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Exosome – Human CD63 Isolation/Detection (from cell culture media)

Protocol for use in Flow Cytometry

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Catalog Number: 10606D **Store at 2°C to 8°C**

Product Description

Exosome – Human CD63 Isolation/Detection (from cell culture media) is primarily intended for isolation of $CD63^+$ human exosome subsets from a pre-enriched exosome solution prepared using Total Exosome Isolation (from cell culture media) reagent or ultracentrifugation for flow cytometry analysis. This product can also be used to prepare exosome subsets for western blots, electron microscopy, and qRT-PCR.

Dynabeads® magnetic beads are uniform, superparamagnetic polystyrene beads (4.5 µm dia.) coated with a primary monoclonal antibody specific for the CD63 membrane antigen expressed on most human exosomes. The Dynabeads® magnetic beads are incubated with samples overnight and captured exosomes are magnetically separated for downstream applications.

Product Contents

Exosome – Human CD63 Isolation/Detection (from cell culture media) reagent is sufficient for processing 7.5 mL of pre-enriched exosome solution.

Components	Amount	No. of Flow Reactions
Exosome – Human CD63 Isolation/Detection (from cell culture media)	3 mL	150

Contains 1×10^7 beads/mL in PBS, pH 7.4, with 0.1% BSA, and 0.02% sodium azide as a preservative. **Caution**: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

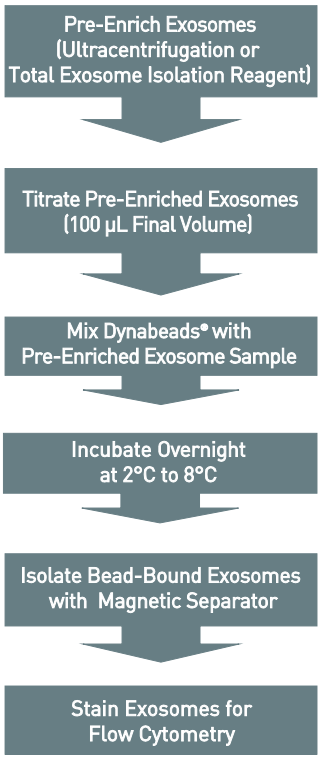
Required Materials

- DynaMag™-2 or DynaMag™-5 Magnetic separators.
- HulaMixer® Sample Mixer or other mixing device (tilting and rotating), or sample shaker (500–1000 rpm).
- Tubes appropriate for the sample volume and the magnet used for isolation (see "General Guidelines").
- Isolation Buffer (PBS with 0.1% BSA, filtered through a 0.2 µm filter).
- Pre-enriched exosomes prepared using the Total Exosome Isolation (from cell culture media) reagent, or by ultra-centrifugation.

General Guidelines

- Good mixing is critical to successful exosome isolation.
  - Use a mixer that tilts and rotates to ensure that the beads do not settle in the tube.
  - Avoid end-over-end rotation for small sample volumes (e.g. 100 µL). See "Guidelines for Optimal Mixing Conditions" for recommendations.
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- The isolation success is dependent on the quality of the sample from the pre-enrichment process.
- If isolating exosome subsets from small volumes (<500 µL), we recommend:
  - Use round or flat-bottomed tubes (e.g. 2-mL Sarstedt tubes).
  - **Do not** use conical sample tubes (e.g. Eppendorf microcentrifuge tubes).

Exosome Isolation and Detection Workflow



Protocol

Pre-enrich exosomes

Use Total Exosome Isolation Reagent, or standard ultra-centrifugation methods to pre-enrich the exosomes.

Note: Pre-enriched exosome solutions may vary in exosome content. Total protein can be used as general guidance, however, the relation between total protein and exosome content may depend on the pre-enrichment method used (e.g. ultracentrifugation or the Total Exosome Isolation reagent).

