**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 4oC (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 4oC 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 4oC. 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 4oC 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 4oC. 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 4oC.

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 deoxylnucleotide transferase positive cells in leukaemia/lymphoma by flow cytometry. Leukemia Research. 11: 509-518 (1987).