Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NB300-105

NB300-105 Protocol

Western Blot protocol for NMDAR2A Antibody (NB300-105)

Western Blot Protocol specific for NMDAR2A Antibody (NB300-105): https://www.novusbio.com/products/nmdar2a-antibody_nb300-105
Western Blot:

**For brain tissue homogenize in hot 1% SDS then run 7.5 % (as 2A is ~ 180 kDa) standard SDS gels and blot.

1. Thoroughly sonicate cell lysates or tissue homogenates to be loaded onto gel then dilute in appropriate sample buffer and boil for 5 minutes at 100C. 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 to more stably fix proteins onto membrane.
5. Block non-specific sites on membrane in 5% NFDM (Non-fat dry milk) or 3% BSA-TTBS (Tris-Buffered Saline + 0.1% Tween-20) for 1 hour while shaking at room temperature.
6. Incubate membrane in primary antibody diluted in 1% NFDM (or BSA)-TTBS while shaking overnight at 4C.
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 -1:20,000 dilution in 1% Milk (or BSA)-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(R) X-100 to reduce excessive background if needed.
10. ECL Detect.