ABCEPTA CUSTOM SERVICES:

Flow Cytometry Protocol

Preparation of Buffer:

PBS buffer: PH=7.4

Blocking Buffer: 0.5% BSA-PBS

Fix Buffer: 2% paraformaldehyde

Penetrating Buffer: 90% methanol

Step-by-step procedure:

1. Cell Collection: Collect the cell suspension, adjust the cell concentration into 1-5 x 10^6 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: Drop the supernatant, then fix cells with 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. Cell penetration: Drop the supernatant, add 1 ml precool penetrating buffer and incubate at room temperature for 10 min. (If it is the extrazellular stain, just 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 at the same condition. Repeat again.

10. Incubate Secondary Antibody: Incubate with FITC-conjugated secondary antibodies for 40 min at room temperature (Keep in dark place).

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 analyze on flow cytometry.

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