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Immunofluorescence Protocol

4%-PFA Fixed Cell Line Slides

Note: Do not let the tissues dry out once they are re-hydrate.
Use separate tubs for antibodies and negative control slides to avoid contamination.

MATERIALS

Coverslips
Slide racks & tray
Staining dishes with lids
Orbital shaker & Transfer pipettes

Deionized water (DI H_2O)
PBS (Phosphate Buffered Saline)
Triton X-100
Primary antibody
Fluorescent secondary antibody
Bovine Serum Albumin (BSA – for blocking)
Glycerol

Permeabilize Membrane (Optional)

1. Add one drop of PBS/0.1% Triton X-100 to each well to permeabilize the cells. Incubate slides for one (1) minute at room temperature.
2. Remove the liquid and wash the slides twice (2x) in PBS, 5 minutes each on the shaker.

Blocking

3. Remove the liquid and add 5% BSA into each well. Incubate overnight at 4°C in a humid chamber.

Primary Antibody

4. Dilute the primary antibody to the recommended concentration in 1% BSA diluent.
5. Remove BSA and incubate with primary antibody for one (1) hour at room temperature.

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6. Remove primary antibody solution and wash slides three (3) times in PBS, 5 minutes each on the shaker.

Secondary Antibody and Detection

7. Dilute the biotinylated secondary antibody to 1:200 in a solution of 1% BSA diluent.
8. Remove fluid and incubate with secondary antibody for one (1) hour at room temperature in dark place.
9. Wash three (3) times 5 minutes in PBS on an orbital shaker. Remove excess fluid.

Cover Slips

10. Add several drops of coverslip solution (50% glycerol / DI H_2O) to the slide.
11. Place coverslip on top of the slide and store slides at 4°C until ready to view.