

Intracellular Cytokine Staining

Cells can be counterstained for surface markers to get cell-specific results. Cytotfix/Cytoperm™, Perm/Wash™ Buffer, and GolgiPlug™ are available in a kit (Cat. No. 555028) or as single reagents from BD Biosciences. Cytokine-specific antibodies are available from multiple vendors.

1. After samples have been stimulated for the appropriate amount of time add 1µL/mL of GolgiPlug™ and incubate for 5 hours at 37°C in 5% CO₂.
2. Resuspend cells in 100µL of Cytotfix/Cytoperm™ solution and incubate on ice for 20 minutes in the dark.
3. Add 100µL of 1x Perm/Wash™ Buffer, centrifuge at 1400rpm for 10 minutes, and repeat wash 2 more times with 200µL of Perm/Wash™ Buffer.
4. Resuspend in 50µL of Perm/Wash™ Buffer and 0.05-0.1µg/mL of anti-cytokine antibody and incubate at 4°C for 30 minutes in the dark.
5. Wash cells 3 times with Perm/Wash™ Buffer and twice with 5% FBS in PBS.
6. Resuspend cells in 2% Formaldehyde/PBS and analyze.
 - a. Alternatively, cells can be stored at 4°C in the dark until acquisition (best within 24hrs).