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FLOW CYTOMETRY (FC, FCM)

Reagents

1) PBS Buffer: pH 7.4
2) Blocking Buffer: 0.5% BSA-PBS
3) Fix Buffer: 2-4% paraformaldehyde
4) Penetrating Buffer: 90% methanol
5) Primary Antibody
6) Secondary Antibody (this is not required if the sample was directly labelled with a conjugated primary antibody)

Procedure

1) Cell Collection: Collect cells and adjust the cell concentration to 1-5 x 106 cells/ml.
2) Wash and Centrifuge: Add 2 ml blocking buffer, then shake slightly and centrifuge at 1500-2000 rpm for 5 min.
3) Cell Fixation: Discard the supernatant, then fix cells in 1 ml fix buffer and incubate at room temperature for 10 min.
4) Wash and Centrifuge: Centrifuge at 1500-2000 rpm for 5 min, then wash cells once with 1 ml blocking buffer and centrifuge again at the same condition.
5) Permeabilisation: Discard the supernatant, add 1 ml precooled penetrating buffer and incubate at room temperature for 10 min (If the target is extracellular, skip this step).
6) Wash and Centrifuge: Centrifuge at 1500-2000 rpm for 5 min, then wash cells once with 2 ml blocking buffer and centrifuge again at the same condition.
7) Blocking: Incubate cells in blocking buffer for 30 min at room temperature.
8) Incubate Primary Antibody: Add primary antibody at 0.025 mg/ml and incubate for 90 min at room temperature.
9) Wash and Centrifuge: Centrifuge at 1500-2000 rpm for 5 min, then wash cells once with 2 ml blocking buffer and centrifuge again in the same conditions. Repeat this once.
10) Incubate Secondary Antibody: Incubate with conjugated secondary antibody for 40 min at room temperature (For direct labelling using a conjugated primary antibody, skip this step).
11) Wash and Centrifuge: Centrifuge at 1500-2000 rpm for 5 min, then wash cells once with 2 ml blocking buffer and centrifuge again at the same condition.
12) FC analysis: Re-suspend cells in 1 x PBS and analyse on flow cytometer.