

Mouse ExoStep™

Exosome FACS analysis and purification kit of exosomes derived from Mouse

REFERENCE	SIZE	DESCRIPTION
MO2ExoS- 25-C9	25 test	Includes CD9 biotin as primary detection antibody.

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).

PRODUCT DESCRIPTION

The kit is a simple immunobead assay for isolation/detection of exosome, using a bead-bound anti-CD63 capture antibody and a fluorochrome conjugated anti-CD9 detection antibody. The kit provides reproducible results and can be run in parallel to exosome immunophenotyping.

Tested application: Flow Cytometry

Species reactivity: Rodent

Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN₃).

Recommended usage: Immunostep's Rodent ExoStep, is intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched CD63⁺ rodent exosomes from cell culture media.

Presentation: liquid

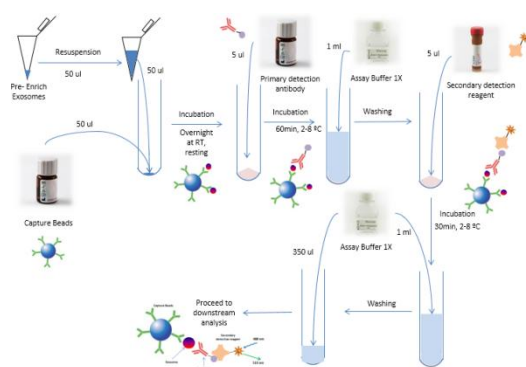


Figure 1: Immunobead assay for isolation/detection of exosome workflow.

REAGENTS PROVIDED

Description	Components	Amount	Nº test/µg
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 test
Primary detection antibody	Anti-CD9 biotin (Clone MZ3)	(5 µl/test)	25 test
Secondary detection reagent	Streptavidin-Phycoerythrin (PE) is useful for detecting biotinylated antibodies. The excitation of PE by 488 nm laser light induces a light emission maximum of 575 nm.	5 µl/test	25 test
Assay Buffer 10X	PBS 10% BSA, pH 7.4 – 10X. Do not freeze. Dilute contents of the 10X Assay Buffer to 1X (PBS 1% BSA) in PBS, for use in this assay.	10 ml	

APPROPRIATE 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.

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.

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

RECOMMENDATIONS AND WARNINGS



- Avoid microbial contamination of the reagent. Assay buffer 1X can be filtered before use.
- Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.
- The samples should be treated with appropriate handling procedures.
- Depending on the type of exosomes used, the number of exosomes may vary with respect to the concentration of the protein.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
- The isolation and detection success is dependent on the quality of the sample pre-enrichment process.

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's sole liability is limited to either the replacement of the products or refund of the purchase price.

SAMPLE PREPARATION

Exostep allows the detection of isolated exosomes from differential ultracentrifugation, precipitation or size exclusion purification as well as direct detection in the sample without previous isolation and just with simple pretreatment.

Stain exosomes for flow cytometry

1. 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.
2. Incubate in the dark 60 minutes at 2-8°C, without stirring.
3. Wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer 1X.
4. 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.2) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100µl of supernatant in the tube.

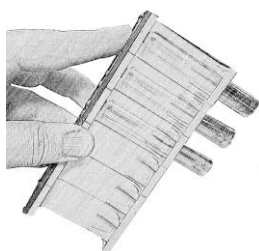


Figure 2: Hand-decanting supernatant using a magnetic rack.

5. Remove the tubes from the magnetic rack and resuspend the microspheres in the remaining 100µL of Assay Buffer 1X. Mix gently by pipetting.
6. Add 5µL of the secondary detection reagent to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.
7. Incubate in the dark 30 minutes at 2-8°C, without stirring.
8. Wash the sample (bead-bound exosomes) by adding 1ml of Assay Buffer 1X.
9. 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.2) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100µl of supernatant in the tube.

10. Resuspend the sample in 350µL Assay Buffer 1X 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.

ASSAY ACQUISITION

An adequate gating strategy FSC / SSC for 6 micron bead size and FL3 / FL4, 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. 3A).
2. Gate on the single population(s) on a FL3 vs. FL4 channel (bead auto fluorescence) in logarithmic scale (Fig. 3B).
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.

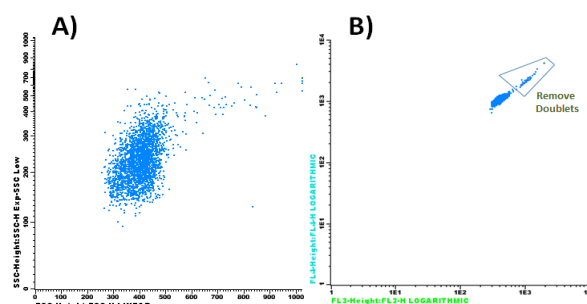


Figure 3: Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and FL3 vs FL4 (B).

FLOW CYTOMETRY ANALYSIS

ExoStep has been used for detection of exosome derived from different sources.

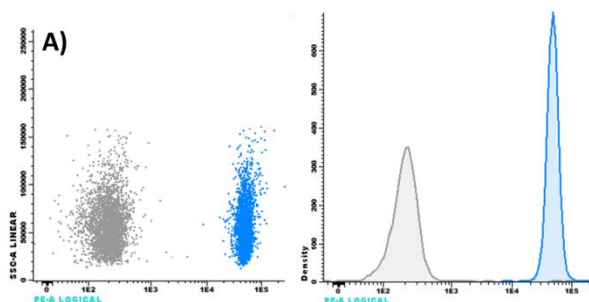


Figure 4: Flow analysis of exosomes bound to ExoStep. Cell culture exosomes, pre-enriched using total exosome isolation from PC3 cell culture media (A), were resuspended in PBS and bound to CD63-capture beads during an overnight incubation. The following day the bead-bound exosomes were indirectly stained with primary detection antibody (CD9 Biotin/CD81 Biotin) and Streptavidin-PE and analyzed by flow cytometry.

PERFORMANCE DATA

Limit of Detection (LOD), dynamic range and linearity of exosome kit was assessed at Immunostep.

LOD is the lowest quantity of exosomes that is distinguished from the absence of analyte (a blank value), and as reference, was determined in $>0,25\mu\text{g}$ which corresponds with $>1.75 \times 10^8$ vesicles. Whilst the upper limit or saturation level was established in $24\mu\text{g}$. For both technical specifications were used exosome from B16F10 cell culture media (7×10^8 particles $/\mu\text{l}$). (Fig. 5)

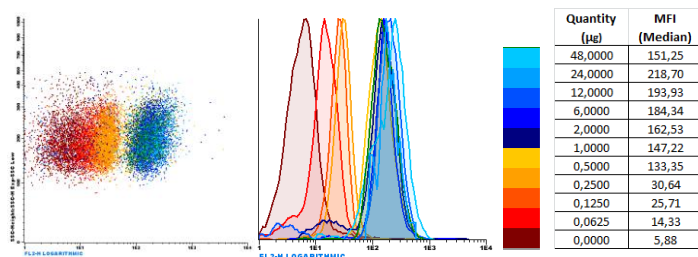


Figure 5: Dynamic range of the assay analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations.

Several measurements of multiple concentrations of lyophilized exosomes were analyzed across the reportable range of the kit, finding the linearity of the kit in a broad range of concentrations, allowing fluorescence interpolation in the estimation of concentrations.

Please, refer to our website technical support <http://www.immunostep.com/content/34-protocols> for further information.

REFERENCES

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