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NB110-56996 Protocol

Protocol specific for mTOR Antibody (NB110-56996)

  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 dH2O. Adjust pH to 7.6.
 Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH2O. Add 1 ml Tween-20 and mix well.

 1.4. Distilled water (dH2O)

 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 (C6H8O7.H2O) in 1 liter of dH2O.
 Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C6H5Na3O7.2H2O) in 1 liter of dH2O.
 Working solution: Add 9 ml of Stock solution A and 41 ml of stock solution B to 450 ml of dH2O. 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. Protocol

 2.1. Deparaffinization/Rehydration

 2.1.1. 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 H2O 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 dH2O.

 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.