# Exosome Isolation Kit, Precipitation Method, For Cell Culture Media

**Cat:** EX0016
**Size:** 50T(10mL)/250T(50mL)
**Storage:** 2-8℃, valid for 1 year.

## Introduction

Exosomes are membrane-coated Extracellular vesicles (EVs), approximately 40-160nm in diameter, with a lipid bilayer structure, naturally occurring in blood, urine, cerebrospinal fluid, and the supernatant of cultured cells. Exosomes are produced and released by almost all types of cells. Exosomes, which can be recognized and fused by nearby or distant cells, are an important medium for mutual regulation between cells. They are involved in the pathogenesis of many diseases, such as cancer, neurodegeneration and inflammatory diseases, and affect various functions of cells.

Exosome Isolation Kit, Precipitation Method, For Cell Culture Media, also known as exosome extraction kit, It is an efficient and convenient kit for the extraction of exosomes or other extracellular vesicles in the supernatant of cell culture medium. The polymeric precipitation method is adopted, which has low equipment requirements, convenient operation, short extraction time, low sample starting amount, high extraction efficiency, and relatively complete exosomes morphology. Exosomes extracted using this kit can be used for protein analysis, nucleic acid analysis, Western blot, PCR, RNA extraction, high-throughput sequencing, exosome size and concentration analysis (Nanoparticle tracking analysis,NTA),electron microscope analysis, cell co-culture and other experiments.

## Protocol(*for reference only*)

**First. Sample Preparation**

1. Culture the required cells under appropriate conditions, when the cell density reaches 70%-80% (in the logarithmic growth phase), add serum culture medium without exosome or appropriate serum-free culture medium, continue to culture for 12-24 hours, and collect the cell supernatant when the cell density reaches 90%-100%.

Note 1: Since the serum contains a large number of exosomes, in order to avoid contamination, it is necessary to add serum without exosomes at this time. Exosom-free serum can be obtained by hypercentrifugation, or exosom-free serum can be used directly. It can also be cultured using serum-free culture medium according to specific experimental conditions, and some cells can grow normally without serum for about 12 hours, or the cell-free culture medium group can be added as a negative control.

Note 2: The amount of exosomes in the cell supernatant varies with the cell type, cell state and number of cells, and the starting amount of the sample must be determined according to the experimental needs.

Note 3: A large number of vesicles are released during the process of apoptosis and death, and these vesicles will contaminate the exosomes produced by living cells during the extraction and purification of exosomes. Please ensure that the cells are in good condition and the proportion of apoptotic or dead cells does not exceed 5%.

2. Centrifuge the collected cell culture medium at 500×g at 4ºC for 5 minutes, and gently and slowly absorb the supernatant into a new centrifuge tube; Centrifuge the supernatant at 4ºC, 10,000-16,500 ×g, for 30 minutes, and gently and slowly draw the supernatant into a new centrifuge tube.
3. Use a 0.22μm needle filter to filter the supernatant, further remove impurities such as large cell vesicles and apoptotic bodies, and transfer the filtered supernatant to a new centrifuge tube.
4. (*Optional*) Use an ultrafiltration tube between 10-100kDa to concentrate the supernatant. The concentrated supernatant was removed from the dead volume collector of the ultrafiltration tube for subsequent exosome extraction.

Note: Some cells (stem cells, nerve cells) secrete less exosomes, so the supernatant liquid can be concentrated about 10 times before subsequent exosomes extraction; For tumor cells with a large amount of exosomes secreted, the concentration can be avoided or the cell supernatant liquid can be concentrated by about 2-5 times for subsequent exosome extraction. The specific enrichment ratio can be adjusted according to the actual situation.

**Second, Exosome Extraction**

1. Every 1ml of supernatant sample prepared in Step 1, 190μl of extractor for extractor of cell supernatant was

added to the sample. After blowing and mixing, it was placed at 4ºC and left for 4 hours or overnight.
Note: Exosome extraction reagent is very viscous, it should be slowly absorbed, slowly added, and ensure that the exosome extraction reagent and the cell supernatant are fully mixed. If the cell secretes fewer exosomes, the exosome yield can be increased by increasing the resting time.
2.10,000×g centrifuge at 4ºC for 30 minutes, carefully remove the supernatant with a 1ml suction head, and absorb the supernatant as much as possible without touching the precipitation, and collect the precipitation, which is the exosome.

Note: The content of exosomes in the cell culture fluid sample is generally small, and the precipitation may not be visible to the naked eye at this time. If the angular rotor is used during centrifugation, note the direction of the centrifuge tube and mark the bottom position with a circle.

3. Exosome precipitates obtained by centrifugation can be re-suspended with appropriate amount of PBS or normal saline. Generally, the initial amount of 10ml cell culture medium is re-suspended with 0.1-1ml of re-suspension. Precipitation can also be directly used for subsequent experiments.

Note: Exosomes can be stored for 1 week at 4ºC, or long-term at -20 ℃ and lower.

4. (*Optional*) There are more non-exosome impurities in some samples, resulting in more precipitation, and the impurities can be removed by re-suspension and brief centrifugation. The precipitate was re-suspended with appropriate amount of PBS, then centrifuged at 12,000×g at 4ºC for 2 min, and the supernatant was taken. If there is too much precipitation, the supernatant can be centrifuged at 4ºC for more than 12,000×g for 2 minutes until there is no obvious precipitation.

Note 1: Precipitation may be difficult to re-hang, need to be blown repeatedly.

Note 2: Appropriate suspensions should be selected according to subsequent purification methods.

**Third. Exosome Purification (*optional*)**

1. The obtained exosomes can be further purified by exosome purification column or affinity chromatography.
2. If sterile exosomes need to be obtained, a 0.22μm needle filter can be used for filtration. In order to reduce the damage, please wash the filter with PBS first. If the extracted exosomes are not used for the time being, they can be stored at -80ºC after packaging.

## Note

1. This product is sticky, it should be completely mixed and absorbed before use, and ensure that the absorption volume is accurate.
2. This product is only used for scientific research by professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary homes.
3. For your safety and health, please wear a lab coat and disposable gloves.

## Related products

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| *K007602P* | *Anti-CD63 Polyclonal Antibody* |
| *K001361M* | *Anti-Hsp70 Monoclonal Antibody* |
| *K106583P* | *Anti-HSPA1A Polyclonal Antibody* |
| *K000385P* | *Anti-CD81 Polyclonal Antibody* |
| *K114124P* | *Anti-TSG101 Polyclonal Antibody* |
| *EX0010* | *Exosome Isolation Kit, EPF Method，For Blood Sample* |
| *EX0011* | *Exosome Isolation Kit, EPF Method，For Cell Culture Media* |
| *EX0012* | *Exosome Isolation Kit, EPF Method，For Urine Sample* |
| *EX0013* | *Exosome Isolation Kit, EPF Method，For Emulsion Sample* |
| *EX0015* | *Exosome Quantitative Detection Kit* |