Immunofluroscent Staining Protocol

Immunofluorescent staining with antibodies against intracellular cytokines and cell surface markers provides a high resolution method to identify the nature and frequency of cells which express a particular cytokines.

Buffers

Cell culture media:	IMDM containing 10% (v/v) FCS
FACS Buffer:	1x PBS containing 2 mM EDTA, 2 mM NaN3 and 2% (v/v) FCS
Paraformaldehyde:	4% (w/v) in 1x PBS

Staining Protocol

- ✓ Harvest cells from tissue and prepare a single cell suspension. (Red blood cells may be removed by lysis or density gradient.)
- ✓ Adjust cell concentration to $1x10^7$/ml.
- ✓ Transfer 100 µl of the cell suspension in a 96-well round bottom plate, corresponding to ~$1x10^6$ cells.
- ✓ Finally, pellet cells by centrifugation (1500 rpm) for 5 min, remove supernatant and vortex plate
- ✓ Thoroughly resuspend cells in 100 µl of FACS Buffer with a fluorochrome-conjugated monoclonal antibody specific for a cell surface antigen for 20-30 min, 4° in the dark.
- ✓ Wash cells 2x with 100 µl FACS Buffer per well, pellet by centrifugation (1500 rpm), and remove supernatant.
- ✓ ***Optional***: Thoroughly resuspend cells in 75 µl of 4% PFA solution and incubate for 5 min at 4°C. Cell aggregation can be avoided by vortexing prior to the addition of the PFA solution.
- ✓ Wash cells 2x with 100 µl FACS Buffer per well, pellet by centrifugation (1500 rpm), and remove supernatant.
- ✓ Finally, resuspend in 200 µl FACS Buffer prior to flow cytometric analysis