



## Phospho-NMDAR1 (Ser890) Antibody

## Data Sheet

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<b>Catalog Number:</b>	RA18011	<b>Host:</b>	Rabbit
<b>Product Type:</b>	Affinity purified antibody	<b>Species Reactivity:</b>	Rat, Mouse, Human
<b>Immunogen Sequence:</b>	Phosphopeptide corresponding to residues around serine 890 of human NMDAR1. Antibodies are purified by protein A and peptide affinity chromatography.	<b>Format:</b>	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol
<b>Applications:</b>	<b>Immunofluorescence</b> 1:100 <b>Immunohistochemistry</b> 1:100 – 1:400 <b>Western blotting</b> 1:1000		
	Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
<b>Storage:</b>	Store at -20°C. Do not aliquot.		

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### 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<sup>2</sup>) 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%).

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