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IMMUNOHISTOCHEMISTRY FOR PARAFFIN EMBEDDED TISSUE PROTOCOL

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

TISSUE PREPARATION (FORMALIN FIXED, PARAFFIN-EMBEDDED SECTIONS)

1. Cut sections at 4um and place on pre-cleaned and positively charged microscope slides.
2. Heat in a tissue-drying over for 45 minutes at 60°C.

DEPARAFFINIZATION AND REHYDRATION

3. Wash slides 2 times in Xylene for 3 minutes each time at RT.
4. Wash slides in Xylene 1:1 with 100% ethanol for 3 minutes at RT.
5. Wash slides 2 times in 100% ethanol for 3 minutes each at RT.
6. Wash slides 2 times in 95% ethanol for 3 minutes each at RT.
7. Wash slides in 70% ethanol for 3 minutes at RT.
8. Wash slides in 50% ethanol for 3 minutes at RT.
9. Rinse slides gently with running distilled water for 5 minutes at RT.

ANTIGEN RETRIEVAL

10. Boil slides in 0.01M sodium citrate buffer (pH6) at 100°C for 15-20 minutes. Remove the slides from heat and allow them to stand at RT in buffer for 20 minutes.
11. Rinse twice with TBST for 5 minutes at RT.

IMMUNOSTAINING

Recommended: Do not allow tissues to dry at any time during the staining procedure.

12. Block with endogenous peroxidase with 3% hydrogen peroxide for 30 minutes.
13. Block with 5% serum or BSA for 2 hours at RT.
14. Drain blocking buffer from slide.
15. Incubate slides with the diluted primary antibody overnight at 4°C with gentle agitation.
16. Wash slides 2 times with TBST for 5 minutes at RT.
17. Incubate slides with diluted conjugated secondary antibody for 2 hour at RT with gentle agitation.
18. Wash slides 2 times with TBST for 5 minutes at RT.
19. Develop with chromogen for 10 minutes at RT.
20. Wash slides in distilled water for 1 minute at RT.
21. Counterstain (if required).
22. Dehydrate when using a chromogen substrate that is alcohol insoluble by washing slides in 80%, 95%, 100% and Xylene each for 1 minute at RT.
23. Mount coverslips.