PROTOCOL FOR IMMUNOHISTOCHEMICAL STAINING OF FROZEN SECTIONS

1. Preparation of Frozen Tissues for sectioning

Materials needed:
2-methylbutane (isopentane); Liquid nitrogen; Dry ice; Peel-Away® base molds; Frozen tissue matrix (OCT® or Cryomatrix®); Long forceps; Necropsy tools; Superfrost Plus slides.

Protocol:
1. Label base mold and partially fill the mold with frozen tissue matrix.
2. Sacrifice animal by prescribed and approved euthanasia techniques.
3. Remove desired tissues and place in pre-labeled base molds filled with frozen tissue matrix. Try to arrange tissue in matrix near the bottom so tissue is easily exposed when sections are cut.
4. Plunge base mold with tissue in frozen tissue matrix into 2-methylbutane prechilled in a dewar of liquid nitrogen until the block ALMOST solidifies (30 seconds).
NOTE: If the block is left in too long, it may crack.
5. Remove tissue block from 2-methylbutane and place blocked tissues on dry ice. (Tissues may be stored in the base molds or transferred to plastic bags.)
6. Store frozen tissue blocks in -70°C freezer until sectioning.
7. For sectioning, attach the frozen tissue block on the cryostat chuck. Allow tissue block to equilibrate to the cryostat temperature (-20°C) before cutting sections. Routine sections are cut at 5 microns and picked up onto slide.
8. Dry at room temperature till the sections are firmly adhered to the slide.
9. Fix sections in cold acetone (-20°C) for 2 min. Dry fixed slides completely (usually 1 hour at room temperature). Store in a -70°C freezer until use.

2. Standard Immunohistochemical Staining Procedure For Frozen Sections
Please read entire procedure before staining sections. Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested.

Materials needed:
Phosphate Buffered Saline (PBS); Blocking buffer; Antibody diluent for IHC; Streptravidin/ HRP

Protocol:
1. Remove frozen slides from freezer and allow to come to RT.
2. Rinse slides 2-3 times in PBS to remove frozen mounting media.
3. Apply the blocking buffer and incubate for 10 min.
4. Rinse slides in 3 changes of PBS, 2 min each.
5. Dilute primary antibody in antibody diluent for IHC (Cat. No. 559148/70991A). Apply to cover tissue sections on slide and incubate 1 hr at RT in a humid chamber.
6. Rinse slides in 3 changes of PBS, 2 min each.
7. Dilute biotinylated secondary antibody in antibody diluent (Cat. No. 559148/70991A). Apply onto tissue sections and allow to incubate at RT for 30 min.
8. Rinse slides in 3 changes of PBS, 2 min each.
9. Apply pre-diluted Strepavidin/HRP to each slide and incubate at RT 30 min.
10. Rinse slides in 3 changes of PBS, 2 min each.
11. Prepare DAB according to manufacturer's specifications.
***SAFETY NOTE: DAB is a suspect carcinogen and must be handled with care. Always wear* gloves.**
12. Drain slides and add DAB solution to the sections. Allow to incubate 5 min or less till the desired color intensity is reached.
13. Drain excess DAB on paper towel and Rinse slides well in water 3 times.
14. Counterstain slides:
a. Dip twice in Hematoxylin.
b. Rinse thoroughly in water.
c. Dip twice in Bluing Reagent or dilute ammonia water.
d. Rinse thoroughly in water.
15. Dehydrate through 4 changes of increasing grades of alcohol to 100%, clear in 3-4 changes of xylene (or xylene substitute) and coverslip.