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Exosome – Streptavidin for Isolation/Detection

Protocol for use in Western Analysis

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Catalog Number: 10608D Store at 2°C to 8°C

Product Description

Exosome – Streptavidin Isolation/Detection is primarily intended for isolation of 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 streptavidin. The Dynabeads® magnetic beads need to be coated with your exosome specific antibody (biotinylated) prior to use. The antibody coupled beads are incubated with samples overnight and captured exosomes are magnetically separated for downstream applications.

Product Contents

Exosome – Streptavidin for Isolation/Detection reagent is sufficient for processing 7.5 mL of pre-enriched exosome solution.

Components	Amount	No. of Western Reactions
Exosome – Streptavidin for Isolation/Detection	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.

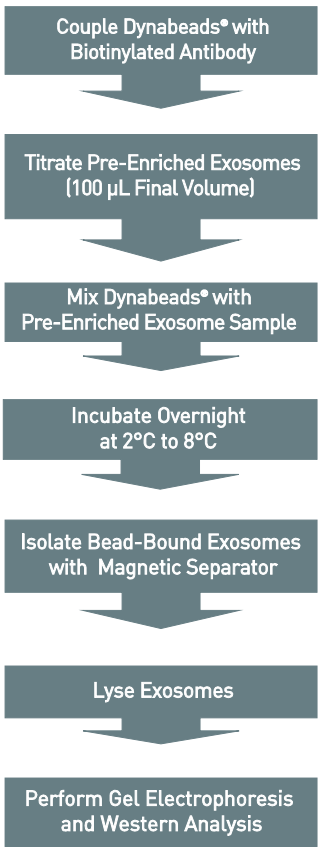
Required Materials

- 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.
- Biotinylated antibody of choice. If your antibody is unconjugated, we recommend using the FluoReporter® Mini-biotin-XX Protein Labeling Kit.
- 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

- Pre-enrich the exosome sample using Total Exosome Isolation (from cell culture media) or by ultracentrifugation.
- 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).
- Choose a good exosome specific primary antibody. Antibodies showing great detection in flow analysis or western analysis, may not be suitable for biotinylation and coupling to Dynabeads® magnetic beads for exosome isolation.
- 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

Couple Dynabeads® magnetic beads with biotinylated antibody

This protocol is for coupling of 1×10^7 Dynabeads® magnetic beads (1 mL) with 4 µg of biotinylated antibody, but can be scaled by adjusting volumes proportionally.

- For coupling of larger volumes, Dynabeads® magnetic bead concentration can range from 1×10^7 to 1×10^8 beads/mL.
- If antibody concentration is low (e.g. 0.01 mg/mL), adjust the volume of Isolation Buffer to achieve a concentration of 1×10^7 beads/mL during coupling.

1. Resuspend the magnetic beads on a mixing device for >10 min or vortex 30 sec.
2. Transfer 1 mL of magnetic beads to a new tube.
3. Place the tube on the magnet for 1 min and discard the supernatant.
4. Remove the tube from the magnet and wash the magnetic beads by adding 1 mL of Isolation Buffer. Mix well.
5. Place the tube on the magnet for 1 min and discard the supernatant.
6. Remove the tube from the magnet and resuspend the magnetic beads in 1 mL of Isolation Buffer. Mix well.
7. Add 4 µg biotinylated antibody. Mix well
8. Incubate for 30–60 min at room temperature with mixing (e.g. on a HulaMixer® Sample Mixer).
9. Place the tube on the magnet for 1 min and discard the supernatant.
10. Remove the tube from the magnet and wash the antibody-coupled magnetic beads by adding 1 mL of Isolation Buffer. Mix well.
11. Place the tube on the magnet for 1 min and discard the supernatant.
12. Repeat steps 10–11 twice.
13. Add 1 mL of Isolation Buffer to restore the magnetic bead concentration to 1×10^7 beads/mL.

Antibody-coupled Dynabeads® magnetic beads can be stored at 2°C to 8°C for several months in Isolation Buffer with 0.02 % sodium azide as a preservative (dependent on the stability of the primary antibody).

Isolate exosome subset from pre-enriched exosome sample

This protocol is for isolating exosome subsets from pre-enriched samples prepared using the Total Exosome Isolation reagent or by ultracentrifugation.

The isolation is based on using 100 µL of Dynabeads® magnetic beads. For larger volumes, scale up all reagents and volumes proportionally.

- Pre-enriched exosome samples can vary in exosome content. Total protein can be used as general estimate of quantity, but the relation between total protein and exosome content may depend on the pre-enrichment method used (e.g. Total Exosome Isolation reagent, or ultracentrifugation).
- Titrate the amount of pre-enriched exosome sample. Start with ~25 µg total protein (≤100 µL) per 100 µL of Dynabeads® magnetic beads.

Day 1

1. Titrate pre-enriched exosome sample to a total volume of 100 µL with Isolation Buffer.

Pre-Enriched Exosome Sample	Isolation Buffer
100 µL	0 µL
50 µL	50 µL
5 µL	95 µL

Note: The sample can be scaled from 100 µL final volume to 5 mL final volume. Refer to the preceding table or use your own calculations, if you have scaled up the protocol.

2. Resuspend the magnetic beads by mixing for >10 min or vortexing for 30 sec.
3. Transfer 100 µL of 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.
4. Wash the magnetic beads by adding 500 µL of Isolation Buffer. Mix well.
5. Place the tube on the magnet for 1 min and discard the supernatant.
6. Remove the tube from the magnet, and add the pre-enriched exosome sample (in a total volume of 100 µL) to the tube containing the magnetic beads. Mix well.
7. Incubate the tube overnight (18–22 hours) at 2°C to 8°C with mixing (e.g. on a HulaMixer® Sample Mixer).

Continued on next page

Day 2

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

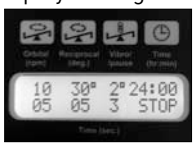
The exosome bound beads are now ready for exosome lysis, gel electrophoresis, and western analysis.

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 exosome subset from pre-enriched exosome sample", step 12). 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 – Human CD63 Isolation/Detection (from cell culture media)	10606D
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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