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

**Notes**

This is a general protocol, which does not concern specific antibodies, however it takes into consideration: Incubation, washing and fixation.

Consider blocking before staining.

In this protocol the incubation is performed at 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.

**MATERIAL**

1. Stain buffer: PBS pH 7.4 with 0.5% BSA and 0.09% Na-azide
2. Fluorocrome conjugated antibodies and maybe isotype controls
3. Cells at 1-5x106/ml in stain buffer
4. A round bottom microwell plate (not for cell culturing)
5. 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. Blocking could be performed now
3. Add antibodies to the wells
4. Transfer 100µl cell suspension to each well and pipette up and down 5 times times to mix cells and antibodies.
5. Incubate in the dark 15-30 min at RT. Some cell types will require incubation 30-60 min at 4 C0
6. Add 100 µl washing buffer to each well.
7. Centrifuge the plate at 350g for 2 min at RT (or cold)
8. Place a thick tissue flat on the table
9. Pour off the supernatant in one sliding movement and press the plate briefly against the tissue before you turn the plate bottom downwards again
10. Loosen the cell pellet by a gentle knocking on the side of the plate
11. Add 200µl stain buffer to each well using a multichannel pipette, resuspend cells using the pipette.
12. Repeat from 7-10 twice
13. Fix and resuspend the cells by adding 200µl of fixation buffer to each well and pipette up and down 5 times
14. Place a lid on the plate and keep it cold and dark until analysis
15. The use of tandem dyes will limit the storage time to 24 hours. We recommend to analyse as quickly as possible.