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

Protocol for use in Western Analysis

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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 Western Reactions
Exosome – Human CD63 Isolation/Detection (from cell culture media)	3 mL	30

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.

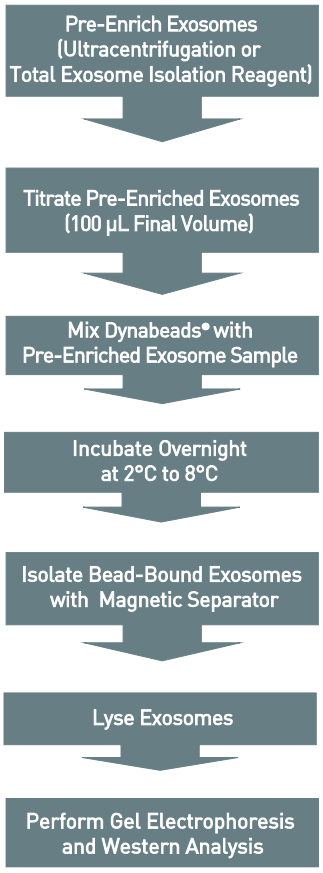
Required Materials

- 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.
- Lysis buffer (e.g. RIPA buffer).
- Protein inhibitor solution (e.g. cOmplete, EDTA-free; Roche Cat. no. 11 837 580 001).
- Protein electrophoresis equipment (e.g. NuPAGE® system).

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).
- To obtain the best possible result, optimize the following parameters:
  - Dynabeads® magnetic bead volume for exosome isolation.
  - Lysis conditions (buffer/volumes).
  - Amount of lysed exosomes loaded into each well on the gel.
- For detection of small exosomal proteins, we recommend using a 10% or 12% polyacrylamide gel.
- For western blot conditions, follow antibody manufacturer instructions (e.g. non-reducing conditions for some exosomal markers, such as CD63 and CD81).
- Use the most sensitive method available for detection (e.g. chemiluminescence and x-ray film). Camera detection may be less sensitive than x-ray film.
- For detection of exosomal markers such as CD81 (25 kDa) that are of equal size to the antibody heavy- or light-chains, use the secondary antibody Mouse TrueBlot® Ultra Ig HRP (eBioscience Cat. no. 18-8817).

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 100 µL magnetic beads if sample prepared with Total Exosome Isolation reagent.
   - Maximum 100 µL per 100 µL magnetic beads if sample prepared by ultracentrifugation.
2. Add Isolation Buffer to 100 µL final volume per 100 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 100 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 100 µL magnetic beads into an appropriate tube.

   Note: To achieve greater depletion of exosomes, increase the number of magnetic beads by 2–5 times per 100 µL (final volume) of sample.
3. Wash the magnetic beads by adding 500 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 and mix well. Refer to the preceding table, or use your own calculations if you have scaled up the protocol.
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.

The exosome bound beads are now ready for exosome lysis, gel electrophoresis, and western analysis western analysis.

Lyse exosomes and prepare sample for gel electrophoresis

1. Add 15 µL of lysis buffer (e.g. 1 x RIPA buffer) to bead-bound exosomes ("Isolate CD63⁺ exosomes", step 11). Mix well.
2. Add 0.5 µL of 25X protein inhibitor solution. Mix well.
3. Incubate at 2°C to 8°C for 15 min to lyse exosomes.
4. Transfer 15 µL of exosome lysate to a new tube (e.g. 1.5-mL Eppendorf tube).
5. Add 15 µL 2X sample buffer. Mix well.
6. Add 1.5 µL loading buffer. Mix well.
7. Incubate at 95°C for 5 min.
8. Place the tube in the magnet and load up to 25 µL of the supernatant on the gel (depending on well capacity).

Follow manufacturer instructions for performing gel electrophoresis and general western blotting conditions.

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

SPEC-07821