Lyophilized Exosome Standards (2nd Generation)

REFERENCE	SIZE	DESCRIPTION
ExoPC3	100 µg	Exosomes from PC-3, a human metastatic prostate cancer cell line
ExoHT29	100 µg	Exosomes from HT-29, a human colon cancer cell line
ExoMCF7	100 µg	Exosomes from MCF-7, a human breast cancer cell line
ExoSERUM	100 µg	Exosomes from human serum
ExoA-375	100 µg	Exosomes from A-375, a human malignant melanoma cell line
EXORPMI	100 µg	Exosomes from RPMI8226, a human myeloma cell line
EXOCaCo2	100 µg	Exosomes from CaCo2 a human colon cancer cell line

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

Lyophilized exosomes (~1x1012) derived from human cancer cell line [8,9].
Exosomes are isolated by differential ultracentrifugation [7].

- **Tested application**: Flow Cytometry (FMC), Nanoparticles Tracking Analysis (NTA, Nanosight), Western Blot (WB), BCAProtein Assay.
- **Species reactivity:** Human
- **Presentation**: Lyophilized
- **Reconstitution of 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. APPROPIATE STORAGE AND HANDLING CONDITIONS

Lyophilized exosomes can be stored between 2°C and 8°C for up to 2 years without functional compromise. Immunostep recommends storing small, single –use aliquots of reconstituted exosomes, at – 20°C for up to one month or at – 80°C 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 4°C 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. BIOSAFETY LEVEL 1

Biosafety classification is based on 2000/54/EC Directive from the European Council.
Customer has to ensure that their facilities comply with biosafety regulations for their own country.

6. 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.

7. PERFORMANCE DATA

All exosome standard batches has been validated using FCM, WB and NTA Analysis, additionaly, in order to compare the effects of lyophilization process we have compared all lyophilized batches with respect to fresh exosomes stored at -20°C. Exosome batches are checked and compared for the presence of the CD63 and CD9, a common exosome marker, by FCM (Fig. I) and WB (Fig. 2).

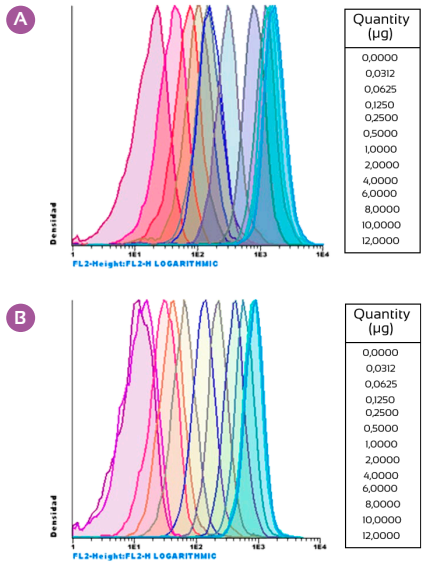

Figure I: Dynamic range of fresh A and lyophilized B PC3 exosomes analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations. Exosomes were captured by CD63+ (Clone TEA3/18) capture beads and subsequently detected by Anti-CD9 PE (Clone VJ1/20).

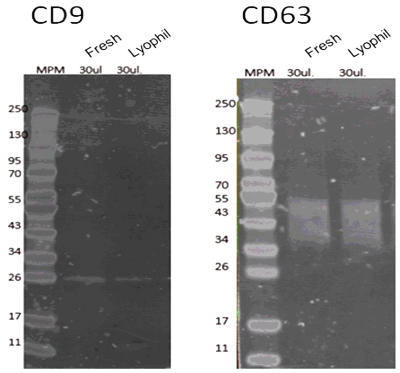

Figure 2: Fresh and lyophilized MCF-7 exosome batches were analyzed and compared by WB in native conditions for exosomal markers, by anti-CD9 (Clone VJ1/20) and anti-CD63 (Clon TEA3/18) antibodies at a 1:1000 dilution(0,1 mg/ml).

All exosome batches are also subjected to NTA Analysis to test for particle size and concentration (Fig. 3).

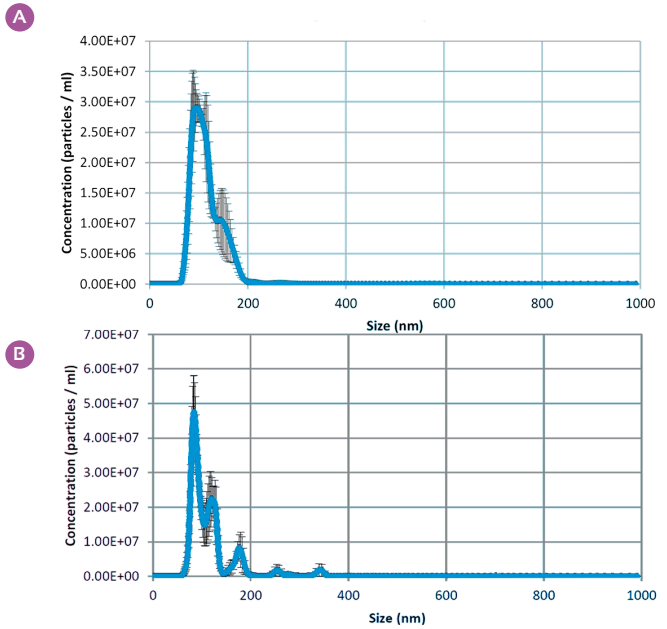

Figure 3: Exosome analysis and comparative of fresh A and lyophilized B serum exosomes for particle size and concentration by NTA, NanoSight LMIOHSB. Analysis was carried out with 1 µl of purified exosomes diluted in 999 µl of HEPES buffer (diution 1:1000). The purified exosomes showed a size distribution profiles, with peak diameters from 50 – 150 nm and concentrations about 1x1010 exosomes/ml.

8. REFERENCES

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