Immunocytochemistry Staining Protocol V.3

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Troubleshooting

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

Sterilization (for 12-well plates with coverslips)

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.
00:20:00

2 Wash quickly three times with PBS.

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

3 Add 1 mL of 0.1 mg/mL Poly-D-lysine solution into a well for 15 minutes at room temperature.
00:15:00

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

Sample Preparation

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.

6 Rinse cells briefly in PBS.

7 Fix cells by incubation with freshly made 1% Paraformaldehyde in PBS for 10 minutes at room temperature.
00:10:00

8 Rinse three times quickly in PBS.

9 For intracellular staining, add permeabilization solution and incubate at room temperature for 10 minutes. Then wash quickly three times in PBS.
00:10:00

Sample Blocking

10 Block samples in 1 mL of blocking buffer at room temperature for 30 minutes.
00:30:00

Sample Staining

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

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.

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.

00:10:00

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.

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

01:00:00

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.

00:10:00

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.

00:20:00

18 Apply anti-fade mounting medium to the cover slip.

19 Seal slides with nail polish.