



The Fleischman Lab

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Title	CFSE Staining of Leukocytes	
Introduction	CFSE is a fluorescent dye that is often used to track cells (e.g. during phagocytosis) or monitor cellular division. It appears in the FITC channel and is thus easily detectable by fluorescence microscopy or flow cytometry. CFSE is able to passively diffuse into cells, and is retained in the cells following quenching with complete medium. CFSE staining may be used to track cell division as its intensity steadily decreases following mitosis. These same properties make CFSE a good method to visualize phagocytosis of cells, as phagocytic macrophages will take up the CFSE dye and then become fluorescent themselves.	
Materials	<ol style="list-style-type: none">1. 2x CFSE (10 μM CFSE in PBS, prepared from 5 mM CFSE in DMSO stock)2. PBS3. Ice-cold complete medium such as R104. FACS tubes5. Swinging bucket centrifuge6. BD Accuri or fluorescence microscope7. 37°C 5% CO₂ incubator with \geq95% humidity	
Protocol	A. Staining	Notes
1.	Harvest cells into a FACS tube and centrifuge at 1200 rpm for 10 minutes at room temperature.	
2.	Discard the supernatant and wash 2x with PBS. Centrifuge at 1200 rpm for 10 minutes at room temperature.	
3.	Prepare 2x CFSE by adding 2 μ l of 5 mM CFSE stock into 1 ml of PBS.	<i>This recipe can be easily scaled up for multiple samples.</i>
4.	Resuspend target cells in 500 μ l PBS. Add 500 μ l 2x CFSE.	
5.	Incubate cells in the dark for 15 minutes at 37°C.	<i>Cells may be incubated up to 30 minutes for brightest staining.</i>
6.	Quench cells with 2 ml ice-cold R10 and centrifuge at 7°C for 10 minutes at 1200 rpm.	<i>Ice-cold complete medium is critical to retain CFSE staining within the cells.</i>
7.	Discard the supernatant and wash with ice-cold R10 and centrifuge at 7°C for 10 minutes at 1200 rpm.	
8.	Resuspend cells in the desired medium. Cells may be analyzed for CFSE staining on the Accuri or on a fluorescence microscope under the FITC channel.	<i>For phagocytosis, resuspend in X-Vivo. For culture, resuspend in an appropriate medium for the specific cell type.</i>