Immunofluorescence Microscopy Protocol with Methanol Fixed Cells

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Abstract

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Protocol

Sample preparation

Step 1.

Grow cultured cells on cover slips or chamber slides overnight or add appropriate amount of cells to poly-L-lysine coated chamber slides and incubate at least 30 minutes at 37°C. At the time of fixation cells should be 50% confluent.

DURATION
00:30:00

Sample preparation

Step 2.

Rinse cells briefly in 1X PBS.

Sample preparation

Step 3.

Fix cells by incubation with cold 100% methanol for 5-15 minutes at -20°C.

DURATION
00:15:00

Sample preparation

Step 4.

Rinse three times in 1X PBS, 5 minutes each.

Sample Blocking

Step 5.

Block samples in 5% FBS/PBS for 1 hour at room temperature.

DURATION
01:00:00

Sample staining

Step 6.

Dilute the primary antibody to the recommended concentration/dilution in 5% FBS/PBS.

Sample staining

Step 7.

For 8 well chamber slides, add 200µl per well and incubate 2-3 hours at room temperature or overnight at 4°C.

DURATION

03:00:00

Sample staining

Step 8.

Rinse three times in 1X PBS, 5 minutes each.

NOTE: If using primary antibodies directly conjugated with fluorophores, skip to step 7.

Sample staining

Step 9.

Prepare fluorophore-conjugated secondary antibody in 5% FBS/PBS according to therecommended manufacturer specification data sheet and add 200µl per well (8 wells) to thechamber slides.

Sample staining

Step 10.

Incubate the samples for 1 hour at room temperature in the dark.

DURATION

01:00:00

Sample staining

Step 11.

Rinse three times in 1X PBS, 5 minutes each.

Sample staining

Step 12.

Coverslip with anti-fade mounting medium.

Sample staining

Step 13.

Seal the edges of the coverslip to the slide to prevent movement of the coverslip while imaging.One sealant that is recommended is a 1:1:1 ratio mixture of vasoline, lanolin and paraffin.

Material and Buffers

Step 14.

Chamber slides and coverslips

Material and Buffers

Step 15.

Fixation solution: 100% methanol stored at -20°C for at least two hours before use.

Material and Buffers

Step 16.

Antibody dilution solution: 5% FBS in 1X PBS.