use 10,000 × g sup. fraction as sample.
※3 When **Large EVs** are needed, use ppt of Large EVs obtained by centrifugation at 10,000 x g as sample after suspending it with TBS.
※4 The concentration step is an option when using a large volume (~ 50 mL) of cell culture supernatant as a sample for purification.

2. Buffer Preparation

This section is the process of preparing the buffers used in each process. The volume of various buffers required for one sample is 5.0 mL (preparation volume : 5.5 mL) for Exosome Immobilizing/Washing Buffer (1×) and 100 μL (preparation volume : 150 μL) for Exosome Elution Buffer (1×). When performing the affinity reaction overnight, prepare the 3 mL of Exosome Immobilizing/Washing Buffer (1×) used for washing just before use.

Buffer	Required amount	Preparation volume
Exosome Immobilizing/ Washing Buffer (1×)	5.0 mL 2.0 mL → for the section 3 3.0 mL → for the section 5	5.5 mL
Exosome Elution Buffer(1×)	100 μL	150 μL

1) Add 0.55 mL of Exosome Immobilizing/Washing Buffer (10×) and 4.95 mL of distilled water to a 50 mL centrifuge tube, and add 11 μL of Exosome Binding Enhancer (500×) to prepare the Exosome Immobilizing/Washing buffer (1×)※5.
2) Add 15 μL of Exosome Elution Buffer (10×) and 135 μL of distilled water to a 1.5 mL microcentrifuge tube (not included in the kit) to prepare the Exosome Elution buffer (1×)※6, ※7.
3) Mix both 1) and 2) well and store at room temperature until use.

※5 Make sure to add 1/500 volume of Exosome Binding Enhancer (500×) to Exosome Immobilizing/Washing Buffer (1×). When not added, the recovery efficiency of exosome is significantly reduced.
※6 By adding EV-Save™ Extracellular Vesicle Blocking Reagent (Code No : 058-09261) (sold separately) to the Exosome Elution Buffer used in this process, the adsorption of EVs to the tube can be suppressed and the recovery efficiency can be increased. However, since this product contains a polymer component, please carefully consider its use for proteomics analysis before use.
※7 When using plasma (heparin, EDTA, and citric acid) as a sample, recovery efficiency may be improved by using 2× Exosome Elution Buffer. In that case, mix 30 μL of Exosome Elution Buffer (10×) and 120 μL of distilled water.

3. Immobilization of Exosome Capture

This is the section to immobilize Biotin-labeled Exosome Capture to Biotin Capture Magnetic Beads. Make sure to use the 1.5 mL Reaction Tube (included) in the downstream experiment using magnetic beads※8.

1) Transfer 60 μL of Biotin Capture Magnetic Beads, which has been well stirred with a vortex mixer, into a new 1.5 mL Reaction Tube.
2) Add 500 μL of Exosome Capture Immobilizing/Washing Buffer (1×) into the 1.5 mL Reaction Tube from step 1) and suspend it by vortexing.
3) Spin down 2), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant.
4) Add 500 μL of Exosome Capture Immobilizing/Washing Buffer (1×) and 10 μL of Biotin-labeled Exosome Capture into the 1.5 mL Reaction Tube from step 3) and mix by vortexing※9.
5) Mix for 10 minutes at 2-10℃ with rotator or tube mixer.
6) Spin down 5), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant.
7) Add 500 μL of Exosome Capture Immobilizing/Washing Buffer (1×) into the 1.5 mL Reaction Tube from step 6)

and mix by vortexing.

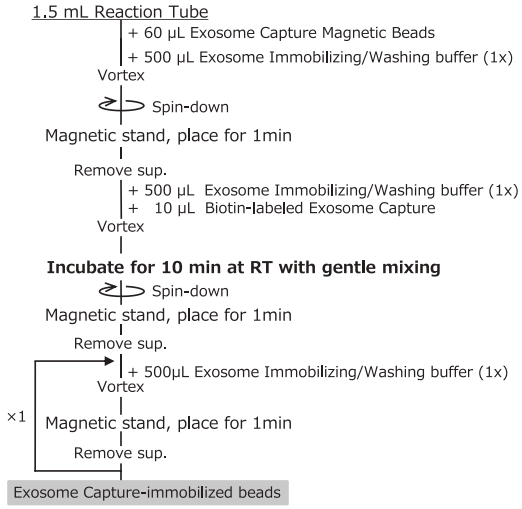
8) Spin down 7), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant.

9) Repeat steps7)-8) again.…**Exosome Capture-immobilized beads**

※8 The 1.5 mL Reaction Tube included in the kit can suppress the decrease in recovery rate due to bead adsorption because it uses a material that magnetic beads do not easily adsorb to the tube.

※9 Depending on the sample, recovery efficiency may be improved by reducing the amount of Biotin-labeled Exosome Capture. When recovery efficiency was low using the standard protocol, reduce the amount of Biotin-labeled Exosome Capture (Example : 10 μL → 2-5 μL).

〔Flowchart of immobilization of Exosome Capture (Section 3)〕



4. Affinity Reaction

This is the affinity reaction section to react the Exosome Capture-immobilized beads with exosomes and other EVs in a sample. The basic protocol use the 1.5 mL Reaction Tube (max volume : 1 mL). When exosomes and other large EVs are needed, use **1,200×g supernatant** as a sample. Additionally, when highly purified exosomes are needed, use **10,000×g supernatant** as a sample.

Note : The affinity reaction protocol for EDTA plasma and citrated plasma are different.

In the case of cell culture medium, serum, and heparin plasma

1) Transfer a sample (max volume : 1 mL)※10 to a new 1.5 mL microcentrifuge tube (not included in the kit) and add 1/500 volume of Exosome Binding Enhancer (500×) to the sample. Mix by vortexing.…**A sample including Exosome Binding Enhancer (500×)**

2) Spin down 1), then transfer it into the 1.5 mL Reaction Tube containing **Exosome Capture-immobilized beads**. Mix by vortexing.

