The Fleischman Lab

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Title: CFSE Staining of *E. coli*

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 detectible by fluorescence microscopy or flow cytometry. CFSE is able to passively diffuse into cells, making CFSE staining a good method to visualize phagocytosis of bacteria. As phagocytic macrophages consume bacteria, they will take up the CFSE dye and then become fluorescent themselves.

Materials

1. *E. coli* grown in LB broth
2. 2x CFSE (10 µM CFSE in PBS, prepared from 5 mM CFSE in DMSO stock)
3. PBS
4. 1.5 ml Eppendorf tubes
5. Microcentrifuge
6. Dry bath for 1.5 ml tubes set at 65°C
7. 37°C 200 rpm orbital shaker

Protocol

	A. Staining	*Notes*
1.	Grow bacteria in LB broth containing an appropriate antibiotic (usually chloramphenicol or ampicillin).	
2.	Harvest 1 ml of bacteria into a 1.5 ml tube and centrifuge at 4000g for 5 minutes to pellet cells.	
3.	Discard supernatant and resuspend cells in 1 ml of 2x CFSE.	*Bacteria may be stained at a higher concentration than leukocytes; we use the same 2x CFSE as 1x here.*
4.	Incubate cells in the shaker at 37°C and 200 rpm for 30 minutes to 1 hour. Be sure to keep cells dark.	
5.	Wash cells 3x with room-temperature PBS. Centrifuge at 4000g for 5 minutes and discard supernatant.	*Wash cells thoroughly to remove excess CFSE.*
6.	Resuspend cells in PBS. Store at 4°C until ready to use.	
7.	*Optional:* To heat-kill cells, incubate at 65°C for 15 minutes. Store at 4°C until ready to use.	