Bangs Laboratories, Inc.
BEADS ABOVE THE REST

Fluorescence Reference Standards

Product Data Sheet 890

DESCRIPTION

Fluorescence Reference Standards are designed for use in the proper set up of all flow cytometers for optimal analysis of stained samples.

Fluorescence Reference Standard microbeads are highly uniform microbeads in size and fluorescence. Their size approximates that of human lymphocytes (7-9µm in diameter) and are suspended in a sterile filtered, isotonic buffered solution (pH 7.2) containing surfactants and preservatives. They are available in a variety of fluorescent dyes (refer to bottle label for specific dye) or as an unstained blank. These standards are designed for use in an application-specific manner when they are selected for having the same fluorochrome(s) as the sample being analyzed. Because application-specific standards bear the same fluorochromes used in the stained sample, they have the same excitation and emission properties as the sample. Refer to bottle label for specific dye to ensure that they match the same spectral properties as cells labeled with the same dye. Analytical artifacts are minimized when application-specific standards are used in setting up a flow cytometers to measure prepared samples. Additionally, because these standards are uniform in size and fluorescence, they may be used for optical alignment.

Referencing your instrument with application-specific standards is the simplest level of "calibration." This involves standardization of the intensity scale of the flow cytometer by adjusting detector high voltage and/or gain setting to reproduce the signal intensity of an application-specific standard as seen on the appropriate fluorescence or light scatter histogram. This signal intensity is indicated by the target (peak) channel of the bead population. This procedure is equivalent to re-setting the instrument to a constant relative fluorescence intensity scale, something which can not necessarily be accomplished by simply re-establishing a constant set of instrument settings.

CHARACTERISTICS

Mean Diameter:	7-9µm
Particle Concentration:	2 x 10^6 particles/mL

MATERIAL

Material Supplied	Fluorescence Reference Standard microbeads (refer to bottle label for specific dye)
Material Required	Isotonic phosphate buffered saline (pH 7.2)
	Appropriate sized test tubes
	Flow cytometer

PROCEDURE

Researchers are advised to optimize the use of particles in any application.

Optical Alignment

1. Vigorously shake the bottle to ensure uniform suspension of microbeads.
2. Add one drop of the suspension to 0.5mL of isotonic phosphate buffered saline (pH 7.2).
3. Run the reference standards in the flow cytometer. Adjust the flow rate, or the suspension concentration, so that the count rate is optimal for your instrument. A count rate of 100 microbeads/second is optimal for tight CV's. Note: Decreasing the flow rate often results in narrower CV's.
4. Align your flow cytometer according to the instrument manufacturer's recommendations.
5. Adjust forward scatter settings so that the peak channel of reference standards falls approximately in the middle of the forward scatter histogram.
6. Place a live gate around the singlet population on the forward vs. side scatter histogram.
7. Repeat Step 5 for all other parameters of interest (e.g. FL1, FL2, etc.).
8. Count a minimum of 5000 microbeads within the live gate.
9. Record the date, lot number, peak channels, and CV's from the histograms, as well as the instrument settings (laser mw and amps, amplifier gains, and PMT settings). Mean channels may be used if peak channels are unavailable. However, the two cannot be used interchangeably.
10. Using a Levy-Jennings plot, establish normal ranges for your laboratory. Any significant variation in PMT or amplifier settings observed in bringing peaks to target channels may indicate an instrument problem.

Set Target Conditions

1. Optically align your flow cytometer to the instrument manufacturer's instructions, or use the Optical Alignment Procedure above.
2. Run a specimen which is representative of the samples you want to analyze.
3. Adjust forward and side scatter signals for optimal visualization of the sample on the histograms.
4. Draw a live gate around the population of interest.
5. Count a minimum of 5000 microbeads inside the live gate.
6. Observe the fluorescence signals on the single-color or dual-color histograms.
7. Adjust PMT/gains for optimal visualization of any parameter you want to analyze, i.e., forward scatter, FL1, etc.

Determine Target Channels using Fluorescence Reference Standards

1. Dispense a drop of the selected Fluorescence Reference Standard into a test tube, add ~0.5mL diluent, and establish a proper flow rate through the instrument (200-600/second recommended).
2. Run the Fluorescence Reference Standard on your flow cytometer at exactly the same instrument settings used to set target conditions in the prior instrument run.
3. Draw a live gate around the singlet population on the forward vs. side scatter histogram.
4. Count a minimum of 5000 events in the live gate.
5. Record the peak (median) channel for forward scatter. The mean channel may be used if the peak channel is not available, but the two are not interchangeable.
6. Observe the fluorescence or light scatter signal on the appropriate histogram(s). Record the peak channel. The peak channel for each parameter is the target channel for the target conditions specific to this sample type. Record this data on your log sheet.

Reset Target Conditions to Analyze Sample

1. Dispense a drop of the selected Fluorescence Reference Standard into a test tube, add ~0.5mL diluent, and establish a proper flow rate through the instrument (200-600/second recommended).
2. Draw a live gate around the singlet population on the forward vs. side scatter histogram.
3. Count a minimum of 5000 events in the live gate.
4. Adjust forward scatter signal, so the peak channel on the population falls in the predetermined forward scatter target channel. This may require moving the live gate to follow the population. Note: Some patient types, or sample preparations, may differ sufficiently from others so as to require a unique set of target channels.
5. Observe the desired fluorescence or light scatter signal on the appropriate histogram. Adjust the PMT or gains setting until the peak channel or 5000 events equals the predetermined target channel for the parameter.
6. Repeat each target channel.
7. Analyze all samples that correspond to these target conditions.

