

## NB110-55435 Protocol

### Protocol specific for 14-3-3 beta/alpha Antibody (NB110-55435)

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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%)
  - 1.3. Washing buffer: TBST washing buffer: 1XTBS/0.1% Tween-20
  - 1.4. 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.
  - 1.5. 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.
  - 1.6. Distilled water (dH<sub>2</sub>O)
  - 1.7. Antigen Retrieval Solution: 0.01M Sodium Citrate Buffer, pH 6.0
  - 1.8. 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.
  - 1.9. 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.
  - 1.10. 3% Hydrogene Peroxide
  - 1.11. 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)
  - 1.12. Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)
  - 1.13. 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.
  - 2.2. 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.
  - 2.3. Antigen Retrieval
    - 2.3.1. Immerse slides into staining dish containing Antigen Retrieval Solution.
    - 2.3.2. Place covered staining dish into the rice cooker. Add 120 ml of dH<sub>2</sub>O.
    - 2.3.3. When cook is turned to warm (about 20 to 30 min), unplug the cooker and remove the staining dish to the bench top.
    - 2.3.4. Allow to cool down, without cover, for 20 min.
    - 2.3.5. Staining
      - 2.3.5.1. Wash slides with TBST for 5 min on a shaker.
      - 2.3.5.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 min.
      - 2.3.5.3. Wash slides three times with TBST (3 min each on a shaker).
      - 2.3.5.4. Block slides with the blocking solution for 1 hour.
      - 2.3.5.5. Dilute primary antibody in the blocking buffer per recommendation on the data sheet.
      - 2.3.5.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4C).
      - 2.3.5.7. Wash slides three times with TBST (3 min each on a shaker).
      - 2.3.5.8. 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.
      - 2.3.5.9. Wash slides three times with TBST (3 min each on a shaker).
      - 2.3.5.10. Add freshly prepared DAB substrate to the sections.
      - 2.3.5.11. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2 to 5 min).
      - 2.3.5.12. Rinse sections with water.
      - 2.3.5.13. Counterstain with Hematoxylin.
      - 2.3.5.14. Rinse sections with water.
      - 2.3.5.15. Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by Xylene (30 dips per rinse).
      - 2.3.5.16. Mount coverslips on slides using Permount medium.