

NBP2-31366 Protocol

Western Blot Protocol for PIM1 Antibody (NBP2-31366)

Western Blot Protocol

1. Perform SDS-PAGE on protein samples to be analyzed, loading 10-40 ug of total protein per lane.
 2. Electro-blot the proteins to a suitable membrane (PVDF or Nitrocellulose) according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
 3. Stain the membrane with Ponceau S (or a similar product) to assess transfer success. Mark molecular weight standards where appropriate.
 4. Thoroughly rinse the membrane of stain with TBST.
 5. Incubate the membrane in blocking buffer (5% non-fat milk in TBST or 5% BSA in TBST) as appropriate, for 60 minutes.
 6. Dilute the primary antibody as appropriate in blocking buffer and incubate for 60 minute at room temperature to overnight at 4 degrees C with gently shaking.
 7. Wash the membrane in TBST three times for 10 minutes each.
 8. Incubate the membrane in the appropriate secondary antibody prepared in blocking buffer (as per manufacturer's instructions) and incubate for 60 minutes at room temperature.
 9. Wash the membrane in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
 10. Incubate the membrane in the appropriate detection reagent in accordance with the manufacturer's instructions and image the blot.
- Note: Tween-20 can be added to the blocking, wash and antibody dilution buffers to a final concentration of 0.05-0.1%.