Immunocytochemistry Staining Protocol V.3

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BEFORE START INSTRUCTIONS

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Reagent List:

■	Fixation solution: 1% Paraformaldehyde, in PBS
■	Chamber slides, coverslips, or 12-well plates
■	Permeabilization solution: 0.5% Triton X-100
■	Phosphate-buffered

saline (PBS)	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..

- 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.