

Description of Methods for the Use of Novocastra Antibodies in Indirect Flow Cytometry

REAGENTS

1. Single cell suspension.
2. Monoclonal antibody; Unconjugated.
3. PBS or Isoton.
4. NCL-SAM-FITC or equivalent secondary reagent (titre for optimum dilution).
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 at various dilutions eg 1:10 upwards to 90µl of cell suspension. Incubate for 30 minutes at 4°C (an isotype control is recommended).
3. Wash twice in PBS.
4. Add NCL-SAM-FITC (or equivalent) in PBS. Incubate for 30 minutes at 4°C.
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 4°C (an isotype control is recommended).
3. Wash twice in PBS.
4. Add NCL-SAM-FITC (or equivalent) in PBS. Incubate for 30 minutes at 4°C.
5. Wash twice with PBS.
6. Resuspend in 300µl of PBS for analysis.

References

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