Western Blot Protocol for PINK1 Antibody (BC100-494)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot of the protein stain.
5. Block the membrane using 5% BSA for at least 1 hour.
6. Dilute anti-PINK1 primary antibody in 1% BSA and incubate 1 hour at room temperature.
7. Wash the membrane in wash buffer three times for 10 minutes each.
8. Incubate in diluted HRP-conjugated Rabbit secondary antibody in 1% BSA (as per manufacturers instructions) and incubate 1 hour at room temperature.
9. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
10. Apply the detection reagent of choice in accordance with the manufacturers instructions.