



Immunofluorescence Protocol for Tissue Sections

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

MATERIALS

Paraffin-embedded tissue slides
Coverslips
Slide racks & tray
Staining dishes with lids
Orbital shaker
PAP pen & Transfer pipettes

Deionized water (DI H₂O)
PBS (Phosphate Buffered Saline)
Primary antibody
Fluorescent secondary antibody
Bovine Serum Albumin (BSA – for blocking)
Glycerol

BUFFERS

Working Citrate Buffer

9mL of 0.1M Citric Acid (10.5g citric acid monohydrate to 500mL DI H₂O)
41mL of 0.1M Sodium Citrate (14.7g sodium citrate dehydrate to 500mL DI H₂O)
450mL of DI H₂O

Deparaffinization and Rehydration (Cover staining dishes with a lid in each step)

1. Dip slides in three (3) changes of xylene or a xylene substitute for 3 minutes each.
2. Dip slides in two (2) change of 100% alcohol for 3 minutes each.
3. Dip slides in one (1) change of 95% alcohol for 3 minutes.
4. Dip slides in one (1) change of 70% alcohol for 3 minutes.
5. Rinse slides twice (2x) in DI H₂O for 5 minutes.



Antigen Retrieval (Microwave Method)

6. Soak the slides in the working citrate buffer and cover with a lid.
7. Microwave until the liquid boils, about 1-5 minutes.
8. Remove from heat and let it stand at room temperature for 20 minutes.
9. Wash three (3) times for 5 minutes in DI H₂O
10. Remove the liquid (do not touch the tissue!) and use a PAP pen to circle around the tissue.

Blocking

11. Apply enough 5% BSA with a transfer pipette to cover the tissues.
12. Incubate the slides overnight at 4°C in a humid chamber.

Primary Antibody

13. Dilute the primary antibody to the recommended concentration in 1% BSA/PBS diluent.
14. Remove the BSA, and incubate with primary antibody solution for 1 hour at room temperature.
15. Wash slides three (3) times 5 minutes each on the shaker.

Secondary Antibody and Detection

16. Dilute the fluorescent secondary antibody to 1:200 in 1% BSA diluent.
17. Incubate with secondary antibody solution for 1 hour at room temperature in dark place.
18. Wash slides in PBS three (3) times 5 minutes each on the shaker.

Cover Slips

19. Apply 50% glycerol/PBS to the middle of the slide.
20. Apply coverslip to slide and store slides at 4°C until ready to view.