3) Mix for 1 hours ～ at RT or 4℃ with rotator※11.

4) Spin down 3), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant※12.…**EVs-binding beads**

In the case of EDTA/citrated plasma

1) Dissolve 10,000 U of sodium heparin (Code No : 085-00134) with distilled water to make 1,000 U/mL heparin sodium solution※13.

2) Transfer a sample (max volume : 1 mL)※10 to a new 1.5 mL microcentrifuge tube (not included in the kit) and add 1/200 volume of heparin sodium solution to the sample. (final conc. : 5 U/mL)

3) Add 1/100 volume of Exosome Binding Enhancer (500×) to 2) and mix by vortexing.…**A sample including Exosome Binding Enhancer (500×)**

4) Spin down 3), then transfer it into the 1.5 mL Reaction Tube containing Exosome Capture-immobilized beads. Mix by vortexing.

5) Mix for 1 hours ～ at RT or 4℃ with rotator※11.

6) Spin down 5), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant※12.…**EVs-binding beads**

※10 When the sample volume is less than 0.5 mL, add TBS to scale up the sample volume to 0.5 mL or more to obtain the better mixture of the Exosome Capture-immobilized beads with a sample. (Example : 100-200 μL → 500 μL)

※11 Appropriate reaction time varies depending on the sample and sample volume. For obtaining a sufficient recovery amount, extend the reaction time as appropriate. Regarding the temperature, select either RT or 4℃ depending on the purpose of use of the purified exosome.

※12 When further recovery of remaining EVs is needed, please store the removed supernatant in another tube.

※13 The maximum usage per kit (for 10 purification) is 100 μL. After preparation, please store at 4℃.

5. Washing of EVs-binding beads

This is the washing section of EVs-binding beads.

Note : Make sure to add Exosome Binding Enhancer (500×) to Exosome Capture Immobilizing/Washing Buffer (1×).

1) Add 1 mL of Exosome Capture Immobilizing/Washing Buffer (1×) into the 1.5 mL Reaction Tube containing **EVs-binding beads** and mix by vortexing.
   In the case of EDTA/citrated plasma
   After adding 1/200 volume of heparin sodium solution to 1 mL of Exosome Capture Immobilizing/Washing Buffer (1×) only at the first washing (final conc. : 5 U/mL), suspend with a vortex mixer.

2) Spin down 1), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant.

3) Add 1 mL of Exosome Capture Immobilizing/Washing Buffer (1×) and mix by vortexing.

4) Spin down 3), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove

the supernatant.

5) Repeat steps 3)-4) one more time.

6) Spin down again the 1.5 mL Reaction Tube from step 6), then places it on the magnetic stand for 1 minute to separate the beads from the solution, and remove the supernatant completely※14.…**Washed EVs-binding beads**

※14 Please remove Exosome Capture Immobilizing/Washing Buffer (1×) in the tube completely, because the remaining buffer causes a reduction of elution efficiency of captured exosomes and other EVs.

6. Elution of Extracellular Vesicles

This section is the elution process of EVs from Washed EVs-binding beads.

1) Add 50 μL of Exosome Elution Buffer (1×) into the 1.5 mL Reaction Tube containing **Washed EVs-binding beads** and mix by vortexing, then spin down※15, ※16, ※17.

2) Places 1) on the magnetic stand for 1 minute to separate the beads from the solution, and transfer the supernatant into a new 1.5 mL microcentrifuge tube (not included in the kit).

3) Add 50 μL of Exosome Elution Buffer (1×) into the 1.5 mL Reaction Tube from step 2) again, and mix by vortexing, then spin down※15, ※16, ※17.

4) Places 3) on the magnetic stand for 1 minute to separate the beads from the solution, and transfer the supernatant into the 1.5 mL microcentrifuge tube from step 2) to pool together※18, ※19. (Total : 100 μL)

※15 If the magnetic beads aggregate and can not be uniformly suspended with only a vortex mixer, suspend the beads evenly while applying tapping or pipetting operation. Please avoid excessive suspension using a tube mixer.

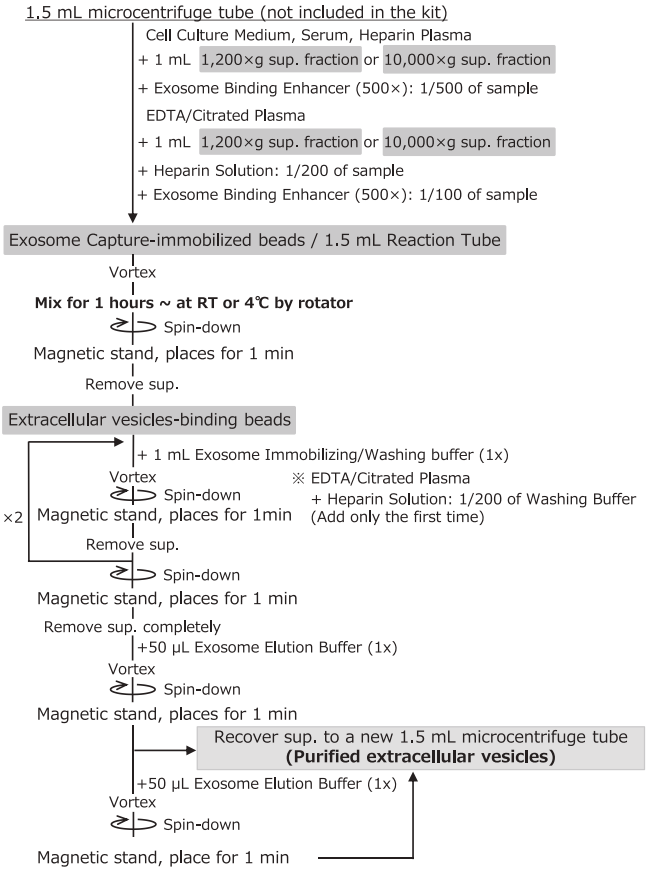
※16 By adding 25 μL of Exosome Elution Buffer (1×), you can obtain a more concentrated sample.

