Mouse CD63 Capture Beads for Flow Detection

Immunobeads for Exosome capture and detection

Reference: MO63CB-25
Size: 25 test

1. INTRODUCTION

Exosomes are small extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB)[1], with the plasma membrane. They are thought to provide a means of intercellular communication[2,3] and of transmission of macromolecules between cells allowing the spread of proteins, lipids, mRNA, miRNA and DNA and as contributing factors in the development of several diseases. Exosomes can also modulate cancer microenvironment[4] and the immune response [5,6].

2. PRODUCT DESCRIPTION

The product consists of a simple bead population, coated with a capture antibody (CD63) for isolation/detection of exosome.

- Tested application: Flow Cytometry
- Species reactivity: Mouse
- Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3).
- Recommended usage: CD63 Capture Beads are intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched CD63+ rodent exosomes from cell culture media.
- Presentation: liquid

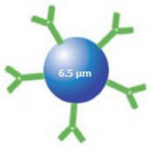

Figure 1: Superparamagnetic Capture Beads.

3. REAGENTS PROVIDED

DESCRIPTION	COMPONENTS	AMOUNT	Nº TEST
Superparamagnetic Capture Beads	CD63+ (Clone NVG-2) capture beads. Polystyrene micro-particles with Mean Diameter (µm) 6.5±0.2 (CV<5%), having discrete fluorescence intensity characteristics	6000 beads/test (50 µl/test)	25

4. APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, ref rigerated between 2 °C and 8 °C. DO NOT FREEZE. The kit is stable until the expiry date stated on the vial label if kept at 2-8°C. Do not use after the date indicated.

5. REAGENTS NOT PROVIDED

- Pre-enriched exosomes by ultra-centrifugation.
- Magnetic Rack; MagneSphere(R) Mag. Sep. Stand 12-hole, 12x75mm (PROMEGA, Ref Z5343).
- 12x75 mm Polystyrene Round Bottom Tubes (cytometer tubes).
- Sterile syringe filter with a 0.45 µm pore (EMD Millipore Millex, Ref: SLHV033RS).
- Syringe of adequate volume.

6. EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

7. RECOMMENDATIONS AND WARNINGS

a. Avoid microbial contamination of the reagent. Assay buffer IX can be filtered before use.
b. Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.
c. The samples should be treated with appropriate handling procedures.
d. Depending on the type of exosomes used, the number of exosomes may vary with respect to the concentration of the protein.
e. Do not use after the expiry date indicated on the vial.
f. Deviations from the recommended procedure could invalidate the analysis results.
g. Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
h. The isolation and detection success is dependent on the quality of the sample pre-enrichment process.

8. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep sole liability is limited to either the replacement of the products or refund of the purchase price.

9. SAMPLE PREPARATION

CD63 capture beads allows the detection of isolated exosomes from differential ultracentrifugation (a) as well as direct detection in the sample without the need for ultracentrifugation, just with simple pretreatment (b).

a) Purification of Exosomes by Differential Ultracentrifugation

The product has been validated for pre-enriched rodent exosomes from cell culture, through an ultracentrifugation protocol (Fig. 2).

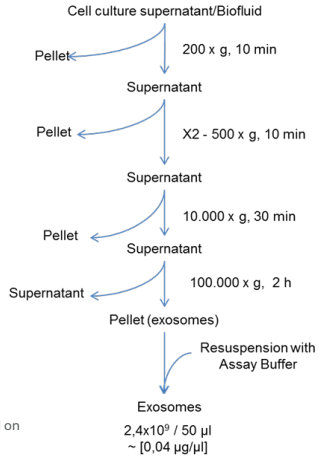

Figure 2: Workflow for the exosome pre-enrichment based on differential ultracentrifugation.

Please, refer to http://www.immunostep.com/content/34-protocols for additional technical information.

b) Sample pretreatment for direct exosome detection

The sample pretreatment for direct exosome detection from cell culture supernatant is not recommended for detection of exosomes from body fluids.

