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ProductInformation

Flow Cytometry Compensation Kit
Product Number **COMP-1**

TECHNICAL BULLETIN

Product Description

The Flow Cytometry Compensation Kit is intended for use in the adjustment of flow cytometer settings to eliminate overlapping fluorescein (FITC) and R-phycoerythrin (PE) signals.

Quality control and standardization of instrument performance is critical in flow cytometry.[1-8] Among the factors which require monitoring and calibration are the overlapping signals of the various fluorophores currently in use (e.g., FITC and PE). Although each fluorophore emits light at a characteristic primary wavelength, overlapping emission spectra can result in interference in the simultaneous detection of more than one fluorophore.[1-9] Thus, an electronic adjustment correcting for these inappropriate signals is necessary for proper data interpretation. This is referred to as "color compensation".[1]

Color compensation levels are established on the initial setup of the flow cytometer and are an integral part of daily performance evaluation. Channel readings are recorded on an appropriate log sheet and monitored to maintain consistent results. Compensation parameters can be test specific and may be altered when high voltage/gain (PMT) settings are significantly changed.[1-3] Optimal compensation levels can be established through the use of labeled cells or microbead particles.[1] Typically, cells (e.g., lymphocytes) stained with antibodies (e.g., FITC-CD3 Antibody, PE-CD19 Antibody) are used for such adjustments. However, the preparation and potential variability of these cells makes the use of reproducible labeled microbeads, displaying spectral properties and fluorescence intensity characteristics of the cells under analysis, the preferred method for such adjustments.[1-3] The Flow Cytometry Compensation Kit provides a series of labelled and unlabelled microbead reagents to facilitate color compensation for two color analysis using FITC and PE antibody conjugates.

Reagents

Microbeads are prepared from hydrophobic, polymeric material, suspended in an isotonic buffered solution with surfactants and 0.1% sodium azide. Microbeads are at a concentration of ~2 x 10^6/ml.

Autofluorescence Standard (A 4938) (Matches the autofluorescence of unstained human periferal lymphocytes)	5 ml
FITC Standard (F 7649) (FITC-labeled standard)	5 ml
PE Standard (P 8689) (PE-labeled standard)	5 ml
FITC/PE Standard (F 7774) (FITC and PE dual-labeled standard)	5 ml

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding safe handling practices. R & D use only.

Storage/Stability

Store at 2-8°C. Do Not Freeze. Store and use protected from light.

Procedure

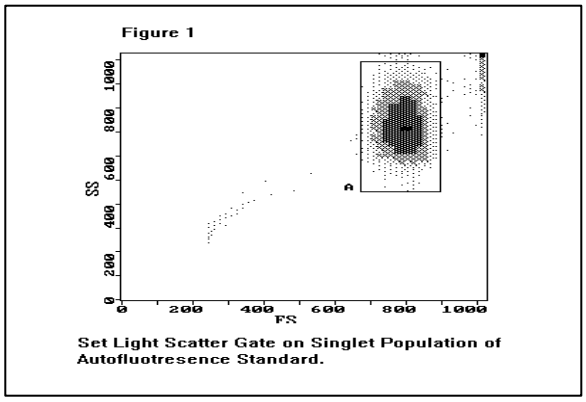
Materials and Equipment Required but not Provided

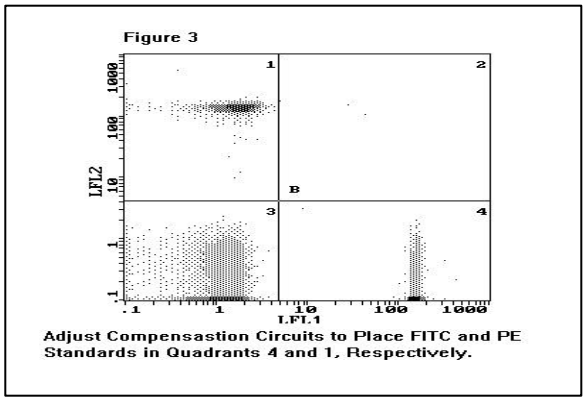
- Alignment Standards
- Log Sheets
- Phosphate Buffered Saline, pH 7.2
- Flow Cytometer
- Optional Reagents
- Microbead Standards M 0162
- Cellular Microspheres C 1557