※17 When using plasma (heparin, EDTA, and citric acid) as a sample, recovery efficiency may be improved by using 2×Exosome Elution Buffer.

※18 Exosome Capture-immobilized beads are reusable after eluting EVs (up to 4 times). Therefore, when you need to recover more remaining EVs from samples, please repeat the section 4-6 to increase the recovered amount. After recovering the eluate, please add the stored supernatant of section 4 (refer to ※12) into the 1.5 mL Reaction Tube containing Exosome Capture-immobilized beads from step 4) of section 6 and repeat from the section 4-3) to the section 6-4) as necessary. When using EDTA/Citrated plasma, repeat from the section 4-5) to the section 6-4). This kit contains the required reagents necessary for reuse up to 4 times.

※19 There is a possibility that the pooled eluate have a small amount of magnetic beads. When EVs are analyzed by Nanoparticle Tracking Analysis (NTA) or electron microscopic analysis, filter pooled eluate with 0.45 μm pore size filter unit before analysis as necessary. (Example : Ultrafree MC 0.45 μm, Code No. UFC30HV25, Millipore)

〔**Flowchart of Affinity Purification (Section4-6)**〕



〔**Optional**〕

1. RNA extraction from Purified EVs

When extracting RNA from eluted EVs, add 100 μL of Exosome Elution Buffer (1×) to pooled eluate to prepare 200 μL of sample in total. Then, extract RNA from EVs with microRNA Extractor® SP Kit (Code No : 295-71701) in accordance with the instruction manual.

2. Recycling of Exosome Capture-immobilized beads

Used Exosome Capture-immobilized beads can be recycled for "A. Repeated extraction of the remaining EVs from the same sample (refer to ※12)" and "B. Purification of EVs from the same lot of culture supernatant sample and body fluid sample." Recycling is up to 4 times. When purifying EVs from the same lot of sample, prepare samples first and proceed to section 4-1) of the affinity reaction.

When storing used Exosome Capture-immobilized beads, add 1×TBS containing 0.05 w/v% of sodium azide to the Reaction Tube containing the used Exosome Capture-immobilized beads. After suspending it, store at 4℃. We recommend using Exosome Capture immobilized-beads as soon as possible.

【Related Products】

Code No.	Description	Size
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)	96 reactions
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin HRP)	96 reactions
299-77603	MagCapture™ Exosome Isolation Kit PS	2 purifications
293-77601		10 purifications
290-80301	PS Capture™ Exosome Isolation Resin Kit	1 Kit (0.5 mL Slurry)
297-79701	PS Capture™ Exosome Flow Cytometry Kit	300 reactions
290-83601	CD63-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 reactions
296-83701	CD9-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 reactions
292-83801	CD81-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 reactions
014-27763	Anti CD9, Monoclonal Antibody (1K)	100 μL
019-28173	Anti CD9, Rat Monoclonal Antibody (77B)	100 μL
017-28211	Anti CD9, Rat Monoclonal Antibody (77B), Biotin-conjugated	50 μL
019-27953	Anti CD9, Rat Monoclonal Antibody (30B), Biotin Conjugated	100 μL
012-27063	Anti CD63, Monoclonal Antibody (3-13)	100 μL
014-27643	Anti CD63, Monoclonal Antibody (3-13), Fluorescein Conjugated	100 tests
017-27753	Anti CD63, Monoclonal Antibody (3-13), Red Fluorochrome (635) Conjugated	100 tests
019-27713	Anti CD63, Monoclonal Antibody (3-13), Biotin Conjugated	100 μL
011-27773	Anti CD81, Monoclonal Antibody (17B1)	100 μL
010-28223	Anti CD81, Rat Monoclonal Antibody (9B)	100 μL
011-28111	Anti CD81, Rat Monoclonal Antibody (9B), Biotin-conjugated	50 μL
052-09301	Exosomes, from COLO201 cells, purified	50 μL
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL
290-35591	Magnet Stand	1 each
295-71701	microRNA Extractor® SP Kit	50 reactions
085-00134	Heparin Sodium	10,000 U
317-90175	10×TBS Buffer (pH 7.4)	500 mL

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产品编号：294-84101 （2 tests）
290-84103（10 tests）

MagCapture™ Exosome Isolation Kit PS Ver.2
MagCapture™ 外泌体提取试剂盒 Ver.2

基因研究用

该中文说明书为国内翻译版本，仅供参考。
与英文版说明书有不符之处，以英文版说明书为主。

【前言】

外泌体属于细胞外囊泡，其表面或内部含有蛋白、mRNA、microRNA、DNA及磷脂等，由细胞分泌并稳定存在于血液、尿液、唾液、脑脊髓液、母乳等体液中。外泌体是细胞间通讯的信使及疾病的生物标记物。提取外泌体的常规方法有：超速离心、使用可识别表面抗原的抗体进行亲和层析、利用多聚物试剂进行沉淀。然而，这些方法在回收率、纯度及可操作性上不尽如人意。

本试剂盒采用磁珠和外泌体表面的磷脂酰丝氨酸（PS）结合的方法（即PS亲和法）进行纯化。该方法可方便、高效地提取高纯度的膜表面PS阳性的外泌体，以及其他细胞外囊泡。若想获得更高纯度的外泌体，需使用10,000×g离心分离获得的样品上清。试剂盒利用pH中性的金属螯合试剂，将捕获到的细胞外囊泡从磁珠上洗脱下来，可以获得完整的外泌体和其他细胞外囊泡。所提取的细胞外囊泡可用于各种下游实验，包括电子显微镜、纳米粒子追踪分析（NTA）、摄取实验、组分（蛋白、脂质、核酸等）分析。

