



The Fleischman Lab

Author	Angela G. Fleischman	May 6, 2014
Title	Intracellular cytokine staining	
Introduction	Intracellular flow cytometry can be used to detect cells producing cytokines. Cells are stimulated with the addition of an agent to block export of proteins (such as brefeldinA or monesin). Cells are then fixed, permeabilized and stained for cell surface markers along with intracellular cytokines.	
Materials	<ol style="list-style-type: none"> 1. Cells for stimulation (for example, mononuclear cells) 2. Stimulant (for example, LPS) 3. Brefeldin A (we use BD Golgi Plug, 1000X concentrated) 4. FACS tubes 5. PBS 6. 4% paraformaldehyde (to make add 10ml 16% paraformaldehyde + 30ml PBS) 7. 10% saponin (5gm saponin in 50ml PBS, filter) 8. PFSB (PBS + 0.5% BSA) 9. Permeabilization buffer (2.5ml 10% saponin + 47.5ml PFSB) 10. FACS Antibodies (cytokine and cell surface) 	
Protocol	A. Stimulate Cells	Notes
1.	Add stimulus to cells	<i>Length of stimulation may vary depending on your experiment</i>
2.	Add Brefeldin A to cells to inhibit extracellular export of proteins	
3.	Incubate at 37°C in tissue culture incubator for 5 hours	<i>Length of time may vary depending on your experiment</i>
	B. Harvest cells	
1.	Harvest stimulated cells from wells into FACS tubes, rinse wells 2x with PBS and add to tube with harvested cells.	
2.	Centrifuge at 1200 rpm (400g) for 5 minutes, aspirate supernatant	
3.	Resuspend pellet in 1ml PBS	
	C. Fix cells	
4.	Add 1ml 4% paraformaldehyde, pipette up and down to mix	
5.	Incubate at room temp x 20 minutes	
6.	Fill tubes with cold PBS and centrifuge at 1200 rpm (400g) for 5 minutes, aspirate supernatant, vortex	
7.	Fill tubes with PFSB	<i>Tubes can be stored overnight at 4°C if needed</i>
8.	Centrifuge at 1200 rpm (400g) for 5 minutes, aspirate supernatant, vortex	

	D. Permeabilize cells	
1.	Add 300µl permeabilization buffer, mix, incubate x 10 minutes at room temperature	
2.	Fill tubes with permeabilization buffer, centrifuge at 1200 rpm (400g) for 5 minutes, aspirate (leave about 100µl), vortex	
	E. Stain cells	
1.	Stain with antibodies in permeabilization buffer	<i>Cell surface antibodies require testing to confirm they can be used after fixation, if not staining of cell surface antigens may need to be performed before fixation</i>
2.	Incubate in dark at room temperature x 20 minutes	
3.	Fill tube with permeabilization buffer, centrifuge at 1200 rpm (400g) x 5 minutes, aspirate, vortex	
4.	Fill tube with PFSB, centrifuge at 1200 rpm (400g) for 5 minutes, aspirate, vortex	
5.	Analyze sample on FACS machine	