INDUSTRY'S BEST-DEFINED EXOSOME STANDARDS

Ready-to-use MCF7-derived extracellular vesicles (EVs) standards purified from serum-free conditioned media. The EVs are extensively characterized to define size, concentration, and molecular markers for exosome biology. The standards can be used as a control for sizing and quantitation assays, characterization assays, functional assays, and biomarker analysis using Nanoparticle Tracking Analysis (NTA), Tunable Resistive Pulse Sensing (TRPS/MRPS), Dynamic Light Scattering (DLS), flow cytometry, electron microscopy (EM), ELISA, Western blot and other techniques.

I. Multicolor fluorescent Nanoparticle Tracking Analysis (Zetaview, Particle Metrix GmbH)

Characterized for size, concentration, purity, and EV-specific biomarkers expression assessed using fNTA. Samples are analyzed in scatter mode for the size distribution and concentration of all particles present in the solution. Sample purity and phenotypic profile are further assessed with fNTA using membrane dye and staining with fluorescently labeled antibodies. Percent labeling is calculated relative to the total particle count in scatter mode.

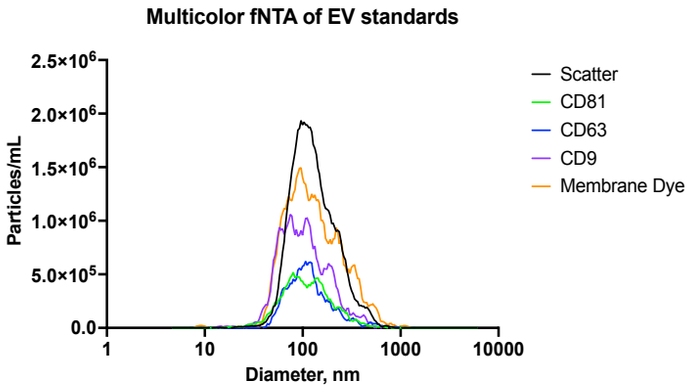

Figure 1. Particle size distribution overlays of NTA in scatter and fluorescent modes of EVs stained with fluorescently labeled antibodies for common EV markers CD9, CD63, and CD81, and a membrane dye.

Label	Median Size (X50),nm	Mode Size, nm	Mean Size, nm	Concentration (particles/mL)	Labeling % of scatter mode
Scatter	113.2	**88.3**	141.8	1.90E+12	
CD81	98.6	**77.5**	120.3	4.10E+11	21.6
CD63	98.2	**79.3**	127.8	4.00E+11	21.1
CD9	95.8	**85.3**	135.1	1.00E+12	55.6
Membrane dye	117.0	**69.4**	160.6	1.66E+12	87.3

Table 1. EV characterization by fluorescent NTA using Zetaview Quatt (Particle Metrix).

II. Cryo-Electron Microscopy (Talos Arctica, Thermo)

CryoEM imaging and analysis are used to ensure the structural integrity of EV standards, as well as an orthogonal technique for size distribution analysis. Hundreds of particles are measured for each lot for adequate statistics.

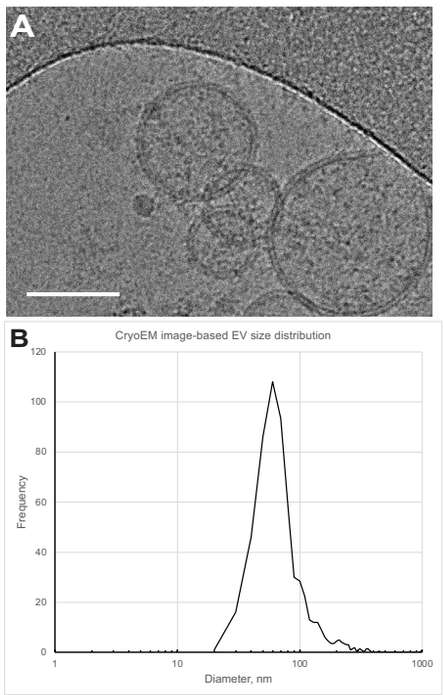

Figure 2. CryoEM microphotograph of EV, scale bar 50 nm (A). CryoEM image-based particle size distribution, N=606 (B).