【特点】

· 新型PS亲和法，可获得更高纯度的细胞外囊泡
· 比超离法获得的外泌体纯度更高，回收率更高
· 可获得完整的细胞外囊泡，可应用于多种实验
· 磁珠法操作简单
· 无需超速离心

【试剂盒组成】

本试剂盒由以下6种试剂构成。

■ 纯化2次量

(1) 生物素捕获亲和磁珠（Biotin Capture Magnetic Beads）	120 µL×1 瓶
(2) 生物素标记的外泌体捕获（Biotin-labeled Exosome Capture）	20 µL×1 瓶
(3) 外泌体固定化/清洗缓冲液（10x）（Exosome Immobilizing/Washing Buffer）(10x)	5 mL×1 瓶
(4) 外泌体结合增强剂（500x）（Exosome Binding Enhancer（500x））	300 µL×1 瓶
(5) 外泌体洗脱缓冲液（10x）（Exosome Elution Buffer）（10x）	300 µL×1 瓶
(6) 反应管（Reaction Tubes）	4 支

■ 纯化10次量

(1) 生物素捕获亲和磁珠（Biotin Capture Magnetic Beads）	600 µL×1 瓶
(2) 生物素标记的外泌体捕获（Biotin-labeled Exosome Capture）	100 µL×1 瓶
(3) 外泌体固定化/清洗缓冲液（10x）（Exosome Immobilizing/Washing Buffer）（10x）	25 mL×1 瓶
(4) 外泌体结合增强剂（500x）（Exosome Binding Enhancer（500x））	1500 µL×1 瓶
(5) 外泌体洗脱缓冲液（10x）（Exosome Elution Buffer）（10x）	1500 µL×1 瓶
(6) 反应管（Reaction Tubes）	22 支

【保存条件】

冷藏（2~10°C）

【其他准备物品】

试剂：

① 纯净水
② TBS（视实际需要而定）

工具：

① 低温离心机（Max＞10,000×g）
② 旋涡混合器
③ 掌上离心机
④ 磁力架（推荐FUJIFILM Wako产品编号290-35591）
⑤ 回转式搅拌机（旋转混合仪）或试管混合器
⑥ 微量移液器
⑦ 移液器吸头
⑧ 1.5 mL微量离心管
⑨ 15 mL离心管（视实际需要而定）
⑩ 50 mL离心管（视实际需要而定）
⑪ 离心式超滤管
如Sartorius品牌的Vivaspin6 Centrifugal Concentrators 100K【产品编号VS0641】或Vivaspin20 Centrifugal Concentrators 100K【产品编号VS2041】（视实际需要而定）

【操作前注意事项】

1. 耗材类

请使用经高温灭菌处理或市售无DNase或RNase的离心管、移液器吸头。实验时请佩戴乳胶或塑胶手套及口罩以防止DNase或RNase污染。

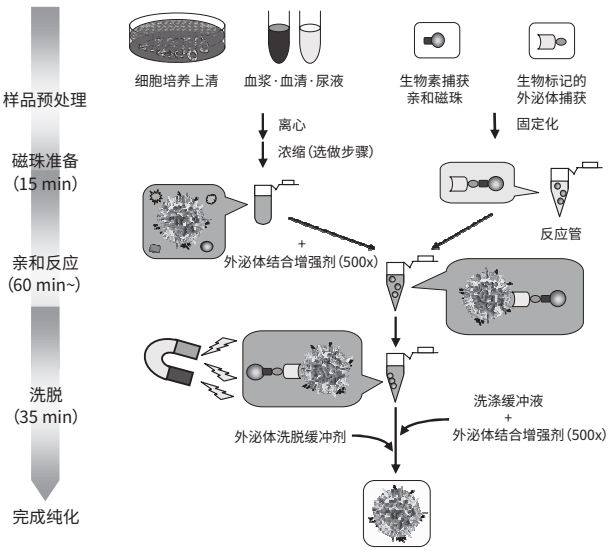
2. 试剂类

如分离外泌体及其他细胞外囊泡后需要进行核酸（RNA、DNA）分析，需注意尽量将试剂保持无菌状态。如分离后需要进行蛋白质谱分析时，需注意避免其他蛋白污染。

3. 实验材料及耗材的处理

人体液样品、组织样品和细胞培养上清样品要作为感染性实验材料处理。提取过程中产生的废液、使用过的移液器吸头及离心管等受污染废弃物应按相应单位的指引进行处理。

【操作流程简要】



【操作流程】

1. 样品预处理

该步骤是样品进行预处理。如果想提取外泌体及其他细胞外囊泡（微囊泡等），请按照以下指示，使用经**1,200×g离心**的上清作为样品。如果想获得更高纯度的外泌体，请使用经**10,000×g离心**的上清作为样品※1。本说明书也说明了细胞培养上清及血清、血浆样品的步骤。如需使用其他液体样品时，请参考血清、血浆样品的操作步骤，探讨合适的前处理操作步骤。

※1 为去除样品中如凋亡小泡和微囊泡等大的EVs，推荐上清液样品使用离心过滤装置（Millipore, Ultrafree-MC, GV 0.22 µm sterile, 产品编号：UFC30GV0S）经10,000×g, 30 min离心。滤液作为测试样本。

细胞培养上清

① 在适当的条件下培养细胞※2。
② 收集细胞培养基。
③ 细胞培养基在4°C条件下300×g离心5 min，通过沉淀去除细胞及碎片。
④ 将步骤③中得到的上清移至新的离心管。
⑤ 4°C条件下1,200×g离心20 min，通过沉淀去除细胞碎片。
⑥ 将步骤⑤中得到的上清移至新的离心管（得到经**1,200×g离心的上清**样品）。
⑦ 4°C条件下10,000×g离心30 min，通过沉淀去除较大细胞外囊泡。
⑧ 将步骤⑦中得到的上清转移至新的离心管（得到经**10,000×g离心的上清**样品）。

