

Quantifying Immunostained Extracellular Vesicles with Spectradyne's ARC™ Particle Analyzer

Extracellular vesicles (EVs) expressing human tetraspanin CD81 were used to demonstrate:

- Antigen-specific immunolabeling of EVs and subpopulation analysis with the ARC particle analyzer
- Measurement of single-particle epitope abundance by quantitative fluorescence

Experimental Design:

EVs expressing human tetraspanin CD81 were labeled with two PE-conjugated antibodies: A monoclonal antibody targeting CD81 and an IgG isotype control. Particle size, concentration and fluorescence were measured for each staining condition using Spectradyne's ARC particle analyzer.

The ARC uses Microfluidic Resistive Pulse Sensing (MRPS)—an electrical technique—to measure particle size and concentration, while simultaneously measuring the fluorescence of each particle as would a flow cytometer. This unique combination of orthogonal measurement methods—optical and electrical—yields accurate measurements of nanoparticle size and concentration for all particles in a sample, as well as for fluorescent subpopulations. Sample purity can therefore be assessed with a single measurement.

Results:

Figure 1 shows the total particle size distribution for both stained samples as measured by MRPS. The overall particle size distribution is not significantly impacted by the antibody staining.

Figure 2 shows a scatterplot (left) of PE fluorescence intensity versus MRPS diameter for particle detection events in each sample. Note that by default, Spectradyne's ARC reports brightness measurements in transferrable, NIST-traceable units of Equivalent Reference Fluorophores (ERF). The limit of detection (LOD) for ARC in the PE channel is shown as the dashed horizontal line on the scatterplot, at 2.5 PE ERF, a brightness equivalent to 2.5 molecules of PE in solution.

Particle concentration as a function of brightness in each sample is shown in the right plot, after gating to include particles with brightness > 2.5 PE ERF. The isotype control stain shows no significant concentration of fluorescent particles, as expected, confirming that the immunostaining is specific to the target, CD-81. From the brightness of particles in the specifically stained sample, the data indicate that for particles larger than 65 nm diameter and having brightness exceeding 2.5 PE (ERF), the EVs express a median of 6.4 copies of CD-81 (assuming a multiplicity of one PE molecule per antibody).

Figure 3 shows the particle size distribution of all particles in the specifically stained sample (blue) and the gated population expressing CD81 with brightness > 2.5 PE (ERF). The total concentration of CD81-positive EVs is 6.37×10^8 particles/mL, representing 41.1% of all particles in the sample.

Discussion: The ARC particle analyzer accurately measures the size and concentration of particles as small as 50 nm diameter using MRPS, while simultaneously measuring fluorescence in up to three channels for each particle. Standard units are reported for each measurement: Size in nanometers, concentration in particles/mL, and fluorescence in (MESF/ERF units). The ARC particle analyzer side-steps the severe limitations of light scatter-based particle analysis methods such as Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), or flow cytometry at the nanoscale, and delivers fast and accurate measurements of complex samples.

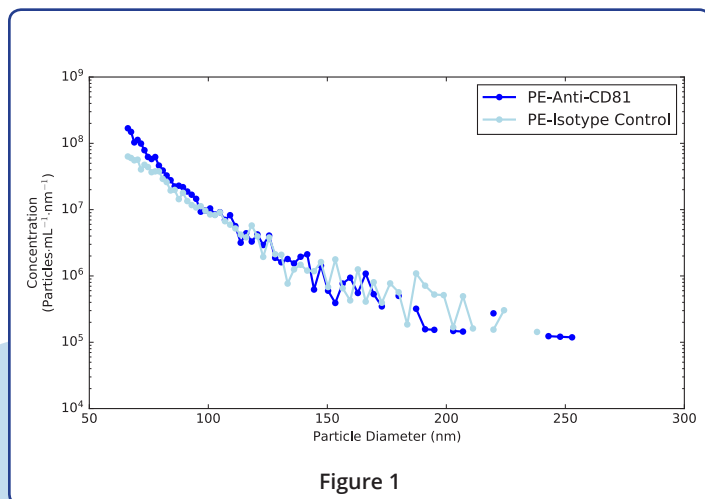


Figure 1

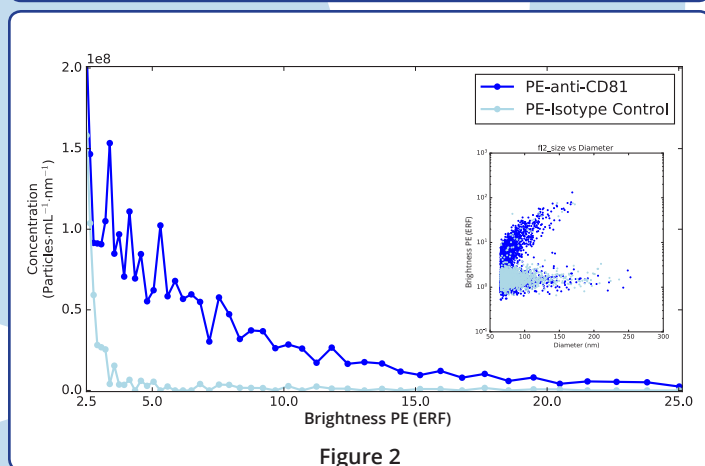


Figure 2

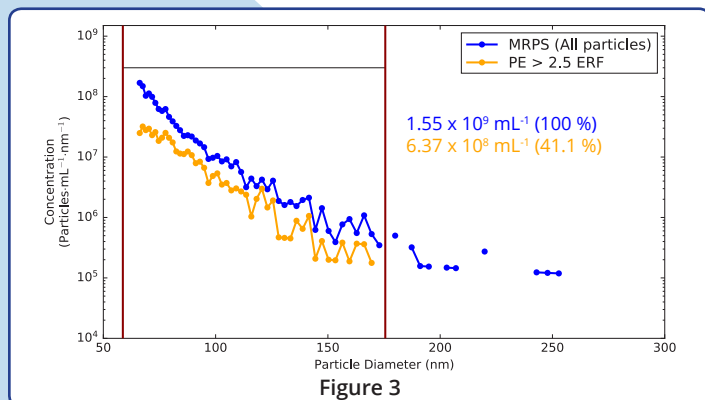


Figure 3