Lyophilized Exosome Standards (2nd Generation)

REF	SIZE	
ExoPC3	100 µg	Exosomes from PC-3 (human prostate adenocarcinoma cell line)
ExoHT29	100 µg	Exosomes from HT-29 (human colon cancer cell line)
ExoMCF7	100 µg	Exosomes from MCF-7 (human breast cancer cell line)
ExoSERUM	100 µg	Exosomes from human serum
ExoA375	100 µg	Exosomes from A-375 (human malignant melanoma cell line)
ExoRPMI	100 µg	Exosomes from RPMI8226 (human myeloma cell line)
ExoCaCo2	100 µg	Exosomes from CaCo2 (human colon cancer cell line)
ExoA549	100 µg	Exosomes from A-549 (human lung cancer cell line)
ExoPANC1	100 µg	Exosomes from PANC-1 (human pancreas cancer cell line)
ExoMSC	100 µg	Adipose-derived Mesenchymal stem/stromal cells (MSCs) derived exosomes.

1. INTRODUCTION

Exosomes are small (~40-100 nm) extracellular vesicles (EVs) released from all cell types upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB) [1] with the plasma membrane. Exosomes found in body fluids and cell culture supernatants. They are though 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 (~1x10[12]) 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.

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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. 1) and WB (Fig. 2).

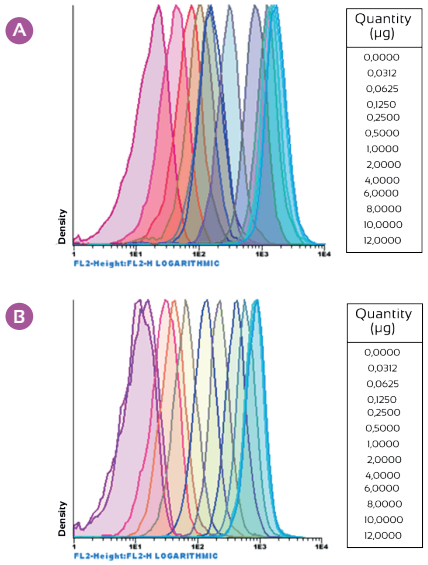

Figure 1: Dynamic range of fresh A and lyophilized B PC3 exosomes analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations. Exosomes were captured by CD63+ (Clone TEA3/18) capture beads and subsequently detected by Anti-CD9 PE (Clone VJ1/20).

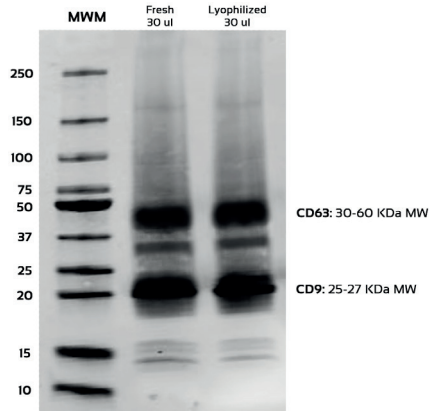

Figure 2: Fresh and lyophilized MCF-7 exosome batches were analyzed and compared by WB in native conditions for exosomal markers, by anti-CD9 (Clone VJ1/20) and anti-CD63 (Clon TEA3/18) antibodies at a 1:1000 dilution(0,1 mg/ml).

