

EAAT1 cytoplasmic domain

Cat.No. 250-11P; control peptide, 100 µg peptide (lyophilized)

Data Sheet

Reconstitution/ Storage	100 µg peptide, lyophilized. For reconstitution add 100 µl H ₂ O to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use. Control peptides should be stored at -20°C when still lyophilized! For detailed information, see back of the data sheet.
Immunogen	Synthetic peptide corresponding to AA 522 to 541 from rat EAAT1 (UniProt Id: P24942)
Recommended dilution	Optimal concentrations should be determined by the end-user.
Matching antibodies	250 113, 250 114, 250 116
Remarks	This control peptide consists of the synthetic peptide (aa 522 - 541 of rodent EAAT 1) that has been used for immunization. It has been tested in preadsorption experiments and blocks efficiently and specifically the corresponding signal in Western blots. The amount of peptide needed for efficient blocking depends on the titer and on the affinity of the antibody to the antigen.

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. Five EAATs with differential expression patterns have been described so far: **EAAT 1**, also referred to as **GLAST** and **SLC1A3**, has neuroprotective potential following ischemia and is expressed by reactive astrocytes and activated microglia. **EAAT 2 (GLT-1, SLC1A2)** is the most abundant and primarily expressed in astrocytes. **EAAT 3 / SLC1A1** is expressed in neurons and has also been shown to be involved in the uptake of extracellular cysteine. EAAT 4 shows weak expression in the forebrain and high levels in Purkinje cells of the cerebellum. EAAT 5 has only been described for humans and is primarily expressed in the retina.

Selected General References

Specificity controls for immunocytochemistry: the antigen preadsorption test can lead to inaccurate assessment of antibody specificity.

Holmseth S, Zhou Y, Follin-Arbelet VV, Lehre KP, Bergles DE, Danbolt NC
The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society (2012) 603: 174-87. .

Reactive astrocytes and activated microglial cells express EAAT1, but not EAAT2, reflecting a neuroprotective potential following ischaemia.

Beschorner R, Simon P, Schauer N, Mittelbronn M, Schluesener HJ, Trautmann K, Dietz K, Meyermann R
Histopathology (2007) 507: 897-910. .

Expression of EAAT1 reflects a possible neuroprotective function of reactive astrocytes and activated microglia following human traumatic brain injury.

Beschorner R, Dietz K, Schauer N, Mittelbronn M, Schluesener HJ, Trautmann K, Meyermann R, Simon P
Histology and histopathology (2007) 225: 515-26. .

Binding and transport of [3H](2S,4R)- 4-methylglutamate, a new ligand for glutamate transporters, demonstrate labeling of EAAT1 in cultured murine astrocytes.

Apricò K, Beart PM, Crawford D, O'Shea RD
Journal of neuroscience research (2004) 756: 751-9. .

Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice.

Watake K, Hashimoto K, Kano M, Yamada K, Watanabe M, Inoue Y, Okuyama S, Sakagawa T, Ogawa S, Kawashima N, Hori S, et al.
The European journal of neuroscience (1998) 103: 976-88. .

Identification of functional domains of the human glutamate transporters EAAT1 and EAAT2.

Mitrovic AD, Amara SG, Johnston GA, Vandenberg RJ
The Journal of biological chemistry (1998) 27324: 14698-706. .

Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1.

Tanaka K, Watake K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, et al.
Science (New York, N.Y.) (1997) 2765319: 1699-702. .

The mouse and human excitatory amino acid transporter gene (EAAT1) maps to mouse chromosome 15 and a region of syntenic homology on human chromosome 5.

Kirschner MA, Arriza JL, Copeland NG, Gilbert DJ, Jenkins NA, Magenis E, Amara SG
Genomics (1994) 223: 631-3. .

High-affinity glutamate transporter GLAST/EAAT1 regulates cell surface expression of glutamine/neutral amino acid transporter ASCT2 in human fetal astrocytes.

Gegelashvili M, Rodriguez-Kern A, Pirozhkova I, Zhang J, Sung L, Gegelashvili G
Neurochemistry international () 486-7: 611-5. .

Access the online factsheet including applicable protocols at <https://sysy.com/product/250-11P> 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 freeze-dried) 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 at 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 freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.