



## NB100-493 Protocol

# Western Blot Protocol for PINK1 Antibody (NB100-493)

### Western Blot

1. Load 10-30 ug of whole cell lysate and mitochondrial fractions\* on an 18% Criterion Polyacrylamide Tris-HCl gel (BioRad).
2. Transfer the proteins at 90V for 2 hours at 4 degrees Celsius.
3. Block the membrane in 5% NFDM + TBSt for 1 hour at room temperature.
4. Dilute the PINK1 primary antibody (NB 100-493) 1:500 in blocking buffer and incubate overnight at 4 degrees Celsius.
5. Wash the membrane for 1 hour in TBST, changing the wash buffer every 5-10 minutes.
6. Dilute the secondary antibody (as per manufacturer's guidelines) in TBST + 1% BSA and incubate for 1 hour at room temperature.
7. Develop the blot using ECL Plus.

\*NOTE: Mitochondria were isolated using Pierce's mitochondria isolation kit (#89874). The protocol for this kit procedure is online at Pierce.