**Antibody Staining of Surface Antigens for Cell Sorting in Flow Cytometry**

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This guideline works in connection with the FACS Core Facility Guidelines:

* Panel Design in Flow Cytometry (1)
* Titration in Flow Cytometry (2)
* Controls in Flow Cytometry (3)
* Compensation in Flow Cytometry (4)
* Preparing cells for sorting (5)

**Notes**

This guideline is for staining cells intended for sorting.

This is a general guideline based on staining of cell lines or other single cell suspensions.

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

You may need to adjust the procedure.

We strongly recommend that you evaluate if blocking is essential for your experiment (6). It depends on the cell type you are working with. If in doubt, make a parallel experiment with and without blocking.

In this guideline the antibody incubation is performed at room temperature (RT). If you need to incubate on ice, the incubation time should be expanded to 30-60 minutes and the centrifuge should be cold (4 °C).

**Materials**

1. Single cell suspension
2. Tubes - see table next page
3. Stain and wash-buffer: PBS pH 7.4 with 0.5% BSA or 2% FBS
4. Alternative to Stain and wash-buffer: Non-stick stain-buffer: HBSS with 1% BSA, 2,5mM EDTA and 25mM HEPES
5. Blocking Reagent: Relevant species serum, commercial Fc Block or blocking reagent (6)
6. Fluorochrome conjugated antibodies.
7. Viability dye

**Procedure**

1. Adjust your single cells to your desired concentration in stain buffer. If your cells are sticky use the non sticky cell buffer all the way through.
2. If necessary, blocking can be added to your cells now, mix and incubate at +4 °C for at least 15 minutes.
3. Distribute your cells to the required number of tubes for staining and controls.
4. Add antibodies and mix.
5. Incubate in the dark 15-30 min at RT. Some cell types will require incubation 30-60 min at +4 °C.
6. Add washing buffer to each tube (according to table below) and mix briefly.
7. Centrifuge the tubes at 350-600xg (depending on cell type) for 5 min at RT (or cold).
8. Pour or pipette off the supernatant.
9. Loosen the cell pellet by gentle knocking the tube.
10. Repeat from 6-9.
11. Resuspend the cells in at least 300 µl washing buffer. For optimal cell concentration for sorting, see Preparing Cells for Sorting (5).
12. Keep the samples cold and dark until sorting. We strongly recommend sorting as quickly as possible.
13. If a live-dead-stain is not already added, we can add PI or DraQ7 (viability dyes) immediately before sorting.

**References:**

1. [FACS Core Facility Guidelines, Panel Design in Flow Cytometry](https://facs.au.dk/facscorefacilityguidelines/)
2. [FACS Core Facility Guidelines, Titration in Flow Cytometry](https://facs.au.dk/facscorefacilityguidelines/)
3. [FACS Core Facility Guidelines, Controls in Flow Cytometry](https://facs.au.dk/facscorefacilityguidelines/)
4. [FACS Core Facility Guidelines, Compensation in Flow Cytometry](https://facs.au.dk/facscorefacilityguidelines/)
5. [FACS Core Facility Guidelines, Preparing cells for sorting](https://facs.au.dk/facscorefacilityguidelines/)
6. Blocking: 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

<https://www.ncbi.nlm.nih.gov/pubmed/27731950>

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| Tubes | Number of cells | Final stain volume | Wash volume |
| 1,5 ml eppendorf tube | ≤ 1x10^6 | 100 μl | 500 μl1 |
| 2 ml eppendorf tube | ≤ 5x10^6 | 100 μl | 1 ml1 |
| 5 ml FACS tube | ≤ 5x10^6 | 100 μl | 2 ml2 |
| 15 ml tube | ≤ 50x10^6 | 100-500 μl | 5-10 ml2 |

1 Carefully aspirating closely to pellet.

2 Pour or aspirate carefully.