

**Product Name**

Name: AdvanCell Exosome Isolation Reagent (from cell culture media)

Cat. No.: AC7001-0050

Size: 50 ml

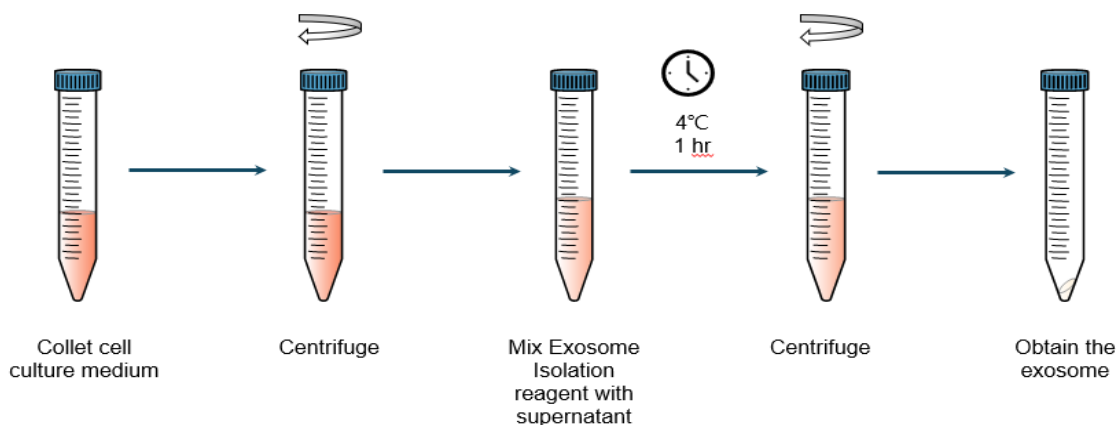
**Product Description**

Exosomes are small vesicles secreted by cells into the extracellular environment. These vesicles play a crucial role in intercellular communication by carrying various molecules such as proteins and RNA from one cell to another. They are involved in processes such as immune response, tissue regeneration, and cell signaling. Exosomes have garnered significant interest in both research and clinical settings due to their potential applications in diagnostics, drug delivery, and therapeutics.

AdvanCell Exosome Isolation Reagent is designed for isolating exosomes from cell culture media samples. Compared to traditional ultracentrifugation methods, this approach only requires low-speed centrifugation, minimizing the impact of centrifugal forces on exosomes and preserving their integrity. Additionally, this method saves experimental time, requires only small sample volumes for efficient isolation, and is simple to operate even for processing large sample volumes. Isolated exosomes can be applied to a variety of downstream applications, such as RNA analysis, high-throughput sequencing, cell co-culture, etc.

**Storage**

Ship at room temperature and store at 4°C or room temperature upon receipt.

**Procedure**

**Sample collection:**

1. To prevent contamination from bovine exosomes present in FBS (Fetal Bovine Serum), serum-free culture media or exosome-depleted serum media can be used when the cell density reaches 50% - 70%. After an additional 12 hours of cell cultivation, the supernatant can be collected and utilized. Alternatively, cells can be initially cultured in exosome-depleted serum media, allowing for direct isolation of exosomes from the collected culture media supernatant without changing the culture media. Wear a laboratory coat and disposable gloves to prevent RNase contamination.
2. Collect cell culture media. Centrifuge at  $3000 \times g$  for 15 minutes to remove cells and cell debris. Transfer the supernatant to a new sterile tube. *Note: Be careful not to touch the pellet.*

**Isolate exosome:**

1. Add 1/5 volumes of the Exosome Isolation Reagent to the cell-free supernatant.

cell-free supernatant	Isolation Reagent
1 ml	200 $\mu$ l
10 ml	2 ml

2. Mix well by inverting or flicking the tube until there is a homogenous solution.
3. Refrigerate 1 hour at  $+4^{\circ}\text{C}$ .

*Note: The tubes should not be rotated or mixed during the incubation period and should remain upright.*

4. Centrifuge isolation reagent/culture media mixture at  $1500 \times g$  for 30 minutes.

*Note: Centrifugation may be performed at either room temperature or  $+4^{\circ}\text{C}$  with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the vessel.*

5. Aspirate supernatant to a new sterile tube. Add 5 ml PBS smoothly and centrifuge at  $10,000 \times g$  for 20 minutes.
6. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated exosomes in pellet.
7. Resuspend exosome pellet in 100-500  $\mu$ l using sterile 1X PBS. We recommend using the precipitated exosomes immediately rather than freezing them for future use.

**Precaution and Disclaimer**

For research use only, not for clinical diagnosis, and treatment.