

# Description of Methods for the Use of Novocastra Antibodies in Direct Flow Cytometry

## REAGENTS

1. Single cell suspension.
2. Monoclonal antibody; FITC-conjugated and/or Biotin-conjugated.
3. PBS or Isoton.
4. Avidin/Streptavidin-Fluorochrome reagent for Biotin-conjugated antibodies.
5. Permeabilisation reagent; eg. saponin (also see references).

## EQUIPMENT

Flow cytometer, laboratory centrifuge, 4°C fridge.

## PROCEDURES

### 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).

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