

User Manual

OriCell™ Exosomes Extraction Kit For Cell Culture Supernatant

Catalog No. EEKC-10001



Introduction

Exosomes are nanosized extracellular vesicles (EVs) secreted by cells, typically ranging from 30 to 150 nm in diameter. They possess a phospholipid bilayer membrane similar to that of cells and display various specific membrane proteins on their surface. Internally, exosomes carry a diverse array of biologically important signaling molecules, including proteins, RNA, and DNA. These vesicles are widely distributed in bodily fluids such as blood, urine, cerebrospinal fluid, saliva, breast milk, and bile.

Currently, research and development in both academia and industry focus primarily on four key areas involving exosomes: their use as biomarkers for early disease diagnosis, therapeutic agents for disease treatment, inhibitors of exosome activity, and drug delivery vehicles. Most studies concentrate on exosomes derived from pluripotent stem cells and tumor cells.

The OriCell™ Exosomes Extraction Kit for Cell Culture Supernatant employs a unique isolation technology that enables rapid and efficient extraction of large quantities of exosome particles from cell culture supernatant. These isolated exosomes are suitable for applications in cell biology, animal studies, and related experimental research.

Note: This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.

When citing our products in academic publications, please use the following format: “OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences.”

Product Information

Components	Catalog Number	Volume
OriCell™ Exosomes Extraction Kit For Cell Culture Supernatant	EEKC-10001-50	50 mL
OriCell™ Exosomes Extraction Kit For Cell Culture Supernatant	EEKC-10001-100	100 mL

Product Stability and Storage Conditions

- This product can be shipped and stored at room temperature and remains stable for up to 24 months.
- Please use the product within its shelf life, as expired components may significantly reduce extraction efficiency.

Precautions

1. To ensure high-purity exosome isolation, we recommend culturing cells with exosome-depleted serum.
2. This product is intended exclusively for isolating exosomes from cell culture supernatants and is not suitable for exosome extraction from other sources.

Instructions

1. Collect the cell culture supernatant into a 50 mL centrifuge tube and centrifuge at $300 \times g$, 4°C for 10 minutes.
2. Collect the supernatant and transfer it to a new 50 mL centrifuge tube, then centrifuge at $3000 \times g$, 4°C for 10 minutes.
3. Collect the supernatant and transfer it to a new 50 mL centrifuge tube, then centrifuge at $10,000 \times g$, 4°C for 30 minutes.
4. Filter the resulting supernatant through a $0.22 \mu\text{m}$ membrane filter and set aside for subsequent steps.

Note: If immediate processing is not possible, store the collected supernatant at -80°C . Before use, thaw at room temperature and proceed with the protocol.

5. Prepare the OriCell™ Exosomes Extraction Kit for Cell Culture Supernatant and mix the reagents thoroughly before use.
6. In a 50 mL centrifuge tube, add the exosome extraction reagent to the filtered supernatant at a ratio of 1:4 (extraction reagent : supernatant). The total volume should preferably not exceed 35 mL.
7. Seal the tube tightly, invert gently to mix for 1 minute, and incubate at 4°C for at least 16 hours.

Note: Longer incubation time can improve exosome yield. For supernatants from stem cells, suspension cells, and similar sources, overnight incubation (not exceeding 24 hours) is recommended. For supernatants from regular cell lines, a minimum incubation time of 4 hours is sufficient.

8. Centrifuge the incubated tube at $10,000 \times g$, 4°C for 60 minutes. After centrifugation, a white exosome pellet should be visible on the tube wall. Mark the pellet location, carefully remove the supernatant with a pipette without disturbing the pellet, and remove any residual liquid.
9. Centrifuge the tube again at $2,000 \times g$, 4°C for 2 minutes to remove remaining liquid.

10. Resuspend the pellet with a small volume of 1× PBS by pipetting up and down for 1 minute. After thorough resuspension, transfer the suspension to a new 1.5 mL centrifuge tube.

Note:

- 1) Exosome particles tend to adhere to tube walls. During resuspension, pipette PBS repeatedly against the walls to maximize recovery. Adjust the PBS volume according to the pellet size.
 - 2) For 200 mL of starting supernatant, use 700–1000 µL of 1× PBS for resuspension to maintain adequate protein concentration.
 - 3) If some pellet remains undispersed, it can be discarded. Transfer the supernatant for the next step.
11. Centrifuge the 1.5 mL tube at 12,000 × g, 4°C for 2 minutes and collect the supernatant.
 12. Filter the supernatant through a 0.22 µm membrane filter (Jet, FPE-204-013) using a 1 mL syringe. The filtrate contains the purified exosomes.
 13. Aliquot and store the purified exosomes for downstream applications. Store at 4°C for up to one week or at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.

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