molecular probes®
by life technologies™

Cell Sorting Set-up Beads

Catalog nos. C16506, C16507, C16508, C16509

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
Cell Sorting Set-up Beads	2 × 1.5 mL dropper vials	Suspension in water containing 0.05% Tween® 20 and 2 mM sodium azide*	• 2–8°C • Protect from light • Sonicate before use • Do not freeze	When stored as directed, reagents are stable for at least 1 year.†
* The Cell Sorting Set-up Bead suspensions contain ~1.7 × 10^7 beads/mL (0.2% solids). † No leaching or signal degradation is expected.				
Approximate fluorescence excitation and emission maxima: See Table 2, page 2.				

Introduction

Life Technologies' Cell Sorting Set-up Beads are reliable standards for the setup and calibration of flow cytometry sorter instruments. The Cell Sorting Set-Up Beads have a diameter of 6 µm (± 10%), and thus approximate the size, emission wavelength, and intensity of many biological samples. As such, the beads can be used to calibrate a flow cytometer's cell sorting system. The Cell Sorting Set-Up Beads are optimized for checking cell sorter settings such as drop delay and efficiency (cell loss during sorting).

The Cell Sorting Set-Up Beads are fluorescent-dye infused microspheres that have been optimized for use with UV, blue, green/yellow and red lasers. Table 2, page 2 details the optimal excitation and emission spectra of each product.

The Cell Sorting Set-Up Beads provide:

- Consistency —in production, creating ideal reference standards
- Confidence—ensure the reliability of optimal daily instrument performance
- Compatibility—with any instrument

The Cell Sorting Set-Up Beads can be used to calibrate a flow cytometer's laser source, optics, and stream flow without wasting valuable and sensitive experimental material.

For optimal verification of the laser alignment of a cell sorter cytometer, please see our selection of Alignflow™ Flow Cytometry Alignment Beads.

Bead Labeling

As with all of our flow cytometry standard microspheres, the Cell Sorting Set-Up Beads are stained internally rather than on the surface. The dyes are therefore insulated from environmental interactions that could cause variable fluorescence output, resulting in excellent signal stability. These fluorescent polystyrene microspheres are supplied as suspensions packaged in dropper vials for convenient dispensation, with a choice of four colors at the 6.0 µm size to match your laser (UV, blue, green/yellow, or red).

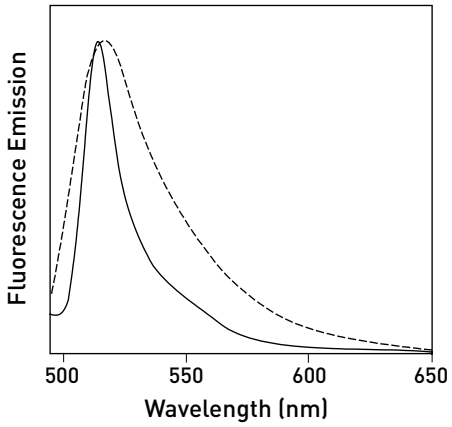
The dyes used in the manufacture of the Cell Sorting Set-up Beads have been carefully selected to provide emission peaks coincident with cells labeled with commonly used fluorescent dyes. The emission profiles for these standards are intentionally narrow in comparison to cells labeled with a corresponding fluorescent dye. Due to their narrow emission profiles, beads of two different fluorescent colors exhibit minimal spectral overlap, and little or no color compensation is needed when setting up for multicolor experiments.

Table 2 Cell Sorting Set-up Beads available from Life Technologies.

Catalog no.	Laser type	Laser excitation*	Emission peak*	Emission matches cells stained with
C16506	UV laser	350–375 nm	460 nm	DAPI, Hoechst dyes
C16507	Red laser	633 nm	680 nm	TOTO®-3, Cy™ 5 dye
C16508	Blue laser	488 nm	515 nm	fluorescein, Oregon Green® 488, Alexa Fluor® 488
C16509	Green/yellow laser	532 or 561 nm	575 nm	R-phycoerythrin, tetramethylrhodamine, Alexa Fluor® 546

* Approximate laser excitation and emission maxima, in nm.

Figure 1 Normalized emission spectra of Cell Sorting Set-up Beads for Blue Lasers (Cat. no. C16508, solid line) and fluorescein-labeled cells (dashed line). The narrow emission spectrum of Cell Sorting Set-up Beads for Blue Lasers is approximately centered on the broader emission spectrum of fluorescein.



Guidelines for Use

Life Technologies' Cell Sorting Set-up Beads serve as a reference standard for calibrating flow cytometers. Experimental protocols depend somewhat on the flow cytometer and software used; please refer also to the reference materials applicable to your particular instrument. Before sampling, be sure that the polystyrene beads are uniformly suspended by vortex mixing and sonicating the suspension. Generally, one drop added to 1 mL of Haema-Line 2 sheath fluid or buffered saline solution provides an appropriate concentration for analysis; mix well before applying the sample.

During the alignment, set the approximate photomultiplier tube (PMT) voltage to place the sample in a convenient range for the flow cytometer, then maximize the signal and minimize the coefficient of variation (CV) by making the appropriate optical adjustments. Follow the instrument manufacturer's manual carefully for optimal performance.

When using the Cell Sorting Set-up Beads as a daily reference source, maximize the peak heights and minimize the CVs, as outlined above. Once the settings are optimized, record all settings and print out the relevant data for your record. Each day, pre-run the flow cytometer using these reference beads and compare the output to the reference record. Deviations from the baseline readings may indicate instrument malfunction.

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

Cat. no.	Product Name	Unit Size
C16506	Cell Sorting Set-up Beads for UV Lasers *6 µm*	2 × 1.5 mL
C16507	Cell Sorting Set-up Beads for Red Lasers *6 µm*	2 × 1.5 mL
C16508	Cell Sorting Set-up Beads for Blue Lasers *6 µm*	2 × 1.5 mL
C16509	Cell Sorting Set-up Beads for Green/Yellow Lasers *6 µm*	2 × 1.5 mL

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