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General Immunostaining Protocol: Indirect methods for bright field

1. A) for fresh frozen sections: Fresh tissues or organs are excised from animals.
   And go to step (3) and thereafter (Case A).
   B) for 4% paraformaldehyde fixed cryosections: Fix tissues either by perfusing animals with 4% paraformaldehyde in PBS(pH7.2) for less than 10 min, or by immersing small tissue blocks with the same fixative for less than 1~2hr (depending on the antigens).
2. The fixed tissues are excised and washed with PBS and further immersed in 20~30% sucrose in PBS overnight at 4°C.
3. Immerse tissue blocks in OCT compound (Tissue-Tek).
4. Make snap-frozen tissue blocks in liquid nitrogen.
5. Keep frozen blocks in sealed boxes at –80°C.
6. Make cryosections (5-10 micron thick) on Poly-L-lysin coated slide glass.
7. Air-dry under an electric fan for 2 hr overnight.
8. Fix in acetone at RT for 10 min, and air-dry.
   (Omit this step, if the sections are prefixed with 4% paraformaldehyde : Case B.)
9. Make marks to surround sections with a PAP pen or a Dako pen.
10. Store the sections in a sealed slide-box at –20°C-80°C, if they are not stained on the day.
11. Thaw sections and Air-dry for at least 30 min.
12. Incubate Rehydrate in PBS (pH7.2) and block with normal serum* or Block-ace (Dainippon-seiyaku) to reduce background stains.
13. Incubate with a specific primary antibody (at a proper concentration).
14. Wash with PBS 5x for 5 min.
15. Incubate with an HRP- or ALP-conjugated secondary antibody (at a proper concentration).
16. Wash with PBS 5x for 5 min.
17. Visualization with a proper painting tool. (e.g. HRP-DAB, AlP-Fast Red, Fast Blue)
18. Wash with distilled water 3x for 5 min.
19. Counterstain with hematoxylin or methyl-green.
20. (Dehydration and) mounting with proper mounting media.
    eg. Aquatex for an aqueous medium, Vector shield for fluorescent staining*.
21. Observation under a microscope.