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

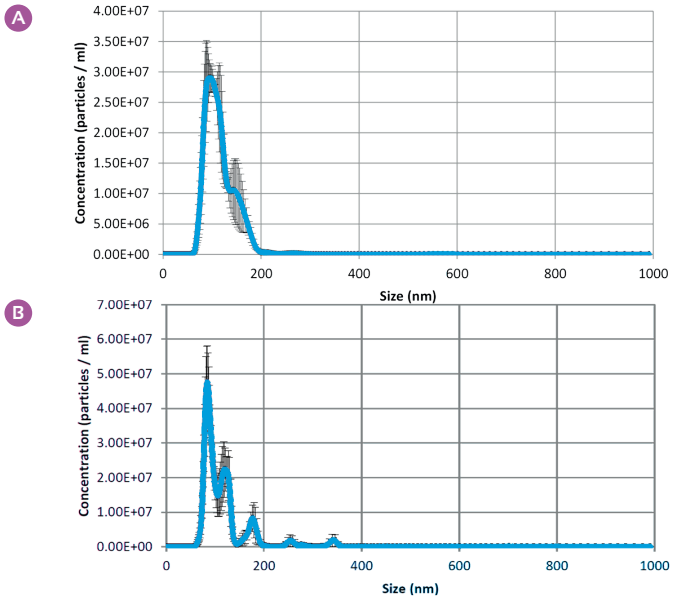

Figure 3: Exosome analysis and comparative of fresh A and lyophilized B rum exosomes for particle size and concentration by NTA, NanoSight LM10HSB. Analysis was carried out with 1 µl of purified exosomes diluted in 999 µl of HEPES buffer (diution 1:1000). The purified exosomes showed a size distribution profiles, with peak diameters from 50 – 150 nm and concentrations about 1x1010 exosomes/ml.

Exosomes have been proposed to provide means for intercellular exchange of macromolecules, allowing the transfer of proteins, lipids, mRNA and miRNA. MiRNAs are a class of 17-24 nt small, noncoding RNAs. Exosomal miRNAs play an important role in disease progression. These miRNAs can stimulate angiogenesis or facilitate metastasis in cancers. Therefore, exosomal miRNAs present potential for uses as noninvasive biomarkers that can indicate the stage of the disease.

The miRNA content of each of our lyophilized exosomes has been analyzed (figure 4). Differential expression analysis was performed with EdgeR software. Besides, sequencing quality was validated with FASTQC software. Below is a series of graphs where the most relevant results of the study are collected.

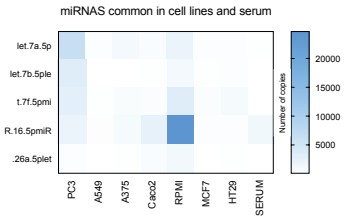

Figure 4: This heatmap shows the common miRNAs in exosomes from our cell lines and serum. Serum samples are non cancer donors, however other pathologies cannot be ruled out. miRNAs selected were those who presented more than 50 copies.

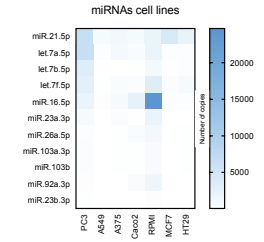

Figure 5: This heatmap shows the common miRNAs in exosomes from our cell lines. miRNAs selected were those who presented more than 50 copies.

In addition, It was made a comparison of the miRNAs with more than 50 copies of readings of each line with respect to the serum (figure 6: A, B, C, D, E, F, and G).

