

**Datasheet for 612-401-D89****NMDA R2A Antibody****Overview**

|                      |                                               |
|----------------------|-----------------------------------------------|
| <b>Description:</b>  | Anti-NMDA R2A (RABBIT) Antibody - 612-401-D89 |
| <b>Item No.:</b>     | 612-401-D89                                   |
| <b>Size:</b>         | 100 µL                                        |
| <b>Applications:</b> | WB, IF                                        |
| <b>Reactivity:</b>   | Rat                                           |
| <b>Host Species:</b> | 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  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) are known as kainate/AMPA receptors (K/AMPA). 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.

|                      |                                                                                                              |
|----------------------|--------------------------------------------------------------------------------------------------------------|
| <b>Synonyms:</b>     | Glutamate [NMDA] receptor subunit epsilon-1, N-methyl D-aspartate receptor subtype 2A, NMDAR2A, NR2A, Grin2a |
| <b>Host Species:</b> | Rabbit                                                                                                       |
| <b>Clonality:</b>    | Polyclonal                                                                                                   |
| <b>Format:</b>       | IgG                                                                                                          |

**Target Details**

**Gene Name:** Grin2a

|                            |                                                                                                                                                                                                                                                                     |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Reactivity:</b>         | Rat                                                                                                                                                                                                                                                                 |
| <b>Immunogen Type:</b>     | Conjugated Peptide                                                                                                                                                                                                                                                  |
| <b>Immunogen:</b>          | 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.                                                                                         |
| <b>Purity/Specificity:</b> | 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. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q00959</a></li><li>• <a href="#">GeneID - 24409</a></li><li>• <a href="#">UniProtKB - Q00959.2</a></li></ul>                                                                                        |

## Application Details

|                                |                                                                                                                                                                                                                                                                                                |
|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b>    | WB                                                                                                                                                                                                                                                                                             |
| <b>Suggested Applications:</b> | IF (Based on references)                                                                                                                                                                                                                                                                       |
| <b>Application Note:</b>       | 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. |
| <b>Assay Dilutions:</b>        | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                |
| <b>IHC:</b>                    | 1:500                                                                                                                                                                                                                                                                                          |
| <b>WB:</b>                     | 1:1000                                                                                                                                                                                                                                                                                         |

## Formulation

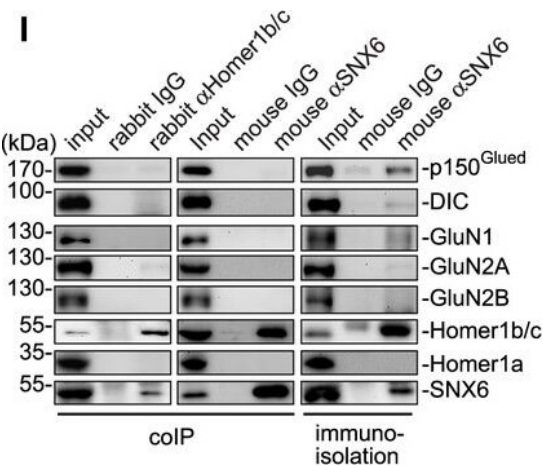
|                        |                                                                                  |
|------------------------|----------------------------------------------------------------------------------|
| <b>Physical State:</b> | Liquid                                               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

## Shipping & Handling

|                            |         |
|----------------------------|---------|
| <b>Shipping Condition:</b> | 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

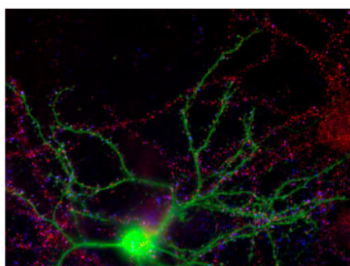


Western Blot

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  $\pm$  SEM, N = 3. Total length 1459  $\mu$ m). (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  $\mu$ m. 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.

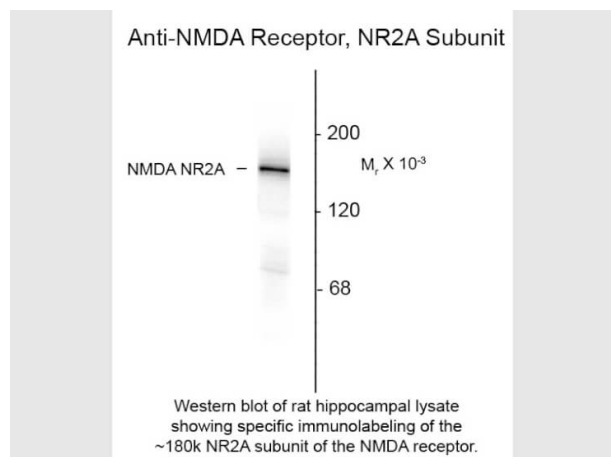
**Anti-NMDA Receptor, NR2A Subunit**



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.

### 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.



#### 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)

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.