SYSY Antibodies

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EAAT1 (GLAST) cytoplasmic domain

Cat.No. 250 113; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 µg specific antibody, lyophilized. Affinity purified with the immunogen. Albumin was added for stabilization. For **reconstitution** add 50 µl H_2O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Antibodies should be stored at +4°C when still lyophilized. Do not freeze! For detailed information, see back of the data sheet.
Applications	**WB**: 1 : 1000 up to 1 : 10000 (AP staining) **IP**: yes **ICC**: 1 : 1000 up to 1 : 5000 **IHC**: 1 : 1000 up to 1 : 5000 **IHC-P (FFPE)**: 1 : 1000
Immunogen	Synthetic peptide corresponding to AA 522 to 541 from rat EAAT1 (UniProt Id: P24942)
Reactivity	Reacts with: human (P43003), rat (P24942), mouse (P56564). Other species not tested yet.
Specificity	K.O. validated
Matching control	250-11P

TO BE USED IN VITRO / FOR RESEARCH ONLY
NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

Background

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. After the release of glutamate from synaptic vesicles into the synaptic cleft during neurotransmission, excitatory amino acid transporters (EAATs) remove extracellular glutamate to avoid excitotoxic levels (1).
Five EAATs with differential expression patterns have been described so far: **EAAT1**, also referred to as GLAST and SLC1A3, has neuroprotective potential following ischemia and occurs in reactive astrocytes and activated microglia. EAAT2 (GLT-1, SLC1A2) is the most abundant isoform and is primarily expressed in astrocytes. Both variants show high levels in brain and retina. EAAT3 / SLC1A1, EAAT4 / SLC1A6 and EAAT5 / SLC1A7 are expressed in neurons (2). EAAT4 shows weak expression in the forebrain and high levels in the cerebellum, where it mainly locates to Purkinje cells (3). EAAT5 primarily occurs in the retina, where it locates very close to glutamate release sites. In K.O. mice flicker resolution is considerably compromised (4). Recent findings suggest that EAAT5 is an abundant isoform, expressed also in non-neuronal peripheral tissues (5).

Selected References for 250 113

Induction of Survival of Motor Neuron (SMN) Protein Deficiency in Spinal Astrocytes by Small Interfering RNA as an In Vitro Model of Spinal Muscular Atrophy.
Leo M, Schmitt LI, Fleischer M, Steffen R, Osswald C, Kleinschnitz C, Hagenacker T
Cells (2022) 113: . . **ICC, IHC; tested species: mouse**

Spinal astrocyte dysfunction drives motor neuron loss in late-onset spinal muscular atrophy.
Schmitt LI, David C, Steffen R, Hezel S, Roos A, Schara-Schmidt U, Kleinschnitz C, Leo M, Hagenacker T
Acta neuropathologica (2023) : . . **WB, IHC; tested species: mouse**

Ataxia-linked SLC1A3 mutations alter EAAT1 chloride channel activity and glial regulation of CNS function.
Wu Q, Akhter A, Pant S, Cho E, Zhu JX, Garner A, Ohyama T, Tajkhorshid E, van Meyel DJ, Ryan RM
The Journal of clinical investigation (2022) 1327: . . **ICC; tested species: drosophila**

Astrocyte dysfunction increases cortical dendritic excitability and promotes cranial pain in familial migraine.
Romanos J, Benke D, Pietrobon D, Zeilhofer HU, Santello M
Science advances (2020) 623: eaaz1584. . **WB; tested species: mouse**

Differences in glutamate uptake between cortical regions impact neuronal NMDA receptor activation.
Romanos J, Benke D, Saab AS, Zeilhofer HU, Santello M
Communications biology (2019) 2: 127. . **WB; tested species: mouse**

Selected General References

Phosphatidylcholine metabolism after transfer from lipid emulsions injected intravenously in rats. Implications for high-density lipoprotein metabolism.
Martins IJ et al. Biochim Biophys Acta (1989) PubMed:2804050

Climbing Fiber-Mediated Spillover Transmission to Interneurons Is Regulated by EAAT4.
Malhotra S et al. J Neurosci (2021) PubMed:34400517

Excitatory amino acid transporter EAAT5 improves temporal resolution in the retina.
Gehlen J et al. eNeuro (2021) PubMed:34772693

Excitatory amino acid transporter 5 is widely expressed in peripheral tissues.
Lee A et al. Eur J Histochem (2013) PubMed:23549460

Association of excitatory amino acid transporters, especially EAAT2, with cholesterol-rich lipid raft microdomains: importance for excitatory amino acid transporter localization and function.
Butchbach ME et al. J Biol Chem (2004) PubMed:15187084

Access the online factsheet including applicable protocols at https://sysy.com/product/250113 or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable without loss of quality at ambient temperatures for several weeks.

Storage of Sealed Vials after Delivery

- **Unlabeled** and **biotin-labeled antibodies** and **control proteins** should be stored at **4°C** before reconstitution. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.