MSCs-derived exosomes

REF	Σ		
ExoMSC	100 µl	Adipose-derived Mesenchymal stem/stromal cells (MSCs) derived exosomes.	RUO

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]. In this sense, it is well known that Mesenchymal stromal/stem cells (MSCs) are clinically useful for cell-based therapy, however some studies have shown that MSC-derived Exosomes emulate the effect of MSCs in various experimental models, stimulating cell proliferation and repair [7].

2. PRODUCT DESCRIPTION

Lyophilized/Frozen exosomes (~1x10^{12}) from adipose-derived Mesenchymal stem/stromal cells (MSCs) [8,9]. Exosomes are isolated by differential ultracentrifugation [10].

- **Tested application:** In-vitro functional assay, Flow Cytometry (FMC), Nanoparticles Tracking Analysis (NTA, Nanosight), Western Blot (WB), BCA Protein Assay, Confocal Microscopy.
- **Species reactivity**: Human
- **Presentation**: Lyophilized/Frozen
- **Reconstitution of lyophilized Exosomes:** For reconstitution, we recommended adding sterile, distilled water to achieve a final exosome concentration of 1µg/µl (e.g., for 100 µg standard, add 100 µl of dH2O). After the addition of water, recap vial and briefly vortex making sure that the liquid has been gently distributed and has covered the entire inside of the vial.
  After vortexing, make sure that the solution is collected at the bottom of the vial, if not, centrifuge shortly the vial solution. Now the standard is ready to use.

3. APPROPRIATE STORAGE AND HANDLING CONDITIONS

Lyophilized exosomes can be stored between 2ºC and 8ºC for up to 2 years without functional compromise. Frozen exosomes can be stored at -20 ºC.

Immunostep recommends storing small, single –use aliquots of reconstituted exosomes, at – 20ºC for up to one month or at – 80ºC for longer periods, preferably in locations in frost-free freezers, without appreciable temperature fluctuation. This will minimize protein denaturation that can occur after multiple freeze/thaw cycles.

Reconstituted exosomes, store properly, are functionally guaranteed for up to six months from date of reconstitution.

Any unfrozen and/or unused exosome standard can be stored at 4ºC for short term use (<1 week), and should not be re-frozen.

4. 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

5. 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.

6. PERFORMANCE DATA

At the time of exosome collection, for each batch, MSCs phenotype analysis is performed by FCM in order, to guarantee the right signature described by The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) (11). Thus MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface molecules (Fig. 1).

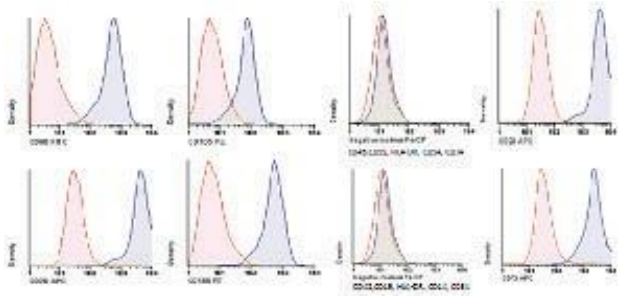
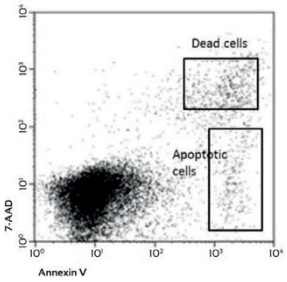


Figure 1: The following histograms correspond to the analysis of antigen expression in an expansion of Adipose-derived Mesenchymal stem/stromal cells MSCs (passage 2) cultured for exosome production.



The percentage of apoptotic and necrotic MSCs at the time of collection is also analyzed by FCM (Fig.2) to verify that exosome preparation is not cross-contaminated with apoptotic bodies.

Exosome batches are checked and compared for the presence of the CD63 and CD9, a common exosome marker, by FCM and WB (Fig.3).

Figure 2: Apoptotic and necrotic MSCs percentage analysis by FCM at the time of supernatant collection.

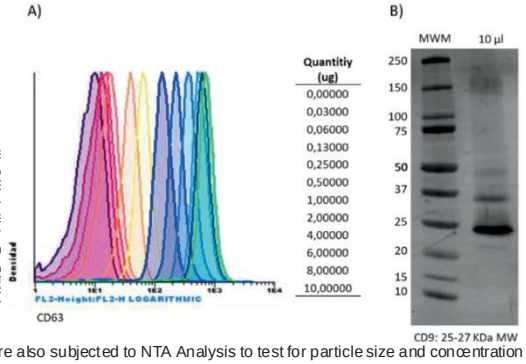


Figure 3: Dynamic range of MSCs-derived exosomes analyzed by flow cytometry (A). Exosomes were captured by CD63+ (Clone TEA3/18) capture beads and subsequently detected by Anti-CD9 PE (Clone VJ1/20). MSCs-derived exosome batches are analyzed by WB in native conditions for exosomal markers, by anti-CD9 (Clone VJ1/20) antibody at a 1:1000 dilution (0,1 mg/ml).

All exosome batches are also subjected to NTA Analysis to test for particle size and concentration (Fig.4).

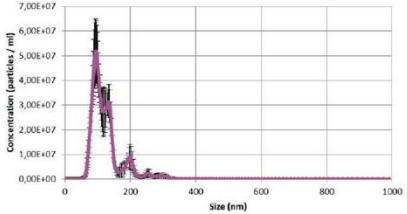


Figure 4: MSCs-derived exosome analysis for particle size and concentration by NTA, NanoSight LM10HSB. Analysis is carried out with 1 µl of purified exosomes diluted in 999 µl of HEPES buffer (dilution 1:1000). The purified exosomes showed a size distribution profiles, with peak diameters from 50 – 150 nm and concentrations about 1x10^{12} exosomes/ml.

Finally, exosomes derived from MSCs are functionally analyzed in an in vitro assay (Fig. 5), in which uptake cell and internalization exosomes are checked on wide range of different cell cultures.

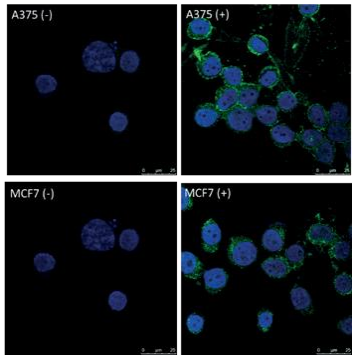


Figure 5: Cellular uptake of exosomes. MSCs-derived exosomes are isolated and labeled with PKH67 and DAPI. The labeled exosomes are cultured with several cell lines (here are shown A375 and MCF7) for 17 hours and analyzed by confocal microscopy C. PKH67 bright spots were seen in the cells cultured with loaded exosomes, and not seen in control cells, whilst DAPI stains nucleic acid in both.

7. EXPLANATION OF SYMBOLS

	Fluorochrome
REF	Product reference
Σ	Content for <n> analysis
	Regulatory Status
	Description
RUO	Research Use Only
	Manufacturer

8. REFERENCES

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