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ProductInformation

PKH26 Reference Microbeads

Product No. P 7458

PKH26 Reference Microbeads are polymer microbeads having fluorescence intensity and light scatter characteristics similar to PKH26-labeled human leukocytes. The reference microbeads are impregnated with PKH26 dye, then carefully formulated as a suspension in buffered saline with surfactants and 0.1% sodium azide (see MSDS*). A lot-specific singlet bead count is given in the enclosed Certificate of Analysis to permit calculation of absolute cell counts when the PKH26 Reference Microbeads are used as an internal standard.

Description

A principle concern in flow cytometry analysis, especially for immunophenotyping, is instrument standardization to ensure reproducibility of instrument performance and to permit valid data comparison over time and between instruments. Instrument standardization can be achieved by monitoring optical alignment, fluorescence sensitivity and linearity, and proper color compensation. To establish a valid basis for data comparison, it is essential to incorporate in every assay protocol a reference standard which will always exhibit the same target values (channel numbers) in parameters of interest (e.g., light scatter, fluorescence) under a given set of target conditions (user defined values for instrument variables such as light scatter, fluorescence high voltage/gains and color compensation). The effect, in practice, is the normalization of data to an established common standard. In immunophenotyping by flow cytometry, the ideal reference material should display characteristics similar to those of labeled cells (e.g., size, fluorescence intensity, and fluorescence spectral properties). This is especially important in the application of microbeads for standardization, as a great deal of variability may be observed, depending on the method by which they are prepared.

The use of microbeads for flow cytometry quality control is gaining wide acceptance and has several demonstrated applications.[1-8] These are of uniform size and are designed to simulate forward scatter and PKH26 fluorescence intensity generated by PKH26-labeled cells. The combination of these properties allows standardization of instrument variables in a single run. Initially, set up the cytometer according to the manufacturer's directions. Unstained cells (no PKH26 or immunophenotyping reagents) are used to select PMT voltage and single-stained cells are used to adjust the compensation circuits. As fluorescence intensity standards, the microbeads can be analyzed as external or internal standards. Record the mean channel number of the microbeads and the cytometer variables. In subsequent assays, the same settings for the instrument variables should place the PKH26 microbeads in the same channel position. Fine adjustments of FL2 PMT voltage to place the PKH26 Reference Microbeads in the same channel position will insure comparable assay data over time. If such adjustments are made, reexamine the compensation requirements. If large adjustments in FL2 PMT voltage are necessary to place the same PKH26 microbeads in the predetermined channel position, it is an indication that the cytometer requires maintenance service or the reference microbeads have deteriorated. Instrument problems can be ruled out by running a known independent fluorescence standard for FL2 such as PE microbeads. This practice insures consistency in instrument setup and that all data are normalized to the same intensity reference.

To maximize the information content of the test sample data files, the PKH26 microbeads are used to obtain absolute cell counts in any model of flow cytometer. This approach allows the investigator to identify proliferation due to a minor fraction of the cell population while most of the cells may have died and disintegrated in culture. The cell number per volume should be maintained as in the original culture. This is an important consideration if the cells are harvested from culture and manipulated before data acquisition on the cytometer.

Procedure

The following procedure should be followed when the PKH26 Reference Microbeads are used as an internal standard and will permit the calculation of absolute cell counts.

1. Shake the bottle of PKH26 microbeads vigorously to obtain a homogeneous suspension.
2. Add an equal volume of the microbeads to the test sample. Caution: The microbeads and expected cell numbers should be similar (on the order of 2 x 10^5). Otherwise, adjust this ratio. The microbeads are stable in 2% paraformaldehyde for up to 2 weeks. The lot-specific microbead concentration may be found on the enclosed Certificate of Analysis.
3. Set up the cytometer as follows:
   a. Use unstained cells to set the PMT voltage.
   b. Use single-stained cells (cells stained with PKH26 alone or cells stained with a single immunophenotyping reagent) to adjust compensation when performing multi-color analyses.

   CAUTION: PKH26 Reference Microbeads are not designed for use in compensation adjustments The PKH26 dye response when embedded in the polymer microbead is different from the dye response when embedded in a cell membrane rendering the PKH26 Reference Microbeads unsuitable for this application.

4. Set a light scatter gate around the microbead singlet population. Set the cytometer counter to acquire 5,000 microbead events.
5. Example for the calculation of absolute cell counts:
   Lot Specific PKH26 microbeads concentration = 200,000 per ml.
   (200,000 is used for example only. The correct lot-specific concentration may be found on the enclosed Certificate of Analysis)
   Volume counted corresponding to 5,000 microbead events (a) = 5,000 x dil x 1/200,000 ml
   Dilution factor for both microbeads and cells = 2
   Number of cells corresponding to 5,000 microbeads = b
   Cell concentration in culture = b x dil x 1/a per ml

Storage

Store at 2-8 °C.

*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

1. 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).
2. Shapiro, H., Practical Flow Cytometry, 2nd Ed., Alan R. Liss, Inc., New York, 267-270 (1988).
3. Keren, D., Flow Cytometry in Clinical Diagnosis, ASCP Press, Chicago, 27-28 (1989).
4. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. National Committee for Clinical Laboratory Standards. Document H42-P, **9**, 789 (1989).
5. Horan, P., and Loken, M., "A Practical Guide to the Use of Flow Systems", In: Flow Cytometry Instrumentation and Data Analysis, Van Dilla, M., et al., (Eds.), Academic Press, NY, pp 260-280 (1985).
6. Horan, P., and Kappler, J., J. Immunol. Meth., **18**, 309 (1977).
7. Giorgi, J., et al., Clin. Immunol. Immunopathol., **55**, 173 (1990).
8. Parker, J., et al., Clin. Immunol. Immunopathol., **55**, 187 (1990).

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