ADVANCED TARGETING SYSTEMS

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CFSE Compensation Kit
FLOW CYTOMETRY REAGENT

Catalog Number: FL-13
Quantity: 2.0 ml negative/unstained microspheres and 1.0 ml CFSE microspheres
Format: Ready to Use, do not dilute

Background:
Fluorescence compensation is the process wherein a portion of the primary dye signal is removed from all non-primary dye channels. Compensation is generally required whenever more than a single dye (color) is used in flow cytometric analysis. Fluorescent dyes emit light over a range of wavelengths and while the filters are optimized to collect the light from the primary dye, some light may spillover into other channels. The evaluation and setting of compensation therefore is needed to determine how much spillover occurs from one dye channel to the next and to adjust for that spillover.

Specificity and Preparation:
This 1-color CFSE (carboxyfluorescein-succinimidyl ester) Compensation Kit contains negative/unstained microspheres [2.0 ml, use 1 drop (≈30 μL) in 5 tubes per set up] and CFSE microspheres [1.0 ml, need 1 drop (~30 μl) in 1 tube per set up], sufficient for 25 tests. The kit contains a commonly used fluorescent dye for *in vitro* or *in vivo* cell labeling. CFSE binds to amine groups on cellular proteins. Cells, once labeled with CFSE, can be stimulated *in vitro* and cell proliferation measured by changes in the staining intensity of the cells. This is due to the fact that as cells divide, the CFSE content per cell is reduced by approximately 50%. Fluorescently-labeled antibodies to cell surface antigens can be used in the analysis to identify specific cell subpopulations. The maximum excitation wavelength is 488 nm and the maximum emission wavelength is 519 nm. The range of emission spillover is 500->650 nm.

Usage and Storage:
The CFSE (carboxyfluorescein-succinimidyl ester) Compensation Kit provides a reproducible and convenient source of CFSE particles for evaluating and correcting for the spectral overlap between CFSE and other dyes. Store at 2-8°C. Note: leaving the labeled microspheres in dilute solution in PBS for > 1 hour may degrade the fluorescence signal.

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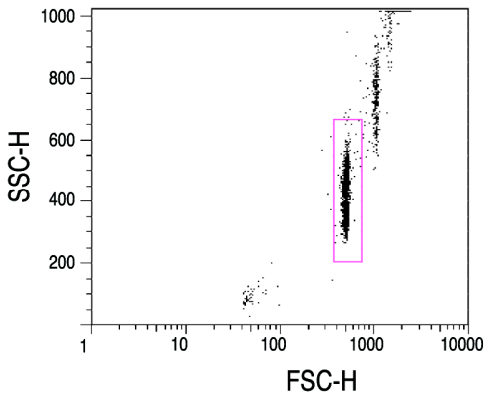

FSC vs. SSC dot plot display showing gate/region of singlet microspheres

References:

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2. Bagwell CB (2005) Hyperlog- a flexible log-like transform for negative, zero, and positive valued data. Cytometry 64:34-42.
3. Tung JW, Parks DR, Moore WA, Herzenberg LA (2004) New approaches to fluorescence compensation and visualization of FACS data. Clin Immunol 110:277-283.
4. Theil A, Scheffold A, Rabbruch A (2004) Antigen-specific cytometry-New tools arrived! Clin Immunol 111:155-161
5. Roederer M (2001) Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. Cytometry 45:194-205.
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7. Lyons AB, Parish CR (1994) Determination of lymphocyte division by flow cytometry. J Immunol Meth. 171:131-137.

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