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NBP2-46888 Protocol

Western Blot protocol for LC3 Antibody (NBP2-46888)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
 2. Transfer protein to 0.2 um 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.
 5. Block the membrane using standard blocking buffer for at least 1 hour.
 6. Dilute anti-LC3 primary antibody in blocking buffer and incubate 1 hour at room temperature.
 7. Wash the membrane in wash buffer three times for 10 minutes each.
 8. Apply the diluted HRP conjugated rabbit secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
 10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
 11. Apply the detection reagent of choice in accordance with the manufacturers instructions.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.