Expected Values

Expected values may vary under specific conditions, therefore establishing normal ranges for your laboratory may be required. Some patient types, or sample preparations, may differ sufficiently from others so as to require a unique set of target channels.

Under the optimal adjustments for your instrument, the peak channels may fall in the middle of the forward scatter histogram and should be reset to meet this position before running samples.

Examples of Fluorescence Reference Standards

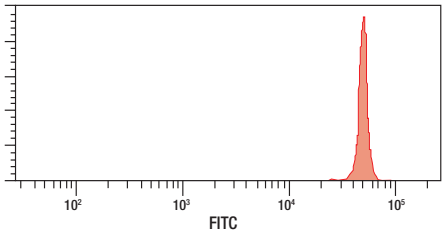

Figure 1: Peak channel of a Fluorescence Reference Standard dyed with Fluorescein (FITC).

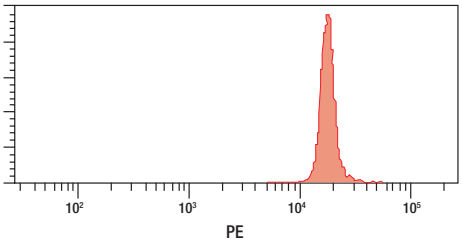

Figure 2: Peak channel of a Fluorescence Reference Standard dyed with R-Phycocerythrin (PE).

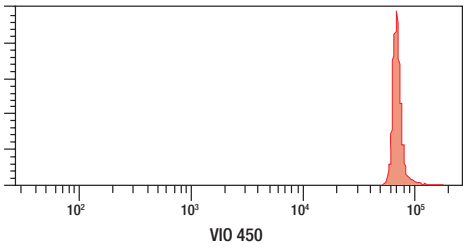

Figure 3: Peak channel of a Fluorescence Reference Standard dyed with Pacific Blue™

NOTES

1. Fluorescence intensity of some fluorochromes (e.g., FITC) can be extremely sensitive to slight changes in pH. It is important to dilute Fluorescence Reference Standards and control cells in the same diluent to maintain comparable spectral properties. Fluorescence Reference Standards are suspended in isotonic buffered solution at physiologic pH (7.2).
2. Vigorous shaking or vortexing is required to uniformly suspend the beads.

REFERENCES

1. Horan, P.K., K.A. Muirhead, S.E. Slezak. 1990. *Standards and controls in flow cytometry.* In Melamed, M.R., T. Lindmo, M.L. Mendelsohn, eds., Flow cytometry and sorting, 2nd ed. New York: Wiley-Liss.
2. Measel, J.W. 1989. *Clinical applications of flow cytometry*. Diagnostics & Clinical Testing, 27:25-29.
3. Paxton, H., P. Kidd, A. Landay, J. Giorgi, N. Flomenberg, E. Walker, F. Valentine, J. Fahey, R. Gelman. 1989. *Results of the flow cytometry ACTG quality control program: analysis and findings.* Clin Immuno Immunopath, 52(1):68-84.
4. Shapiro, H.M. 1988. *Practical flow cytometry*. New York: Wiley-Liss.
5. Sisken, J.E. 1989. *Fluorescent standards.* In Taylor, D.L., Y. Wang, eds., Methods in cell biology (Vol. 30). San Diego, CA: Academic Press Inc.

TRADEMARKS AND REGISTERED TRADEMARKS

1. Autofluor™ and Certified Blank™ are trademarks of Bangs Laboratories, Inc.
2. Cy™, including Cy5 and Cy7, are trademarks of GE Healthcare Limited. These products are manufactured under license from Carnegie Mellon University under U.S. Patent Number 5,268,486 and related patents.
3. Alexa Fluor®, Texas Red®, & Pacific Blue™ are trademarks of Life Technologies Corporation.

STORAGE AND STABILITY

Store at 2-8˚C. Freezing of particles may result in irreversible aggregation and loss of binding activity. Stable for 6 or 12 months from date of purchase, as noted on the product label, provided the product is handled in accordance with the manufacturer's recommendations.

Standards should be kept in the bottles they are shipped in. Exposure of this product to room light, even for limited periods, may substantially reduce performance.

SAFETY

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Safety Data Sheet for more information.

These products are for research use only and are not intended for use in humans or for in vitro diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
890	Certified Blank™	1mL, 5mL, or 14mL
897	Acridine Orange	1mL, 5mL, or 14mL
886	Alexa Fluor® 488	1mL, 5mL, or 14mL
887	Alexa Fluor® 647	1mL, 5mL, or 14mL
901	Allophycocyanine (APC)	1mL, 5mL, or 14mL
914	APC-Cy™7	1mL, 5mL, or 14mL
898	Chlorophyll	1mL, 5mL, or 14mL
895	Cy™5	1mL, 5mL, or 14mL
906	DAPI	1mL, 5mL, or 14mL
891	Fluorescein	1mL, 5mL, or 14mL
916	Pacific Blue™	1mL, 5mL, or 14mL
894	Hoechst 33342	1mL, 5mL, or 14mL
908	PE-Cy™5	1mL, 5mL, or 14mL
889	PE-Cy™7	1mL, 5mL, or 14mL
909	PE-TR	1mL, 5mL, or 14mL
892	Propidium Iodide	1mL, 5mL, or 14mL
899	R-Phycoerythrin	1mL, 5mL, or 14mL
905	T.M. Rhodamine	1mL, 5mL, or 14mL
893	Texas Red®	1mL, 5mL, or 14mL
915	Violet Laser	1mL, 5mL, or 14mL

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