Exosome – Human CD81 Flow Detection (from cell culture)

invitrogen

USER GUIDE Pub. No. MAN0010737 Rev. C.0

Print Options

Package Contents

Catalog Number	Size
10622D	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 minutes
- Incubation time: 16–24 hours
- Staining for flow cytometry: 45 minutes

Selection Guide

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

Product Description

- Exosome – Human CD81 Flow Detection (from cell culture) is intended for isolation of CD81-positive human exosome subsets from a pre-enriched exosome sample.
- 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.
- Results are dependent on the level of exosomes present in the pre-enriched exosome sample.

Online Resources

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

Protocol outline

1. Pre-enrich exosomes.
2. CD81 positive isolation.
3. Flow cytometry 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.

Very high levels of CD81-positive exosomes in the pre-enriched exosome solution may exceed the binding capacity of Dynabeads™ magnetic beads, while very low levels can lead to flow cytometry results close to the background fluorescence signal of Dynabeads™ magnetic beads.

Pre-enriched Exosome sample	Assay Buffer	Dynabeads	Final Volume (after buffer exchange)
200 µL	0 µL	40 µL	200 µL
100 µL	0 µL	20 µL	100 µL
10 µL*	90 µL	20 µL	100 µL
1 µL	99 µL	20 µL	100 µL

* 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.

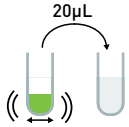
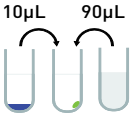
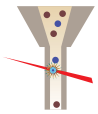
Example of CD81 flow cytometry analysis

Limited product warranty and disclaimer details

ThermoFisher SCIENTIFIC

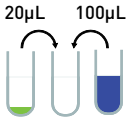
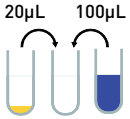
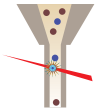
Exosome – Human CD81 Flow Detection (from cell culture) CD81 positive isolation protocol

This protocol is designed for one isolation sufficient for a single positive staining and background control. The protocol below describes an exosome input of 10 µL pre-enriched exosome solution. Scale the protocol according to the number of analyses to be performed.

Timeline		Step	Action
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 20 µL bead solution to a tube containing 1 mL Assay 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 Assay 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. Centrifuge sample tube briefly 1–2 sec. 2. Add 300 µL of Assay Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant. 3. Remove tube from magnetic separator.
	5 	**Wash bead-bound exosomes**	1. Add 300 µL of Assay Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant. 2. Remove tube from magnetic separator. 3. Add 300 µL Assay Buffer.
	6 	**Proceed to downstream analysis**	▪ **Flow cytometry** ▪ Electron microscopy

Flow cytometry analysis after CD81 positive exosome isolation

- Include a matched isotype control as a background control.
- Use 100 µL of sample for each staining reaction.
- Titrate staining antibodies to ensure optimal staining (high levels of staining reagent are generally required).

Timeline		Step	Action
Day 2	1 	**Prepare target specific sample**	1. Add 20 µL of anti-human CD81-RPE, clone JS-81 (BD Cat. No. 555676). 2. Add 100 µL of bead-bound exosome sample.
	2 	**Prepare isotype control**	1. Add 20 µL of mouse IgG1-RPE (BD Cat. No. 559320). 2. Add 100 µL of bead-bound exosome sample.
	3 	**Stain samples**	1. Incubate tubes at room temperature for 45 min on an orbital shaker at 1000 rpm. 2. Protect samples from light during incubation.
	4 	**Wash samples**	1. Add 300 µL of Assay Buffer to each tube, and place the tubes in a magnetic separator for 1–2 min before removing buffer. 2. Remove the tubes from the magnetic separator and repeat the wash step.
	5 	**Perform flow cytometry analysis**	1. Add 300 µL of Assay Buffer to each sample (adjust volume according to instrument and tubes used). 2. Perform flow cytometry analysis.