T cell CFSE staining

1. Purify T cells using the manner of your choosing
2. Calculate the number of T cells that will be required post-staining (5 x 10^4/well, if doing *ex vivo* T cell stimulation in 96-well format). Transfer cells to sterile 1.7 mL tube
3. Pellet T cells (350 x g, 5 minutes, 4 C), decant, and carefully aspirate solution down to 100 µL.
4. Vortex to resuspend pellet, then dilute with FACS buffer (PBS + 2% FBS) to 1 mL
5. Repeat step 3

6. Prepare a 10 µM (2X) solution of CFSE in PBS, and quickly add to cell suspension to a final concentration of 5 µM (1X)

    a. The CFSE stock is 5 mM, so 1 mL of 10 µM CFSE – 998 µL PBS, 2 µL CFSE
    b. CFSE reacts quickly in aqueous solution, so quick dispersion through the cell suspension is desired
    c. This can be achieved by adding the CFSE while gently pulse vortexing the tube containing the cells
    d. If using an older stock of CFSE (>6 months old), a concentration of 20 µM (10 µM final) can be used

7. Incubate 5 minutes at room temperature, protected from light
8. Dilute cells with ten volumes of FACS buffer (1 mL)
9. Pellet, decant, vortex to resuspend, and dilute in 1 mL FACS buffer. Repeat 1 additional times.
10. Resuspend cells in an appropriate volume of RPMI 1640 + 10% FBS and count

    a. Ideally, 20% losses during staining can be expected, but it may be wise to plan for 50%

Notes for flow: <24 hours after staining, the CFSE signal is VERY intense, and will likely saturate the FITC detector as well as bleed into many other nearby channels that FITC would not typically spill into. Typically, wait at least 24 hours before acquiring any samples, as much of the initial labeling intensity decays over this time period down to a stable level.

At subsequent time points, CFSE intensity should decrease by ~1/2 with each cell division. With a good stain, 6-7 divisions should be readily discernible before resolution is lost.

If surface staining will be performed on CFSE-stained cells, and these surface stains exhibit spectral overlap with CFSE, the initial CFSE stain should include extra cells to be used as a compensation control. Unstained cells should also be included, and mixed with the singly-stained CFSE cells to generate a compensation control with positive and negative populations.

Cells stained with a FITC conjugated antibody are NOT a suitable replacement for a CFSE-labeled comp

Much of this protocol is derived from Quah, B., et. al (2007) *Nature Protocols*

CFSE -- https://www.biolegend.com/en-us/products/cfse-cell-division-tracker-kit-9396