

Fixation and Immunostaining of Cells

Cells for fixation can be grown on either glass coverslips or glass-bottomed dishes. When using coverslips, washes should be performed in wells of 6-well or 12-well plates, and antibody treatments made by inverting the coverslips on to drops of the antibody dilution spotted on to parafilm on an even surface. When using glass-bottomed dishes, all steps should be carried out in the dish.

A) Methanol fixation

Fix for 4 min in methanol at -20°C

Wash 3x in PBS each 3 min

1st Ab 30 min diluted with PBS

Wash 3x PBS each 3 min

2nd Ab 30 min diluted with PBS

Wash 3x PBS each 3 min

Mount with Mowiol

B) PFA fixation / TX-100 permeabilisation

Fix for 20 min in 3% PFA at room temperature

Quench for 5 min in 30 mM glycine / PBS, pH: 7.5

Wash 3x in PBS each 3 min

Permeabilise for 5 min in 0.1% TX-100 / PBS

Wash 3x in PBS each 3 min

1st Ab 30 min diluted with PBS

Wash 3x in PBS each 3 min

2nd Ab 30 min diluted with PBS

Wash 3x in PBS each 3 min

Mount with Mowiol

C) PFA fixation / saponin permeabilisation

Fix for 20 min in 3% PFA at room temperature

Wash 3x in PBS / 2% BSA each 3 min

Permeabilise for 5 min in 0.05% saponin / PBS

Wash 3x in PBS / 2% BSA each 3 min

1st Ab 30 min diluted with PBS / 2% BSA

Wash 3x in PBS / 2% BSA each 3 min

2nd Ab 30 min diluted with PBS / 2% BSA

Wash 2x in PBS / 2% BSA each 3 min

Wash 2x in PBS each 3 min

Mount with Mowiol