Flow Cytometry Size Calibration Kit (F-13838)

Quick Facts

Storage upon receipt:
- 4°C
- Sonicate before use
- Do not freeze

Introduction

One of the primary functions of a flow cytometer is to measure the scatter of light caused by a particle. Forward scatter (FSC) is light from the illuminating laser beam that has been bent (refracted or otherwise deflected) at a small angle as it passes through a particle, e.g., a cell or microsphere. The intensity of the FSC signal is proportional to the particle’s size. Therefore, it is possible to analyze the size of many bioparticles using a flow cytometer by comparing their FSC signals with that of a population of microsphere standards that have known diameters.

Molecular Probes’ Flow Cytometry Size Calibration Kit provides a set of microsphere suspensions to serve as reliable size references for cytometry users. The kit contains six suspensions of unstained polystyrene microspheres, each with a known diameter, determined by transmission electron microscopy. The size of cells in an experimental sample can be estimated by comparing the FSC signals with those of the reference microspheres. The microspheres function as reproducible size markers (Figure 1) and can be used intermixed with the experimental sample or in parallel runs.

We caution, however, 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 the same refractive index (1.591 at 590 nm), therefore the differences in the FSC intensities truly reflect their relative sizes. Because the refractive index of cells may differ from that of the microspheres, the sizes estimated by using the Flow Cytometry Size Calibration Kit may not be the actual cell sizes. Furthermore, the physiological state of a cell may affect its refractive index. For example, dead cells typically have a lower refractive index due to leaky outer membranes, give lower FSC signals and thus appear smaller than healthy cells.

Materials

Contents
The Flow Cytometry Size Calibration Kit contains six vials of highly uniform polystyrene microspheres:

- Component A: 1.0 µm-diameter microspheres
- Component B: 2.0 µm-diameter microspheres
- Component C: 4.0 µm-diameter microspheres
- Component D: 6.0 µm-diameter microspheres
- Component E: 10 µm-diameter microspheres
- Component F: 15 µm-diameter microspheres

The microspheres within a given vial are very 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. The spheres are provided as 1 mL suspensions in water containing 0.05% Tween® 20 and 2 mM sodium azide. The 1.0 µm size is supplied at a density of ~6 × 10^7 beads/mL; the 2.0 µm and 4.0 µm sizes, at ~3 × 10^7 beads/mL; and the 6.0 µm, 10 µm and 15 µm sizes, at ~2 × 10^7 beads/mL.

Storage
Upon receipt, the kit should be stored at 4°C until required for use. When stored properly, these reagents are stable for at least two years from the date of purchase. SONICATE BEFORE USE. DO NOT FREEZE.

Experimental Applications

Experimental protocols depend somewhat on the flow cytometer and software used; please also refer to the reference materials applicable to your particular flow cytometer.

Before sampling any of the kit components, uniformly suspend the microspheres by vortex mixing and sonicating the suspensions. The larger microspheres will settle out within...
minutes. To use, prepare a mixed suspension of the six standard microspheres by adding one drop of each to approximately 2 mL of sheath fluid or buffered saline. Alternatively, it may be desirable to use only a subset of the six suspensions in a given experiment, depending on the size markers needed. Be sure that the microspheres in the mixture are well suspended, and apply the sample.

Follow the manufacturer’s instructions for optimizing the instrument for size measurement. Due to the wide range of sizes represented in the Flow Cytometry Size Calibration Kit, it may be necessary to select the log mode of the FSC amplifier and then adjust the gain setting in order to have all six sizes on scale at the same time (see Figure 1).

References


Product Summary

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

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<th>Cat #</th>
<th>Product Name</th>
<th>Unit Size</th>
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<td>F-13838</td>
<td>Flow Cytometry Size Calibration Kit</td>
<td>1 kit</td>
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Flow Cytometry Size Calibration Kit – *nonfluorescent microspheres*

Contact Information

Further information on Molecular Probes’ products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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