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NB400-127 Protocol

Western Blot protocol for Niemann-Pick type C1 Like-1 Antibody (NB400-127)

[[URL: https://www.novusbio.com/products/niemann-pick-type-c1-like-1-antibody_n...]][[Caption:Niemann-Pick type C1 Like-1 Antibody]]
Western Blot Protocol

1. Perform SDS-PAGE (3-8%) on samples to be analyzed, loading 50ug of total protein per lane.
2. Transfer proteins to Nitrocellulose 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 proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk in TBS for 1 hour at room temprature.
6. Dilute the rabbit anti-NPC1L1 primary antibody (NB 400-127) in 5% BSA and incubate overnight at 4 degreesCelcius.
7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody inblocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reducebackground).
10. Apply the detection reagent of choice in accordance with the manufacturers instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

**Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere
with antibody-antigen binding.