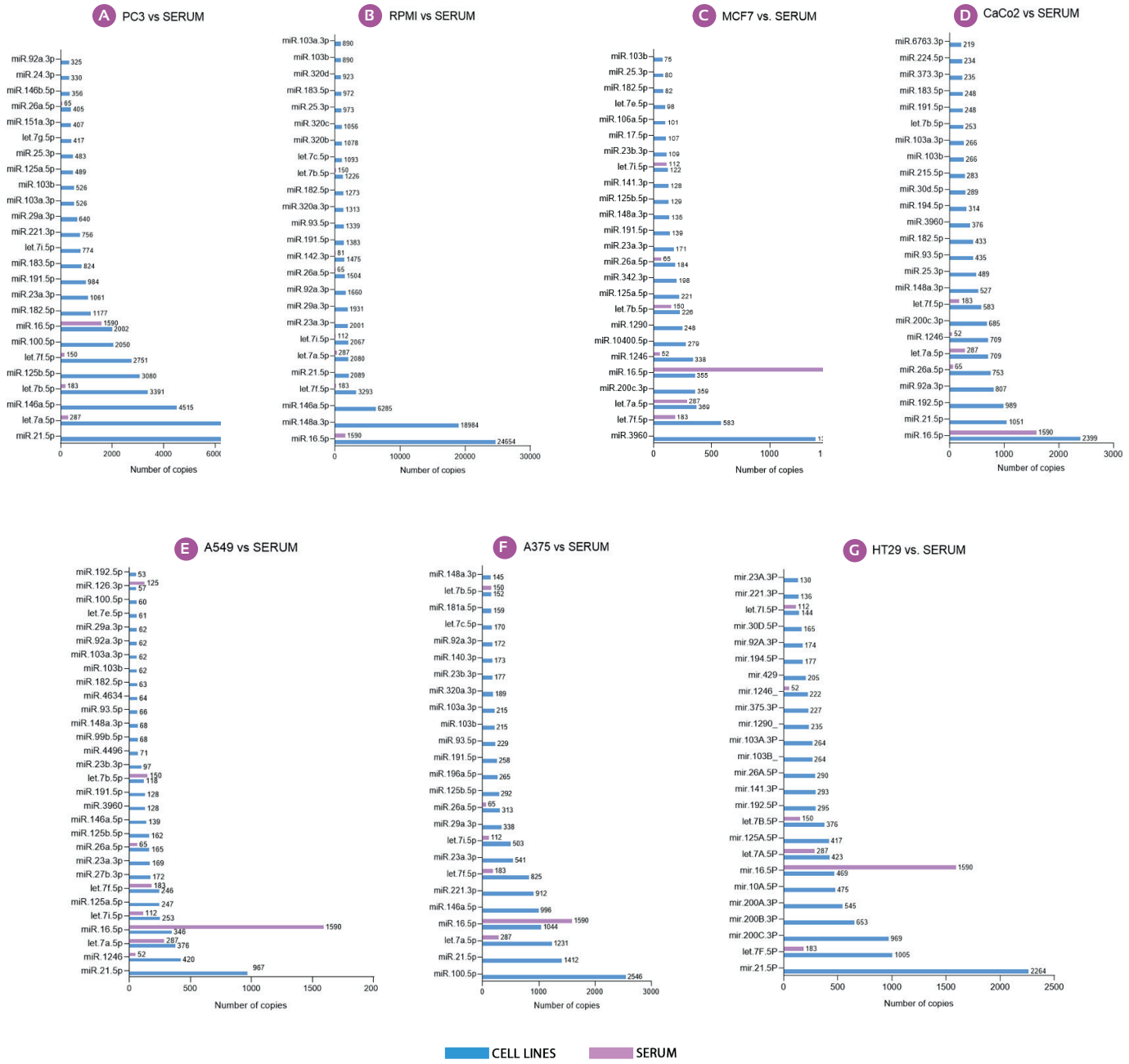

Figure 6 (A, B, C, D, E, F, and G): Comparison of miRNAs of each cell line with respect to serum.

8. REFERENCES

1. Yáñez-Mó M, Siljander P, Andreu Z, Bedina Zavec A, Borràs F, Buzas E et al. Biological properties of extracellular vesicles and their physiological functions. Journal of Extracellular Vesicles. 2015;4(1):27066.
2. Pitt JM, André F, Amigorena S, Soria JC, Eggermont A, Kroemer G, Zitvogel L. Dendritic cell-derived exosomes for cancer therapy. J Clin Invest. 2016.
3. Tkach M, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. 2016 Cell 10;164(6):1226-32.
4. Becker A, Thakur BK, Weiss JMI, Kim HS, Peinado H, Lyden D Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell 2016 Dec 12;30(6):836-848.
5. López-Cobo S, Campos-Silva C, Valés-Gómez M. Glycosyl-Phosphatidyl-Inositol (GPI)-Anchors and Metalloproteases: Their Roles in the Regulation of Exosome Composition and NKG2D-Mediated Immune Recognition. Front Cell Dev Biol. 2016 Sep 12;4:97.
6. Jonathan M. Pitt, Guido Kroemer, Laurence Zitvogel Extracellular vesicles: masters of intercellular communication and potential clinical interventions. 2016 J Clin Invest. 2016;126(4):1139-1143
7. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006 Apr;Chapter 3:Unit 3.22.
8. Campos S, Suárez H, Jara-Acevedo R, Linares-Espinós E, Martínez-Piñeiro L, Yáñez-Mó M, Valés-Gómez M. High sensitivity detection of extracelular vesicles immune-captured from urine by conventional flow citometry. Sci Rep. 2019; Feb 14;9(1):2042.
9. Jara-Acevedo R, Campos-Silva C, Valés-Gómez M, Yáñez-Mó M, Suárez H, Fuentes M. Exosome beads array for multiplexed phenotyping in cancer. J Proteomics. 2019; Apr 30;198:87-97.

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