

Intracellular Cytokine staining protocol

▪ Stimulation of cells

- 1/ Stimulate cells following the appropriate protocols in presence of Brefeldin A (1 µg/ml). Collect the cells and transfer the cell suspension to a centrifuge tube.
- 2/ Centrifuge cell preparations at 828 g for 5 min

▪ Fixation of cells

- 3/ Incubate $20 \cdot 10^6$ cells /ml in 4% paraformaldehyde for 20 min at 4°C
- 4/ Centrifuge 5 min at 828g and remove the supernatant

▪ Permeabilization and intracellular staining

- 5/ Adjust the cell concentration to $4 \cdot 10^6$ cells/ml in 5% FCS, 0.3% Saponin, 1X PBS
- 6/ Dilute the antibody in 5% FCS, 0.3% Saponin, 1X PBS. Titration of the mAb is recommended to obtain an optimal staining on activated cells and none staining on resting cells.
- 7/ Incubated $2 \cdot 10^5$ cells with R-PE or FITC conjugated monoclonal antibody for 30 min at 4°C
- 8/ Wash cells in 5% FCS, 0.1% Saponin, 1X PBS (100µl/well)
- 9/ Wash cells in 1X PBS (100µl/well)
- 10/ Resuspend the cells in 250µl of 1X PBS

▪ Analysis by flow cytometry

Recommendations

Any fluorochrome conjugated mAb will be suitable to detect an antigen expressed at high level.

The PE conjugated mAb will be recommended to detect an antigen expressed at lower density.

Positive cells will be more separate from negative cells using a PE conjugated mAb.

The non-specific binding will be determined with an irrelevant isotype matched mAb.

To avoid artifactual staining of Fc binding sites, a serum incubation may be performed before staining.