Exosome – Human CD81 Isolation (from cell culture)

Package Contents

Catalog Number	Size
10616D	2 mL

Storage Conditions

- Store at 2°C to 8°C.
- When stored as instructed, expires two year from date of receipt unless otherwise indicated on product label.

Required Materials

List of Materials

Timing

- Hands-on time: 45–60 minutes
- Incubation time: 16–24 hours

Selection Guide

Exosome Research Products
Magnetic Separators
Go online to view related exosome products and magnets.

Product Description

- Exosome – Human CD81 Isolation (from cell culture) is intended for isolation of CD81-positive human exosome subsets from a pre-enriched exosome sample.
- After isolation, exosomes can be characterized by downstream applications including western blot, qRT-PCR, and sequencing.
- Dynabeads® are uniform, superparamagnetic polystyrene beads (2.7 µm dia.) coated with a primary monoclonal antibody specific for the CD81 membrane antigen expressed on most human exosomes. The Dynabeads® magnetic beads are incubated with your samples overnight and isolated exosomes are magnetically separated.

Important Guidelines

- Follow the recommended pipetting volumes and incubation times.
- Avoid air bubbles (foaming) during pipetting.
- The western analysis is dependent on the level of exosomes present in the pre-enriched exosome sample, the protein transfer efficiency, the quality of the western blotting antibody and detection system (chromogenic detection is not recommended).

Online Resources

Visit our product pages for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

6 June 2014

invitrogen™ by life technologies™

Print Options

Protocol outline

1. Pre-enrich exosomes.
2. CD81 positive isolation.
3. Protein electrophoresis.
4. Western blot analysis.

Pre-enriched exosome sample input

Pre-enriched exosome solution can be prepared using Total Exosome Isolation (from cell culture media) reagent, (Cat no 4478359) or ultracentrifugation.

Pre-enriched Exosome sample	Isolation Buffer	Dynabeads	Final Volume (after buffer exchange)
200 µL	0 µL	80 µL	200 µL
100 µL	0 µL	40 µL	100 µL
10 µL*	90 µL	40 µL	100 µL
1 µL	99 µL	40 µL	100 µL

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

* Titration of exosome input is recommended: starting with 100 mL conditioned cell culture medium, concentrated to 2 mL after pre-enrichment (50x concentrated), use 10 µL pre-enriched exosomes as starting sample (equals 500 µL conditioned cell culture medium).

Guidelines for optimal mixing conditions

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.

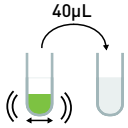
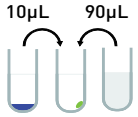
Example of CD81 western blot analysis

Limited product warranty and disclaimer details

life technologies™

CD81 positive isolation

This protocol is designed for one isolation. The protocol can be scaled according to the desired number of analyses to be performed. The protocol below describes an exosome input of 10 µL pre-enriched exosome solution with 40 µL of bead solution.

Timeline		Steps	Actions
Day 1	1 	**Prepare exosome – human CD81 isolation beads**	1. Place vial of beads on a roller for >10 minutes or vortex for 30 sec to resuspend. 2. Transfer 40 µL bead solution to a tube containing 1 mL Isolation Buffer. 3. Place the tube in magnetic separator for 1–2 min. 4. Remove the buffer.
	2 	**Mix isolation beads with pre-enriched exosome sample**	1. Add 90 µL Isolation Buffer to tube containing beads. 2. Add 10 µL pre-enriched exosome sample.
	3 	**Incubate beads and exosomes**	Incubate at 2–8°C overnight with end-over-end mixing (tilting and rotation).
Day 2	4 	**Isolate bead-bound exosomes with magnetic separator**	1. Spin sample tube briefly 1–2 sec. 2. Add 1 mL of Isolation Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant. 3. Remove tube from magnetic separator. 4. Add 0.5 mL of Isolation Buffer and place tube in magnetic separator for 1–2 min before removing supernatant.
	5 	**Proceed to downstream analysis**	▪ **Western blot analysis** ▪ qPCR ▪ Sequencing

Western blot analysis after CD9 positive exosome isolation

- Electrophoresis should be performed using a 5–15% gradient gel, or 12% homogeneous gel.
- Exosomal markers such as CD9, CD81, and CD63 should be separated under non-reducing conditions.
- For detection of proteins that are equal in size to antibody heavy- or light-chains (e.g. CD81) we recommend the Mouse TrueBlot® Ultra Ig HRP Secondary antibody (eBioscience Cat. no. 18-8817).

Timeline		Steps	Actions
Day 2	1 	**Lyse exosomes**	1. Add 10 µL 1X RIPA buffer with protease inhibitors to bead bound exosomes. 2. Sonicate for 10 sec. 3. Incubate on ice for 15 min.
	2 	**Denature protein sample**	1. Add 10 µL 2X sample buffer (with or without reducing agent). 2. Add 1 µL loading buffer. 3. Incubate at 95°C for 5 min.
	3 	**Load sample on polyacrylamide gel**	1. Spin sample tube briefly 1–2 sec. 2. (*Optional*) Place tube on magnetic separator. 3. Pipette sample into well.
	4 	**Perform electrophoresis**	1. Add appropriate molecular weight markers. 2. Perform electrophoresis at 200 V for 30 min (or according to your standard protocol).
Day 3	5 	**Perform western blot**	1. Perform wet transfer at 100 V for 1 hour on ice. 2. Incubate with primary antibody overnight. 3. Detect protein by chemiluminescence.