Revised: 04–April–2003

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.

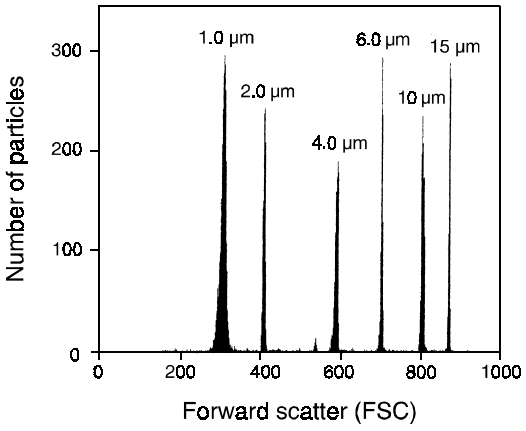

Figure 1*. Histogram analysis of the forward scatter intensity (FSC) log channel values of the six polystyrene microsphere samples supplied in our Flow Cytometry Size Calibration Kit. FSC measurements were performed on a Becton Dickinson FACScan® flow cytometer using excitation at 488 nm.*

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

1. Flow Cytometry, A Practical Approach, 2nd Edition, M.G. Ormerod, Ed., IRL Press (1994); **2.** Givan A.L., Flow Cytometry, First Principles, Wiley-Liss (1992).

Product Summary

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

Cat #	Product Name	Unit Size
F-13838	Flow Cytometry Size Calibration Kit *nonfluorescent microspheres*	1 kit

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.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 ● Fax: (541) 344-6504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 465-8338 ● Fax: (541) 344-6504 ● order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 ● Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 ● Fax: (541) 465-4593 ● tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl