

Description of Methods for the Use of Monosan Antibodies in Direct Flow Cytometry

Surface Staining:

1. Prepare cell suspension in PBS .
2. Add 10µl of antibody to 90µl of the cell suspension. Antibodies have been pre-titred to provide optimum staining. Incubate for 30 minutes at 4°C (An appropriate isotype control is recommended).
3. Wash twice in PBS or Isoton. Resuspend in 300µl PBS for analysis.
4. For the detection of Biotin-conjugated antibodies add 100µl of pre-titred avidin or streptavidin-fluorochrome conjugate. Incubate for 30 minutes at 4°C and wash prior to analysis.

Intracellular Staining:

1. Prepare cell suspension in PBS (containing 0.5% (w/v) saponin). Prepare fresh saponin solution on day of use.
2. Add 10µl of appropriate antibody to 90µl of the cell suspension. Antibodies have been pre-titred to provide optimum staining. Incubate for 30 minutes at 4°C. An appropriate isotype control is recommended.
3. Wash twice in PBS or Isoton. Resuspend in 300µl PBS for analysis.
4. For the detection of Biotin-conjugated antibodies add 100µl of pre-titred avidin or streptavidin-fluorochrome conjugate. Incubate for 30 minutes at 4°C and wash prior to analysis.

Dual Labelling:

1. Prepare cell suspension in PBS (containing 0.5% (w/v) saponin if either antigen is internal).
2. Add 10µl of appropriate Biotin-conjugated antibody to 90µl of the cell suspension, then add 10µl of the FITC-conjugated second antibody and mix. (Antibodies have been pre-titred to provide optimum staining). Incubate for 30 minutes at 4°C. Appropriate isotype controls are recommended.
3. Wash twice in PBS or Isoton. Add 100µl of pre-titred avidin or streptavidin-fluorochrome conjugate. Incubate for 30 minutes at 4°C.
4. Wash twice in PBS. Resuspend in 300µl PBS for analysis.

References:

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- Rigg K M, Shenton B K, Murray I A, et al.. A flow cytometric technique for simultaneous analysis of human mononuclear cell surface antigens and DNA. *Journal of Immunological Methods*. 123: 177-184 (1989).
- Hirata M, and Okamoto Y. Enumeration of terminal deoxynucleotide transferase positive cells in leukaemia/lymphoma by flow cytometry. *Leukemia Research*. 11: 509-518 (1987).