Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 20-30 ug of total protein per lane.
2. Transfer proteins to PVDF membrane 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 protein. Rinse the blot in water to remove excess stain and mark the lane and molecular weight markers location using a pencil.
4. Rinse the blot in TBST for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk in TBST for 1 hour.
6. Dilute the rabbit anti-Beclin primary antibody (NB500-249) in 1% milk-TBST and incubate for 2 hours at room temperature or overnight.
7. Wash the membrane 3x for 10 min each in TBST and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in 1% milk-TBST and incubate for 2 hours at room temperature or overnight.
8. Wash the blot 3x for 10 min each in in TBST.
9. Apply the detection reagent of choice in accordance with the manufacturer's instructions.