CFSE Labeling Protocol

CFSE Cat. No. 13-0850-U500

***Note**:* CFSE is provided as 500 ug of lyophilized powder. CFSE may be reconstituted to a stock concentration of 5 mM with 180 uL of anhydrous DMSO. It is recommended to aliquot smaller volumes and store at -20°C with dessicant and protected from light. Avoid repeated freeze-thaw cycles.

Other Materials Required

- 1X PBS
- RPMI complete medium (RPMI with 10% FBS, 1% pen/strep, 50 uM 2-ME)

1. Prepare cells as a single cell suspension.
2. Wash cells twice with 1-2 mL 1X PBS to remove serum. Spin at 300-400 x *g* for 5 minutes at room temperature and decant supernatant.
3. Resuspend cells at 1-10 x 10^6/mL in room temperature 1X PBS.
4. Prepare a working solution of CFSE at 2X the desired final concentration in room temperature 1x PBS (ie: for a final concentration of 5 uM CFSE, prepare a 2X working solution by adding 2 uL of 5mM stock to 1 mL 1X PBS).

   ***Note**:* CFSE can be used to label cells at concentrations ranging from 0.5-20 uM, depending on the cell type and assay. It is recommended that the investigator determine the optimal concentration of CFSE for the assay of interest. Over-labeling of cells with high concentrations of CFSE can obstruct normal cell functions and interfere with compensation in multicolor experiments.
5. Add an equal volume of 2X CFSE working solution to cell preparation.
6. Mix immediately and incubate in the dark for 10-20 minutes at room temperature.
7. Quench the labeling reaction by adding 5 volumes of cold complete media and incubate on ice for 5 minutes, protected from light.
8. Centrifuge the cells at 300-400 x *g* for 5 minutes at room temperature and decant supernatant. Wash cells 2 times with 1-2 mL complete media.
9. CFSE labeled cells are ready for assay.