







	<b>Package Contents</b>	<b>Catalog Number</b> 10620D	<b>Size</b> 2 mL
	<b>Storage Conditions</b>	<ul style="list-style-type: none"> <li>Store at 2°C to 8°C.</li> <li>When stored as instructed, expires two year from date of receipt unless otherwise indicated on product label.</li> </ul>	
	<b>Required Materials</b>	 <b>List of Materials</b>	
	<b>Timing</b>	<ul style="list-style-type: none"> <li>Hands-on time: 45 minutes</li> <li>Incubation time: 16–24 hours</li> <li>Staining for flow cytometry: 45 minutes</li> </ul>	
	<b>Selection Guide</b>	<a href="#">Exosome Research Products</a> <a href="#">Magnetic Separators</a> Go online to view related exosome products and magnets.	
	<b>Product Description</b>	<ul style="list-style-type: none"> <li>Exosome – Human CD9 Flow Detection (from cell culture) is intended for isolation of CD9-positive human exosome subsets from a pre-enriched exosome sample.</li> <li>Dynabeads™ are uniform, superparamagnetic polystyrene beads (2.7 µm dia.) coated with a primary monoclonal antibody specific for the CD9 membrane antigen expressed on most human exosomes. The Dynabeads™ magnetic beads are incubated with your samples overnight and isolated exosomes are magnetically separated.</li> </ul>	
	<b>Important Guidelines</b>	<ul style="list-style-type: none"> <li>Follow the recommended pipetting volumes and incubation times.</li> <li>Avoid air bubbles (foaming) during pipetting.</li> <li>Results are dependent on the level of exosomes present in the pre-enriched exosome sample.</li> </ul>	
	<b>Online Resources</b>	Visit our <a href="#">product pages</a> for additional information and protocols. For support, visit <a href="http://thermofisher.com/support">thermofisher.com/support</a> .	

## Protocol outline

1. Pre-enrich exosomes.
2. CD9 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 CD9-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

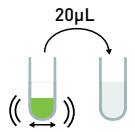
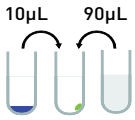



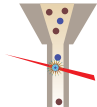
Good mixing is critical to successful exosome isolation. Use a mixer that tilts and rotates to ensure that the beads do not settle in the tube.

## Example of CD9 flow cytometry analysis

## Limited product warranty and disclaimer details

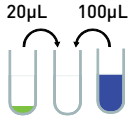
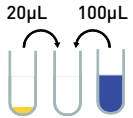


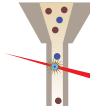
## Exosome – Human CD9 Flow Detection (from cell culture) CD9 positive isolation protocol

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  $\mu$ L pre-enriched exosome solution. Scale the protocol according to the number of analyses to be performed.

	Timeline	Step	Action
Day 1		<b>Prepare exosome – human CD9 isolation beads</b>	<ol style="list-style-type: none"> <li>1. Place vial of beads on a roller for &gt;10 minutes or vortex for 30 sec to resuspend.</li> <li>2. Transfer 20 <math>\mu</math>L bead solution to a tube containing 1 mL Assay Buffer.</li> <li>3. Place the tube in magnetic separator for 1–2 min.</li> <li>4. Remove the buffer.</li> </ol>
		<b>Mix isolation beads with pre-enriched exosome sample</b>	<ol style="list-style-type: none"> <li>1. Add 90 <math>\mu</math>L Assay Buffer to tube containing beads.</li> <li>2. Add 10 <math>\mu</math>L pre-enriched exosome sample.</li> </ol>
		<b>Incubate beads and exosomes</b>	Incubate at 2–8°C overnight with end-over-end mixing (tilting and rotation).
Day 2		<b>Isolate bead-bound exosomes with magnetic separator</b>	<ol style="list-style-type: none"> <li>1. Centrifuge sample tube briefly 1–2 sec.</li> <li>2. Add 300 <math>\mu</math>L of Assay Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant.</li> <li>3. Remove tube from magnetic separator.</li> </ol>
		<b>Wash bead-bound exosomes</b>	<ol style="list-style-type: none"> <li>1. Add 300 <math>\mu</math>L of Assay Buffer and place tube in magnetic separator for 1–2 min before removing all supernatant.</li> <li>2. Remove tube from magnetic separator.</li> <li>3. Add 300 <math>\mu</math>L Assay Buffer.</li> </ol>
		<b>Proceed to downstream analysis</b>	<ul style="list-style-type: none"> <li>▪ Flow cytometry</li> <li>▪ Electron microscopy</li> </ul>

## Flow cytometry analysis after CD9 positive exosome isolation

- Include a matched isotype control as a background control.
- Use 100  $\mu$ L 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	<b>1</b> 	<b>Prepare target specific sample</b>	1. Add 20 $\mu$ L of anti-human CD9-RPE, clone ML-13 (BD Cat. No. 555372). 2. Add 100 $\mu$ L of bead-bound exosome sample.
	<b>2</b> 	<b>Prepare isotype control</b>	1. Add 20 $\mu$ L of mouse IgG1-RPE (BD Cat. No. 559320). 2. Add 100 $\mu$ L of bead-bound exosome sample.
	<b>3</b> 	<b>Stain samples</b>	1. Incubate tubes at room temperature for 45 min on an orbital shaker at 1000 rpm. 2. Protect samples from light during incubation.
	<b>4</b> 	<b>Wash samples</b>	1. Add 300 $\mu$ L 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.
	<b>5</b> 	<b>Perform flow cytometry analysis</b>	1. Add 300 $\mu$ L of Assay Buffer to each sample (adjust volume according to instrument and tubes used). 2. Perform flow cytometry analysis.