Capturem™ Exosome Isolation Kit (Cell Culture) Protocol-At-A-Glance

I. Introduction

This protocol is provided for the Capturem Exosome Isolation Kit (Cell Culture) (Cat. No. 635723) which is designed for the easy and rapid isolation of exosomes and yields up to 2×10^{11} of purified exosomes per column, depending on cell type and cell culture conditions. This kit is suitable for the isolation of exosomes from precleared mammalian cell culture supernatants in under 30 min.

II. Materials and Reagents

A. Included

- 6 Capturem Maxiprep Exosome Isolation Columns (Cell Culture) with green inserts in 50-ml collection tubes
- 6 Exosome Isolation Pre-Clearing Columns with purple inserts
- 70 ml Exosome Isolation Wash Buffer
- 4 ml Exosome Isolation Elution Buffer

B. Not Included

- Collection tubes

  Each isolation will require three additional standard 50-ml collection tubes that are suitable for centrifugation up to 2,000*g*. These tubes should be used throughout the protocol to collect precleared cell culture supernatant, flowthrough, wash, and eluate samples as described in the protocol for downstream analysis, such as nanoparticle tracking analysis.

NOTE: We strongly recommend using a swinging bucket centrifuge for use with spin columns for the complete removal of solution, which may not be possible with the use of a fixed angle centrifuge. The following formula can be used to convert RPM to RCF or G-force, if not available on the centrifuge:

$$\textbf{RCF or G-force} = 1.12 \times R \times (RPM/1000)^2$$

R is the distance (in millimeters) from the center of the rotor to the end of the spin bucket when held horizontally and away from the center.

III. Sample Preparation

A. General Considerations

- Use either serum-free media or media containing exosome-depleted serum when culturing cells for isolation of exosomes, to prevent sample contamination.
- The cell culture supernatant must be prefiltered using the supplied Exosome Isolation Pre-Clearing Column (purple insert) to remove any large membrane fragments, apoptotic bodies, smaller cell fragments, etc., in order to avoid any possible blocking of the Capturem Maxiprep Exosome Isolation Columns.

- For the best possible yield, we strongly recommend using freshly isolated cell culture supernatant for the procedure. For the same-day isolation of exosomes, supernatants can be stored at 2–8˚C until ready for processing. For long-term storage, supernatant should be kept at –80˚C. However, the yield of exosomes may be compromised upon long-term storage and the freezing and thawing of the cell culture supernatant.

  NOTE: Refrigerated or frozen supernatant should be warmed using a water bath or incubator at 37˚C before applying to the Exosome Isolation Pre-Clearing Column (purple insert).

- All steps in the protocol should be performed at room temperature.

B. Collecting Cell Culture Supernatant

1. **For adherent cells:** Aspirate the cell culture supernatant into a collection tube and centrifuge at 3,000*g* for 10 min to remove any dead cells or larger cell fragments. Carefully transfer the centrifuged cell culture supernatant to a new tube without disturbing the pellet.

   For non-adherent cells: First centrifuge the cell suspension at 500*g* for 5 min to separate cells from the growth media, then perform a second centrifugation step at 3,000*g* for 10 min. Carefully transfer the centrifuged cell culture supernatant to a new tube without disturbing the pellet.

2. Place the Exosome Isolation Pre-Clearing Column, with purple insert, into a 50-ml collection tube (supplied by the user; see Section II.B) and load the cell culture supernatant from Step 1 above (up to 25 ml). Centrifuge at 1,000*g* for 2–4 min at room temperature and use the precleared cell culture supernatant for exosome isolation.

   NOTES: If exosomes are present at low concentrations, additional cell culture supernatant can be applied to the Exosome Isolation Pre-Clearing Column in 25-ml increments.

IV. Exosome Isolation

1. Load the precleared cell culture supernatant (up to 25 ml) onto the Capturem Maxiprep Exosome Isolation Column (green insert) in the provided collection tube. Centrifuge at 1,000*g* for 2–4 mins at room temperature. Save the collection tube containing the sample flowthrough for any downstream particle analysis.

   NOTES:
   - If exosomes are present at low concentrations, additional fresh precleared cell culture supernatant can be applied to the isolation column in 25-ml increments.
   - Alternatively, the flowthrough can be reapplied to the isolation column to ensure optimal capture of the exosomes.

2. Place the Capturem Maxiprep Exosome Isolation Column into a new collection tube (supplied by the user) and add 10 ml of Exosome Isolation Wash Buffer to the spin column. Centrifuge at 1,000*g* for 2 min at room temperature. Save the collection tube containing the wash flowthrough for any downstream particle analysis.

3. Insert the Capturem Maxiprep Exosome Isolation Column into a new collection tube (supplied by the user) and add 500–600 µl of Exosome Isolation Elution Buffer to the column. Centrifuge at 1,000*g* for 2 min at room temperature to collect the eluate.

4. For better yield, repeat the elution by re-applying the eluate from Step 3 to the Isolation Column and centrifuge at 1,000*g* for 2 min at room temperature to collect the final eluate. The eluted exosomes are now ready for downstream analysis.

NOTES:

- Eluted exosomes can be stored at 4°C for up to 1 week. For long-term storage, we advise storage at –20°C or –80°C. If exosomes will need to be thawed more than once, we recommend aliquoting them into multiple tubes to avoid any damage/loss of exosomes due to repeated freeze/thaw cycles.
- Exosomes are eluted using a phosphate-based buffer containing inorganic salts. The eluted particles can be directly used for physical particle analysis such as nanoparticle tracking analysis (NTA) and exosome labeling for cellular uptake assays. NTA requires appropriate sample dilution depending on the yield of eluted exosomes (i.e., 1:10–1:200), which should be carried out using Dulbecco's PBS without $CaCl_2$ or $MgCl_2$ immediately prior to analysis while keeping the tube containing the eluted exosomes at 4°C.
- Some downstream applications require concentrated and buffer-exchanged exosomes for protein analysis (e.g., Western blotting and proteomics) and small RNA analysis. For these applications we strongly recommend an ultrafiltration method using the Sartorius Vivaspin 500 concentrator with a 100-kDa cut-off (Cat. No. VS0141). We recommend Dulbecco's PBS without $CaCl_2$ and $MgCl_2$ for the buffer exchange step. We do not recommend an ultracentrifugation method to concentrate exosomes, as this can damage exosomes, resulting in poor recovery and yield.
- We strongly recommend using the following protocol for optimal Western blot results: Isolate exosomes and concentrate using 100-kDa Vivaspin filter (Sartorius); wash once with PBS to recover approximately 40 µl. Mix the concentrated exosomes with 10 µl of 5X RIPA lysis buffer (Alfa Aesar) containing 1X protease inhibitor cocktail (Roche) and vortex. Next, sonicate at 20°C for 5 min in a water sonicator (Ultrasonic Cleaner, JSP). After sonication, incubate the tube on ice for 20 min with intermittent mixing. Next, centrifuge the mixture at 10,000*g* for 10 min at 4°C. Transfer the isolated clear lysate to a fresh tube. Estimate the protein amount using the Micro BCA Protein Estimation Kit (Thermo Fisher Scientific) and load at least 10 µg of protein per lane for Western blotting.

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