Immunocytochemistry Staining Protocol V.4

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Guidelines

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

General Tips and FAQ:

Are fluors such as BV570™, BV605™, BV650™, BV711™, BV785™, PE/Cy7, PE/Cy5, APC/Cy7, PE, APC, PerCP, and FITC good for IF?

- These dyes due to their vulnerability to photo bleaching are not recommended for IF. Alexa Fluor® and Dylight® fluors, as well as BV421™ and BV510™, are good alternatives for IF application.

Which fixation method is most suitable for my antibody?

- The fixation and permeabilization method used will depend on the epitope and the sensitivity of the antibody, and may require some optimization.

  Fixation can be done using crosslinking reagents, such as paraformaldehyde. These are better at preserving cell structure, but may reduce the antigenicity of some cell components, as the crosslinking will obstruct antibody binding (antigen retrieval techniques may be required).

  Another option is to use organic solvents such as methanol, ethanol and acetone. These remove lipids while dehydrating the cells. They also precipitate proteins on the cellular architecture.

Sterilization (for 12-well plates with coverslips):

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.

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.

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.

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.

Sample Blocking:

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

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 an additional two times.Note: If using primary antibodies directly conjugated to fluorochromes, then skip to step 13.

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.

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 an additional two times.

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.

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

19 Seal slides with nail polish.