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NB500-243 Protocol

Immunoprecipitation and Western Blotting protocol specific for TRPM7 Antibody (NB500-243)

TRPM7 Antibody: https://www.novusbio.com/products/trpm7-antibody_nb500-243
Immunoprecipitation and Western Blotting Procedure
[Buffer recipes to follow protocol]

1. Prepare fresh 1X Lysis buffer (pH 7.4): Need 0.35 ml/pt. + 3 ml/pt.
2. Collect cells by washing them down, trypsinizing or scraping them (as appropriate).
3. Pellet 5 minutes at 1K rpm
4. Remove media.
5. Break pellet by gently tapping it.
6. Wash with X ml cold 1X PBS (pipet up/down once). (X = # of pts. (lanes) to be derived from the cell line.)
7. Aliquot cells 1ml/microfuge tube.
8. Spin cells for 30 seconds at 12K rpm then place on ice.
9. Aspirate off the supernatant.
10. Lyse cells with 0.35 ml 1X Lysis buffer (added to each tube).
11. Pipet up/down 3-4X to completely resuspend the cells. (**Do not vortex)
12. Incubate for 30 minutes at 4C, on rotator.
13. Pellet unlysed nuclei down, 5 minutes at 12K rpm, 4C.
14. Set up anti-TRPM7, NB 500-243 (primary antibody) aliquots into fresh tubes.
15. Transfer the sup into assigned tubes containing the pre-aliquotted primary.
16. Rotate for 2 hours at 4C on a rotator.
17. Add 0.015 ml of pre-washed Protein-G-Sepharose capturing beads. (Washing protocol provided by bead manufacturer)
18. Rotate 45 minutes at 4C.
19. Remove from 4C and place on ice.
20. Spin cells for 30 seconds at 12K rpm.
21. Aspirate off the supernatant.
22. Wash 3x by centrifuging for 1 minute at 12K rpm and aspirating and resuspending in 1 ml Lysis buffer.
23. Centrifuge for 1 minute at 12K rpm, 4C.
24. Aspirate off supernatant.
25. Dry pellet with Hamilton syringe (careful not to lose any beads).
26. Resuspend pellet with 0.075 ml (5X stock) reducing SDS-PAGE sample loading buffer.
27. Poke a hole in the top of each tube and boil the samples in a 95C for 8 minutes.
**At this point the samples are stable indefinitely at 4C.
28. Quick spin the condensation for 30 seconds at 12K rpm.
29. Load a 10% gel with the sample.
30. Run the gel overnight at 60V or 6 hours at 140V.
31. Transfer the proteins from the gel to a PVDF membrane (activated by methanol), at 1.4 constant Amp for 3 hours, 20 minutes.
32. Block the membrane in 5% NFDM for >1 hour at RT, gently shaking.
33. Incubate the membrane with the anti-TRPM7, NB 500-243 overnight @ 4C or for 2 hours @ RT, gently shaking.
34. Wash the blot 5X for 5 minutes, each time, in TBS-T (TBS + 0.05% Tween-20).
35. Incubate the membrane with the anti-rabbit IgG secondary antibody (dilution determined by manufacturer's suggestion), diluted in TBS-T + 0.5% NFDM for 45-60 minutes @ RT, gently shaking.
36. Wash the blot 4X for 5 minutes, each time, in TBS-T (TBS + 0.05% Tween-20).
37. Expose the membrane to the ECL reagents of choice.

200 ml 2X Lysis Buffer
20 ml 1X Lysis Buffer
10 ml 2X lysis buffer
8.8 ml dH2O
0.1 ml PMSF
0.1 ml Vanadate
111.5 mg Na-pyrophosphate
1 ml 10% Triton X-100

150 mM NaCl
80 mM NaF
20 mM Iodocetamide
100 mM HEPES
bring up to 200 ml w/ dH2O
sterile filter, store at 4C

5X Reducing Sample Loading Buffer
6 ml 1M Tris (pH 6.8)
50 ml 50% Glycerol
10 ml 20% SDS
0.1% (w/v) Bromophenol Blue
5 ml 2-Mercaptoethanol (BME)

Primary Antibody Diluent
1X TBS
0.05% Tween-20

* Add BSA to a final concentration of 0.5% before using with the primary antibody