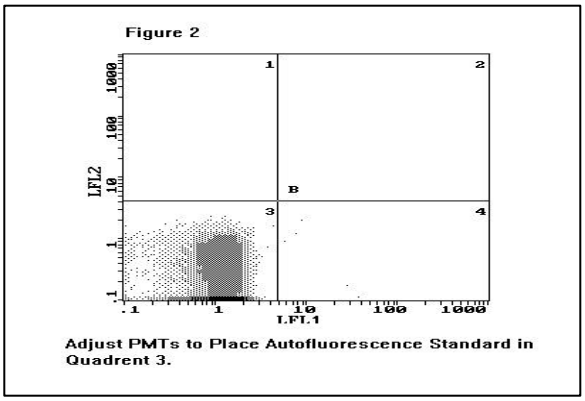
The guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS) caution against potential differences which may be observed in fluorescence intensities of "particles" vs. stained cells and recommends verification of optimal compensation levels with appropriately stained lymphocyte populations.[1] The labeled Compensation Kit microbeads are expected to demonstrate the fluorescence characteristics of brightly labeled cells.

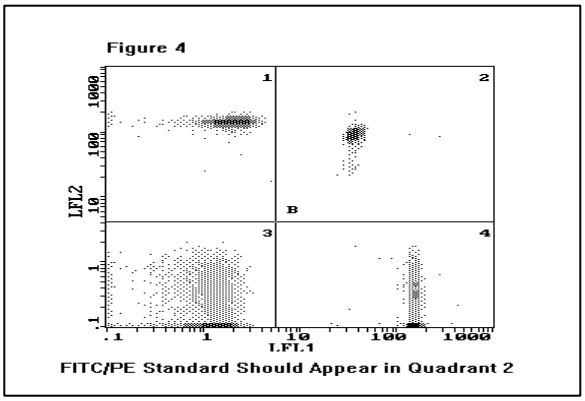
1. Align flow cytometer using manufacturer's alignment methods.
2. Vigorously mix each reagent by shaking to ensure an adequate uniform suspension of microbeads.
3. Place one drop (50 µl) of the Autofluorescence Standard to 1 ml of isotonic phosphate buffered saline, pH 7.2 in an appropriately sized test tube.
4. Run the Autofluorescence Standard on the instrument. Flow rate should be adjusted to the optimal count rate for the flow cytometer. A rate of 100-400 particles/sec is recommended.
5. Construct a live gate around the singlet population of the Autofluorescence Standard using the dot plot display of forward scatter vs. side scatter. Data should appear as in Figure 1.
6. Change the display parameters to FL1 vs. FL2 still using the dot plot.
7. Adjust the FL1 and FL2 PMT voltage to position the Autofluorescence Standard population in the lower left corner (Quadrant 3) of the display (Figure 2). This is the region in which unlabeled leukocytes are expected to appear.
8. Add one drop (50 µl) of the FITC Standard and one drop (50 µl) of the PE Standard to the remaining Autofluorescence Standard. Mix vigorously.
9. Run the three bead mixture on the flow cytometer.
10. Adjust the color compensation circuits such that the FITC Standards appear in Quadrant 4 and the PE Standards appear in Quadrant 1. Both should be in line with the Autofluorescence Standards, which should still appear in Quadrant 3 (Figure 3).
11. Add one drop (50 µl) of the FITC/PE Standard to the suspension prepared in Step 8. Mix vigorously.
12. Run the bead suspension (Step 11) on the instrument.
13. The FITC/PE Standard population should appear in Quadrant 2, toward the middle to upper right corner, as would dual labeled cells. The PE Standard, Autofluorescence Standard and FITC Standard populations should still be in line and detected in Quadrants 1, 3, 4, respectively. Data should appear as in Figure 4.

Run the four microbead suspension (Step 11), collecting 10,000 events. Record all appropriate instrument settings as well as the histogram peak channels from each of the four standards.





