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Datasheet for 612-401-D89

NMDA R2A Antibody

Overview

Description:	Anti-NMDA R2A (RABBIT) Antibody - 612-401-D89
Item No.:	612-401-D89
Size:	100 µL
Applications:	WB, IF
Reactivity:	Rat
Host Species:	Rabbit

Product Details

Background:	NMDA R2A Antibody detects NMDA R2A protein. The ion channels activated by glutamate are typically divided into two classes. Glutamate receptors that are activated by kainate and α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) are known as kainate/AMPA receptors (K/AMPAR). Those that are sensitive to N-methyl-D-aspartate (NMDA) are designated NMDA receptors (NMDAR). The NMDAR plays an essential role in memory, neuronal development and it has also been implicated in several disorders of the central nervous system including Alzheimer's, epilepsy and ischemic neuronal cell death. The NMDA receptor is also one of the principal molecular targets for alcohol in the CNS. The NMDAR is also potentiated by protein phosphorylation. The rat NMDAR1 (NR1) was the first subunit of the NMDAR to be cloned. The NR1 protein can form NMDA activated channels when expressed in Xenopus oocytes but the currents in such channels are much smaller than those seen in situ. Channels with more physiological characteristics are produced when the NR1 subunit is combined with one or more of the NMDAR2 (NR2 A-D) subunits. Anti-NMDA R2A Antibody is ideal for investigators involved in Neuroscience, Cell Signaling, and Signal Transduction research.
Synonyms:	Glutamate [NMDA] receptor subunit epsilon-1, N-methyl D-aspartate receptor subtype 2A, NMDAR2A, NR2A, Grin2a
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Grin2a

Reactivity:	Rat
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-NMDA R2A Antibody was produced by repeated immunizations with a synthetic peptide corresponding to amino acid residues from the N-terminal region of the NR2A subunit.
Purity/Specificity:	Anti-NMDA R2A antibody is directed against NMDA R2A protein. The antibody was affinity purified from monospecific antiserum by immunoaffinity purification. Expect reactivity with the following species based on 100% sequence homology: bovine, canine and mouse.
Relevant Links:	• UniProtKB - Q00959 • GeneID - 24409 • UniProtKB - Q00959.2

Application Details

Tested Applications:	WB
Suggested Applications:	IF (Based on references)
Application Note:	Anti-NMDA R2A Antibody is tested for use in Western Blotting and ICC. Specific conditions for reactivity should be optimized by the end user. Expect a band of approximately 180 kDa in size corresponding to the NR2A subunit of the NMDA receptor in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IHC:	1:500
WB:	1:1000

Formulation

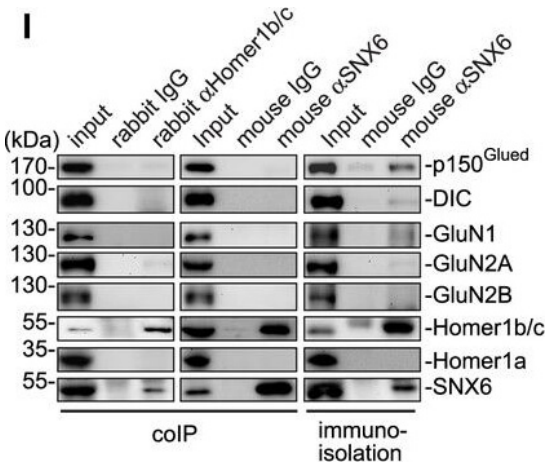
Physical State:	Liquid
Buffer:	0.01 M HEPES, 0.15 M Sodium Chloride, pH 7.5
Stabilizer:	0.1 mg/ml Bovine Serum Albumin (BSA) - IgG and Protease free, 50% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Dry Ice

Storage Condition:	Store vial at -20° C prior to opening. This product is stable at 4° C as an undiluted liquid. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot

