Nuclear Factor Fixation and Permeabilization Staining Protocol

Kelsey Miller

Abstract

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Guidelines

Prior to following the staining procedure, dilute one (1) part Nuclear Factor Fixation Buffer (4X) with three (3) parts PBS, and dilute one (1) part Nuclear Factor Permeabilization Buffer with nine (9) parts PBS.

Protocol

Step 1.

Prepare target cells of interest and perform surface staining as described in BioLegend's Cell Surface Immunofluorescence Staining Protocol

Step 2.

Fix the cells with 1 ml/tube BioLegend's Nuclear Factor Fixation Buffer, at room temperature in the dark for 20 minutes.

DURATION
00:20:00

Step 3.

Centrifuge at 250 x g for 5 minutes; discard supernatant.

DURATION
00:05:00

Step 4.

Wash once with 1 ml Biolegend's Nuclear Factor Permeabilization Buffer (1x).

Step 5.

Resuspend cells in 1 ml BioLegend's Nuclear Factor Permabilization Buffer (1x), incubate at room temperature in the dark for 20 minutes, spin down cells and discard the supernatant; then resuspend the pellet in 100 ul of BioLegend's Nucelar Factor Permeabilization Buffer (1x).

DURATION
00:20:00

Step 6.

Add appropriate amount of flurochrome-conjugated antibodies for nuclear target of interest, and incubate at room temperature in the dark for 30 minutes.

DURATION
00:30:00

Step 7.

Wash twice with Cell Staining Buffer, and resuspend in 0.5 ml Cell Staining Buffer, then analyze with flow cytometer with appropriate instrument settings.