Immunocytochemistry Staining Protocol Version 3

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Abstract

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Before start

Reagent List:

- Chamber slides, cover slips, or 12-well plates
- Phosphate-buffered saline (PBS)
- Fixation solution: 1% Paraformaldehyde, in PBS
- Permeabilization solution: 0.5% Triton X-100 in PBS
- Blocking buffer: 5% FBS in PBS

Protocol

Sterilization (for 12-well plates with coverslips)

Step 1.

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

DURATION
00:20:00 :

Sterilization (for 12-well plates with coverslips)

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.

Sample Preparation

Step 6.

Rinse cells briefly in PBS.

Sample Preparation

Step 7.

Fix cells by incubation with freshly made 1% Paraformaldehyde in PBS for 10 minutes at room temperature.

DURATION

00:10:00 :

Sample Preparation

Step 8.

Rinse three times quickly in PBS.

Sample Preparation

Step 9.

For intracellular staining, add permeabilization solution and incubate at room temperature for 10 minutes. Then wash quickly three times in PBS.

DURATION

00:10:00 :

Sample Blocking

Step 10.

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

DURATION

00:30:00 :

Sample Staining

Step 11.

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

Sample Staining

Step 12.

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.

Sample Staining

Step 13.

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 17.

DURATION
00:10:00 :

Sample Staining

Step 14.

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 15.

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

DURATION
01:00:00 :

Sample Staining

Step 16.

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 17.

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.

DURATION
00:20:00 :

Sample Staining

Step 18.

Apply anti-fade mounting medium to the cover slip.

Sample Staining

Step 19.

Seal slides with nail polish.