

Flow cytometry analysis for intracellular protein

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Abstract

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Protocol

Step 1.

Harvest cells and wash with PBS.

Step 2.

Fix cells with 1% paraformaldehyde in PBS for 5 min.

Step 3.

Wash cells once in PBS.

Step 4.

Permeabilize cells with 0.1% saponin in PBS containing 5% (w/v) nonfat milk for 30 min on ice.

Step 5.

Add antibody conjugated to fluorochrome or unlabelled primary antibody and incubate for 30 min on ice in the dark.

Step 6.

Wash cells twice with 0.1% saponin in PBS containing 5% (w/v) nonfat milk, dilute in PBS and proceed to analysis.

In case of using unlabelled primary antibody, stain cells with secondary antibody conjugated to fluorochrome for 30 min on ice in the dark, wash twice and analyze.