Capture Beads for Flow Detection

Immunobeads for Exosome capture and detection

REFERENCE	SIZE
63CB-25	25 test
9CB-25	
8ICB-25	
274CB-25	
326CB-25	
MO63CB-25 (mouse)	
IGGICB-25 (negative control)	
IGG2ACB-25 (negative control)	

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 for isolation/ detection of exosomes.

- **Tested application:** Flow Cytometry [7,8].
- **Species reactivity**: Human / Mouse (MO63CB-25)
- **Storage buffer**: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3).
- **Recommended usage**: Capture Beads are intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched human exosomes from biofluids (plasma, urine) or cell culture media.
- **Presentation**: Liquid

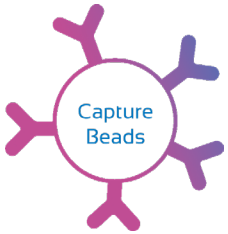


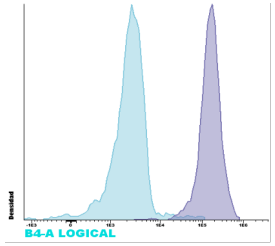
Figure 1: Superparamagnetic Capture Bead Graphical Representation.

3. REAGENTS PROVIDED

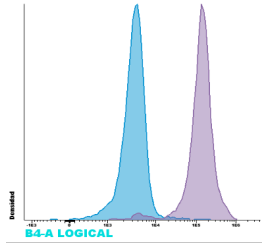
CAPTURE BEADS CLONE	DESCRIPTION	AMOUNT
63CB-25 (TEA3.18)	Superparamagnetic Capture Beads Polystyrene micro-particles with Mean Diameter (µm) 6.5±0.2 (CV<5%), having discrete fluorescence intensity characteristics	6000 beads/test (50ul/test)
9CB-25 (VJ/20)		
8ICB-25 (M38)		
IGGICB-25 (BI1/6)		
274CB-25 (29E.2A3)		
326CB-25 (VU-ID9)		
MO63CB-25 (NVG-2)		
IGG2ACB-25 (BI2/8)		

4. ISOTYPES CONTROLS

Immunostep's isotypes controls IgG1 (clone BI1/6) and IgG2a (clone BI2_8) are monoclonal antibodies produced against a synthetic hapten, which is normally not present in humans or animals using in flow cytometry. These are often used as negative controls.
IgG1 is coated in the population beads number while IgG2a is in population number 2. Both populations do not differ in size but in average fluorescence intensity.



IgG1 + (CD63 or CD81) capture beads



IgG2a + CD9 capture beads

5. APPROPIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated 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.

6. 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.
- Assay Buffer: PBS-BSA 1% pH 7.4. Assay buffer 10X can be purchased as it appears in the catalog with the following reference IMS0515.

7. 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

8. RECOMMENDATIONS AND WARNINGS

a. Avoid microbial contamination of the reagent. Assay buffer 1X 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.
i. Pay attention if the kit is used in combination with annexin assays. The buffer used to work with annexin generates non-specificity with the Capture Beads.

9. 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.

10. SAMPLE PREPARATION

Exostep 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 kit has been validated for pre-enriched human exosomes from cell culture and bodily fluids, such as serum/plasma, and urine, through an ultracentrifugation protocol (Fig. 2)[9].

The principle for exosome purification is the same for cell culture and bodily fluids, but due to the viscosity of some fluids it is necessary to dilute them with an equal volume of PBS, before centrifugations.

