**SOP:** Flow cytometry intranuclear staining (mouse)

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**Reagents:**
- Foxp3/Transcription factor staining buffer set (eBioscience 00-5523-00)

**Working dilutions:**  
Fixative: 1 part Fix/Perm Concentrate, 3 parts fixation diluent  
Perm: 1 part Perm Buffer, 9 parts DI water

**Procedure:**
1. After staining surface markers, resuspend cells in 200µl fixation buffer (pre-diluted 1:4 in diluent) and incubate for 20 minutes on ice, protected from light.  
2. Centrifuge at 1500 rpm for 5 minutes at 4 degrees C and discard supernatant.  
3. Resuspend in 200µl perm buffer (pre-diluted 1:10 in DI water) and centrifuge at 1500 rpm for 5 min at 4 degrees C.  
4. Resuspend in 100µl perm buffer with appropriate dilutions of IC antibodies. Incubate for 30 minutes on ice, protected from light.  
5. Centrifuge samples at 1500 rpm for 5 minutes at 4 degrees and discard supernatant.  
6. Resuspend cells in 200µl perm buffer, centrifuge, and discard supernatant. Repeat a second time.  
7. Resuspend cells in 200µl FACS buffer for flow cytometry.