Western Blot Protocol for APE1 Antibody (NB100-101)

1. Proteins are separated on 4-20% Tris-glycine (SDS/PAGE) gels at 125V for 1.5 hours.
2. Proteins are transferred to 0.45 mm nitrocellulose sheets by electroblotting in a Novex XCell II transfer apparatus, using Novex Transfer Buffer. Gels, Whatman paper, and NC membranes are wet in electroblotting buffer prior to transferring. The transfer is carried out for 1.5 hours at 25V.
3. Following the protein transfer, the filters are blocked with 3% BSA in PBS for 1 hour at room temperature, gently shaking.
4. The filters are then washed 3x5 minutes in washing buffer [0.5% BSA and 0.01% Tween-20 in PBS] at room temperature, gently shaking.
5. NB100-101 (APE/ref-1 primary antibody) is diluted 1:1,000 in dilution buffer [0.5% BSA in PBS] and incubated with the filters for 1 hour at room temperature, gently shaking.
6. The filters are then washed 3x5 minutes in washing buffer at room temperature, gently shaking.
7. Secondary antibody [HRP conjugated goat anti-rabbit, BioRad] is diluted 1:10,000 in dilution buffer and incubated with the filters for 1 hour at room temperature, gently shaking.
8. The filters are then washed 3x5 minutes in washing buffer at room temperature, gently shaking. Cross-reacting proteins are detected using the Amplified Opti-4CN Western Blotting kit (BAR and streptavidin-HRP incubations and colorimetric detection) from BioRad.

NOTE: HeLa whole cell extracts (NB800-PC1) were used a 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. Preblock the cells for 30 min. with 10% goat serum in TBS (200 ul).
6. Aspirate blocking solution and add NB100-101 (APE/ref-1 primary antibody) at a dilution of 1:100 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 20 antibody (anti-rabbit 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