

Human Immunology Center Core Laboratory
David H. Smith Center for Vaccine Biology and Immunology
Aab Institute of Biomedical Sciences

STANDARD OPERATING PROCEDURE: Carboxyfluorescein Diacetate Succinimidyl Ester (CFDA SE) labeling of Lymphocytes for CFSE proliferation Studies

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Signature:

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1.0 Purpose:

To label lymphocyte populations with the intracellular fluorescent dye, carboxyfluorescein diacetate succinimidyl ester (CFDA SE) to monitor cell proliferation.

2.0 Principle:

CFSE is used to study the division progress in proliferating cells. CFDA SE passively crosses cell membranes and covalently binds to free amine groups of intracellular proteins where intracellular processing changes it to fluorescent CFSE. The cell population to be labeled is isolated and resuspended at 10×10^6 cells per mL. Cells are labeled with CFDA SE for 10 minutes at 37°C. The reaction is stopped using medium or PBS/5%FBS. After incorporation of the CFSE in the cytoplasm, cell proliferation can be monitored using flow cytometric analysis as the fluorescence in daughter cells is sequentially halved with each cell division.

3.0 Materials and Reagents:

Phosphate buffered saline, Biowhittaker, Catalog# 17-512F or equivalent
Fetal Bovine Serum, Hyclone, Catalog# SH30396.03
CFSE, Molecular Probes, Catalog# C1157
Human peripheral blood mononuclear cells
Digital 20-200 μ L Multichannel Pipettor, VWR, Catalog # 40000-294
Finnpipette 5-50 μ L single channel pipet, Thermo Electron, Cat # 4600220
Finnpipette 1-10 μ L single channel pipet, Thermo Electron, Cat # 4600180
Finnpipette 30-300 μ L single channel pipet, Thermo Electron, Cat # 4600240
Sterile 20-200 μ L disposable pipet tips, Eppendorf, Catalog # 2249193-8
Sterile 30-300 μ L disposable pipet tips, Eppendorf, Catalog # 2249194-6
Sterile 0.1-20 μ L disposable pipet tips, Eppendorf, Catalog # 2249113-0
1 mL sterile disposable serological pipets, Costar, Catalog # 4012
5 mL sterile disposable serological pipets, Costar, Catalog # 4051
10 mL sterile disposable serological pipets, Costar, Catalog # 4101
25 mL sterile disposable serological pipets, Costar, Catalog # 4251
Pipet Aid, Drummond, Catalog # 4-000-101
15 mL sterile polypropylene conical tubes, Falcon, Catalog # 35-2096

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50 mL sterile polypropylene conical tubes, Falcon, Catalog # 35-2070

37 °C waterbath, Sheldon, Model # 1255

Household bleach for decontamination, VWR, Catalog # 37001-056

Biological safety cabinet, Class II, Baker, Model # SG403

3.1 Reagent preparation:

- 3.1.1 1 X PBS/5% FBS: Add 25mL of FBS to 475mL of 1X PBS. Store at 4°C. Handle using sterile technique.

4.0 Procedure:

- 4.1 Isolate the cell population of interest.
- 4.2 Resuspend the cells at a 10×10^6 cells/mL in sterile PBS with 5% FBS
- 4.3 Dilute 5mM stock of CFDA SE to 1/1000 in PBS. Add 110 μ L of diluted CFDA SE per mL of cells. This will give a final concentration of 0.5 μ M per mL. The CFDA SE **MUST** be added quickly and evenly to ensure uniform labeling of the cells. Tilt the tube containing cells until almost horizontal. Add the CFDA SE to the dry area at the top of the tube. Place cap on tube and mix.
- 4.4 Incubate the cells for 10 minutes in a 37°C water bath.
- 4.5 Stop reaction by filling the tube with medium or PBS/5%FBS. Centrifuge for 10 minutes at 300xg at 22°C.
- 4.6 Decant liquid from tube and repeat step 4.5.
- 4.7 Resuspend cells in appropriate culture medium and proceed with experiment.