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NB300-151 Protocol

Western Blot Protocol for GABA A Receptor gamma 2 Antibody (NB300-151)

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Western Blot Protocol

1. Pour lower gels according to recipes layer with about 300 ul ethanol and allow to polymerize at least 45 minutes. 7.5% SDS-PAGE gels work well for Synapsin, NR2A, 2B,and 2C antibodies.

2. Rinse off ethanol with water. Shake and/or use a kimwipe to remove excess water. Pour upper gels (stacks) and insert combs. Let polymerize about 15-20 minutes. Remove combs, making sure you pull them straight out, and rinse with water.

3. Attach gel holders to running electrode apparatus and fill chamber with 1X running buffer.

4. Load gels beginning with 10 ul of the kaleidoscope molecular weight marker.

5. Attach electrodes to power source and run gels at 200 Volts for about 45 minutes or until dye front runs down past gray gasket.

6. Turn off power source and remove gel holders from running apparatus first and then carefully remove plates from holders. Remove one plate and leave gel attached to the other plate. Use a spacer or the green scraper to cut off stacks and discard.

7. Place plate with attached gel in some 1X transfer buffer and let equilibrate while you assemble the transfer genie. Transfer buffer + 20% Methanol is standard for many antibodies. NMDA antibodies seem to look a little better in transfer buffer with 5% Methanol + .05% SDS.

8. Wearing gloves cut PVDF membranes to gel size and wet in Methanol to activate for about 30 seconds. Rinse 2-3 times in water. Be sure to keep membrane wet at all times. Put membrane in some 1X transfer buffer until you are ready to use it.

9. Assemble the genie transfer apparatus per instructions on wall. Fill with 1X transfer buffer.

10. Carefully place gels on filter paper and then place PVDF membranes on top of gels making sure there are no air bubbles. Use the sawed-off pipette to roll over sandwich. Complete the assembly of transfer apparatus making sure there is enough buffer to come to the top of the scotch-brite pads.

11. Clean off electrodes with a Q-tip and attach to battery charger. Plug charger in and set to 6 Volts for the mini-genie and 12 Volts for the large genie. Transfer gel for 1.5-2 hours.

12. Take down transfer apparatus and rinse blot a couple of times in water. Place blot on kimwipe and let air dry about 10-15 minutes to fix proteins. Reactivate membranes by rewetting in Methanol and rinsing in water.

13. Block blots in 5% Non-fat dry milk-TTBS for 30 minutes while shaking at room temperature. Blocking time may be increased to an hour if blots look dirty. It is not necessary to block when working with the Synapsin antibody. Milk works great for the NMDA antibodies, but when working with phospho-site or other antibodies that don't like milk, use 3% BSA-TTBS to block.

14. Incubate blots overnight in cold-room in primary antibody diluted in 1% milk TTBS or 1% BSA-TTBS.

15. Decant unbound primary antibody solution (save in fridge) and wash blot 3 x 10 minutes in TTBS.

16. Incubate blots in secondary antibody at a 1:10,000 1:30,000 dilution in 1% Milk or 1% BSA for 1 hour while shaking at room temperature. Use Goat Anti-Rabbit HRP for polyclonals and Goat Anti-Mouse HRP for monoclonals.

17. Decant secondary antibody solution and wash blots 3 x 15 minutes in TTBS or use TTBS + 0.1% Triton X-100 to reduce excessive background if needed.

18. ECL Detect---- Mix equal volumes of each reagent in the Pierce Super Signal ECL kit using just enough to cover blots (0.125 ml/cm of membrane). I use 1 ml of each for a total of 2 mls per standard size blot. Vortex ECL solution briefly and incubate blots in substrate for 1 minute only. Pour off excess ECL solution and blot with a kimwipe to further remove excess. Place blot on a piece of plastic sheet protector and put into the Alpha Chemi-Imager to visualize bands. Set exposures from roughly 15 seconds to 4 minutes.