Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NB100-116

NB100-116 Protocol

Western Blot Protocol for APE1 Antibody (NB100-116)

APE Antibody (13B8E5C2): https://www.novusbio.com/products/ape-antibody-13b8e5c2_nb100-116
Western Blot
1. Gels, Whatman paper, and membranes are soaked in electroblotting buffer (25 mM Tris-HCl; 193 mM glycine; 20% methanol) for 15 minutes prior to transferring
2. Proteins separated on SDS-polyacrylamide gels are transferred onto 0.22 micron nitrocellulose sheets by electroblotting in a Transblot BioRad transfer apparatus in 25 mM Tris, 192 mM Glycine, 20% Methanol at 150 mA (70 V). The transfer is carried out for 1 hour at 4 degrees C.
3. Following protein transfer, the filter is blocked with Blotto [1X TBST (10X TBST = 1.5 M NaCl; 100 mM Tris-HCl, pH 8.0; 0.5% Tween 20; 2% NP-40; 0.2% SDS); 5% Carnation dried milk; 0.02% sodium azide] for 1 hour at room temperature on a rotator.
4. Dilute NB 100-116 (anti-APE/ref-1) in Blotto and incubate with the filter, at 4 degrees C overnight, on a rotator.
5. Wash filter 3 times in 1X TBST (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.5) for 10 minutes at 4 degrees C. Secondary antibody (peroxidase conjugated anti-mouse) is incubated with the blot for 30 minutes at room temperature. Cross-reacting proteins are detected using the Chemiluminescence Western Blotting Kit from Boehringer-Mannheim.
NOTE:
HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.
Immunohistochemistry/Immunocytochemistry
The description that follows is for cultured cells but can be used for cytospins.
1. Split cells into 3.5 cm culture dishes for growth.
2. Wash cells with 5 ml PBS.
3. Fix cells with approx. 3 ml Histochoice (Amresco) for 30 min (Cryostat tissues for 45 min) or use 10% formalin for 30 minutes.
4. Rinse cells with 5 ml TBS, wipe plate dry leaving a small circle of buffer and cells. Mark with red pencil.
5. Pre-block the cells for 30 min. with 10% goat serum in TBS (200 ul).
6. Aspirate blocking solution and add NB 100-116 (anti-APE/ref-1), dilution in 10% goat serum.
7. Incubate in humidified chamber for 3 hours (overnight for tissue at 4 degrees C).
8. Incubate the cells with 1:100 diluted secondary antibody (anti-mouse IgG made in goat) in 10% goat serum and TBS for 1 hour in humidified chamber.
9. Wash 2 times with 5 ml TBS for 5 min each.
10. Block with ABC solution for 30 min.
11. Wash 2 times with 5 ml TBS for 5 min each.
12. Incubate with DAB solution until signal develops. Place into dH2O. Add coverslip with Aqua-mount. TBS: 50 mM Tris, 150 mM NaCl, pH 7.5, ABC and DAB solutions: Vector laboratories