

IF Staining with Parafilm Section Slide (Double Staining)

Procedure:

1. Heat slides in an oven at 60°C for 45 minutes. (usually do a day before)
2. Deparaffinize/hydrate using the following series of washes:

Xylenes	3X	5 min
100% EtOH	2X	2 min
95% EtOH	2X	2 min
80% EtOH	1X	2 min
70% EtOH	1X	2 min
dH ₂ O	2X	3 min + shaker
PBS	1X	5 min + shaker
3. Microwave slide in boiling 10mM Sodium Citrate Buffer. Fill chamber to brim with buffer. Microwave 10 minutes on high power in buffer and allow 20 minutes to cool down at RT.
4. Fix with 200ul of 4% paraformaldehyde/PBS for 20 min at RT (fix time)
5. Wash with PBS
6. Permeablize with 0.1% Triton x-100/PBS for 3 min at RT (fix time)
7. Wash with PBS
8. Treated with goat serum/PBS (1:200) for 30 min at RT.
9. Wash with PBS
10. Added primary Antibody in goat serum/PBS for 1 hr at RT
11. Wash with PBS
12. Added goat anti-rabbit 594 or anti-mouse for 30 min at RT.
13. Wash with PBS
14. Treated with goat serum/PBS (1:200) for 30 min at RT.
15. Wash with PBS
16. Added 2nd primary Antibody in goat serum/PBS for 1 hr at RT
17. Wash with PBS
18. Added 2nd goat anti-rabbit 594 or anti-mouse for 30 min at RT.
19. Added goat anti-rabbit 594 or anti-mouse for 30 min at RT.
20. Mounting the slide with reagent contain DAPI.
21. Store at dark box at 4°C. Good for one week.