

ABGENT 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 $1-5 \times 10^6$ 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.