

## NB300-106 Protocol

### Western Blot protocol for NMDAR2B Antibody (NB300-106)

[[URL:[https://www.novusbio.com/products/nmdar2b-antibody\\_nb300-106](https://www.novusbio.com/products/nmdar2b-antibody_nb300-106)]][[Caption:NMDAR2B Antibody ]]  
Western Blot Protocol

Thoroughly sonicate tissue in the following buffer:

1 % SDS (hot, but not boiling, ~ 90C )  
10 mM Tris pH 8.0  
1 mM EDTA

1. Dilute in appropriate SDS PAGE sample buffer and boil for 5 minutes at 100 C. Let samples cool to room temperature then load onto gel.
2. Run SDS-PAGE per gel apparatus manufacturer's instructions.
3. Transfer proteins to nitrocellulose or PVDF membrane (if using PVDF, be sure to activate membrane in Methanol prior to use).
4. After transfer, air-dry blot (~15 min.) to more stably fix proteins onto membrane.
5. Block non-specific sites on membrane in 5% NFDM (Non-fat dry milk) -TTBS(Tris-Buffered Saline + 0.1% Tween-20) for 1 hour while shaking at room temperature.
6. Incubate membrane in primary antibody diluted 1:1,000 in 1% NFDM -TTBS while shaking overnight at 4 C.
7. Decant unbound primary antibody solution and wash blot 3 x 10 minutes in TTBS.
8. Incubate blots in appropriate HRP-conjugated (for ECL detection) secondary antibody at a 1:10,000 dilution in 1% NFDM-TTBS for 1 hour while shaking at room temperature.
9. Decant secondary antibody solution and wash blots 3 x 10 minutes in TTBS or use TBS + 0.1% Triton X-100 to reduce excessive background if needed.
10. ECL Detect. (We recommend Pierce's Super Signal West Dura)