Exosome Isolation Kit for Cell Culture Media

Introduction

Exosomes are a class of vesicle-like structures with a diameter of about 30-150 nm, which are released into the extracellular environment by cells through exocytosis. They are found in the body fluids of almost all living organisms, including blood, urine, saliva, milk, and cell supernatants cultured in vitro. Exosomes are important tools for cell-to-cell communication, which can carry and deliver a variety of biomolecules such as proteins, lipids, RNA, and DNA, thus playing an important role in regulating immune responses, promoting tissue repair, and transmitting genetic information.

Exosome Isolation Kit for Cell Culture Media is a kit for efficient, simple, and rapid extraction of exosomes from cell culture medium using polymer precipitation. This kit does not require ultracentrifugation or ultrafiltration, and a large number of intact and high-purity exosomes can be obtained directly from cell culture supernatants with a simple lower centrifugation. This kit has low equipment requirements, takes less time, and is free of RNases/DNases/proteases. The exosomes extracted using this kit can be used for protein analysis, nucleic acid analysis, Western Blot, PCR, RNA extraction, high-throughput sequencing, exosome particle size and concentration analysis (Nanoparticle tracing analysis, NTA), electron microscopy analysis, and cell co-culture.

Components and Storage

Components	K2707-10 mL
Exosome Isolation Reagent for Cell Culture Media	10 mL

Store the kit at -20°C, stable for 2 years. Or store the kit at 4°C, stable for 1 year. Or store the kit at room temperature, stable for 1 month.

Protocol

1. Sample preparation

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

***Note:**

1. Serum contains a large number of exosomes. To avoid contamination of exosomes in serum, exosomes can be removed from serum by ultracentrifugation, or directly use exosome-free serum. A serum-free medium can also be used directly or by using a

cell-free medium as a negative control.

2. The amount of exosomes in the supernatant varies depending on the cell types, so the start amount of samples also varies depending on the experiment.
3. Processes such as apoptosis and death release a large number of vesicles, which will interfere with the extraction of exosomes, so it is necessary to ensure that the cells are in a good state and the proportion of apoptotic or dead cells does not exceed 5%.

2) Centrifuge the collected cells at 4°C, 500 g for 5 min, gently and slowly pipette the supernatant into a new centrifuge tube, and then centrifuge the newly obtained supernatant at 4°C, 10000-16500 g for 30 min, gently and slowly aspirate the supernatant to a new centrifuge tube.

3) Filter the resulting supernatant through a 0.22 μm syringe filter and transfer the filtered supernatant to a new centrifuge tube.

***Note:** This step is to remove impurities such as larger cellular vesicles and apoptotic bodies.

4) (Optional) Use an ultrafiltration tube (10-100KDa) to concentrate the supernatant to some extent. The concentrated supernatant is then collected from the dead volume collector of the ultrafiltration tube for subsequent exosome extraction.

***Note:** For some cells, such as stem cells and nerve cells, secrete fewer exosomes, so the supernatant volume can be concentrated 10-fold before extraction. For cells with a large number of exosome secretions, such as tumor cells, the supernatant may not need to be concentrated or just concentrated 2-5 fold before extraction. The specific concentration fold can be adjusted as needed.

2. Exosome extraction

1) Exosome Isolation Reagent for Cell Culture Media is viscous. Mix it well before use.

2) Add 190 μL of Exosome Isolation Reagent for Cell Culture Media per 1 mL prepared supernatant, mix gently and incubate at 4°C for 4 h or overnight.

***Note:** Exosome Isolation Reagent for Cell Culture Media is very viscous and should be pipetted slowly and it needs to be well mixed with the supernatant. If there are few exosomes, the incubation time can be appropriately extended to improve the yield of exosomes.

3) Centrifuge at 10000 g for 30 minutes at 4°C, carefully discard the supernatant with a 1 mL pipette tip, taking care not to touch the pellet. Exosomes are contained in the pellet.

***Note:** The pellet may not be visible at this time. If using an angled rotor, you can pay attention to the direction of the tube before centrifugation and draw a mark at the bottom.

4) The obtained exosomes can be resuspended with an appropriate amount of PBS or normal saline, and 10 mL of cell culture medium can be resuspended with 0.1-1 mL of resuspension. Precipitation can also be used directly for subsequent experiments.

***Note:** Exosomes can be stored at 4°C for 1 week and at -20°C or lower for long periods.

5) (Optional) Some samples have more non-exosomal impurities that may cause a large precipitate, and the impurities can be removed by centrifugation. Resuspend the pellet with PBS, then centrifuge at 12000 g for 2 min at 4°C and collect the supernatant. If the pellet is large, the supernatant can be centrifuged

briefly several times until there is no obvious precipitation.

***Note:**

1. The pellet may be difficult to resuspend and may need repeated pipetting.
2. The appropriate exosome resuspension needs to be selected according to the subsequent purification method.

3. **(Optional) Exosome purification**

   1) The collected exosomes can be further purified by exosome purification columns or affinity chromatography.

   2) If sterile exosomes are required, filter them using a 0.22 μm syringe filter. To minimize losses, the filter can be rinsed with PBS in advance. The exosomes can be stored at -80°C after aliquoting for long-term storage.

Note

1. The reagent in this kit is viscous and needs to be thoroughly mixed and pipetted slowly to ensure accurate aspiration.
2. This kit is suitable for the extraction of exosomes from cell culture medium, and is not suitable for the purification of exosomes from serum/plasma.
3. When stored at -80°C for long-term storage, the exosomes can be aliquoted to avoid repeated freeze-thaw cycles.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com