



phospho-NMDAR1 (Ser896)

Datasheet

Catalog Number:	RA18012	Host:	Rabbit
Product Type:	Affinity purified antibody	Species Reactivity:	Rat, Mouse, Human
Immunogen Sequence:	Phosphopeptide corresponding to the residues around Ser896 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:	Western blotting 1:1000 Immunohistochemistry 1:100 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at -20°C. Do not aliquot.		

Application Notes

Phospho-NMDAR1 (Ser896) Antibody detects over-expressed NMDAR1 only when phosphorylated at serine 896.

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 μ l per well of 6-well plate or 500 μ l 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 μ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ l 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 cm²) 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.

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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%).

Tissue Fixation

Fix tissues by intracardiac perfusion with ice-cold PBS for 1 minute followed by ice-cold 4% paraformaldehyde in phosphate buffer delivered with a peristaltic pump at 50 ml/min for 10 minutes. Remove tissues and keep in the same fixative solution at 4°C for 24 hours, then section with a Vibratome at a thickness of 50 µm. Vibratome sections can be stored in an antifreeze solution at -20°C for at least several months.

ABC-DAB

1. Wash tissue sections in TBS/Triton.
2. Treat sections with freshly made 1% H₂O₂ (0.1 ml of 30% H₂O₂ in 3 ml TBS) for 30 minutes.
3. Wash the sections with TBS/Triton three times for 30 minutes each at room temperature.
4. Block nonspecific binding sites with 3% BSA in TBS/Triton for 30 minutes to 1 hour.
5. Incubate the sections with primary antibody diluted in
6. 3% BSA in TBS/Triton overnight at 4°C.
7. Wash the sections in TBS/Triton three times for 10 minutes each at room temperature.
8. Incubate the sections in biotinylated anti-rabbit secondary antibody (for polyclonal primaries) or biotinylated anti-mouse secondary antibody (for monoclonal primaries) diluted in 1%
9. BSA in TBS/Triton for 1 hour at room temperature.
10. Prepare ABC Reagent solution and leave it at room temperature for at least 15 minutes.
11. Wash the sections in TBS/Triton three times for 10 minutes each at room temperature.
12. Incubate the sections in the ABC reagent for 1 hour at room temp.
13. Wash the sections in TBS/Triton three times for 10 minutes each at room temperature.
14. Incubate the sections in DAB reagent until staining is optimal as determined by light microscopic examination.
Note: Handle DAB reagent with gloves.
15. Wash the sections in TBS three times for 5 minutes each.
16. Mount the sections on gelatin-coated slides and dry them at room temperature.
17. Dehydrate the sections sequentially in 50%, 70%, 95% and 100% ethanol for 2 minutes each, 50%:50% ethanol/xylenes for 2 minutes and 100% xylenes for 5 minutes.
18. Mount the coverslides using Permount.

Fluorescence

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1. Wash tissue sections in TBS/Triton.
2. Block nonspecific binding sites with 3% BSA in TBS/Triton for 30 minutes to 1 hour.
3. Incubate the sections with a first primary antibody (e.g., phospho--specific rabbit antibody) diluted in 3% BSA in TBS/Triton overnight at 4°C.
4. Wash the sections in TBS/Triton three times for 10 minutes each at room temperature.
5. Incubate the sections with a second primary antibody from a different species than the first primary antibody (e.g., mouse) diluted as above for 2 hours at 4°C.
6. Wash the sections in TBS/Triton three times for 30 minutes each at room temperature.
7. Incubate the sections in a fluorescent secondary antibody mixture containing fluorescence-labeled secondary antibody against the first primary (i.e., fluorescence-labeled anti-rabbit) and fluorescence-labeled secondary antibody against the second primary antibody (i.e., fluorescence-labeled anti-mouse) each at a dilution of 1:200 in 1% BSA in TBS/Triton for 1 hour at room temperature. Incubation chambers should be covered with foil to avoid exposure to light.
8. Wash the sections in TBS three times for 30 minutes each at room temperature.
9. Mount and coverslip the sections using Gelvatol. Add a small drop of Gelvatol to sections. Carefully place coverslips on the drops, avoiding air bubbles.
10. The mounting media will set overnight at 4°C or within 2–3 hours at room temperature.

Solutions

Solutions and Reagents

Phosphate Buffer:

0.1 M Na₂HPO₄/NaH₂PO₄ (pH 7.5)

Fixative Solution:

Phosphate buffered saline (PBS), 4% paraformaldehyde in phosphate buffer

Antifreeze Solution:

320 ml 1X PBS (pH 7.4), 240 ml ethylene glycol (30%), 240 ml glycerol (30%)

Tris Buffered Saline (TBS):

0.1 M Tris-HCl (pH 7.4), 0.15 M NaCl

Wash Buffer:

1X TBS, 0.1% Triton X-100 (TBS/Triton)

1% and 3% Bovine Serum Albumin (BSA)

ABC Reagent (avidin-biotin-peroxidase complex):

(Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) 1 drop reagent A and 1 drop reagent B in 5 ml TBS with 1% BSA, 0.1% Triton X-100

DAB Reagent (0.5 mg/ml):

Use 10 ml TBS, 500 µl of 10 mg/ml DAB stock solution, 50 µl of glucose oxidase (30 mg/10 ml TBS), 20 µl NH₄Cl (2.0 g/10 ml TBS) and 50 µl D (+) glucose (2.5 g/10 ml TBS).

Gelvatol Preparation:

- 1) Add 2.4 g of polyvinyl alcohol (Mol. Wt. 30,000–70,000) to 6 ml of glycerol. Stir well to mix. Add 6 ml of dH₂O and leave for at least 2 hours at room temperature.
- 2) Add 12 ml of 0.2 M Tris (pH 8.5). Heat to 50°C for 10 minutes with occasional mixing. After polyvinyl alcohol is dissolved, clarify by centrifugation (5000 x g) for 15 minutes. Collect supernatant liquid.
- 3) Add DABCO (1,4-diazabicyclo [2.2.2] octane; Sigma #D2522) to 2.5% as antifade medium. Aliquot in microtubes and store at –20°C. Stocks of Gelvatol are stable at room temperature for several weeks after thawing.

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