



Description of Methods for the Use of Vector 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 10 μ l of antibody to 90 μ l 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 300 μ l PBS for analysis.
4. For the detection of Biotin-conjugated antibodies add 100 μ l 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 10 μ l of appropriate antibody to 90 μ l 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 300 μ l PBS for analysis.
4. For the detection of Biotin-conjugated antibodies add 100 μ l 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 10 μ l of appropriate Biotin-conjugated antibody to 90 μ l of the cell suspension, then add 10 μ l 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 100 μ l of pre-titred avidin or streptavidin-fluorochrome conjugate. Incubate for 30 minutes at 4 °C.
4. Wash twice in PBS. Resuspend in 300 μ l PBS for analysis.

References

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