Bangs Laboratories, Inc.
BEADS ABOVE THE REST

FITC / PE Compensation Standard

Product Data Sheet 820

DESCRIPTION

The FITC / PE Compensation Standard is to be used in conjunction with hardware or software to remove spectral overlap from fluorochromes into secondary fluorescence detectors of a flow cytometer.

Flow cytometers are designed to have a primary detector for each fluorochrome label (e.g. FL1 - FITC, FL2 - PE, FL3 - Cy™5). Fluorescent signals emitted by fluorochromes can bleed or overlap into the secondary fluorescence detectors. In order to remove this overlap, the proper amount of signal must be subtracted from the secondary detector as a percentage of fluorescence intensity measured in the primary detector. This subtraction is performed by the electrical circuits prior to collecting sample data or by software when analyzing the list mode files. When the mean fluorescence of 2 populations of labeled standards are adjusted such that they have equal intensities in the secondary fluorescence detectors, then the data from the samples will be accurately compensated.

The FITC/PE Compensation Standard is a mixture of 4 populations of microspheres: FITC, PE, FITC/PE, and AutoFluor™. The AutoFluor™ population consists of unlabeled microspheres that exhibit autofluorescence approximating that of unstained lymphocytes. The microsphere standards are suspended in a sterile filtered, pH buffered PBS solution containing surfactant and preservatives. As the microspheres are labeled with the same fluorochromes used to label the samples, the standards will have spectral properties that closely match the cells being analyzed. These matching properties permit accurate adjustment of color compensation across the intensity range of the analysis.

The FITC/PE Compensation Standard allows you to: 1) set quadrant boundaries for unlabeled, FITC-, PE-, and FITC/PE-labeled samples, 2) eliminate the effects of spectral overlap from each fluorescence detector by subtracting the proper percentage of fluorescence signal from each secondary fluorochrome, and 3) determine instrument stability by monitoring the separation between the AutoFluor™ and the fluorescently-labeled microspheres.

CHARACTERISTICS

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

MATERIAL

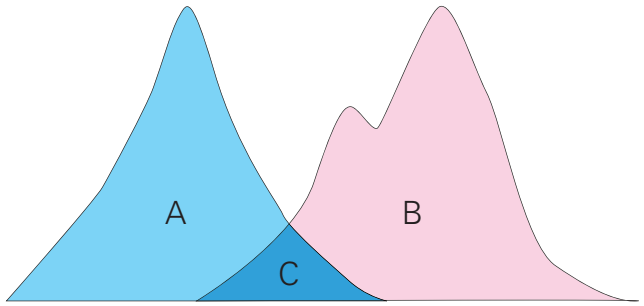
Material Supplied

FITC / PE Compensation Standard kit: 4 bottles included (3 labeled, 1 blank)

Material Required

Cell samples
Cell suspension solution
Sample test tubes
Vortex mixer
Flow cytometer

Fluorescence carryover (C) is the region of overlap of the two emission spectra (A,B)



PROCEDURE

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

Optical Alignment

1. Vortex each bottle to ensure uniform suspension of microspheres.
2. Place 1 drop (~50µL) of the AutoFluor™ microspheres into a tube containing 500µL of cell suspension solution.
3. Analyze the microspheres on a flow cytometer. Adjust the flow rate or the suspension concentration, such that the count rate is optimal for your instrument. A count rate of 100-200 beads per second is recommended.
4. Using your forward scatter versus side scatter dot plot, construct a live gate around the singlet population of the AutoFluor™ microspheres. (Figure 1)
5. Adjust the PMT settings to position the AutoFluor™ population in the lower left corner (quadrant 3) of the FL1 versus FL2 dot plot. (Figure 2)
6. Place 1 drop (~50µL) of both the FITC- and PE-labeled microspheres to the AutoFluor™ microsphere suspension.
7. Adjust the color compensation settings, such that the PE-labeled microspheres appear in quadrant 1, in line with the AutoFluor™ microspheres, and the FITC-labeled microspheres appear in line with the AutoFluor™ microspheres in quadrant 4. (Figure 3)
8. Place 1 drop (~50µL) of the FITC/PE-labeled microspheres to the suspension. Verify that the FITC/PE-labeled microspheres appear in quadrant 2, as dual-labeled cells would. (Figure 4)

RECOMMENDATIONS

For consistency of data across instruments and time, it is recommended that a unified range of analysis (Unified Window of Analysis) be used. The Unified Window of Analysis may be achieved by setting the PMT's of the detectors with Quantum™ QC when performing your daily set-up.