Titrate pre-enriched exosome sample

1. Titrate the volume of the pre-enriched exosome solution. Start with approximately 25 µg of total protein.
   - Maximum 50 µL per 20 µL magnetic beads if sample prepared with Total Exosome Isolation reagent.
   - Maximum 100 µL per 20 µL magnetic beads if sample prepared by ultracentrifugation.
2. Add Isolation Buffer to 100 µL final volume per 20 µL magnetic beads (as originally pipetted from the vial). Refer to the following table.

Total Exosome Isolation Reagent		Ultracentrifugation	
Exosome Solution	Isolation Buffer	Exosome Solution	Isolation Buffer
50 µL	50 µL	100 µL	0 µL
25 µL	75 µL	50 µL	50 µL
5 µL	95 µL	5 µL	95 µL

Note: The protocol can be scaled up from 100 µL to 5 mL final volume by adjusting all volumes proportionally.

Isolate CD63$^+$ exosomes

The protocol is based on isolation using 20 µL of Dynabeads® magnetic beads. For larger volumes, scale up reagents and volumes proportionally.

Day 1

1. Resuspend the magnetic beads by mixing for >10 min or vortexing for 30 sec.
2. Transfer 20 µL magnetic beads into an appropriate tube.
3. Wash the magnetic beads by adding 200 µL of Isolation Buffer. Mix well.
4. Place the tube on the magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet, and add pre-enriched exosome solution titrated with Isolation Buffer (100 µL final volume) to the magnetic beads. Mix well. Refer to the preceding table, or use your own calculations if you have scaled the protocol up.
6. Incubate the tube overnight (18–22 hours) at 2°C to 8°C with mixing (e.g. on a HulaMixer® Sample Mixer).

Day 2

7. Centrifuge the tube for 3–5 sec to collect the sample at the bottom of the tube.
8. Wash the bead-bound exosomes by adding 300 µL of Isolation Buffer. Mix gently by pipetting (**do not vortex**).
9. Place the tube on the magnet for 1 min and discard the supernatant.
10. Remove the tube from the magnet, and add 400 µL of Isolation Buffer. Mix gently by pipetting (**do not vortex**).
11. Place the tube on the magnet for 1 min and discard the supernatant.
12. Resuspend the bead-bound exosomes in 300 µL Isolation Buffer.

The exosome bound beads are now ready to be stained for flow cytometry.

Stain exosomes for flow cytometry

The staining antibody should be titrated for optimal signal to noise ratio, starting with the manufacturer's recommended concentration for staining 1×10^6 cells.

1. Transfer desired staining antibodies (e.g. CD63-PE) to a flow tube.
2. Add 100 µL bead-bound exosomes to the tube (from "Isolate CD63$^+$ Exosomes", step 12). Mix gently by pipetting.
3. Incubate for 45–60 min at room temperature protected from light on a sample shaker (~1000 rpm).
4. Wash the bead-bound exosomes by adding 300 µL of Isolation Buffer. Mix gently by pipetting (**do not vortex**).
5. Place the tube on the magnet for 1 min and discard the supernatant.
6. Repeat the washing steps (step 4 and 5) once, and resuspend in the desired volume of Isolation Buffer for flow cytometry analysis.

Guidelines for Optimal Mixing Conditions

Device	Mixing conditions
HulaMixer® Sample Mixer	Display settings:  Speed: 650 rpm
Roller	Tilting: 5 cm up per 50 cm length 
Plate Shaker	Speed: 650 rpm

Related Products

Product	Cat. No.
Exosome – Streptavidin for Isolation/Detection	10608D
Total Exosome Isolation (from cell culture media)	4478359
Total Exosome RNA and Protein Isolation Kit	4478545
Exosome Immunoprecipitation (Protein A)	10610D
Exosome Immunoprecipitation (Protein G)	10612D
HulaMixer® Sample Mixer	15920D
DynaMag™-2 Magnet	12321D
DynaMag™-5 Magnet	12303D

Visit www.lifetechnologies.com/magnets to view the full range of magnetic separators.

Explanation of Symbols

Symbol	Description
REF	Catalog number

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