Protocol for CFSE

CFSE is a reagent useful for cell tracking, proliferation studies, and cell motility studies. After passively diffusing into cells, it is converted to a fluorescent carboxyfluorescin molecule as its acetate groups are cleaved by intracellular esterases. The fluorescent CFSE is retained inside the cell for extended periods, because the molecules forms stable covalent crosslinks with the intracellular proteins. At each cell division, the intensity of the CFSE fluorescence is halved. The peak excitation of the molecule is 494 nm and the peak emission is 521 nm and it may be used with standard fixatives containing formaldehyde and permeabilization buffers that contain saponin.

Reconstitute CFSE to a stock concentration of 10 mM with 90 uL of anhydrous DMSO. After reconstitution, the product should be stored at -20°C with desiccant and protected from light. Freeze-thaw cycles should be avoided and the reagent should be used within 6 months.

1. Arrange a single-cell suspension of cells of interest.
2. Wash cells twice in sterile 1 X PBS solution to remove serum and resuspend the cells in room temperature PBS at 1-10 x 10^6 cells/mL.
3. Add CFSE solution to the chosen final concentration. If a final concentration of 5 uM is desired, add .5 uL of the 10mM reconstituted solution per mL of cells.
4. Immediately mix and incubate at room temperature in the dark for 10-20 minutes.
5. To quench the staining process, add 4-5 volumes of cold complete media and incubate on ice for 5 minutes
6. Pellet the cells by centrifugation. Discard supernatant.
7. Wash the cells twice with complete media. and cul