EXPECTED VALUES

Figures 1-4 illustrate the expected performance of the FITC / PE Compensation Standard. Once compensation circuits have been correctly adjusted using the product, the effect of spectral overlap into secondary fluorescence channels is eliminated. Thus, single-labeled cell populations will appear in quadrants 1 and 4, and will be separated from dual-labeled populations in quadrant 2.

NOTES

1. If cells are not properly compensated, follow these steps:
   Drain and fill the flow cell several times to eliminate air bubbles and debris.
   Wash fluidics system by running a fresh solution of 10% household bleach.
   Follow manufacturer's instructions.
   Check system for pressure leaks.
   Check alignment of the instrument.
   Consult your service engineer.
2. Proper storage and handling are essential. Exposure to direct light, even for limited periods, may result in photobleaching of the fluorochromes, substantially affecting performance.
3. Fluorescence intensity of the fluorochromes (e.g., FITC) is extremely sensitive to changes in pH. It is therefore important to resuspend the microspheres in the same cell suspension solution used with cell samples in order to maintain comparable spectral properties.

Figure 1

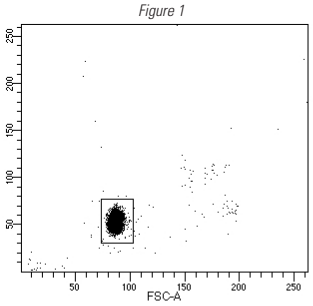


Figure 2

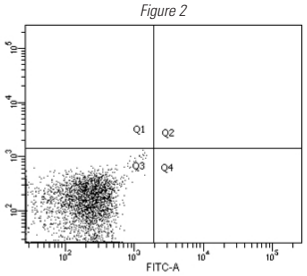


Figure 3

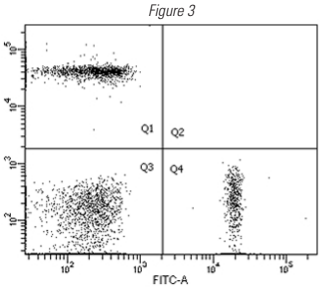
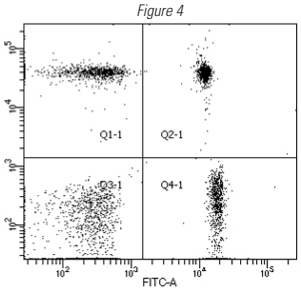


Figure 4



REFERENCES

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2. Shapiro, H.M. 1988. *Practical flow cytometry*. New York: John Wiley & Sons, Inc.
3. National Committee of Clinical Laboratory Standards. 1992. *Clinical applications of flow cytometry: quality assurance and immunophenotyping of peripheral blood lymphocytes*. Tentative Guideline. NCCLS document H42-T (ISBN 1-56238-155-5). NCCLS, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085.
4. National Committee of Clinical Laboratory Standards. 1992. *Clinical applications of flow cytometry: immunophenotyping of lymphocytic cells*. Proposed Guideline. NCCLS document H43-P (ISBN 1-56238-219-5). NCCLS, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085.

TRADEMARKS

1. AutoFluor™, Quantum™ QC, and Right Reference Standard™ are trademarks of Bangs Laboratories, Inc.
2. Cy™, including Cy5, is a trademark 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.

STORAGE AND STABILITY

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

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. Number	Description	Sizes
820	FITC/PE Compensation Standard	1mL, 5mL, or 14mL

RELATED PRODUCTS

Cat. Number	Description	Size
725	Quantum™ QC, 8 Peak Beds	5mL
512	Right Reference Standard™ Fluorescein, High Intensity	5mL
515	Right Reference Standard™ Phycoerythrin, High Intensity	5mL
518	Right Reference Standard™ PE-Cy™5, High Intensity	5mL
521	Right Reference Standard™ APC, High Intensity	5mL

Order online anytime at www.bangslabs.com.