CFSE Loading

CFSE (Molecular Probes) allows the tracking of stimulated populations' division status. Its name is an acronym for 5, 6-Carboxyfluorescein diacetate succinimidyl ester, which is a fluorescent probe (FL-1) that can directly label cell populations in order to distinguish them from other populations or for use with FACS. Once inside the cell, CFSE is converted to a compound that cannot diffuse out of the cell. When a cell divides, each new cell retains half of the original amount of the dye allowing analysis to determine the cell division status of a stained population.

CFSE comes as a powder and is dissolved in DMSO. A 5mM stock solution is made and then further diluted at 1/250 in RPMI to make the loading solution of CFSE. Lymphocytes, whole spleen populations, or any cell suspension can be loaded with CFSE.

1. Suspend cells to a working concentration of $1x10^7$cells/mL in RPMI.
2. Add an equal amount of CFSE, mix, and incubate at 37°C in 5% CO_2 for 5 minutes.
3. After incubation add an equal volume of complete media to stop the reaction and centrifuge at 1000rpm for 10 minutes.
4. Resuspend the cells in complete media.
5. For in vivo experiments, inject the cells into host animals, or, for in vitro experiments, plate cells accordingly.