ADMEbio
for proteomic research

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Immunohistochemistry (IHC) Protocol

(Formalin-fixed and paraffin-embedded tissue)

I. Materials and Reagents:

- Glass slide and coverslip
- Deparaffinize and rehydrate solution: xylene and ethanol
- Double-distilled water (dd.H2O)
- Antigen retrieval solution: 10mM sodium citrate (pH 6.0)
- Washing buffer: 1xPBST (0.05% tween 20)
- Blocking buffer: 3% BSA/1xPBS
- Primary antibody and HRP-conjugated second antibody
- DAB staining buffer: DAB KIT
- Counterstain: hematoxylin
- 0.5% Hydrochloric-alcohol
- Mounting solution

II. Experimental Procedure:

1. Deparaffinize and rehydration:
   - Incubate section with xylene three times for 10 minutes each.
   - Incubate section with 100% ethanol once for 5 minutes.
   - Incubate section with 95% ethanol once for 5 minutes.
   - Incubate section with 80% ethanol once for 5 minutes.
   - Wash section with dd.H2O three times for 5 minutes each.
2. Antigen unmasking: Add 10mM sodium citrate (pH 6.0) into section, microwave for 8-15 minutes and then cool section down to room temperature (about 20-30 minutes).
3. Block endogenous peroxidase activity: Soak section in a solution of 3% H2O2-methanol at room temperature (RT) for 15 minutes. Wash section twice with dd.H2O for 5 minutes each followed by once wash with PBS for 5 minutes.
4. Protein block: Block section by soaking slide in a solution of 3% BSA-PBS at room temperature (RT) for 30 minutes.
5. Primary Antibody: Add primary antibody diluted in 3% BSA-PBS onto section and incubate at 37°C for 1 hour or 4°C overnight in a humidified chamber.

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6. Wash: Wash section three times with PBST for 5 minutes each.

7. Secondary Antibody: Add HRP-conjugated secondary antibody diluted in 3% BSA-PBS onto section and incubate at RT for 30-60 minutes in a humidified chamber.

8. Wash: Remove secondary antibody from section and wash slide 3 times with PBST for 5 minutes each.

9. Colorimetric staining: Add fresh DAB staining buffer according to DAB KIT PROTOCOL onto section and wait for color change (approximately 30 seconds-5 minutes).

10. Wash: Immediately remove DAB staining buffer from section and wash the slide with dd.H2O for 5 minutes.

11. Counterstain:
    - If necessary, counterstain section with hematoxylin for 3-8 minutes and wash slide three times with dd.H2O for 5 minutes each.
    - Add 0.5% Hydrochloric-alcohol onto section and react at RT for 3-5 seconds. Wash slide with PBS for 10-15 minutes.

12. Dehydrate:
    - Incubate section with 70% ethanol once for 5 minutes.
    - Incubate section with 80% ethanol once for 5 minutes.
    - Incubate section with 95% ethanol once for 5 minutes.
    - Incubate section with 100% ethanol twice for 5 minutes each.
    - Incubate section with xylene 2X for 10 minutes each.

13. Mount: Mount coverslip with preferred mounting solution to seal the section.