SOP: Flow cytometry intranuclear staining (mouse)
Author: Brianna Traxinger
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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.