Immunocytochemistry Staining for Methanol Fixed Cells

Version 3

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Abstract

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Before start

Reagent List:

- Chamber slides or cover slips
- Fixation solution: 100% methanol stored at -20°C for at least two hours before use
- Blocking soluion: 5% FBS in PBS
- 70% Ethanol

Protocol

Sterilization

Step 1.

Transfer a single cover slip into a 12-well plate, then add 1 mL of 70% Ethanol into a well for 20 minutes at room temperature.

DURATION
00:20:00 :

Sterilization

Step 2.

Wash quickly three times with PBS.

Poly-Lysine Coating for 12-Well Plates (optional; for loosely attached cells)

Step 3.

Add 1 mL of 0.1 mg/mL Poly-D-lysine solution into a well for 15 minutes at room temperature.

DURATION
00:15:00 :

Poly-Lysine Coating for 12-Well Plates (optional; for loosely attached cells)

Step 4.

Wash quickly three times with PBS and let dry before plating cells.

Sample preparation

Step 5.

Grow cultured cells on cover slips or in wells overnight at 37°C. At the time of fixation, cells should be 70-80% confluent in single layer.

DURATION
00:30:00 :

Sample preparation

Step 6.

Rinse cells briefly in PBS.

Sample preparation

Step 7.

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

DURATION
00:15:00 :

Sample preparation

Step 8.

Rinse three times quickly in PBS.

Sample Blocking

Step 9.

Block samples in 1 mL of blocking buffer at room temperature for 30 minutes.

DURATION
00:30:00 :

Sample staining

Step 10.

Dilute the primary antibody to the recommended concentration/dilution in blocking buffer.

Sample staining

Step 11.

For 8-well chamber slides, add 200 µL per well. For 12-well plates, add 500 µL per well. Incubate two to three hours at room temperature or overnight at 4°C. If using conjugated antibodies, perform this step in the dark.

DURATION
03:00:00 :

Sample staining

Step 12.

For surface staining, rinse 3 times quickly in PBS. For intracellular staining, quickly wash once

followed by incubation with wash buffer for 5-10 minutes. Then quickly wash additional two times.

Note: If using primary antibodies directly conjugated to fluorochromes, then skip to step 12.

DURATION
00:10:00 :

Sample staining

Step 13.

Prepare fluorochrome-conjugated secondary antibody in blocking buffer according to the manufacturer's specification data sheet, and add 200 µl per well to the 8-well chamber slides. For 12-well plates, add 500 µL per well.

Sample staining

Step 14.

Incubate the samples for one hour, at room temperature, in the dark.

DURATION
01:00:00 :

Sample staining

Step 15.

For surface staining, rinse three times quickly in PBS. For intracellular staining, quickly wash once followed by incubation with wash buffer for 5-10 minutes, then quickly wash additional two times.

DURATION
00:10:00 :

Sample staining

Step 16.

Optional: To stain F-actin, prepare a working solution of Flash Phalloidin™ by diluting it 1:20-1:100 in PBS. Add 200 µL per well for an 8-well plate or 500 µL per well for a 12-well plate. Stain for 20 minutes at room temperature in the dark.

Sample staining

Step 17.

Apply anti-fade mounting medium to the cover slip.

Sample staining

Step 18.

Seal slides with nail polish.