Product Datasheet

TRPM7 Antibody
NB500-243SS

Unit Size: 0.025 ml

Store at -20C. Avoid freeze-thaw cycles.

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NB500-243SS

TRPM7 Antibody

Product Information	
Unit Size	0.025 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Whole antisera

Product Description	
Host	Rabbit
Gene ID	54822
Gene Symbol	TRPM7
Species	Human, Mouse (Negative)
Species Reactivity	NB 500-243 recognizes overexpressed human TRPM7. It does not appear to recognize overexpressed mouse TRPM7. It does not work on endogenous samples tested for western analysis (human, rat and mouse cell lines. Other species have not been tested.
Immunogen	A synthetic peptide made to the C-terminal region of rat TRPM7. [UniProt# Q925B3]

Product Application Details	
Applications	Western Blot, Immunoprecipitation
Recommended Dilutions	immunoprecipitation 5 ul, Western Blot 1:1000
Application Notes	This TRPM7 antibody is useful for Western blot on immunoprecipitated samples only, does not work on endogenous samples tested to date. The investigator should determine the optimal working dilution.

Procedures

Immunoprecipitation and Western Blotting protocol specific for 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 min. @ 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 @ 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 @ 4C, on rotator.
13. Pellet unlysed nuclei down, 5 minutes @ 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 @ 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 @ 4C.
19. Remove from 4C and place on ice.
20. Spin cells for 30 seconds @ 12K rpm.
21. Aspirate off the supernatant.
22. Wash 3x by centrifuging for 1 minute @ 12K rpm and aspirating and resuspending in 1 ml Lysis buffer.
23. Centrifuge for 1 minute @12K rpm, 4 dgrees Celcius.
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 95 degree Celcius block for 8 minutes.
**At this point the samples are stable indefinitely @ 4C.
28. Quick spin the condensation for 30 seconds @ 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 @ 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
150 mM NaCl 10 ml 2X lysis buffer
80 mM NaF 8.8 ml dH2O
20 mM Iodocetamide 1 ml 10% Triton X-100
100 mM HEPES 0.1 ml PMSF
bring up to 200 ml w/ dH2O 0.1 ml Vanadate
sterile filter, store @ 4 degrees C 111.5 mg Na-pyrophosphate

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

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.