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Immunohistochemistry Protocol for Paraffin-embedded Tissues

1. Solutions and reagents

1.1. Xylene

1.2. Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%, 50%)

1.3. Washing buffer/TBST: 1X TBS/0.1% Tween-20, pH to 7.6.

1.4. Distilled water (dH_2O)

1.5. Antigen Retrieval Solution: 0.01M Sodium Citrate Buffer, pH 6.0

To prepare Antigen Retrieval stock solutions:

10X Stock: Dissolve 29.4 g sodium citrate trisodium salt dehydrate (C 6H 5Na 3O 7 ·$2H_2O$ in 1 liter of dH2O. Add 5mL Tween-20.

1X Working Solution: Mix 200mL 10X stock with 1800mL dH2O; pH to 6.0

1.6. 3% Hydrogen Peroxide

1.7. Blocking Buffer: 10% serum in PBS (serum origin depends on the host of the secondary antibody)

1.8. Primary Antibody Diluent: 5% serum in PBS (serum origin depends on the host of the Secondary antibody)

1.9. Hematoxylin

1.10. Permanent Mounting Medium

2. Protocol

2.1. Deparaffinization/Rehydration

2.1.1. Heat slides in an oven at 65 °C for 1 hour.

2.1.2. De-paraffinize/hydrate using the following series of washes: two Xylene washes (3 min each), followed by two 100% ethanol rinses (3 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by TBST wash for 3 min on a shaker.

2.2. Antigen Retrieval

This is recommended Heat Induced Epitope Retrieval (HIER) using Decloaking Chamber/Pressure Cooker. Hot water bath or Microwave with temperature sensor can be also used (protocol would vary depending on the method used).

2.2.1. Add 500 ml of dH 2O to Decloaker/Pressure Cooker.

2.2.2. Immerse slides into staining dish containing Antigen Retrieval Solution. Place staining

dish into decloaking chamber.

2.2.3. Program to run for 30 seconds at 125° C, followed by 10 seconds at 90° C.

2.2.4. Let it cool down to room temperature (10 – 20 minutes).

2.2.5. Removes slides and rinse in TBST.

2.2.6. Proceed to Staining step.

2.3. Staining

2.3.1. Wash slides with TBST for 3 min on a shaker.

2.3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 5 min.

2.3.3. Wash slides three times with TBST (3 min each on a shaker).

2.3.4. Block slides with the blocking solution for 1 hour.

2.3.5. Dilute primary antibody in primary antibody diluent per recommendation on data sheet.

2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4 ºC).

2.3.7. Wash slides three times with TBST (3 min each on a shaker).

2.3.8. Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturer's recommendation; incubate for 30 min at room temperature.

2.3.9. Wash slides three times with TBST (5 min each on a shaker).

2.3.10. Add freshly prepared DAB substrate to the sections and incubate until stain develops (generally 1 min).

2.3.11. Rinse sections with water.

2.3.12. Counterstain with Hematoxylin (generally 10 seconds).

2.3.13. Rinse sections with water.

2.3.14. Dehydrate samples using two washes with 100% Ethanol (3 min each), followed by two rinses with Xylene (3 min each).

2.3.15. Mount coverslips on slides using permanent mounting medium.