Western Blot/Anti-pCREB

David Ginty

1. Treat cells. For PC12 or NIH 3T3 cells a moderately confluent 60 mm plate is used.


3. Lyse cells in 200 uL of SDS sample buffer (100ºC). Immediately scrape the cells off the plate with a rubber policeman.

4. Put the extract into an eppendorf and boil for 5 min., cool on ice.

5. Load onto 10% SDS-PAGE. I usually load about 1/5 of the lysate from a 60 mm dish. This is about 100 ug protein for PC12 cells.

6. After electrophoresis, electrotransfer to nitrocellulose membrane. I include in the SDS-PAGE prestained molecular weight markers to check the efficiency of the transfer and for M.W. determination.

7. After transfer, rinse the blot 2 times with TBST (10 mM Tris (pH 7.4), 150 mM NaCl, 0.05% Tween-20).

8. Block with 4% BSA in TBST for 45–60 minutes. I use RIA grade BSA from USB (United States Biochemical; Catalog number 10868). I also include 0.02% NaN3 in this block and reuse it at least 5 times (keep in 4ºC between uses). We have had problems obtaining good signals when we've used poor quality BSA. Others have told me that 10% nonfat dry milk works well for anti-pCREB, but in my hands milk is not as good as BSA from USB (it seems to mask the epitope).

9. Remove block solution and add primary antibody. For anti-pCREB (IgG, 0.7 mg/ml), I use 1:5,000–1:20,000 dilution in 4% BSA in TBST. This should be titrated for optimal results. I incubate with primary Ab 3 hours - overnight at room temp with shaking.

10. Remove the antibody solution and wash the blot 3 times, 5–7 minutes each, with 0.5% BSA in TBST.

11. Incubate with Goat anti-Rabbit/alkaline phosphatase (from Promega; 1:7500 in TBST with 5% BSA) for 1 hour at room temp with shaking. I have also used successfully Amersham ECL detection, which employs goat anti-Rabbit HRP.

12. Wash filter 3 times 7 minutes with 0.5% BSA in TBST.

13. Perform alkaline phosphatase reaction using NBT and BCIP substrates. I find that CREB usually comes up within several minutes of adding the AP substrates, however a one-hour reaction time may be necessary.