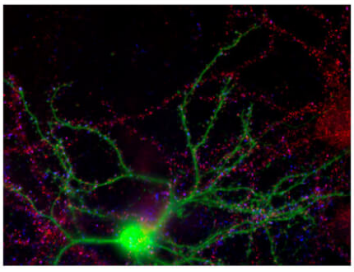
SNX6 interacts with Homer1b/c and colocalizes with Homer1b/c on endosomes.(A) Hippocampal neurons were transfected with pLL3.7.1 on DIV14 to express DsRed as volume marker, fixed on DIV17 and immunostained with antibodies to SNX6 and vesicular markers. DsRed is pseudocolored for presentation. White arrowheads indicate overlapped signals. (B) Quantification of colocalization in (A) from 45 dendritic segments of 15 neurons (mean ± SEM, N = 3. Total length of dendrites: 1568 µm for EEA1; 1447 µm for Rab5; 1637 µm for Rab4; 1489 µm for Rab7; 1319 µm for Rab11; 1207 µm for Golgi97 and 1462 µm for TGN46). (C) Mouse brain lysates were incubated with His-SNX1-N or His-SNX6-N immobilized on Ni-NTA agarose. Bound proteins were subjected to SDS-PAGE and mass spectrometry analysis. The table shows the number of Homer1b/c unique peptides identified by mass spec analysis and their sequence coverage. (D) Schematic representation of the domain structure of Homer1 isoforms and Homer1c fragments used in this study. (E) Upper panels: immunoblotting of bound proteins in (C). Lower panel: coomassie brilliant blue (CBB) stained SDS-PAGE gel shows purified recombinant proteins. (F) Mapping of SNX6-Homer1b/c interaction sites by in vitro binding assay. (G) In vitro binding assay of SNX6 and Homer family members. (H) Lysates from HEK293 cells overexpressing Flag-SNX6 and mEmerald-Homer1c were subjected to co-IP with Flag M2 beads, followed by immunoblotting with antibodies to Flag and Homer1b/c. (I) Total lysates and membrane fractions from mouse brain lysates were subjected to IP and immunoisolation with antibodies to Homer1b/c or SNX6, and antibodies to SNX6 coupled to Dynabeads Protein G, respectively. Shown are immunoblots probed with antibodies to SNX6, p150Glued, DIC, GluN1, GluN2A, GluN2B, Homer1b/c and Homer1a. (J) DIV18 neurons were immunostained with antibodies to Homer1b/c and SNX6. (K) Quantification of colocalization in (J) from 45 dendritic segments of 15 neurons (mean ± SEM, N = 3 independent experiments. Total length 1677 µm). (L)

DIV18 neurons were immunostained with antibodies to Homer1b/c and EEA1. (M) Quantification of colocalization in (L) from 45 dendritic segments of 15 neurons (mean ± SEM, N = 3. Total length 1459 µm). (N) DIV18 neurons were immunostained with antibodies to EEA1, Homer1b/c, and SNX6. Superresolution images were captured by structured illumination microscopy (SIM). White arrowheads indicate overlaps of signals from different channels. Bars: 2 µm.DOI:http://dx.doi.org/10.7554/eLife.20991.007 Colocalization analysis of superresolution images of triple-stained neurons captured by 3D-SIM (15 neurons for each immunostaining experiment).Unlike colocalization analysis of 2D confocal images of double-stained neurons in other figures, we adopted the methodology developed by Fletcher et al. to measure voxel-based colocalization between three fluorophores in the 3D images (Fletcher et al., 2010). (A) Quantification result of colocalization among EEA1, SNX6 and Homer1b/c in Figure 4N. (B) Quantification result of colocalization among p150Glued/DIC, SNX6 and Homer1b/c in Figure 6D. Overlaps of signals from three channels are shown as voxel colocalization values (%). The statistical significance of colocalization values was evaluated by estimation of those occur by chance in randomized images generated by Monte Carlo Simulation as described in (Fletcher et al., 2010) and the results are shown in Table 1.DOI:http://dx.doi.org/10.7554/eLife.20991.008 Figure provided by CiteAb. Source: Elife, PMID: 28134614.

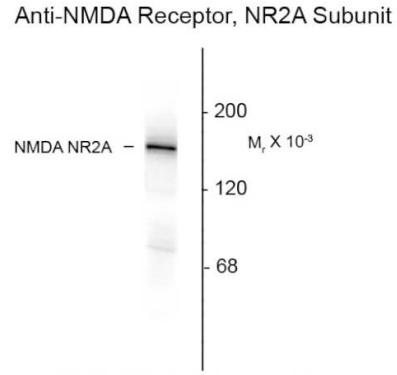
Immunohistochemistry

Immunohistochemistry of Rabbit anti-NMDA R2A antibody. Tissue: striatal and cortical neurons. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: NMDA R2A antibody at 1:500 for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: NMDA R2A is located in the neurons. Staining: NMDA R2A labeled red.

Anti-NMDA Receptor, NR2A Subunit



IHC staining of 21 DIV mouse striatal neurons (green) co-cultured with cortical neurons showing nice punctuate labeling (red) of the N-terminal NMDA NR2A subunit. Photo courtesy of Dr. A.J.Milnerwood, Dr. Lynn Raymond Lab, UBC.



Western blot of rat hippocampal lysate showing specific immunolabeling of the ~180k NR2A subunit of the NMDA receptor.

Western Blot
Western Blot of Rabbit anti-NMDA R2A antibody. Lane 1: rat hippocampal lysate. Lane 2: none. Load: 10 µg per lane. Primary antibody: NMDA R2A antibody at 1:1,000 for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 180 kDa for NMDA R2A. Other band(s): NMDA R2A splice variants and isoforms.

References

- Guo Z et al. Activity-dependent PI4P synthesis by PI4KIIIα regulates long-term synaptic potentiation. *Cell Rep.* (2022)
- Wang et al. Post-acute delivery of memantine promotes post-ischemic neurological recovery, peri-infarct tissue remodeling, and contralesional brain plasticity. *Journal of Cerebral Blood Flow & Metabolism* (2017)
- Niu et al. Ablation of SNX6 leads to defects in synaptic function of CA1 pyramidal neurons and spatial memory. *Elife* (2017)

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