# Exosome – Human EpCAM Flow Detection (from cell culture)

invitrogen

USER GUIDE Pub. No. MAN0010738 Rev. C.0

Print Options

## Package Contents

| Catalog Number | Size |
|---|---|
| 10624D | 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 EpCAM Flow Detection (from cell culture) is intended for isolation of EpCAM-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 EpCAM 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 www.lifetechnologies.com/support.

## Protocol outline

1. Pre-enrich exosomes.
2. EpCAM 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 EpCAM-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

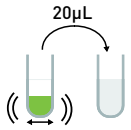
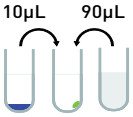
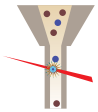
Good mixing is critical to successful exosome isolation. Use a mixer that tilts and rotates to ensure that the beads do not settle in the tube.

## Example of EpCAM flow cytometry analysis

## Limited product warranty and disclaimer details

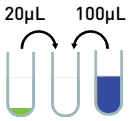
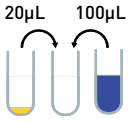
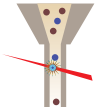
## EpCAM positive isolation

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 EpCAM isolation beads** | 1. Place vial of beads on a roller for >10 minutes or vortex for 30 sec to resuspend.<br>2. Transfer 20 µL bead solution to a tube containing 1 mL Assay Buffer.<br>3. Place the tube in magnetic separator for 1–2 min.<br>4. Remove the buffer. |
| | 2   | **Mix isolation beads with pre-enriched exosome sample** | 1. Add 90 µL Assay Buffer to tube containing beads.<br>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.<br>2. Add 300 µL of Assay Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant.<br>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.<br>2. Remove tube from magnetic separator.<br>3. Add 300 µL Assay Buffer. |
| | 6  | **Proceed to downstream analysis** | ▪ **Flow cytometry**<br>▪ Electron microscopy |

## Flow cytometry analysis after EpCAM 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 EpCAM/CD326-RPE, clone EBA-1 (BD Cat. No. 347198).<br>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).<br>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.<br>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.<br>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).<br>2. Perform flow cytometry analysis. |