



**ApogeeMix for Flow Cytometer
Performance Assessment**

Datasheet for product #1493 & #1527

Introduction

The complex relationship between particle size and the amount of light scattered at different collection angles makes it difficult to infer particle size from a flow cytometer's light scatter data. A population may be described as scattering an amount of light equal to a reference particle (e.g. a polystyrene or silica bead of known size) but same sized particles of different refractive index (η) can give very different signal strengths. When comparing data between flow cytometers the difficulties are compounded by differences in light scatter collection angles. Ideally it would be possible to produce stable reference particles of known size and of a refractive index and structure similar to the bacteria or microvesicles of interest but such particles are not commercially available.

Due to the refractive index difference, polystyrene beads on their own do not offer an accurate means to assess a flow cytometer's light scatter performance for the study of biological particles. Silica (SiO_2) beads can be used as a better reference particle because silica's refractive index is closer to the refractive index of biological vesicles^{1,2,3,4}.

The *ApogeeMix* products (Cat #1493 & 1527) are a convenient mixture of non-fluorescent silica beads and fluorescent polystyrene beads with sizes from 80nm to 1300nm which can be used to prepare flow cytometers for the analysis of small biological particles. The products differ only in that #1493 does not include the 80nm polystyrene beads. They offer an easy means to assess the sensitivity and resolution of the flow cytometer's light scatter and fluorescence optics and the silica beads offer a means to calibrate a flow cytometers light scatter optics at a refractive index of approximately 1.43.

A more precise calibration for assessment of bacteria and biological vesicle size (refractive index in the range 1.36 to 1.42) is offered by ApogeeFlow's Light Scatter Calibration Module (Cat# 1492). This offers scales calibrated for particular refractive indexes, on the light scatter datagrams.

Materials Supplied

The *ApogeeMix* contains 25ml of an aqueous mixture of spheres with diameters 180nm, 240nm, 300nm, 590nm, 880nm and 1300nm diameter with refractive index $\eta=1.43$ (silica, SiO_2). It also contains 80nm, 110nm and 500nm green fluorescent beads with refractive index $\eta=1.59$ (polystyrene). Product lot numbers after CAL0200 also contain 80nm polystyrene green fluorescent spheres. The product is intended to be used to assess a flow cytometer's light scatter and fluorescence performance (both sensitivity and resolution). Shown below are typical data from the *ApogeeMix* analyzed on a "Micro" flow cytometer (FL1=Green fluorescence). The ApogeeFlow "Micro-PLUS" cytometer offers roughly 30x higher sensitivity.

The fluorescent latex beads may be used to assess the fluorescence sensitivity and to assess the performance of the flow cytometer's optics at a different refractive index.

Approximate particle concentrations (lots vary, see product enclosure):

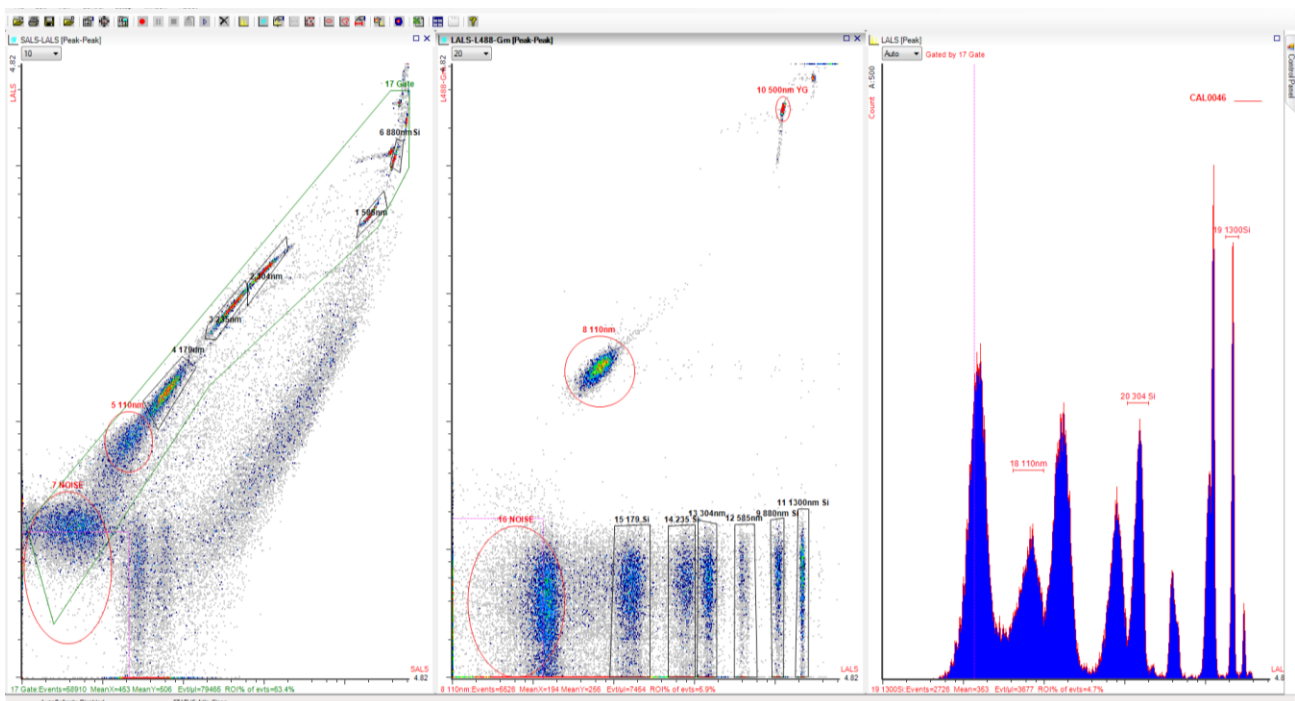
Particle Size (nm)	Approximate number per microlitre	Fluorescence
80 (#1527 only)	5000	Green from L405
110	5000	Green from L488
180	5000	None
240	10000	None
300	9000	None
500	3600	Green from L488
590	2700	None
880	3900	None
1300	3400	None



Typical Data from ApogeeFlow standard “Micro” Cytometer

The resolution of the peaks indicates the flow cytometer's performance. The below image is from #1493 ApogeeMix measured on a standard ApogeeFlow “Micro” Cytometer. A “Micro-PLUS” model would offer more than an extra decade of light scatter sensitivity. Ideally eight populations will be resolved from each other and resolved from instrument noise:

- 6 populations with refractive index 1.43 (silica, SiO₂) and
- 2 green fluorescent (488nm laser) populations (110nm and 500nm) with refractive index 1.59 (polystyrene), middle graph. Product #1527 also contains 80nm polystyrene beads (green fluorescent from 405nm laser excitation, not shown in data below).



Product Safety

Caution: Product contains 0.05% sodium azide.
MSDS available on request (info@ApogeeFlow.com)

References

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A new microparticle size calibration standard for use in measuring smaller microparticles using a new flow cytometer Chandler, W., Yeung, Wandy, Tait, Jonathan;
2. Water Research 42 (2008) 3757 – 3766
Use of silica microspheres having refractive index similar to bacteria for conversion of flow cytometric forward light scatter into biovolume Paola Foladori, Alberto Quaranta, Giuliano Ziglio
3. J Thromb Haemost 2014; DOI:10.1111/jth.12602 van der Pol E, Coumans FAW, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, Sturk A, van Leeuwen TG, Nieuwland
Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing.
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Refractive index determination of nanoparticles in suspension using nanoparticle tracking analysis