〔选做步骤：培养液上清的超滤浓缩〕

当**1,200×g上清或10,000×g上清**超过1 mL时，建议使用离心式超滤浓缩管进行超滤浓缩至1 mL以下。可浓缩的最大体积是50 mL（50倍浓缩）。然而，尽管样品浓缩时能很有效地取得细胞外囊泡，由于可能会产生物理的损伤造成生物性失活，请根据目的来进行适当地处理。

超滤浓缩注意事项

细胞外囊泡可能会吸附在超滤离心装置上，导致回收量减少，添加EV-Save™细胞外囊泡保存稳定剂（产品编号：058-09261）（单独出售）到样品中可减少损失。但是，EV-Save™含有聚合物，不推荐用于需要做蛋白质组学分析的样品中。

※2 请根据不同细胞系要求在适当的条件下培养细胞。因培养基中细胞外囊泡的数量很少，请尽可能地增加培养体积（如 20 mL 或更多）。另外，为确保所分离的外泌体来自目标细胞，请使用去除外泌体的 FBS。可用超离法（例如：110,000×g, 18 h）制备去除外泌体FBS或使用商品化的去除外泌体FBS。
※3 进行蛋白组学分析时，请使用合适的前处理去除聚合物后再进行分析。

处理血清、肝素血浆

在本工艺使用的血清、肝素血浆，请事先用1,200×g进行离心操作，或者使用经过1,200×g处理的样品。
若要获得外泌体+Large EVs，可使用1,200×g的上清作为纯化用的样品，但经1,200×g处理后的样品中可能会存在悬浮物。若出现这种情况，需再次进行1,200×g离心处理，然后作为外泌体+Large EVs纯化样品使用。
① 10,000×g, 4°C, 30 min 离心分离（以去除Large EVs※4）
② 将①的上清移至新的样品管（**10,000×g离心上清**）

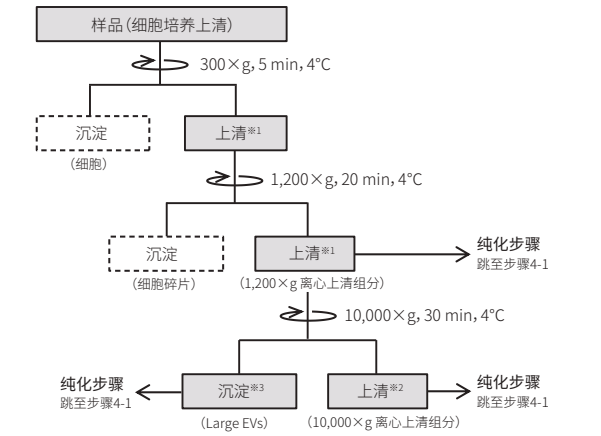
※4 若要取得Large EVs，将10,000×g离心分离得到的“沉淀”悬浮于500 µL～1 mL的TBS中，作为样品使用。

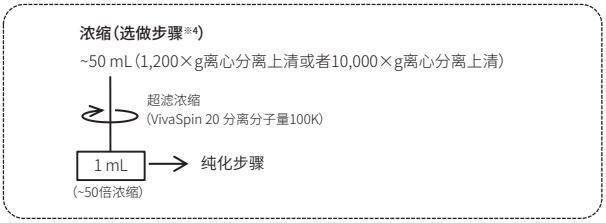
处理EDTA血浆、柠檬酸血浆

在本工艺使用的EDTA血浆及柠檬酸血浆，请事先用1,200×g进行离心操作，或者使用经过1,200×g处理的样品。
对于EDTA血浆及柠檬酸血浆，样品中含有的抗凝固剂会抑制细胞外囊泡和外泌体捕获磁珠的结合。因此，需在亲和反应前即时调配肝素钠溶液，然后添加至经离心分离处理的样品中，再进入纯化步骤。
详细请参考“**4.亲和反应的EDTA血浆、柠檬酸血浆**”项目中所记载的内容。
①10,000×g, 4°C 30 min离心分离（以去除Large EVs※4）
②将①的上清移至新的样品管（**10,000×g离心上清**）

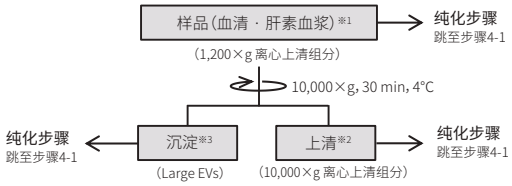
〔样品制备流程（步骤1）〕

细胞培养液上清

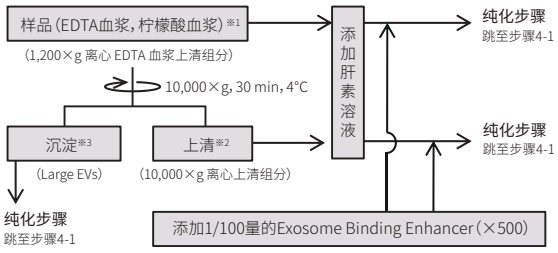




血清和肝素血浆



EDTA血浆、柠檬酸血浆



※1 **提取外泌体+Large EVs时**，请使用1,200×g上清作为提取样品。
※2 **提取外泌体时**，请使用10,000×g上清作为提取样品。
※3 **提取Large EVs 时**，将10,000×g离心所得的沉淀，用TBS 悬浮后，作为样品使用。
※4 该选做步骤适用于体积较大的样品，例如50 mL细胞培养上清。

2. 缓冲液制备

本步骤制备各步骤用到的缓冲液。处理1个样品所需的各缓冲液用量如下：Exosome Immobilizing/Washing Buffer（1×）5 mL（制备量：5.5 mL），Exosome Elution Buffer（1×）100 µL（制备量：150 µL）。另外，若亲和反应需进行过夜操作，请于实验前调配洗涤所需量（3 mL）。

