

## Flow cytometry

1. Fix cells with 2% Paraformaldehyde in PBS at 37°C for 10 min.
2. Wash cells with 1% FCS-PBS.
3. Permeabilize cells with 90% methanol on ice for 30 min.
4. Wash cells with 1% FCS-PBS.
5. Incubate cells with normal serum from the species of the 2nd antibody at room temperature for 30 min to block unspecific binding of the antibodies.
6. Add 1st mAb (0.5-2 µg) and incubate at room temperature for 1 hr.
7. Wash cells with 1% FCS-PBS.
8. Add 2nd Ab and incubate at room temperature for 1 hr..
9. Wash cells with 1% FCS-PBS.
10. Analyze by flow cytometer.