Cellular Microspheres
Catalog Number C1557
Storage Temperature 2–8 °C

Product Description
Cellular Microspheres are synthetic microspheres of uniform size with goat anti-mouse IgG covalently bound to the surface of the beads. They may be used for monitoring stability of fluorochrome labeled mouse monoclonal antibodies in flow cytometric analysis.

Standardization and reproducibility of instrument performance is critical in flow cytometry. There are a variety of means for monitoring instrument parameters. However, of equal importance is the stability of the fluorochrome labeled antibodies employed in the staining procedures.

Positive reagent and method controls can give some indication of the effectiveness of monoclonal antibodies. Yet, these procedures may still leave some ambiguity as to whether decreases in observed fluorescence intensity are due to the biology of the cell sample being stained or diminished antibody fluorescence of the reagents used. One method of consistently tracking such labeled antibody parameters is through the use of microspheres coated with an appropriate antigen or antibody. While antigen-coated microspheres are useful, they are restricted to only antigen-specific antibodies. Antibody labeled microspheres afford the versatility of application to monoclonals of virtually any specificity. The functional instability of the fluorochromes relative to the Ig portion of a conjugate also argues for the practicality of using anti-antibody coated microspheres.

Treated as cell surrogates, Cellular Microspheres can be stained by incubating with the conjugated IgG monoclonal reagent in question. An initial titration curve is established for a specific lot of the antibody reagent. A typical titration curve is shown in Figure 1 (see Appendix). Subsequently, the performance of the monoclonal reagent can be verified against the initial titration curve using the same lot of Cellular Microspheres. In the case of a cell sample giving lower than expected staining intensity, this antibody reagent quality control practice permits one to choose whether the observation is due to reagent deterioration, sample preparation, and/or biology of the cell sample.

Intensity of fluorochrome labeled antibodies as a reflection of their stability may be monitored by this method. Peak channels obtained should be recorded and followed over the useful life of the antibody reagent. Significant decreases in the intensity of fluorescence may indicate a deterioration of antibody performance by flow cytometric methods.

Reagent
The product is provided as a suspension in 100 mM borate, pH 8.5, containing 0.1% bovine serum albumin, 0.05% TWEEN® 20, and 10 mM EDTA.

Materials Required but Not Provided
- Appropriately sized test tubes
- Fluorochrome conjugated mouse IgG monoclonal antibody
- Flow cytometer

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store at 2–8 °C. Do Not Freeze. Proper storage (2–8 °C) and handling are essential. Once labeled antibody is bound to the Cellular Microspheres, exposure to light for even limited periods may substantially alter results.

Procedure
This reagent, as well as the fluorescence intensity of the fluorochromes on the labeled monoclonals employed, is extremely sensitive to slight changes in pH. Cellular 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.

Vigorous mixing of the microspheres prior to use is essential in obtaining a uniform suspension.
Although Cellular Microspheres are primarily intended for use with direct fluorochrome conjugated monoclonal antibodies, the indirect methodology may be used to verify the stability of the fluorochrome conjugated secondary antibody. Refer to manufacturer's literature for proper treatment of these reagents.

1. Vigorously shake the Cellular Microspheres to achieve a uniform suspension.
2. Add 100 µL (2-3 drops or $2 \times 10^5$ microspheres) to an appropriately sized test tube.
3. Add the monoclonal antibody to be tested to the 100 µL of Cellular Microspheres in the volume or concentration recommended by the manufacturer for staining cells. It is advisable to vary this volume/concentration to establish a titration curve for future reference. A minimum of 30 minutes staining time is suggested. Protect from light at this and all subsequent steps.
   **Note:** With some reagents, additional antibody beyond the manufacturer's recommended concentration for staining cells may need to be added to the Cellular Microspheres to obtain saturation conditions.
4. Wash labeled Cellular Microspheres according to the manufacturer's recommended procedure for washing stained cells.
5. Optically align flow cytometer according to manufacturer's specifications.
6. Optional instrument performance QC procedures:
   a. If the reagent is routinely used in 2 color analysis, fluorescence PMT and color compensation using established reagent/protocol.
   b. Analyze Microbead Standards (Catalog Number M0162). 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.
      **Note:** Establishment of consistent, reproducible mean channel performance data for an appropriate reference standard, i.e., Microbead Standards, is crucial to comparisons and proper interpretation of antibody quality control results obtained with Cellular Microspheres.
   c. Alternatively, adjust light scatter and fluorescence PMT using unlabeled Cellular Microspheres.
7. Run the labeled Cellular Microspheres on the flow cytometer, collecting at least 10,000 gated singlet events. Figures 2a and 2b (see Appendix) show histograms of unlabeled and Quantum Red™ labeled Cellular Microspheres.
8. Record the peak (median) channel for the appropriate fluorescence parameter. Mean channel may be alternatively used; however, mean and median channels are not interchangeable.

**References**

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Appendix

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