**Description of Methods for the Use of Monosan Antibodies in Indirect Flow Cytometry**

Surface Staining:

1. Prepare cell suspension in PBS .

2. Add 10µl of antibody at various dilutions eg 1:10 upwards to 90µl of cell suspension. Incubate for 30 minutes at 4oC (an isotype control is recommended).

3. Wash twice in PBS.

4. Add Secondary FITC Ab (or equivalent) in PBS. Incubate for 30 minutes at 4oC.

5. Wash twice with PBS.

6. Resuspend in 300µl of PBS for analysis.

Intracellular Staining:

1. Prepare cell suspension in PBS (containing 0.5% (w/v) saponin).

2. Add 10µl of antibody at various dilutions eg 1:10 upwards to 90µl of cell suspension. Incubate for 30 minutes at 4oC (an isotype control is recommended).

3. Wash twice in PBS.

4. Add secondary FITC Ab (or equivalent) in PBS. Incubate for 30 minutes at 4oC.

5. Wash twice with PBS.

6. Resuspend in 300µl of PBS for analysis.