SOP: Flow cytometry staining
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Reagents:

- Staining/Wash buffer: PBS with 2% FBS.
- Brilliant stain buffer: Required for when using more than one polymer dye (i.e. Brilliant Violet, Brilliant UV, or Brilliant Blue or anything alike). Use BD #566349 if antibodies make up less than half of the cocktail, BD #566385 if antibodies make up more than half of the cocktail.
- Live-Dead/Fc-Block solution: BioLegend TruStain FcX #422302 1:25, and ThermoFisher Fixable L/D, #L-34962 for the Blue UV 1:500
- Fixation buffer: Cytofix (BD #554722) or freshly made 2% PFA

Procedure:

1. Transfer cells (see SOPs for generating single-cell suspensions) into either 5 ml polystyrene tubes or 96-well round bottom/V-bottom plates. Depending on your application you have to use approximately 1-10 x 10^6 cells.
2. Centrifuge for 5 min at 400g (which corresponds to 1500 rpm in a standard desktop centrifuge), flick the supernatants into liquid waste and dry remaining liquid by carefully tapping on a paper towel.
3. Resuspend the cells in 50µl of *freshly prepared* Live-Dead/Fc-Block solution and incubate for 15-20 min at room temperature.
4. Wash by adding 250µl of staining buffer (if staining in plates) or 1ml of staining buffer (if staining in tubes), centrifuge for 5 min at 400g, flick supernatants and dry remaining liquid by carefully tapping on a paper towel.
5. Add 50µl of appropriate antibody mix diluted in Brilliant Stain Buffer, resuspend the cells and incubate for 20-30 min at room temperature.
6. Wash by adding 250µl of staining buffer (if staining in plates) or 1ml of staining buffer (if staining in tubes), centrifuge for 5 min at 400g, flick supernatants and dry remaining liquid by carefully tapping on a paper towel. *Repeat this wash for a total of two times*.
7. Fix your sample for 10-20 minutes using 2% PFA or Cytofix (BD #554722), wash with staining buffer and store the samples resuspended in staining buffer at 4°C in the dark for up to 5 days. For intracellular/intranuclear staining see the corresponding protocols.

Notes:

- Fixation buffers: Cytofix/Cytoperm for intracellular targets (cytokines) BD #554722, Foxp3 fixation buffer for any intranuclear target (all transcription factors), ThermoFisher #00-5523-00.
- Perm/Wash: dilute 10x concentrate with DI water to obtain a 1x working dilution, either BD #554723 or ThermoFisher #00-5523-00.
- For any complex panel (>10 fluorophores) follow best practices for panel design (Ashhurst, Smith and King, Curr Protocols Immunology, 2017) and test the panel on PBMCs prior to the actual experiment.
- The L/D staining reagent is aminoreactive and thus has to be diluted in PBS prior to the actual staining. Fresh preparation is required since the dye will lose reactivity over time. Combining with Fc-Block has been tested and does not interfere.
- When working with myeloid cells, Fc-Block is essential (see Andersen et al, Cytometry PartA, 2017). Not necessarily required for T/B cell-centric stains.
- Quality of single-stained controls is essential. Use antibody capture beads (BD #552843/552844 and BD#560499) and treat these control samples the same way as your sample (fixation/washing steps). For more information: Maciorowski and Chattopadhyay, Current Protocols Immunology, 2017)