NEUROMICS

Phospho-NMDAR1 (Ser890) Antibody Data Sheet

Catalog Number:	RA18011	**Host:**	Rabbit
Product Type:	Affinity purified antibody	**Species Reactivity:**	Rat, Mouse, Human
Immunogen Sequence:	Phosphopeptide corresponding to residues around serine 890 of human NMDAR1. Antibodies are purified by protein A and peptide affinity chromatography.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol

Applications:
Immunofluorescence 1:100
Immunohistochemistry 1:100 – 1:400
Western blotting 1:1000
Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store at -20ºC. Do not aliquot.

Application Notes

Phospho-NMDAR1 (Ser890) Antibody detects over-expressed and endogenous NMDAR1 only when phosphorylated at serine 890.

Western Blot Protocol

Sample Preparation

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape cells from plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose (or PVDF) membrane.

Membrane Blocking and Antibody Incubations
Note: Volumes for 10 cm x 10 cm (100 cm^2) membrane; for different sized membranes, adjust vol. accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.
8. Process membranes using enhanced chemiluminescence.

FOR RESEARCH USE ONLY

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Solutions and Reagents for Western Blot
Note: Prepare solutions with Milli-Q or equivalently purified water.

1X Phosphate Buffered Saline (PBS)

1X SDS Sample Buffer:
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

10X Tris Buffered Saline (TBS):
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

Nonfat Dry Milk (weight to volume [w/v])

Blocking Buffer:
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)

Bovine Serum Albumin (BSA)

Primary Antibody Dilution Buffer:
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).

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