

## FOXP3 Intracellular Staining Protocol

1. Perform cell surface staining as described in BioLegend's *Cell Surface Immunofluorescence Staining Protocol*, page 22.
2. Add 1 ml of 1X BioLegend's FOXP3 Fix/Perm solution to each tube, vortex and incubate at room temperature in the dark for 20 minutes, then spin down the cells and remove the supernatant.
3. Wash once with cell staining buffer (Cat. No. 420201) by spinning at 250 X *g* for 5 minutes and remove the supernatant.
4. Wash once with 1ml 1X BioLegend's FOXP3 Perm buffer.
5. Re-suspend cells in 1ml 1X BioLegend's FOXP3 Perm buffer, incubate at room temperature in the dark for 15 minutes, spin down cells and discard the supernatant, then resuspend the pellet in 100  $\mu$ l of 1X BioLegend's FOXP3 Perm buffer.
6. Add appropriate amount of fluorochrome conjugated anti-FOXP3 antibody and incubate at room temperature in the dark for 30 minutes.
7. Wash twice with cell staining buffer, and resuspend in 0.5ml cell staining buffer then analyze with flow cytometer with appropriate instrument setting.

**NOTE:** BioLegend's FOXP3 Fix/Perm Buffer Set (Cat. No. 421403) is specially developed and formulated for intracellular staining FOXP3 with minimum effect on surface fluorochrome staining and is highly recommended for optimal FOXP3 intracellular immunofluorescent staining results.

### Related Information:

1. Roncador, G., et al., 2005 *Eur. J. Immunol.* 35:1681.

### Reagent List:

1. Cell Staining Buffer (Cat. No. 420201)
2. FOXP3 Fix/Perm Buffer Set (Cat. No. 421403)