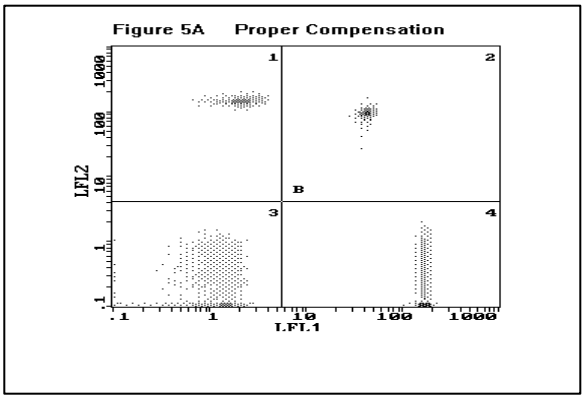


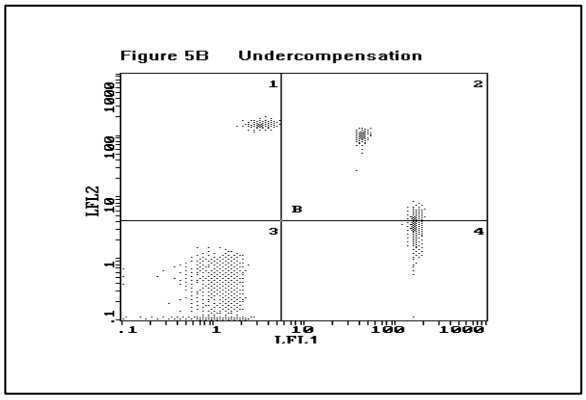


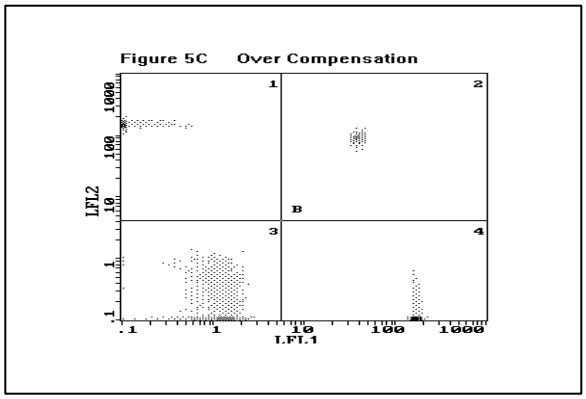
Results

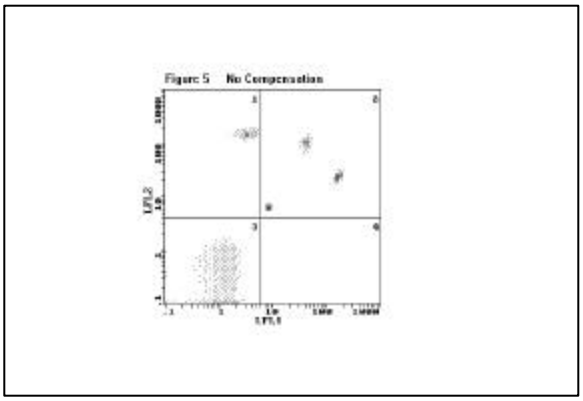
With proper color compensation, the PE Standard should appear in Quadrant 1, the FITC/PE Standard in Quadrant 2, the Autofluorescence Standard in Quadrant 3 and the FITC Standard in Quadrant 4. The resulting histograms will roughly describe the vertices of a rectangle. The mean channel in FL-1 for the PE Standard will be approximately equal to the corresponding value of the Autofluorescence Standard. Similarly, the mean channel in FL-2 for the FITC Standard will be approximately equal to that of the Autofluorescence Standard.

The Autofluorescence, FITC, PE and dual FITC/PE Standards allow proper reference points for electronic compensation for overlapping emission signals. Examples of overcompensated (electronically eliminating too much of the appropriate fluorochrome signal), under-compensated (not eliminating enough of the overlapping fluorochrome signal) and uncompensated results are shown in Figure 5.









Limitations

1. Proper storage (2-8°C) and handling are essential. Exposure of these reagents to room light for even limited periods of time may substantially reduce performance.
2. Vigorously mixing beads prior to use is essential in obtaining a uniform suspension.
3. Flow cytometer must be properly aligned prior to making compensation adjustments. The extent of adjustment necessary and the settings employed may vary with the type of instrument.
4. Some labeled reagents may present spectral characteristics or fluorescence intensities that fall outside the levels set using the FITC/PE Compensation Kit. In these cases, cells stained with the fluorochrome-labelled antibodies should be used to establish proper color compensation.[1]
5. Compensation adjustments should be made daily to insure consistency of flow cytometer performance.

Instrument stability and sensitivity may be monitored by analysis of the channel separation between the Autofluorescence Standard, FITC Standard and PE Standard on the FL1/FL2 histogram. Significant deviation from the expected ranges may be indicative of an instrument problem. The degree of regular channel separation observed is dependent on the type of instrument in use and individual test-specific parameters. Labs should establish the optimal color compensation settings for each flow cytometer and/or type of test.

The establishment of adequate color compensation should be verified as part of a daily start up procedure. Values should be recorded and com-pared with previous results to maintain consistent flow cytometer performance. The labeled microbeads will display fluorescence intensity of brightly labeled cells.

References

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