Staining for Transcription Factors (e-Bioscience)

eBioscience **Fixation/Permeabilization Concentrate (cat. 00-5123)**: 30 ml. Store at 4°C. eBioscience **Fixation/Permeabilization Diluent (cat. 00-5223)**: 100 ml. Store at 4°C. Dilute 1 part **Concentrate** with 3 parts **Diluent** to make the **Fix/perm buffer**. eBioscience **Permeabilization/Wash Buffer (10X) (cat. 00-8333)**: 100 ml. Store at 4°C. Dilute to 1X with deionized/distilled water and store at 4°C.

The Foxp3 Staining Buffer (00-5523-00) has been formulated and optimized for staining against Foxp3 and also other transcription factors such as Nanog, Tbet, Gata-3, Ror gamma as well as cytokines.

1. Do surface staining normally. Use (when possible) 1 – 5x10^6 cells/wells.
2. Prepare the **Fix/perm buffer** by diluting **1** part **Concentrate** with **3** parts **Diluent**. Add **Fix/perm buffer** in 200ul for 30 min on ice.
3. Wash 1x in PBS.
4. Prepare the **Permeabilization/Wash Buffer** by diluting to 1X with deionized/distilled water. Wash twice in 200ul **Perm/Wash buffer**.
5. Incubate 30min in 50ul FC block at 1/100 with 5% normal mouse serum (NMS) in **Perm/Wash buffer**.
   Incubate with anti-transcription factors Ab for 30min on ice diluted in **Perm/Wash buffer**. To do so, without washing add 50ul **Perm/Wash buffer** containing either of the following antibody at the given concentration.
   + Foxp3 Pacific Blue 1/25
   + Helios Pacific Blue 1/10 (weak)
   + Helios PE 1/20
   + GATA-3 PE 1/25
   + GATA-3 Alexa Fluor 600 1/15
6. Wash 2x in 200ul **Perm/Wash buffer**.
7. Wash 1x in FACS buffer. Staining is ready and you can go to the flow cytometer.