Median diameter (X50), nm	60.1
Mean diameter, nm	79.2
Mode diameter, nm	53.4
X10 diameter, nm	37.5
X90 diameter, nm	138.4

Table 2. CryoEM image-based size distribution analysis of EVs

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III. Microfluidic Resistive Pulse Sensing (nCS1, Spectradyne)

Measurement of particle size and concentration of EVs size and concentration are additionally evaluated with MRPS method. In this method, the sample is loaded in a disposable microfluidic cartridge with an MRPS aperture of a certain size through which particles flow one at a time. When a particle flows through the aperture, it changes the electrical resistance of the constriction by occluding part of the current, by an amount proportional to the ratio of the nanoparticle volume to that of the aperture.

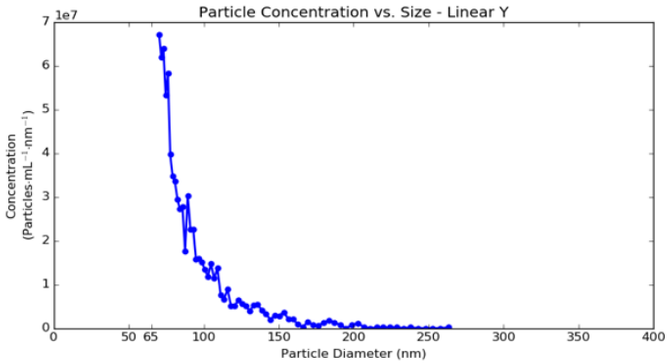

Figure 3. MRPS-based particle size distribution

Chip type	C-400
Total concentration (/mL)	1.43E+12
Number of particles measured	1524
Statistical error in concentration (%)	2.8%
D10 (nm)	75.1
D50 (nm)	88.5
D90 (nm)	145.4

Table 3. EV Analysis by MRPS using Spectradyne nCS1

IV. Super resolution direct Stochastic Optical Reconstruction Microscopy (dSTORM).

Populations of single EVs are characterized using ONI nanoimager. EVs are first identified as cell dye-positive, and cluster analysis is performed to using CODI platform to identify EV populations.

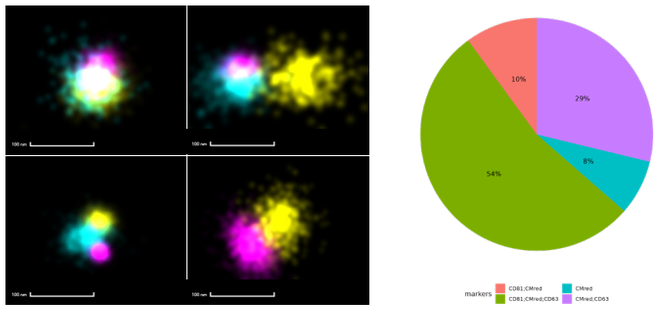

Figure 4. (A) dSTORM super resolution images of MCF7 EVs stained with (1) membrane dye (magenta); anti-CD63 antibodies (yellow), and anti CD81 antibodies (cyan). (B) Cluster analysis of EV populations.

V. Western Blot analysis for positive/negative EV markers (Jess, Protein Simple)

EVs are characterized using capillary western blot with total protein normalization using a panel of biomarker controls, including membrane proteins (CD9, CD81), cytosolic proteins (TSG101, HSP70), and negative controls (CANX, GM130).

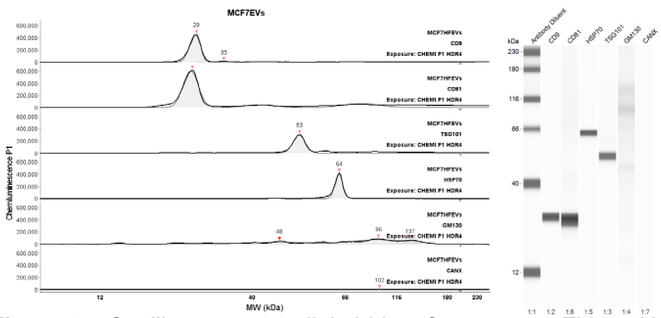

Figure 5. Capillary western digital blots for common EV positive and negative biomarkers.

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