

**NB100-1869 Protocol** 

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Western blot protocol for SLC1A3 antibody (NB100-1869)

[[URL:https://www.novusbio.com/products/eaat1-glast-1-slc1a3-antibody nb100-1869]][[Caption:SLC1A3

- antibody]]Western Blot Protocol 1. Perform SDS-PAGE on protein samples to be analyzed, loading 10-40 ug of total protein per lane.
- 2. Electro-blot the proteins to a suitable membrane (PVDF or Nitrocellulose) according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
- 3. Stain the membrane with Ponceau S (or a similar product) to assess transfer success. Mark molecular weight standards where appropriate.
- 4. Thoroughly rinse the membrane of stain with TBST.
- 5. Incubate the membrane in blocking buffer (5% non-fat milk in TBST or 5% BSA in TBST) as appropriate, for 60
- 6. Dilute the SLC1A3 primary antibody as appropriate in blocking buffer and incubate for 60 minute at room temperature to overnight at 4 degrees C with gently shaking.
- 7. Wash the membrane in TBST three times for 10 minutes each.
- 8. Incubate the membrane in the appropriate secondary antibody prepared in blocking buffer (as per manufacturer's instructions) and incubate for 60 minutes at room temperature.
- 9. Wash the membrane in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
- 10. Incubate the membrane in the appropriate detection reagent in accordance with the manufacturer's instructions and image the blot.

Note: Tween-20 can be added to the blocking, wash and antibody dilution buffers to a final concentration of 0.05-0.1%.