缓冲液名称	用量	制备量
Exosome Immobilizing/Washing Buffer（1×）	5.0 mL 2.0 mL→步骤3使用 3.0 mL→步骤5使用	5.5 mL
Exosome Elution Buffer（1×）	100 µL	150 µL

① 在50 mL离心管加入0.55 mL的Exosome Immobilizing/Washing Buffer（10×）和4.95 mL的纯净水，再加入11 µL 的Exosome Binding Enhaner（500×），制备成Exosome Immobilizing/Washing Buffer（1×）※5。
② 用非本套装附带的1.5 mL试管，加入15 µL Exosome Elution Buffer（10×）和135 µL的纯净水，制备成Exosome Elution Buffer（1×）※6, ※7。
③ 将①和②混合，放在室温保存直到使用。

※5 本步骤Exosome Immobilizing/Washing Buffer（1×）必须添加1/500的Exosome Binding Enhaner（500×）后使用，否则外泌体回收效率会明显下降。
※6 向本步骤所用的Exosome Elution Buffer中，添加我司另售的EV-Save™细胞外囊泡保存稳定剂（产品编号：058-09261），可避免细胞外囊胞吸附于回收管，提高回收率。但本品含有聚合物成分，如用于蛋白质分析，使用前仔细考虑。
※7 对于使用血浆（肝素、EDTA、柠檬酸）等样品的情况，用2×Exosome Elution Buffer可提高回收率。此时，加入30 µL的Exosome Elution Buffer（10×）和120 µL纯净水，混合制备使用。

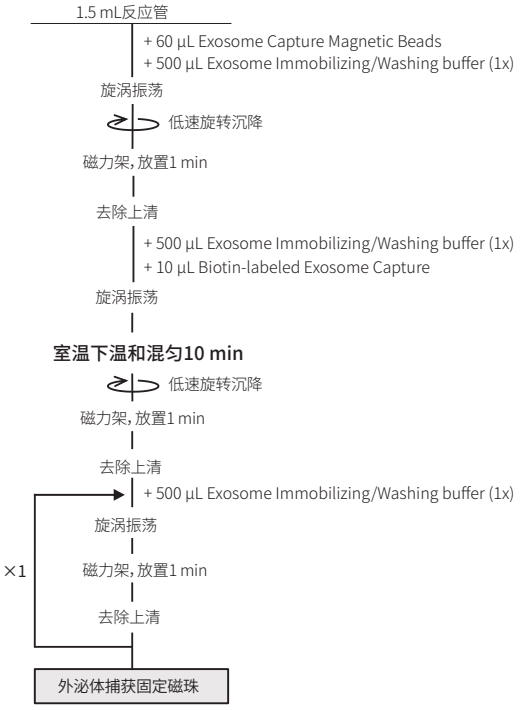
3. 外泌体捕获的固定

本步骤将Biotin-labeled Exosome Capture固定于Biotin Capture Magnetic Beads。**此步骤之后的操作，如需使用磁珠，请务必使用本试剂盒所提供的1.5 mL反应管**※8。

① 用旋涡混合器将60 µL Biotin Capture Magnetic Beads混合均匀后移至1.5 mL反应管。
② 将500 µL Exosome Immobilizing/Washing Buffer（1×）加入1.5 mL反应管，用旋涡混合器充分混合。
③ 将反应管固定于专用磁力架，静置约1 min，待磁珠完全吸附于管壁后，使用微量移液器去除上清。
④ Exosome Immobilizing/Washing Buffer（1×）500 µL和Biotin-labeled Exosome Capture 10 µL加入③的1.5 mL反应管，将其从磁力架取出，使用旋涡混合器进行混合※9。
⑤ 室温状态下在回转式搅拌机（旋转混合仪）或试管混合器上混合10 min。
⑥ 将1.5 mL反应管放在掌上离心机上稍微离心旋转后，固定在磁力架上，静置1 min。待磁珠完全贴附于管壁后，使用微量移液器去除上清。
⑦ 将500 µL Exosome Immobilizing/Washing Buffer（1×）添加到反应管，用旋涡混合器充分混合。
⑧ 将1.5 mL反应管放在掌上离心机上稍微离心旋转后，固定在磁力架上，静置约1 min。待磁珠完全贴附于管壁后，使用微量移液器去除上清。
⑨ 重复操作一次⑦～⑧。· · · **即得外泌体捕获固化磁珠**

※8 试剂盒中的1.5 mL反应管采用了减少磁珠吸附的材质，可改善因磁珠吸附而导致的回收率降低问题。
※9 可能会出现通过减少Biotin-labeled Exosome Capture的固定化量使回收率上升的情况（视乎不同的样品）。对于回收效率低的样品，可以在常规的操作方法上减少Biotin-labeled Exosome Capture的添加量（如10 µL →2~5 µL）。

〔外泌体捕获固定流程（步骤3）〕



4. 亲和反应

在此步骤中，外泌体捕获固定化磁珠与样品发生反应。使用1.5 mL反应管的反应体系（最大体积为1 mL）作为基础的步骤。若想取得外泌体及其他的细胞外囊泡（微囊泡等），请使用经**1,200×g离心的上清**作为样品。如果想获得更高的纯度，请使用经**10, 000×g离心的上清**作为样品。
请注意：EDTA血浆和柠檬酸盐血浆的亲和反应步骤不同。