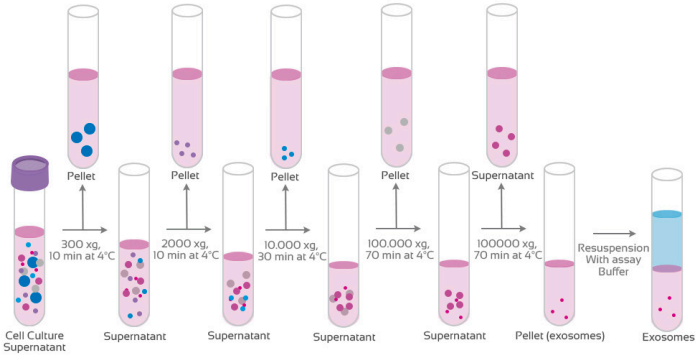


Figure 2: Workflow for the exosome pre-enrichment based on differential ultracentrifugation. Please, refer to https://ww.immunostep.com/content/34-protocols for additional technical information.

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. Specific sample pretreatment protocols are available for body fluids (plasma, urine) each optimized for its specific type of biological sample.
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.

a) 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.

b) Plasma, serum and urine

About 100–1000µL of plasma/urine typically provides enough exosomes for most standard types of analysis.

- 10 min. at 500xg collect supernatant and discard pellet.
- 10 min. at 2000xg collect supernatant and discard pellet.
- 30 min. at 14000xg collect supernatant and discard pellet.

11. PROTOCOL

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). If an isotypic control needs to be added, please, do it at this point.
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. After incubation if only exosome isolation is needed, whithout FACS analysis, go directly to step 12.

Stain exosomes for flow cytometry

5. After overnight incubation, add the suggested volume indicated of the primary detection antibody (not supplied. Visit our website: www.immunostep.com) 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 1X.
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.
9. Go to step 13 for direct stain protocol (using direct detection antibody labeled with fluorochrome) for indirect stain protocol go to next step (10).

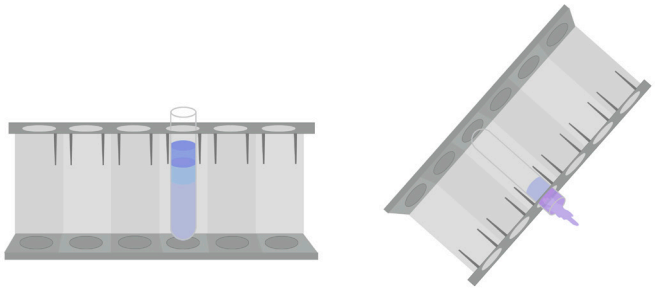


Figure 4: Hand-decanting supernatant using a Magnetic Rack.

10. Add 5µL of the secondary detection reagen (not supplied) t to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.
11. Incubate in the dark 30 minutes at 2-8°C, without stirring.
12. Wash the sample (bead-bound exosomes) by adding 1 ml of PBS-BSA 1% pH 7.4.
13. 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. For exosome magnetic isolation without FACS analysis, protocol ends her, on the contrary, continue with next step.
14. Resuspend the sample in 350µLPBS-BSA 1% pH 7.4. 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

12. 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. 5A).
2. Gate on the single population(s) on a PerCP vs. APC channel (bead auto fluorescence) in logarithmic scale (Fig. 5B)
3. Using the PE channels, determine whether or not any bead populations tested "positive" for the exosome. Note: A positive bead will produce a fluorescent peak in the PE channel.

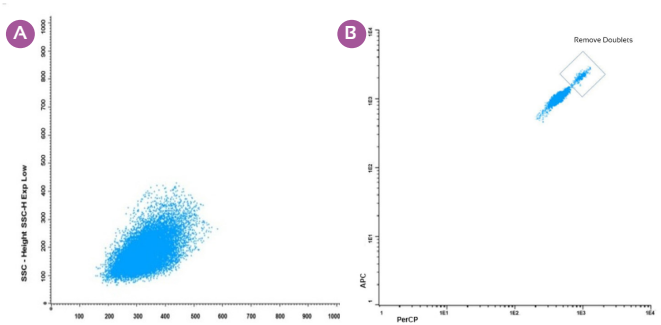


Figure 5: Dot-plot gating strategy for acquisition and analysis. FSC vs SSC and A PerCP vs APC B

13. REFERENCES

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