

AlignFlow™ Flow Cytometry Alignment Beads

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
AlignFlow™ beads	2 × 1.5 mL dropper vials	Suspension in water containing 0.05% Tween® 20 and 2 mM sodium azide*	<ul style="list-style-type: none"> • 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 AlignFlow™ suspensions contain ~1.2 × 10 ⁸ beads/mL (0.1% solids). † No leaching or degradation is expected.				
Approximate fluorescence excitation and emission maxima: See Table 2, page 2.				

Introduction

To help ensure accurate and reproducible results, flow cytometers should be checked daily for proper performance. Reference standards are indispensable for conducting these tests. AlignFlow™ flow cytometry alignment beads are reliable references for aligning, focusing, and calibrating flow cytometers. These fluorescently stained, polystyrene microspheres are highly uniform with respect to size and fluorescence intensity. The AlignFlow™ beads are available in three versions: for 350–370-nm excitation with UV lasers, for 488-nm excitation with blue lasers, and for 633-nm excitation with red lasers, and each version is available in two sizes: 2.5 µm–diameter and 6.0 µm–diameter (see Table 2, page 2).

The fluorescent dyes have been carefully selected for optimal excitation by laser sources commonly used in flow cytometry. The 488 nm–excitable beads for blue lasers emit broadly from 515 nm to 660 nm. The UV-excitable beads emit from 400 nm to 470 nm, and the 633 nm–excitable beads for red lasers, from 645 nm to 680 nm. Because the dyes are contained inside each microsphere, instead of merely on the bead surface, AlignFlow™ beads exhibit superior signal stability.

Using AlignFlow™, an operator can reproducibly adjust parameters crucial to flow cytometry before an experiment is started. The beads approximate the size, emission wavelength, and intensity of many biological samples and permit the calibration of the flow cytometer's laser source, optics, and stream flow without wasting valuable and sensitive experimental material. Because of their excellent stability, these beads can also serve as a daily reference standard by which an instrument's performance and reliability are evaluated.

Table 2 AlignFlow™ flow cytometry alignment beads.

Catalog no.	Bead diameter	Ex *	Em range †	Laser type
A16500	2.5 µm	488 nm	515–660 nm	Blue laser
A16503	6.0 µm			
A16501	2.5 µm	633 nm	645–680 nm	Red laser
A16504	6.0 µm			
A16502	2.5 µm	350–370 nm	400–470 nm	UV laser
A16505	6.0 µm			

* Fluorescence excitation, in nm, for which the beads were designed. † Approximate useful emission range, in nm.

Guidelines for Use

Life Technologies' AlignFlow™ 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 AlignFlow™ 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
A16500	AlignFlow™ Flow Cytometry Alignment Beads for Blue Lasers *2.5 µm*	2 × 1.5 mL
A16501	AlignFlow™ Flow Cytometry Alignment Beads for Red Lasers *2.5 µm*	2 × 1.5 mL
A16502	AlignFlow™ Flow Cytometry Alignment Beads for UV Lasers *2.5 µm*	2 × 1.5 mL
A16503	AlignFlow™ Flow Cytometry Alignment Beads for Blue Lasers *6 µm*	2 × 1.5 mL
A16504	AlignFlow™ Flow Cytometry Alignment Beads for Red Lasers *6 µm*	2 × 1.5 mL
A16505	AlignFlow™ Flow Cytometry Alignment Beads for UV Lasers *6 µm*	2 × 1.5 mL

Purchaser Notification

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www.lifetechnologies.com

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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