



CFSE labeling of lymphocytes

PROTOCOL 1

Reagents:

DMSO anhydrous >99%

5-(and 6-) CFSE (Molecular Probes, cat. no. C1157)

1× PBS

1× PBS containing 2% (v/v) heat-inactivated FCS

Procedures:

1. Resuspend lymphocytes (PBMCs, LNs, splenocytes,...) in 500 μ L PBS 2% FCS at a concentration of $0.5 \times 10^6 - 50 \times 10^6$ cells/ml for CFSE labelling in a FACS tube.

2. Dilute CFSE to a 10 μ M concentration in 500 μ L of 1× PBS solution. Thoroughly resuspend this solution by pipetting.

Critical Point: CFSE will react rapidly upon exposure to aqueous solutions. It is, therefore, essential that such exposure be avoided until immediately prior to cell labeling.

3. Transfer the CFSE solution into the cell suspension tube and thoroughly resuspend the cells by pipetting or in the vortex. Final CFSE concentration is thus 5 μ M.

Critical Point: As CFSE will react quickly in aqueous solution, it is critical that it is dispersed rapidly in the cell suspension, and that the cells are as close to a homogeneous suspension of single cells as possible, to ensure even labeling of lymphocytes.

4. Cover the tube containing the CFSE-labeled lymphocytes with aluminum foil and leave cells to label with CFSE by incubating for 5 minutes at 37°C. Agitate the tube every minute.

5. Wash the cells by diluting in 5 mL of PBS 2% FCS, sediment by centrifugation at 1400 rpm for 5 min and discard the supernatant. Wash the cell pellet in the same manner two more times.

6. Count the cells and assess viability through trypan blue counterstaining.

PROTOCOL 2

Reagents:

DMSO anhydrous >99%

5-(and 6-) CFSE (Molecular Probes, cat. no. C1157)

RPMI culture medium

RPMI culture medium containing 2% (v/v) heat-inactivated FBS

Procedures:

1. Dilute CFSE stock (5mM) to working concentration (5 μ M; dilute 1/1000 – in 3 times 1/10) in 37°C in RPMI without serum.

Critical Point: CFSE will react rapidly upon exposure to aqueous solutions. It is, therefore, essential that such exposure be avoided until immediately prior to cell labeling.

2. Wash cells in RPMI w/o serum and discard supernatant.

3. Resuspend cells at 10^5 to 10^7 /ml in 1 ml of diluted dye solution and incubate for 10 minutes at 37°C, in the dark. Agitate the tube every 2 minutes.

Critical Point: As CFSE will react quickly in aqueous solution, it is critical that it is dispersed rapidly in the cell suspension, and that the cells are as close to a homogeneous suspension of single cells as possible, to ensure even labeling of lymphocytes.

4. Add equal volume of pure ice-cold FBS to quench labeling and place on ice for 30 sec.

5. Wash 2x in RPMI 10% FBS.

6. Transfer CFSE-labelled cells to 96-well plates (vacuum gas-plasma untreated), at the concentration of 1×10^6 cells/mL.