www.biossusa.com
support@biossusa.com
800.501.7654 (DOMESTIC)
+1.781.569.5821 (INTERNATIONAL)

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

This method utilizes frozen tissues that are fixed after snap-freezing and sectioning with a cryostat.

1. Immediately snap freeze fresh tissue with liquid nitrogen or dry ice, or for tissue stored at -80°C, remove from freezer and equilibrate at -20°C in cryostat for about 15 minutes before sectioning. Note: Equilibrate at -20°C. This prevents cracking of the block during sectioning. Do not allow frozen tissue to thaw before cutting.
2. Embed the tissue completely in OCT compound prior to cryostat sectioning.
3. Cut sections at 4-8 um and place on pre-cleaned and positively charged microscope slides.
4. Air dry sections on bench for a few minutes to help the sections adhere to the slides.
5. Fix sections in precooled acetone for 10 minutes at 4°C.
6. 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.

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