



# Immunocytochemistry Staining for Methanol Fixed Cells V.3

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## Before start


### Reagent List:

<ul style="list-style-type: none"><li>▪ Chamber slides or cover slips</li><li>▪ Fixation solution: 100% methanol stored at -20°C for at least two hours before use</li></ul>	<ul style="list-style-type: none"><li>▪ Blocking solution: 5% FBS in PBS</li><li>▪ 70% Ethanol</li></ul>
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## Sterilization


- 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.

 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.

 00:30:00

- 6 Rinse cells briefly in PBS.


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

 00:15:00

- 8 Rinse three times quickly in PBS.





## Sample Blocking

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

 00:30:00

## Sample staining



- 10 Dilute the primary antibody to the recommended concentration/dilution in blocking buffer.
- 11 For 8-well chamber slides, add 200  $\mu$ L per well. For 12-well plates, add 500  $\mu$ L 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.  
 03:00:00
- 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.  
 00:10:00
- 13 Prepare fluorochrome-conjugated secondary antibody in blocking buffer according to the manufacturer's specification data sheet, and add 200  $\mu$ L per well to the 8-well chamber slides. For 12-well plates, add 500  $\mu$ L per well.
- 14 Incubate the samples for one hour, at room temperature, in the dark.  
 01:00:00
- 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.  
 00:10:00
- 16 Optional: To stain F-actin, prepare a working solution of Flash Phalloidin™ by diluting it 1:20-1:100 in PBS. Add 200  $\mu$ L per well for an 8-well plate or 500  $\mu$ L per well for a 12-well plate. Stain for 20 minutes at room temperature in the dark.
- 17 Apply anti-fade mounting medium to the cover slip.
- 18 Seal slides with nail polish.