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# NB110-57320 Protocol

**Protocol specific for cleaved p25 PARP Antibody (NB110-57320)**

  Immunohistochemistry Protocol for Paraffin-embedded Tissues<br /> <br /> 1. Solutions and reagents<br /> <br /> 1.1. Xylene<br /> <br /> 1.2. Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%)<br /> <br /> 1.3. Washing buffer:<br /> TBST washing buffer: 1XTBS/0.1% Tween-20<br /> 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.<br /> Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH2O. Add 1 ml Tween-20 and mix well.<br /> <br /> 1.4. Distilled water (dH2O)<br /> <br /> 1.5. Antigen Retrieval Solution:<br /> 0.01M Sodium Citrate Buffer, pH 6.0<br /> <br /> To prepare stock solutions:<br /> Solution A. 0.1 M citric acid solution: dissolve 21.0 g of citric acid, monohydrate (C6H8O7.H2O) in 1 liter of dH2O.<br /> Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C6H5Na3O7.2H2O) in 1 liter of dH2O.<br /> 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.<br /> <br /> 1.6. 3% Hydrogene Peroxide<br /> <br /> 1.7. Blocking buffer:<br /> 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)<br /> <br /> 1.8. Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)<br /> <br /> 1.9. Permanent Mounting medium (VectaMount, catalog# H-5000 Vector Laboratories, Inc.) <br /> <br /> 2. Protocol<br /> <br /> 2.1. Deparaffinization/Rehydration<br /> <br /> 2.1.1. Heat slides in an oven at 65C for 1 hour.<br /> <br /> 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.<br /> <br /> 2.2. Antigen Retrieval<br /> <br /> 2.2.1. Immerse slides into staining dish containing Antigen Retrieval Solution.<br /> <br /> 2.2.2. Place covered staining dish into the rice cooker. Add 120 ml of dH2O.<br /> <br /> 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.<br /> <br /> 2.2.4. Allow to cool down, without cover, for 20 min.<br /> <br /> 2.3. Staining <br /> <br /> 2.3.1. Wash slides with TBST for 5 min on a shaker.<br /> <br /> 2.3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 min.<br /> <br /> 2.3.3. Wash slides three times with TBST (3 min each on a shaker).<br /> <br /> 2.3.4. Block slides with the blocking solution for 1 hour.<br /> <br /> 2.3.5. Dilute primary antibody in the blocking buffer per recommendation on the data sheet.<br /> <br /> 2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4C).<br /> <br /> 2.3.7. Wash slides three times with TBST (3 min each on a shaker).<br /> <br /> 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.<br /> <br /> 2.3.9. Wash slides three times with TBST (3 min each on a shaker).<br /> <br /> 2.3.10. Add freshly prepared DAB substrate to the sections.<br /> <br /> 2.3.11. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2 to 5 min).<br /> <br /> 2.3.12. Rinse sections with water.<br /> <br /> 2.3.13. Counterstain with Hematoxylin.<br /> <br /> 2.3.14. Rinse sections with water.<br /> <br /> 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).<br /> <br /> 2.3.16. Mount coverslips on slides using Permount medium.  