AlignFlow™ and AlignFlow™ Plus Flow Cytometry Alignment Beads

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
AlignFlow™ * and AlignFlow™ Plus † beads	3 mL dropper vials	Suspensions in water containing 0.05% Tween 20 and 2 mM sodium azide	• 2–6°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). **†** The AlignFlow™ Plus suspensions contain ~1.7×10^7 beads/mL (0.2% solids). **‡** No leaching or degradation is expected.

Approximate fluorescence excitation and emission maxima: See Table 2.

Introduction

In order to ensure accurate and reproducible results, flow cytometers should be checked daily for proper performance. Reference standards are indis-pensable for conducting these tests. Molecular Probes AlignFlow™ and AlignFlow™ Plus 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 2.5 µm–diameter AlignFlow™ beads and the 6.0 µm–diameter AlignFlow™ Plus beads are each available in four versions: for UV (350–370 nm) excitation, for 488 nm excitation, for 633 nm excitation, and for 630–660 nm excitation (Table 2). The fluorescent dyes have been carefully selected for optimal excitation by laser sources commonly used in flow cytometry. The 488 nm–excitable beads emit broadly from 515 nm to 660 nm. The UV-excitable beads emit from 400 nm to 470 nm; the 633 nm–excitable, from 645 nm to 680 nm; and the 630–660 nm–excitable, from 670 nm to 720 nm. Because the dyes are contained inside each microsphere, instead of merely on the bead surface, AlignFlow™ beads exhibit superior signal stability.

Using AlignFlow™ or AlignFlow™ Plus beads, 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 1. Molecular Probes AlignFlow™ and AlignFlow™ Plus flow cytometry alignment beads.

Cat #	Bead Diameter	Ex *	Em range †
A7304	2.5 µm	350–370	400–470
A7305	6.0 µm		
A7302	2.5 µm	488	515–660
A7303	6.0 µm		
A7312	2.5 µm	633	645–680
A7313	6.0 µm		
A14835	2.5 µm	630–660	670–720
A14836	6.0 µm		

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

Guidelines for Use

Molecular Probes AlignFlow™ and AlignFlow™ Plus 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™ or AlignFlow™ Plus 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 #	Product Name	Unit Size
A7304	AlignFlow™ flow cytometry alignment beads, 2.5 µm *for UV excitation*	3 mL
A7302	AlignFlow™ flow cytometry alignment beads, 2.5 µm *for 488 nm excitation*	3 mL
A7312	AlignFlow™ flow cytometry alignment beads, 2.5 µm *for 633 nm excitation*	3 mL
A14835	AlignFlow™ flow cytometry alignment beads, 2.5 µm *for 630-660 nm excitation*	3 mL
A7305	AlignFlow™ Plus flow cytometry alignment beads, 6.0 µm *for UV excitation*	3 mL
A7303	AlignFlow™ Plus flow cytometry alignment beads, 6.0 µm *for 488 nm excitation*	3 mL
A7313	AlignFlow™ Plus flow cytometry alignment beads, 6.0 µm *for 633 nm excitation*	3 mL
A14836	AlignFlow™ Plus flow cytometry alignment beads, 6.0 µm *for 630-660 nm excitation*	3 mL

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