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NB100-617 Protocol

Protocol specific for KAT3B / p300 Antibody (NB100-617)

KAT3B/p300 Antibody (RW109): https://www.novusbio.com/products/kat3b-p300-antibody-rw109_nb100-617
Western Blot Protocol

1. Perform SDS-PAGE (3-8% Tris-acetate) on samples to be analyzed, loading 50ug of total protein per lane.

2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.

3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.

4. Rinse the blot in TBS for approximately 5 minutes.

5. Block the membrane using 5% non-fat dry milk in TBS for 1.5 hours.

6. Dilute the mouse anti-p300 primary antibody (NB 100-617) in blocking buffer and incubate overnight at 4 degrees Celsius.

7. Wash the membrane in water for 5 minutes and apply the diluted mouse-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.

8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.

9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).

10. Apply the detection reagent of choice in accordance with the manufacturer's instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.