

## NB110-57102 Protocol

### Protocol specific for CD11b Antibody (NB110-57102)

&nbsp;

**Immunohistochemistry Protocol for Paraffin-embedded Tissues**

**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.** 1XTBS/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 1 liter 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 1 liter 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 (Dulbeccos 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. Deparaffinization/Rehydration**

**2.1. Heat slides in an oven at 65C 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. Immerse slides into staining dish containing Antigen Retrieval Solution.**

**2.2.2. Place covered staining dish into the rice cooker.** Add 120 ml of dH<sub>2</sub>O.

**2.2.3. When cook is turned to warm (about 20 to 30 min), unplug the cooker and remove the staining dish to the bench top.**

**2.2.4. 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 (4C).**

**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 manufacturers 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 to 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.**