

Product Data Sheet

Product Name: Exosome Isolation Kit (from Cell Culture Media)
 Cat. No.: GK30020

Components

Component name	Specification	Specification
Exosome Concentration Solution	24 mL	120 mL
Exosome Purification Filter	2.0 mL Tube, 4 Tubes	2.0 mL Tube, 20 Tubes

You need to Prepare

1. high-speed centrifuge (can reach 10000 g centrifugal force), vortex oscillator; 50 mL or 15 mL centrifugal rotor, 2 mL centrifugal rotor; 50 mL or 15 mL centrifuge tube, 1.5 mL centrifuge tube;
2. 1 × PBS buffer;

Protocol

Step1: sample pretreatment

1. Sampling: If the sample is frozen, thaw the sample in 25 ° C water bath, and place the completely melted sample on ice; if it is a fresh sample, collect the sample and place it on ice;
- 2.The initial amount of sample: (the amount of sample in a single extraction)

Sample name Minimum value

Cell Culture Media 20 mL

3. Centrifuge to remove cell debris: Transfer the sample to a centrifuge tube and

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centrifuge at 3000 g for 10 min at 4 °C to remove cell debris from the sample;

4. Transfer supernatant: Transfer the centrifuged supernatant to a new centrifuge tube;

Step 2: exosomes extraction

1. Supernatant pretreatment: Add Exosome Concentration Solution (ECS reagent) to the centrifuged supernatant to remove impurities. The specific dosage is as follows:

Sample name	Sample dose	Add ECS dose
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Cell culture Media	20 mL	5 mL
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Note: For other dosage specifications, please convert according to the proportion of reagent in the table.

2. Solution mixing: after adding ECS reagent, close the centrifuge tube tightly, mix the fluid by a vortex shaker for 1 min, placed at 2°C to 8 °C for 2 h;

3. Precipitate: centrifuge the centrifuge tube containing the mixed solution at 10000 g for 60 min at 4 °C, discard the supernatant, and the precipitate is enriched of exosomes; (Note: absorb the supernatant as much as possible)

4. Resuspension: resuspend the pellet in 100 µL volume of 1× PBS, and after it was uniformly suspended in PBS, transfer the suspension to a new 1.5 mL centrifuge tube;

5. Harvesting exosomes: A 1.5 mL centrifuge tube containing the resuspension should be centrifuged at 12000 g for 2 min at 4 °C, and the supernatant was retained. This supernatant is enrich of exosome particles.

Step3: exosomes purification

1. Purification of exosomes: transfer the harvested pre-extracted exosomes liquid to the upper chamber of Exosome Purification Filter (EPF column), centrifuged at 3000 g for 10 min at 4 °C. The highly purified exosome particles is retained in the liquid on the bottom

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of the EPF column.

2. Preservation of exosomes: purified exosomes should be stored in a -80°C low temperature refrigerator, those exosomes can be used for subsequent experiments.

Storage Conditions

This product can be stored stably for 24 months at room temperature, please mix well before use.

Frequently Asked Questions [FAQ]

1. What is the storage condition for this kit?

Store at room temperature, low temperature is not necessary.

2. Can this kit separate and purify vesicles with a diameter of 200-1000 nm?

No, this kit cannot be used to separate vesicles larger than 200 nm.

3. Which type of samples can be used for exosome extraction in this kit?

Besides cell supernatants, the kit can also be used for exosome extraction of urine and other low-density body fluids (such as cerebrospinal fluid, ascites, amniotic fluid, breast milk, saliva, etc.).

4. What instruments and consumptive materials are required in the extraction process of this kit?

Low temperature high speed centrifuge, vortex shaker (Vortex), water bath, pipette, centrifuge tube (1.5 mL, 50 mL).

5. Can the sample be stored at a low temperature before extracting the exosomes?

Yes. Store at -80°C for a long time, no need to add frozen solution; short-term (1-2 days) can be stored at 4°C.

6. How to deal with samples with excessive viscosity?

If the viscosity of the sample is too large (due to a large amount of cell secretion), the sample can be diluted in an equal volume with 1× PBS buffer, exosomes can be extracted with this kit after mix the diluted sample.

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7. How to remove serum-derived exosomes when culturing cells?

