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## Immunohistochemistry Protocol for Paraffin-embedded Tissues

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### 1. Solutions and reagents

1.1. Xylene

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

1.3. Washing buffer:

TBST washing buffer: 1XTBS/0.1% Tween-20

To prepare stock solution of 10X TBS: add 24.2 g Trizma base and 80 g sodium chloride to 1L of dH<sub>2</sub>O. Adjust pH to 7.6.

Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH<sub>2</sub>O. Add 1 ml Tween-20 and mix well.

1.4. Distilled water (dH<sub>2</sub>O)

1.5. Antigen Retrieval Solution:

0.01M Sodium Citrate Buffer, pH 6.0

To prepare stock solutions:

Solution A. 0.1 M citric acid solution: dissolve 21.0 g of citric acid, monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·H<sub>2</sub>O) in 100 ml of dH<sub>2</sub>O.

Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>·2H<sub>2</sub>O) in 100 ml of dH<sub>2</sub>O.

Working solution: Add 9 ml of Stock solution A and 41 ml of stock solution B to 450 ml of dH<sub>2</sub>O. Adjust pH to 6.0.

1.6. 3% Hydrogene Peroxide

1.7. Blocking buffer:

PBS (Dulbecco's Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.) + 10% serum (serum origin depends on the host of the secondary antibody)

1.8. Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)

1.9. Permanent Mounting medium (VectaMount, catalog# H-5000 Vector Laboratories, Inc.)

### 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 (5 min each), followed by two 100% ethanol rinses (5 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by H<sub>2</sub>O and a TBST wash for 5 min on a shaker.

#### 2.2. Antigen Retrieval

2.2.1. Consult the product data sheet for the necessity of using the antigen retrieval procedure. If not using this procedure, skip to the "Staining (2.3)" step.

- 2.2.2. Immerse slides into staining dish containing Antigen Retrieval Solution.
- 2.2.3. Place covered staining dish into the rice cooker. Add  $\frac{3}{4}$  cup of dH<sub>2</sub>O and press “cook”.
- 2.2.4. When “cook” is turned to “warm” (about 20–30 min), unplug the cooker and remove the staining dish to the bench top.
- 2.2.5. Allow to cool down, without cover, for 20 min.

### 2.3. Staining

- 2.3.1. Wash slides with TBST for 5 min on a shaker.
- 2.3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 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 the blocking buffer per recommendation on the data sheet.
- 2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4 °C).
- 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 1 hour at room temperature.
- 2.3.9. Wash slides three times with TBST (3 min each on a shaker).
- 2.3.10. Add freshly prepared DAB substrate to the sections.
- 2.3.11. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2–5 min).
- 2.3.12. Rinse sections with water.
- 2.3.13. Counterstain with Hematoxylin.
- 2.3.14. Rinse sections with water.
- 2.3.15. Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by two rinses with Xylene (30 dips per rinse).
- 2.3.16. Mount coverslips on slides using Permount medium.