ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Excitatory Amino Acid Transporter 4 (EAAT4) Antibodies

Cat # EAAT41-P	Rat EAAT4 control/blocking peptide #1	**SIZE**: 100 ug
Cat # EAAT41-S	Rabbit Anti-Rat EAAT4 antiserum #1	**SIZE**: 100 ul
Cat # EAAT41-A	Rabbit Anti-Rat EAAT4 IgG #1, aff pure	**SIZE**: 100 ug

Glutamate is the main excitatory neurotransmitter in the brain. To date five glutamate Transporters have been cloned: **GLAST (EAAT1), GLT1 (EAAT2), EAAC1 (EAAT3), EAAT4, and EAAT5**. These transporters are believed to be critical in reducing potentially toxic extracellular concentration of glutamate by rapid uptake into nerve terminals and glial cells. Glutamate transporters (525-573 AA) display about 55% homology and are predicted to contain up to 6-10 transmembrane domains. Immunolocalization studies indicate that **GLT1** is localized in astroglial cells throughout the brain and spinal cord. **EAAC1** is specific for certain neurons and purkinje cells, and specifically enriched in cortex, hippocampus, and caudate-putamen and confined to presynaptic and postsynaptic elements. It is also expressed in kidney, heart, lung and muscle. **GLAST** has been observed in both neuron and astroglia. It is most abundant in Bergmann glia, cortex, hippocampus and cerebellum. **EAAT4** has properties of ligand gated Cl-channel. It is localized mainly in cerebellar Purkinje cells in rat and human CNS. **EAAT5** has only been cloned from human. It is primarily expressed in retina.

Source of Antigen and Antibodies

Antigen	21-aa peptide from **rat EAAT4 (1)**; **Designation (EAAT41-P, control/blocking peptide**) conjugated to KLH. **Epitope location** ~ C-terminus, Cytoplasmic domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**EAAT41**-S) and IgG, purified over antigen-agarose (Cat # **EAAT41**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
□ 100 ul □ Solution □ Lyophilized powder
Supplied in Buffer: 0.1% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
□ 100 ug/100 ul □ Solution □ Lyophilized powder
Supplied in **Buffer**: PBS + 0.1% BSA
Reconstitute powder in PBS at 1 mg/ml

Control/blocking peptide
□ 100 ug/100 ul □ Solution □ Lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml

Storage

Short-term: unopened, undiluted liquid vials at 20°C and powder at 4°C or -20°C..

Long-term: at –20°C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20°C or below.
Shipping: 4°C for solutions and room temp for powder

Recommended Usage

Western Blotting We recommend the use of 1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using Chemiluminescence technique (2). EAAT4 monomers in freshly made rat brain extracts appeared as fuzzy ~65 kDa protein (2). High mol. Wt bands may be see after oxidation (2). (not tested)

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: we recommend the use of affinity purified IgG at 2-10 ug/ml in paraformaldehyde fixed sections of tissues (2). Not tested

Specificity & Cross-reactivity

The EAAT41-P peptide was found unique to EAAT4 without significant homology to other glutamate transporters. It is 95% homologous in Mouse**,** 90% in human and 85% in dog EAAT4. Antibody crossreactivity in various species is not established. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at:the web site).

General References:

Lin C (1997) gene accession # g3023680; Maeno-Hikichi Y (1997) Brain Res. Mol. Brain. Res. 48, 176-180; Fairbanks WA et a (1995) Nature 375, 599-603. Dehnes Y (1998) J Neurosci. 18, 3606-3619;

(2) Citations of ADI's Antibodies (see web site for updated list)

Levenson J, 2002 Nature Neuroscience 5, 155 – 161, WB,
Petralia RS, 2004 Eur. J. Neurosci. 19, 2017-2029, IHC
Pignataro L, 2005, Mol. Cell. Neurosc. 28, 440-451, WB, IHC IP
Dunlop, J 1999, Brain Res. 839, 235-242 WB,

Rodriguez-Kern A 2003, Neurochem Intl. 43, 363, WB

**This product is for In vitro research use only.*

Related material available from ADI
EAAT41-S-A-P 180228AC

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