In most cases, cells require serum in vitro, and serum usually contains exosomes. To remove serum exosomes, the following two methods are applicable:

- (1) The serum for cell culture is subjected to ultracentrifugation at 1×10^5 g for 10 h to remove serum exosomes;
- (2) Select serum-free medium for cell culture.

8. When to use the exosome-free serum medium (or serum-free medium)?

After the cells are cultured in normal serum-containing medium for a certain period of time, when the cell fusion degree is about 60%-70%, the original serum-containing medium should be removed and then replaced by fresh exosome-free serum medium (or serum-free medium), then culture for 24-48h and collect the supernatant when the cell fusion degree reaches 80%-95%. The supernatant can be used for exosomes extraction.

9. Will dead cells in the cell culture process affect the extraction of exosomes?

Yes. When harvesting cells, it should be guaranteed that the proportion of dead cells not exceed 5%. A large number of vesicles of different size are released during apoptosis/death. They can contaminate the exosomes produced by living cells during the extraction and purification of exosomes, meanwhile, it may also cause blockage of the EPF purification column.

10. For NGS sequencing of exosome Small RNA, how much exosome do I need to prepare for the initial sample?

It is recommended to use an initial sample amount of 40 mL or more for a common tumor cell line. Due to the low content of exosomes in some cells (such as suspension cells, stem cells, nerve cells, etc.), it is recommended to concentrate with a 10kD ultrafiltration column, prepare more than 40mL of concentrate, and then use this kit to extract exosomes.

11. There is no precipitation after centrifugation with ECS solution. Is this normal?

Due to the low yield of exosomes derived by some cells (such as suspension cells, stem cells, nerve cells, etc.), it is possible that precipitation is difficult to be observed by the naked eye after centrifugation with ECS solution. When resuspending, use PBS solution to blow and elute (avoid severe blows) outer of the centrifuge wall. Extracted exosomes are first subjected to NTA particle size detection or BCA protein quantitative detection, and then decide whether to do the subsequent experiments or not.

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12. Why is the EPF column blocked during the purification process?

The column may be blocked from the large amount of cell debris which are produced due to excessive cell culture time (the sample is not fully centrifuged and pre-treated, and there are still cell debris remaining). It is recommended to collect the supernatant for about 24-48 hours cell incubation.

13. Can the EPF column be used multiple times?

No. Excessive samples will exceed the limits of the purification column and affect the separation.

14. How to save exosomes?

Purified exosome can be stored at 4 °C for no more than one week, and can be stored for a long time at -80 °C.

15. How to identify extracted exosomes?

Extracted exosomes are usually identified by transmission electron microscopy (morphology), particle size detection (size), and Western blot by detect marker proteins such as CD63, CD9, CD81, TSG101, etc.

16. Does the extracted exosome need to be lysed with RIPA before Western blotting?

Yes it is needed. The RIPA reagent is generally added in a ratio of 1:1.

17. Is there any internal reference protein available for selection by Western blot analysis of exosome?

Currently there is no internal reference proteins This test is a qualitative test.

18. How to extract tissue exosomes?

In a biological clean environment, cut the tissue into small pieces (smaller pieces give better results), then incubate in serum-free medium for 12 h; transfer the culture media to a centrifuge tube, centrifuge at 3000 g for 20 min at 4 °C to remove impurities in the culture media. To remove the cell debris, the supernatant should be transferred to a new centrifuge tube; the supernatant is filtered using a 0.45 µm filter, then percolate the supernatant using a 0.2 µm filter, then exosome is extracted according to the kit instructions.

Background

Exosomes are small vesicles (30-150 nm) containing RNA and protein secreted by cells,

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and are abundant in body fluids such as blood, saliva, urine, and breast milk. Exosomes are regarded to have the function of intercellular messengers, transferring their effectors or signaling molecules between specific cells; however, the structure, composition, and biological pathways involved are not clear yet.

In the research of the biological function of exosome, it is necessary to purify intact exosomes, while the traditional ultracentrifugation method is cumbersome, requiring specific hardware and is difficult to operate. This rapid extraction kit for exosome independently is developed by GlpBio Technology. The components are optimized for exosome extraction in cell culture supernatants (urine, etc.), and with a purification filter device. This kit can be used to quickly and efficiently obtain high-purity exosome particles for electron microscopy analysis, NTA particle size analysis, nucleic acid analysis, protein analysis, cytology experiments, and animal experiments.

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