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EAAT2 extracellular domain

Cat.No. 250 203; Polyclonal rabbit antibody, 50 µg specific antibody (lyophilized)

Data Sheet

Reconstitution/ Storage	50 μ g specific antibody, lyophilized. Affinity purified with the immunogen. Albumin and azide were added for stabilization. For reconstitution add 50 μ l H ₂ O 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 (AP staining) IP: not tested yet ICC: 1: 500 (see remarks) IHC: 1: 1000 up to 1: 2000 (see remarks) IHC-P: 1: 500 (see remarks)
Immunogen	Synthetic peptide corresponding to AA 146 to 161 from mouse EAAT2 (UniProt Id: P43006)
Reactivity	Reacts with: rat (P31596), mouse (P43006). Other species not tested yet. Predicted to cross-react with human (P43004) due to high sequence homology.
Specificity	K.O. validated
Matching control	250-2P
Remarks	ICC: This antibody yields excellent results on mouse cells, but is not recommended for rat samples, as it produces non-specific nuclear staining in those specimens. IHC: This antibody yields excellent results on mouse tissue but is not recommended for rat samples, as it produces non-specific nuclear staining in those specimens.
	Fixation with 4% PFA and 0,1% glutaraldehyde according to <u>Danbolt et al. 1998</u> is recommended. IHC-P: This antibody yields excellent results on mouse tissue but is not recommended for rat samples, as it produces non-specific nuclear staining in those specimens.

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 250 203

Chronic Toxoplasma infection is associated with distinct alterations in the synaptic protein composition.

Lang D, Schott BH, van Ham M, Morton L, Kulikovskaja L, Herrera-Molina R, Pielot R, Klawonn F, Montag D, Jänsch L, Gundelfinger ED. et al.

Journal of neuroinflammation (2018) 151: 216. . WB, IHC; tested species: mouse

Pentylenetetrazole-induced Seizure Susceptibility in the Tau58/4 Transgenic Mouse Model of Tauopathy.

Van Erum J, Valkenburg F, Van Dam D, Paul De Deyn P Neuroscience (2019):.. **IHC-P; tested species: mouse**

Region-specific changes in gene expression are associated with cognitive deficits in the alpha-synuclein-induced model of Parkinson's disease: A transcriptomic profiling study.

Manchinu MF, Pala M, Palmas MF, Diana MA, Maschio A, Etzi M, Pisanu A, Diana FI, Marongiu J, Mansueto S, Carboni E, et al. Experimental neurology (2024) 372: 114651. IHC; tested species: rat

Crym-positive striatal astrocytes gate perseverative behaviour.

Ollivier M, Soto JS, Linker KE, Moye SL, Jami-Alahmadi Y, Jones AE, Divakaruni AS, Kawaguchi R, Wohlschlegel JA, Khakh BS Nature (2024):..IHC; tested species: mouse

Depressed glutamate transporter 1 expression in a mouse model of Dravet syndrome. Hameed MQ, Hui B, Lin R, MacMullin PC, Pascual-Leone A, Vermudez SAD, Rotenberg A Annals of clinical and translational neurology (2023) 109: 1695-1699. WB; tested species: mouse

Glutamate transporters EAAT2 and EAAT5 differentially shape synaptic transmission from rod bipolar cell terminals.

Tang FS, Yuan HL, Liu JB, Zhang G, Chen SY, Ke JB

eNeuro (2022):.. IHC; tested species: mouse

Glioblastoma hijacks neuronal mechanisms for brain invasion.

Venkataramani V, Yang Y, Schubert MC, Reyhan E, Tetzlaff SK, Wißmann N, Botz M, Soyka SJ, Beretta CA, Pramatarov RL, Fankhauser L, et al.

Cell (2022):.. IHC; tested species: mouse

Rapid recycling of glutamate transporters on the astroglial surface.

Michaluk P, Heller JP, Rusakov DA

eLife (2021) 10: . . WB; tested species: mouse

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

Access the online factsheet including applicable protocols at https://sysy.com/product/250203 or scan the QR-code.



FAQ - How should I store my antibody?

Shipping Conditions

 All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freezedried) and are stable in this form 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. They must not be stored in the freezer when still lyophilized!
 Temperatures below zero may cause loss of performance.
- Fluorescence-labeled antibodies should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle
 between freezing and thawing (to reduce frost-build-up), which is exactly what should be
 avoided. For the same reason, antibody vials should be placed in an area of the freezer that
 has minimal temperature fluctuations, for instance towards the back rather than on a door
 shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl)
 and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock
 concentration is affected by evaporation and adsorption of the antibody to the surface of the
 storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of
 activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

• Store at -20°C to -80°C.

Monoclonal Antibodies

- Ascites and hybridoma supernatant should be stored at -20°C up to -80°C. Prolonged storage at 4°C is not recommended! Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Polyclonal Antibodies

- Crude antisera: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- Affinity purified antibodies: Less robust than antisera. Storage at -20°C up to -80°C is
 recommended. Adding a carrier protein like BSA will increase long term stability. Most of our
 antibodies already contain carrier proteins. Please refer to the data-sheet for detailed
 information.

Fluorescence-labeled Antibodies

• Store as a liquid with 1:1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add
 the amount of deionized water given in the respective datasheet. If higher volumes are
 preferred, add water as mentioned above and then the desired amount of PBS and a
 stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies
 already contain albumin. Take this into account when adding more carrier protein.
 For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the
 solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled
 with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1:1 (v/v) glycerol to a final
 concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in
 liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freezethaw cycles.
- Please refer to our tips and hints for subsequent storage of reconstituted antibodies and control peptides and proteins.