

## Flow Cytometry Protocol

### Buffers and Reagents:

**Wash solution:** FCS-PBS.

FCS <http://www.fitzgerald-fii.com/fetal-calf-serum-88-nc10.html>

PBS <http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.html>

**Primary antibody:** See our selection of monoclonals at <http://www.fitzgerald-fii.com/monoclonal-antibodies.html> or polyclonals at <http://www.fitzgerald-fii.com/polyclonal-antibodies.html>

**Secondary antibody:** See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

**Some other useful reagents:** <http://www.fitzgerald-fii.com/diluents.html>

### General Procedure:

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