

Flow Cytometry Sub-micron Particle Size Reference Kit

Catalog no. F13839

Table 1 Contents and storage

Material*	Amount	Composition	Storage [†]
0.02 µm-diameter microspheres (Component A)	2 mL	~1 × 10 ⁶ beads/mL in deionized water containing 2 mM sodium azide	<ul style="list-style-type: none"> • 2–8°C • DO NOT FREEZE • Vortex and briefly sonicate before use
0.1 µm-diameter microspheres (Component B)	2 mL		
0.2 µm-diameter microspheres (Component C)	2 mL		
0.5 µm-diameter microspheres (Component D)	2 mL		
1.0 µm-diameter microspheres (Component E)	2 mL	~1 × 10 ⁶ beads/mL in deionized water containing 2 mM sodium azide and 0.05% Tween® 20 solution	
2.0 µm-diameter microspheres (Component F)	2 mL		

* The microspheres within a given vial are homogeneous in size. The sizes listed above are nominal diameters; the actual diameters are determined by transmission electron microscopy and are printed on the labels.

† When stored as directed, these reagents are stable for at least two years from the date of purchase.

Introduction

The Flow Cytometry Sub-micron Particle Size Reference Kit provides a set of green fluorescent microsphere suspensions to serve as reliable size references for flow cytometry users. The kit contains six suspensions of green fluorescent polystyrene microspheres, each with a known diameter as determined by transmission electron microscopy.

All the microspheres in this kit are green (excitation and emission maxima at 505 nm and 515 nm, respectively) and the beads show excitation and emission profile similar to Alexa Fluor® 488 or FITC stained cells (see Figure 1, page 2). The green fluorescent dye molecules are localized within the bead polymeric matrix, which makes the beads brighter and more photostable than conventional surface-stained beads.

The size (or size range) of bioparticles in an experimental sample can be estimated by comparing the forward scatter signals (FSC) with those of the reference microspheres. The microspheres in each component functions as reproducible size markers and can be used individually (one size), pre-mixed (two to six sizes), intermixed with the experimental sample or in parallel runs.

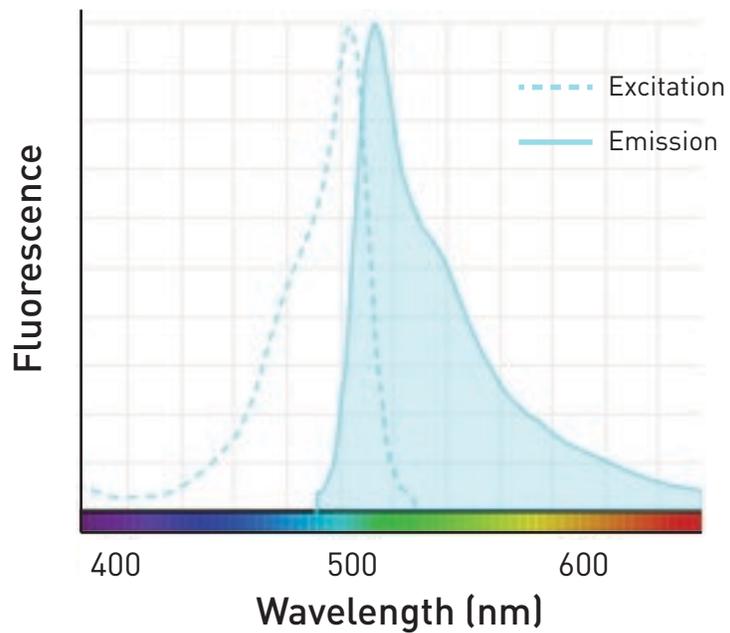
For Research Use Only. Not for use in diagnostic procedures.

The Flow Cytometry Sub-micron Particle Size Reference Kit can also be used as tool to verify the instrument's performance and to establish parameters that are suitable for analyzing sub-micron particles. For example, the kit can be used to check:

- Particle size resolution limit and dynamic range
- Sensitivity of forward and side scatter PMTs
- Level of instrument baseline noise
- Laser and optical alignment and stability
- Stability of the fluidics system

Note that FSC signals are related not only to size but also to other factors, such as the refractive index of a particle. The microspheres in this kit all have equivalent refractive indices (1.591 at 590 nm); therefore, the differences in the FSC intensities reflect their relative sizes. Because the refractive index of cells and many types of bioparticles may differ from that of the microspheres, the sizes estimated by using this kit may not reflect the actual bioparticle sizes. Furthermore, the physiological state of a cell and other bioparticles may also affect their refractive indices.