细胞培养上清液、血清和肝素血浆

① 将样品（最大体积1 mL）※10移至非本试剂盒的1.5 mL离心管，将样品体积1/500的外泌体结合增强剂（500×）添加至样品，使用旋涡混合器进行混合。得到含有外泌体结合增强剂（500×）的样品。
② 将步骤①中得到样品的离心管在掌上离心机上稍微离心旋转，将样品移至含有外泌体捕获固定磁珠的1.5 mL反应管，使用旋涡混合器进行混合处理。
③ 在室温（或4°C）的状态下，使用回转式搅拌机（旋转混合仪）或试管混合器进行混合反应1 h以上。
④ 将1.5 mL反应管放于掌上离心机上稍微离心旋转后，固定在磁力架上，静置1 min。待磁珠完全吸附于管壁后，使用微量移液器去除上清※12，得到细胞外囊泡结合磁珠。

EDTA血浆/柠檬酸盐血浆

① 用纯水溶解10, 000 U的肝素钠（产品编号：085-00134），得到1, 000 U/mL的肝素钠溶液※13。
② 将样品（最大体积1 mL）※10移至非本试剂盒的1.5 mL离心管，将样品体积1/200的肝素钠溶液加至样品（终浓度：5 U/mL）。
③ 将1/100体积的外泌体结合增强剂（×500）加入②样品溶液中，使用旋涡混合器进行混合处理，得到含有外泌体结合增强剂的样品。
④ 将上一步中得到的含有外泌体结合增强剂的样品放于掌上离心机上稍微离心旋转，将样品移至含有外泌体捕获固定磁珠的1.5 mL反应管，使用旋涡混合器进行混合处理。
⑤ 在室温（或4°C）状态下，使用回转式搅拌机（旋转混合仪）或试管混合器进行混合反应1 h以上。
⑥ 在掌上离心机上稍微离心旋转后，将反应管固定在磁力架上，静置1 min。待磁珠完全吸附于管壁后，使用微量移液器去除上清※12，得到细胞外囊泡结合磁珠。

※10 样品体积不足0.5 mL时，可能会出现外泌体捕获固定磁珠与样品无法充分混匀的情况，必要时请添加TBS缓冲液将样品体积增加至0.5 mL以上。（例：100~200 µL → 500 µL）。

※11 根据样品及样品量反应时间不同，若想提高回收量，可以适当延长反应时间。关于温度，根据回收外泌体的用途，选择室温或者4℃下反应。

※12 若需要进一步回收反应后细胞上清中剩余的细胞外囊泡，请勿丢弃本步骤取出的上清，应将其移至另外的容器保管。

※13 每个试剂盒（10次纯化）的最大用量为100 µL。配制好后，请保存于4℃下。

5. 细胞外囊泡结合磁珠的清洗

此步骤清洗细胞外囊泡结合磁珠。**此步骤使用的外泌体捕获固定化/洗涤缓冲液（1×）必须添加外泌体结合增强剂（500×）方可使用。**

① 将1 mL的外泌体捕获固定化/洗涤缓冲液（1×）添加至含细胞外囊泡结合磁珠的1.5 mL反应管中，使用旋涡混合器将其混合。
对于EDTA血浆/柠檬酸盐血浆：
在第一次清洗时，将1/200体积的肝素钠溶液加入到1 mL洗涤缓冲液（含外泌体结合增强剂）中（终浓度：5 U/mL），使用旋涡混合器进行混合。

② 将1.5 mL反应管放在掌上离心机上稍微离心旋转后，固定在磁力架上静置1 min。待磁珠完全吸附于管壁后，使用移液器去除上清。

③ 加入1 mL外泌体捕获固定化/洗涤缓冲液（1×），使用旋涡混合器进行混合。

④ 将1.5 mL离心管在掌上离心机上稍微离心旋转后，固定在磁力架上静置1 min。待磁珠完全吸附于管壁后，使用微量移液器去除上清，得到细胞外囊泡结合磁珠。

⑤ 重复步骤③~④一次。

⑥ 将1.5 mL反应管放在掌上离心机上稍微离心旋转后，固定在磁力架上，静置1 min。磁珠完全粘附于管壁后，使用移液器去除剩余上清※14。得到已清洗细胞外囊泡结合磁珠。

※14 洗涤缓冲液（1×）残留于1.5 mL反应管会降低洗脱率，请完全去除干净。

6. 细胞外囊泡的洗脱

此步骤是从已清洗的细胞外囊泡结合磁珠中洗脱细胞外囊泡。

① 向含有已清洗的结合了细胞外囊泡的磁珠的1.5 mL反应管中，添加外泌体洗脱缓冲液（1×）50 µL，从磁力架取出，使用旋涡混合器混合，然后在掌上离心机上稍微离心旋转※15※16※17。

