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NB110-74570 Protocol

Protocol specific for SLC5A7 Antibody (NB110-74570)

CHT1 Antibody (62-2E8): https://www.novusbio.com/products/cht1-antibody-62-2e8_nb110-74570
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading ~30 ug of total protein per lane.
2. Transfer proteins to PVDF according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then 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 PBS for approximately 5 minutes.
5. Block the membrane using 5% NFDM in PBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [PBS + 0.5% Tween] 3 times for 10 minutes each.
7. Dilute the mouse anti-hCHT primary antibody (NB 110-74570) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [PBS + 0.5% Tween] 3 times for 10 minutes each.
9. Apply the diluted mouse-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [PBS + 0.5% Tween] 3 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 (Amersham ECL Plus).

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