**Antibody staining of surface antigens on cells using a 96 well plate**

**Notes**

This is a general protocol based on staining mononuclear cells from peripheral blood.

It does not concern specific antibodies, however, it takes into consideration: Incubation, washing and fixation.

For other cell types you might need to optimize the procedure.

We recommend blocking before staining.

For more details, read *Elimination of erroneous results in flow cytometry caused by antibody binding to Fc receptors on human monocytes and macrophages* by Andersen et al 2016, [Cytometry A.](https://www.ncbi.nlm.nih.gov/pubmed/27731950) 2016 Nov;89(11):1001-1009

In the following protocol the antibody incubation is performed at room temperature (RT). If you want to incubate on ice the incubation time should be expanded to 30-60 min and the centrifuge should be cold.

In each well there will be approximately 15µl left after pouring off your supernatant from a round bottom plate.

**MATERIAL**

1. Stain buffer: PBS pH 7.4 with 0.5% BSA and 0.09% Na-azide
2. Blocking Reagent: Human Ig
3. Fluorocrome conjugated antibodies.
4. Cells at 1-5x106/ml in stain buffer
5. A round bottom or V bottom microwell plate
6. Fixation buffer: PBS pH 7.4 with 0.9% formaldehyde

**PROCEDURE**

Be cautious at any time not to mix fluid from one well to another when pouring fluid off.

Even a tiny bit of antibody can stain your cells.

1. Make a scheme showing which antibodies in which wells. If viability test is not a part of your panel you should make a sample just with your viability marker
2. Adjust your cell suspension to 1-5x 106/ml in stain buffer.
3. Blocking can be performed now: Add HuIg, 100g/ml cell suspension, mix and incubate at +4 °C for at least 15 minutes.
4. Add antibodies to the wells
5. Transfer 100µl cell suspension to each well and pipette up and down 5 times to mix cells and antibodies.
6. Incubate in the dark 15-30 min at RT. Some cell types will require incubation 30-60 min at 4 °C
7. Add 100 µl washing buffer to each well.
8. Centrifuge the plate at 350xg for 2 min at RT (or cold)
9. Place a thick paper tissue flat on the table
10. Pour off the supernatant in one sliding movement and press the plate briefly against the paper tissue before turning the plate bottom downwards again
11. Loosen the cell pellet by a gentle knocking on the side of the plate
12. Add 200µl stain buffer to each well using a multichannel pipette, resuspend cells using the pipette.
13. Repeat from 8-12 once
14. Then repeat from 8-11 once (makes a total of 3 washes ending up with pellets)
15. Fix and resuspend the cells by adding 200µl or 250µl of fixation buffer to each well and pipette up and down 10 times
16. Place a lid on the plate and keep it cold and dark until analysis
17. The use of tandem dyes will limit the storage time to 24 hours.
We recommend analysing as quickly as possible.