ProductInformation

Product No. C-1432
Certified Blank

Lot 093H0022

The Certified Blank reagent contains unlabelled synthetic microspheres of uniform size. These serve as a qualitative control to determine the "noise" level of individual flow cytometers. Certification verifies the detection of the blank beads in fluorescence channels below that of unstained human leukocytes. The product is offered in buffered saline with stabilizer and 0.1% sodium azide (see MSDS)* as preservative.

Description

Standardization and reproducibility of instrument performance is critical in flow cytometry.[1-8] There are a variety of means for monitoring instrument parameters. It is necessary to define fluorescein threshold in order to ensure that a given flow cytometer has the necessary sensitivity for immunophenotyping of cell surface antigens expressed at low density. Instruments should be maintained at a sensitivity level greater than that detected for autofluorescent cells. Fluorescence threshold is defined as the level above which a fluorescence signal can be measured by an instrument. Non-fluorescent particles can be used to make such determinations.

Procedure

Materials required but not provided:
Appropriately sized test tubes.
Unstained human peripheral blood with erythrocytes lysed using a commercial lysing reagent.

Instrumentation required but not provided:
Flow cytometer.

Optically align flow cytometer per manufacturer's specifications.
Other optional instrument performance QC procedures:
If FITC/PE two color analysis is routine practice in the laboratory, adjust fluorescence PMT (Photomultiplier tube) and color compensation using established reagent(s)/protocol or Flow Cytometry Compensation Kit (Sigma Stock No. COMP-1).
Analyze Microbead Standards (Sigma Product No. M-0162). Record the PMT voltages and mean channel fluorescence on an appropriate log sheet. The results should be consistent with established target conditions and target channels. The use of Microbead Standards permits the direct comparison of fluorescence intensity data over time.
Vigorously shake Certified Blank to achieve a uniform suspension.
Add 0.5 ml (approximately 10-15 drops or 200,000 microspheres) to an appropriately sized test tube.
Run the Certified Blank on the flow cytometer. It may be necessary to adjust the fluorescence PMT(S) so that the peak(s) of the blank microbeads falls on scale. If adjustments are made, repeat data collection in Step 2.
Collect data gated on singlets of the Certified Blank for 10,000 events.
Record the peak (median) channel for each of the fluorescence parameters. Mean channel may be alternatively used, however, note that mean and median channels are not interchangeable.
Run the lysed, unstained whole blood sample on the flow cytometer, set a gate for lymphocytes and collect 10,000 events. Record the mean or median channel at each PMT.
Compare the mean or median channels of the unstained human lymphocytes to that of the Certified Blank. If the mean channels of the unstained lymphocytes is above the values for the Certified Blank microbeads, the instrument has sufficient sensitivity for immunophenotyping assay.

Results

The Certified Blank (unshaded peak) should appear less fluorescent than unstained leukocytes

(shaded peak). The Certified Blank serves as a qualitative control for monitoring flow cytometer sensitivity and the level of background fluorescence. Figures 1 and 2 are histograms illustrating the difference between Certified Blank microbeads and the autofluorescence of lymphocytes.

Limitations

Proper storage, handling and use of Certified Blank is essential. This reagent is extremely sensitive to changes in pH. The Certified Blank microspheres are maintained at physiological pH (7.2). It is, therefore, critical that pH be carefully controlled in performing quantitative and qualitative flow cytometry analyses. Vigorously mixing microspheres prior to use is essential in obtaining a uniform suspension. Establishment of consistent, reproducible mean channel performance data for an appropriate reference standard, i.e., Microbead Standards, is crucial to proper interpretation of results obtained with Certified Blank.

Storage

Store at 0-5?C. **Do Not Freeze**.

*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 hazards and safe handling practices.

References

Proposed Guidelines: Clinical Applications of Flow Cytometry - Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. National Committee for Clinical Laboratory Standards. Document H42-P, **9**, 13 (1989).

Shapiro, H., Practical Flow Cytometry, 2nd Ed., Alan R. Liss, Inc., New York, 267-270 (1988).

Keren, D., Flow Cytometry in Clinical Diagnosis, ASCP Press, Chicago, 27-28 (1989).

Horan, P., and M. Loken, "A Practical Guide to the Use of Flow Cytometry and Flow Cytometric Instrumentation and Data Analysis, M. Van Dilla, et al., (Eds), Academic Press, NY, 260-281 (1985).

References (cont.)

Horan, P., and J. Kappler, J. Immunol. Meth., **18**, 307 (1977).

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Prepared for Sigma

Figure 1
Overlay of Fluorescence Histograms of the Certified Blank and unstained lymphocytes in Fl-1.

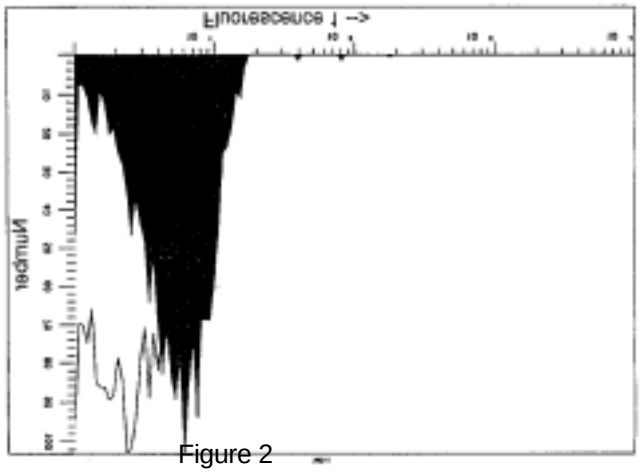

Figure 2
Overlay of Fluorescence Histograms of the Certified Blank and unstained lymphocytes in Fl-2.