To ensure that detected exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS), because normal FBS contains extremely high levels of exosomes that will contaminate the cell derived exosomes.

b.1) Cell culture

Prepare samples by 4 centrifugation steps:

- 5 min at 200xg and 4°C. Collect supernatant and discard pellet.
- 10 min. at 14000xg and 4°C. Collect supernatant and discard pellet.

10. PROTOCOL

a) Isolate exosomes

1. Resuspend the capture beads by vortex for approximately 20 seconds.
2. Add 50µL of the capture bead to each 12x75mm Polystyrene Round Bottom tube (cytometer tube).
3. Add 50µL of sample previously prepared according to "Sample Preparation" to the appropriate tubes. Mix the reactions gently by pipetting up and down several times with a pipette and vortexing for few seconds.
4. Incubate in the dark overnight at room temperature (RT). NO STIRRING.

b) Stain exosomes for flow cytometry

5. After overnight incubation, add the suggested volume indicated of the primary detection antibody (5µL of the supplied antibody) to the bead-bound exosomes tube. Mix gently by pipetting and/or by tapping. It is advisable to prepare an additional tube with the appropriate isotype control or without exosomes, for background determination.
6. Incubate in the dark 60 minutes at 2-8°C, without stirring.
7. Wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer IX.
8. Collect the magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2500xg for 5 minutes. Remove supernatant from tubes by hand-decanting in the case of using the magnetic rack (Fig. 3) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100ul of supernatant in the tube.

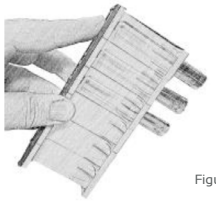

Figure 3: Hand-decanting supernatant using a Magnetic Rack.

Go to step 13 for direct stain protocol (using direct detection antibody labeled with fluorochrome, for indirect stain protocol go to next step (9).

9. Add 5µL of the secondary detection reagent to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.
10. Incubate in the dark 30 minutes at 2-8°C, without stirring.
11. Wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer IX.
12. Collect the magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2500xg for 5 minutes. Remove supernatant from tubes by hand-decanting decanting in the case of using the magnetic rack (Fig. 3) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100 ul of supernatant in the tube.
13. Resuspend the sample in 350µL Assay Buffer IX and acquire on a flow cytometer or store in the dark max up to 2 hours at 2-8°C, until the analysis is carried out.

11. ASSAY ACQUISITION

An adequate gating strategy FSC / SSC for 6 micron bead size and PerCP/APC, PerCP-Cy5/APC or PerCP-Cy5. 5/APC helps bead population identification and discrimination of doublets on flow cytometer.

1. Gate on the single population(s) on a Forward Scatter vs. Side Scatter plot in linear scale. (Fig. 4A).
2. Gate on the single population(s) on a FL3 vs. FL4 channel (bead auto fluorescence) in logarithmic scale (Fig. 4B).
3. Using the FL2 channels, determine whether or not any bead populations tested "positive" for the exosome. Note: A positive bead will produce a fluorescent peak in the FL2 channel.

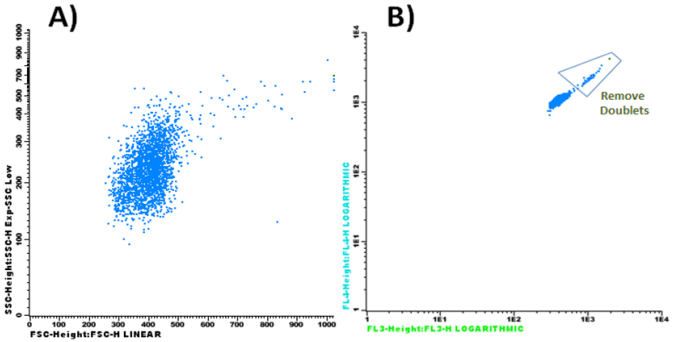

Figure 4: Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and FL3 vs FL4 (B).

14. REFERENCES

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