SOP: Flow cytometry staining with tetramers (human)
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Reagents:

- Staining/Wash buffer: PBS with 2% FBS.
- 1x Phosphate Buffered Saline (PBS with no Calcium Chloride and no Magnesium Chloride): use Gibco Ref # 20012-27
- Staining/Wash buffer: PBS with 2% FBS.
- Live-Dead/Fc-Block: Mix together LIVE/DEAD™ Fixable Dead Cell Stain (Invitrogen) diluted 1:500 in PBS with 10μL per test of Fc-Block (Human TruStain FcX Fc Receptor Blocking Solution – Biolegend, Cat. 422302)
- Compensation Beads: Anti-mouse Ig, κ/Negative Control Compensation Particles Set (BD PN 552843) or Anti-rat Ig, κ/Negative Control Compensation Particles Set (BD PN 552844).
- Live/Dead Compensation Beads: ArC™ Amine Reactive Compensation Bead Kit (ThermoFischer Cat # A10346)
- Brilliant stain buffer: Required for stainings with more than one polymer dye (i.e. Brilliant Violet, Brilliant UV, or Brilliant Blue); 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.

Procedure:
All tetramers need to be titrated prior to use!

1. Transfer cells into 96-well round bottom plates (1-10 x 10^6 cells).

2. Spin down 5 min at 400g (1500 rpm in a standard desktop centrifuge), flick the supernatants into liquid waste (no double flick!)

3. Resuspend the cells in 50µl of *freshly prepared* Live-Dead/Fc-Block solution and incubate in dark for 15-20 min at room temperature (RT).

4. Wash by adding 250μL of staining buffer, spin down for 5 min at 400g, flick supernatants

5. Resuspend cells in 50μL of diluted Tetramer and incubate in dark for 30 minutes at RT.

a. Tetramer titration: Serially dilute Tetramer from 1:100-1:1600. If possible use a negative control (Tetramer with irrelevant peptide loaded).
   *Depending on the tetramer, the incubation conditions may need to be tested and adjusted during titration. Add working conditions to the bottom of this protocol in the section titled “Current working Tetramer Conditions”

6. Prepare single stain controls alongside samples. Add 0.5μL of antibody to single stain controls when staining the sample with that antibody. Tetramers do not bind to compensation beads, so use an antibody with the same fluorophore.

7. Wash 2x by adding 250μL of staining buffer, centrifuge for 5 minutes at 400xg, flick supernatant.

8. Resuspend cells and compensation beads by adding 50μL of surface antibody mix diluted in the appropriate Brilliant Stain Buffer or single stain and incubate in the dark at RT for 20 minutes.

9. Wash 2x by adding 250μL of staining buffer, centrifuge for 5 minutes at 400xg, flick supernatant.

10. To Fix: resuspend with 50μL of 1% paraformaldehyde in PBS and incubate in the dark at RT for 20 minutes.

11. Wash 1x by adding 250μL of staining buffer, centrifuge for 5 minutes at 400xg, flick supernatant.

12. Resuspend cells in staining buffer, store at 4°C in the dark until acquisition.