

CFSE Staining

CFSE binds to proteins; stain cells in buffers that have decreased serum concentration (0.1% FBS). Cells which incorporate too much CFSE will have reduced protein function and therefore reduced viability. A short efflux step using 100% serum is included to allow cells to "purge" some CFSE and washing the cells at least 3 times helps produce a tighter initial CFSE peak. CFSE should be prepared in DMSO and stored in small aliquots at -80. It decays quickly, yellow discoloration indicates the CFSE will no longer conjugate to proteins.

PROTOCOL (optimized for human lymphocytes):

Label: Cell suspension should be 20 million cells per ml in 0.1% FBS/PBS. Add CFSE to a final concentration of 1.5 μ M, vortex gently and let suspension sit for 8 min at room temp. (Larger cells will require more CFSE as will phagocytic cell types. Some researchers use as much as 50 μ M.)

Stop Label and Efflux: Add an equal volume of pre-warmed FBS (100%, filtered). Incubate cells in a 37° water bath for 10 minutes for efflux.

Wash: Centrifuge cells 5min, 400g. Discard supernatant and vortex pellet to obtain single cell suspension. Wash cells by suspending in 1 ml of 2%FBS/PBS for every million cells. Centrifuge cells 5 min, 400g. Discard supernatant and vortex pellet to obtain single cell suspension. Repeat wash 3 times.

Take a day zero CFSE sample to determine initial labeling. Run cells immediately or fix in 1-4% paraformaldehyde.

Culture: Culture cells in appropriate growth media and take samples as determined by your protocol. Run cells immediately or fix in 1-4% paraformaldehyde.

Stain: Stain for surface markers at this point. When planning to surface stain, take into consideration that CFSE reads on the Flow Cytometer as FITC. Run cells immediately or fix in 1-4% paraformaldehyde.