② 将1.5 mL反应管固定在磁力架上静置1 min。待磁珠完全吸附于管壁后，使用移液器将上清回收至非自带的1.5mL离心管中。

③ 再往步骤②中的磁珠添加50 µL外泌体洗脱缓冲液，使用旋涡混合器进行混合，然后在掌上离心机上稍微离心旋转※15※16※17。

④ 将1.5 mL反应管固定在磁力架上并静置1 min。待磁珠完全吸附于管壁后，将上清转移至步骤②中的1.5 mL微量离心管中与上一次合并※18,※19。

※15 若磁珠凝集，使用旋涡混合器无法使其均匀重悬时，可轻轻敲打管壁或使用移液器吹打混匀。请避免使用混合器过度重悬。

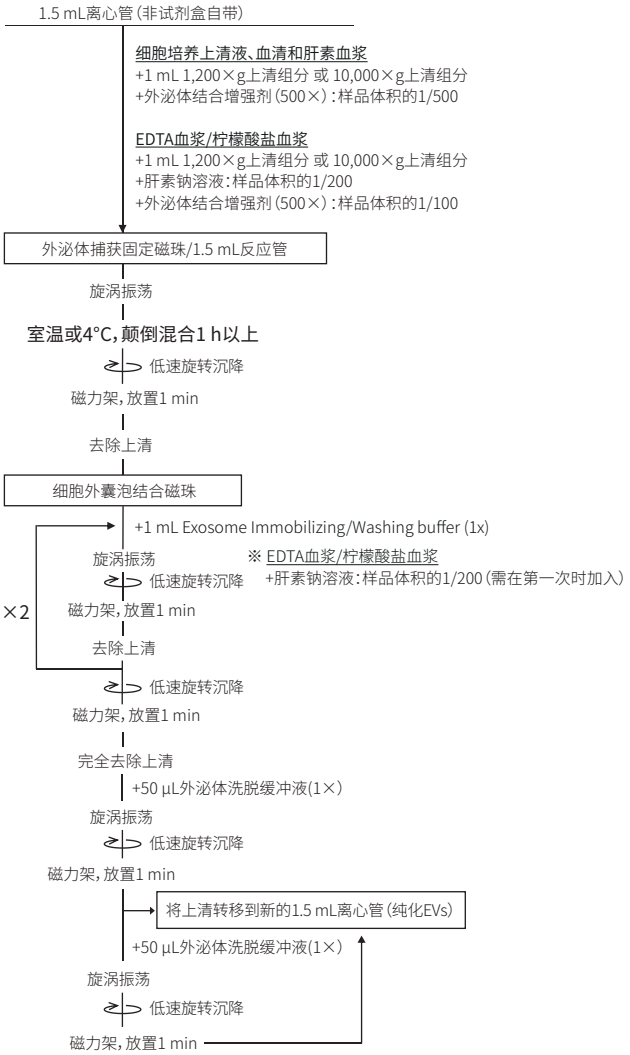
※16 添加的外泌体洗脱液25 µL是为了得到浓缩状态的样品。

※17 使用血浆（肝素、EDTA、柠檬酸）样品时，用2×的外泌体洗脱液进行洗脱步骤，可以提高回收率。

※18 纯化细胞外囊泡后的外泌体固定磁珠可以循环使用（最多4次）。若想回收残留在样品中的细胞外囊泡，可重复第4至第6部分的亲和操作，以增加回收量。洗脱液回收后，含有外泌体固定磁珠（步骤6-4）的 1.5 mL离心管添加第4部分中在另外的容器中保存的剩余样品（参考※12），可根据需要重复操作步骤4-3至步骤6-4操作。处理EDTA血浆、柠檬酸血浆请重复步骤4-5至步骤6-4，本试剂盒已配有可循环使用4次的试剂量。

※19 回收后的样品可能含有少量的磁珠。在进行细胞外囊泡纳米粒子跟踪分析（NTA）或电子显微镜分析时，可根据需要使用0.45 µm孔径的过滤装置（如Merck Millipore® 公司的【产品编号：UFC30HV25】）对回收的洗脱液，进行过滤处理后，再进行分析。

〔亲和纯化流程（步骤4-6）〕



[选做步骤]

1. 从纯化的细胞外囊泡中提取RNA

若想从获得的细胞外囊泡中纯化RNA，在以上步骤6中回收的洗脱液取100 µL添加100 µL外泌体洗脱缓冲液（得到200 µL样品），然后使用microRNA Extractor SP Kit（产品编号：295-71701），按照说明书进行RNA纯化。

2. 细胞外囊泡洗脱后回收外泌体捕获固定磁珠

对于“相同样品中重复提取细胞外囊泡（参考※12）”和“从同一批次的同一体液样品或同一批次的细胞培养上清液纯化外囊泡”，外泌体捕获固定磁珠可以重复使用。最多重复使用4次。请先准备好同批次样品，细胞外囊泡的纯化从亲和反应开始（步骤4-1）。

重复使用的外泌体捕获固定磁珠储存：向含有外泌体捕获固定磁珠的反应管中加入含0.05%叠氮化钠的1×TBS。重悬后，4°C冷藏保存。请尽快使用。

【相关产品】

产品编号	产品名称	包装
297-79201	PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD)	96 tests
298-80601	PS Capture™ Exosome ELISA Kit (Streptavidin HRP)	96 tests
299-77603	MagCapture™ Exosome Isolation Kit PS	2 tests
293-77601		10 tests
290-80301	PS Capture™ Exosome Isolation Resin Kit	1 kit
297-79701	PS Capture™ Exosome Flow Cytometry Kit	300 tests
290-83601	CD63-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 tests
296-83701	CD9-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 tests
292-83801	CD81-Capture Human Exosome ELISA Kit (Streptavidin HRP)	96 tests
014-27763	Anti CD9, Monoclonal Antibody (1K)	100 µL
019-28173	Anti CD9, Rat Monoclonal Antibody(77B)	100 µL
017-28211	Anti CD9, Rat Monoclonal Antibody(77B), Biotin-conjugated	50 µL
019-27953	Anti CD9, Rat Monoclonal Antibody (30B) , Biotin Conjugated	100 µL
012-27063	Anti CD63, Monoclonal Antibody (3-13)	100 µL
014-27643	Anti CD63, Monoclonal Antibody (3-13), Fluorescein Conjugated	100 tests
017-27753	Anti CD63, Monoclonal Antibody (3-13), Red Fluorochrome(635) Conjugated	100 tests
019-27713	Anti CD63, Monoclonal Antibody (3-13), Biotin Conjugated	100 µL
011-27773	Anti CD81, Monoclonal Antibody (17B1)	100 µL
010-28223	Anti CD81, Rat Monoclonal Antibody(9B)	100 µL
011-28111	Anti CD81, Rat Monoclonal Antibody (9B), Biotin-conjugated	50 µL
052-09301	Exosomes, from COLO201 cells, purified	50 µL
058-09261	EV-Save™ Extracellular Vesicle Blocking Reagent	1 mL
299-36421	MAGNET STAND	1个
295-71701	microRNA Extractor® SP Kit	50 tests
085-00134	Heparin Sodium	10,000 U
317-90175	10 x TBS (pH 7.4)	500 mL

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