Figure 1 Excitation and emission profile of the green fluorescence particles in the Flow Cytometry Sub-Micron Particle Size Reference Kit.



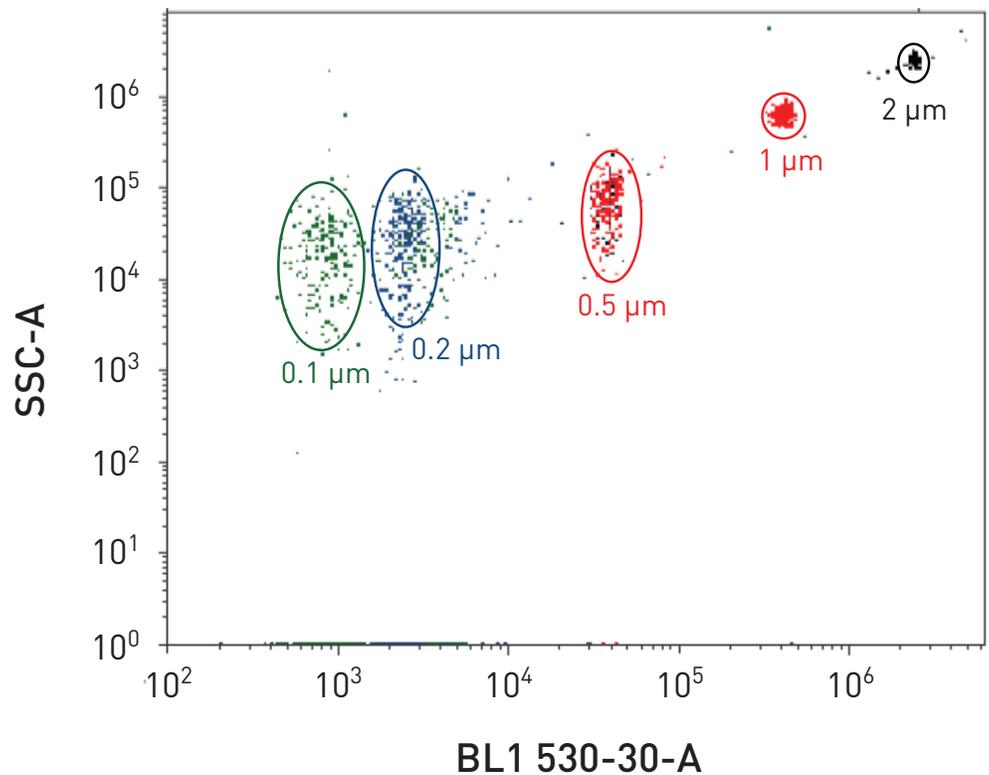
Experimental Applications

Experimental protocols depend on the flow cytometer and software used; refer to the reference materials applicable to your particular instrument. Before conducting size analysis, thoroughly wash your flow cytometer with filtered sheath fluid to avoid any residual particles or carry-over from previous experiments.

Before sampling any of the kit components, uniformly suspend the microspheres by vortex mixing and brief sonication (15 to 30 second) of the dropper bottle(s) in a water bath sonicator.

The microspheres in each component are at a density of $\sim 1 \times 10^6$ beads/mL. For a particle size analysis assay, you can add one drop of beads suspension from each component to 1 mL of preferred buffer. We recommend that you run each size of beads individually first, and then use pre-mixed mixture of beads of two to six sizes. In general, a lower flow rate (e.g., 25 μ L per minute) results in improved resolution.

Figure 2 Five components of the Flow Cytometry Sub-micron Particle Size Reference Kit were analyzed using an Attune[®] Acoustic Focusing Cytometer (Blue/Violet). The diameter of the five different green fluorescent microspheres are as marked.



Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
F13839	Flow Cytometry Sub-Micron Size Reference Kit.	1 kit

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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