

**Western Blotting Protocol
NDJ 6.28.04 V.2**

1. Take samples from cell culture or cerebellar protocol after Bradford.
2. Calculate and add equal amounts of protein. Dillute with lysis buffer.
3. Turn on heat block to 70°C.
4. Add 4x sample buffer.
5. Add 10x Reducing Agent
6. Heat 10 min at 70°C.
7. Load in Gradient Bis-Tris gel with MES buffer (invitrogen).
8. 30ul total in 10well gel. Add 0.1% Antioxidant.
8. Run 100v (few hours) until leading edge of dye front in new bottom.
9. Take out of cassette. Load into transfer cassette with transfer buffer (Invitrogen) with 0.1% antioxidant. Assemble: 3x sponge, filter paper, gel, nitrocellulose, filter paper, 3x sponge. Soak before assembly.
10. Bathe in buffer and make sure no buffers are trapped in cassette assembly.
11. Run ON at 100mAmp/cassette. (MeOH may be used to shorten transfer time. See Current Protocols in Molecular Biology (red books).
12. Block 2 hours in 5% milk. All WB incubART on rocker.
13. Add 1° antibody in block. (1:1000 standard) 2 hours.
14. Quick rinse. 2x ten minute wash in PBS-T
15. Add 2° antibody in block according to 1° antibody. Standard is 1:2000 HRP
16. Rock 45 minutes
17. Quick rinse. 2X ten minute wash PBS-T
18. Use Western lightning HRP detection kit to assess HRP levels.

PBS-T (0.1%) 1 liter	
PBS 10X	100ml
Tween 20	1ml
Fill sterile H2O to 1L	

5% Block 100ml	
Dry Milk	5g
Fill PBS-T 0.1% to 100ml	
Shake to dissolve milk	