

IMMUNOHISTOCHEMISTRY PROTOCOL FOR FROZEN TISSUE

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)

Note: For tissue stored at -80°C , remove from freezer and equilibrate at -20°C for about 15 minutes before sectioning. This prevents cracking of the block during sectioning.

1. Cut sections at 4-8 μm and place on pre-cleaned and positively charged microscope slides.
2. Air dry sections on bench for a few minutes to help the sections adhere to the slides.
3. Fix sections in precooled acetone for 10 minutes at 4°C .
4. Wash with Tris-Buffered Saline (TBS) to remove all traces of acetone.

IMMUNOSTAINING

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

5. Rinse slides 2 times with Tris-Buffered Saline + Tween (TBST) for 5 minutes each at RT.
6. Block with 5% serum or BSA for 2 hours at RT.
7. Drain blocking buffer from slide.
8. Incubate slides with the diluted primary antibody overnight at 4°C with gentle agitation.
9. Wash slides 2 times with TBST for 5 minutes at RT.
10. Incubate slides with diluted conjugated secondary antibody for 2 hour at RT with gentle agitation.
11. Wash slides 2 times with TBST for 5 minutes at RT.
12. Develop with chromogen for 10 minutes at RT.
13. Wash slides in distilled water for 1 minute at RT.
14. Counterstain (if required).
15. Mount coverslips.