Cytometry Research, LLC

# Intracellular Staining

1. ____ Prepare target cells in cold PBS. 1 x $10^6$ cells/sample is enough for analysis.

2. ____ If staining a cell surface receptor, stain with antibody in 100 µl of PBS before fixing cells. Wash 2X with PBS. Stain with secondary antibody if necessary. Wash 2X with PBS.

3. ____ Fix cells in 0.5 ml of 4% paraformaldehyde solution and incubate at 22°C for 20 minutes **in the DARK**. Vortex gently at intermittent times to avoid clumping of cells.

4. ____ Wash the fixed/stained cells 2X with cold PBS.

5. ____ Add 2 ml of blocking buffer and block for 20 minutes at 22°C.

6. ____ Add appropriate amount of conjugated anti-cytokine Mab.

7. ____ Incubate at 22°C for 20 minutes **in the DARK.**

8. Wash once with PBS containing 0.1% saponin, remove supernatant and resuspend the cell pellet with 0.5 ml 2% formaldehyde solution.

9. ____ To analyze the results, call Cytometry Research for a FACS analysis appointment.

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