

Product Specification Sheet

EAT-4 Antibodies

Cat # EAT45-P	Rat EAT-4 control/blocking peptide #1	SIZE: 100 ug
Cat # EAT45-S	Rabbit Anti-Rat EAT-4 antiserum # 1	SIZE: 100 ul
Cat # EAT45-A	Rabbit Anti-Rat EAT-4 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. Most recently, vesicular type transporters for glutamate, termed **VGLUTs (VGLUT1/BNPI, VGLUT2/DNPI, and EAT-4)**, have been cloned and characterized that are related to phosphate transporters. Although neurons exhibit Na-dependent Pi transport, the biological role of Pi uptake is not clear. Proximal tubules in the kidney reabsorb Pi in the glomeruli by the action of a group of phosphate transporters (**Type 1-NaPi** related, **type 2-NaPi-2** related, and **type 3-viral** receptor-related). These receptors show weak (~20% identity) between various subtypes. A distinct type of **brain specific Na⁺-dependent phosphate (Pi) transporter (BNPI)**, originally characterized as a plasma membrane transporter has been localized in a subset of glutamatergic neurons (amygdala, cerebellar cortex, and hippocampus) and identified as **VGLUT1** (rat and human 560 aa; ~60 kDa, ~30% homology with type-1 Pi-transporters). VGLUT1 shows strong sequence homology to **EAT-4**, a C. elegans protein that appears to have specific presynaptic role in glutamatergic transmission. Loss of function mutations in EAT-4 affects multiple glutamatergic neurotransmission pathways. EAT4 encodes a protein of 563 aa (~48% identity with BNPI and ~30% identity with known NaPi-transporters found in mammalian kidney). Like BNPI, EAT-4 is also expressed in glutamatergic neurons.

Source of Antigen and Antibodies

Antigen	An 18 AA synthetic peptide (designated EAT45-P; control peptide). synthesized, conjugated to KLH. Epitope location ~N-terminus of C. elegans EAT-4
Ab Host/type	Rabbit, polyclonal Unpurified antiserum (cat # EAT45-S), and Aff pure IgG1 (cat # EAT45-S -A) purified over the antigen column
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)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG

100 ug/100ul solution lyophilized powder

Supplied in **Buffer:** PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/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 1:1K-5K for antiserum and 1-10 ug/ml for affinity pure antibody using Chemiluminescence technique. EAT-4 is ~65 kDa protein.

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: not tested.

Specificity & Cross-reactivity

The EAT45-P peptide has no significant sequence with mammalian VGLUT1/2 or other NaPi-related (Type-1 to Type-III) transporters. 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:

(1) Lee RYN et al (1999) J. Neurosci. 19, 159-167

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

Related material available from ADI

Anti-GLAST, EAAC1, GLT1, EAAT4, EAAT5, GTRAP41, EAT45, VGLUT1/BNPI, VGLUT-2/DNPI & GABA Transporters (GAT1-3)

Ant-NaPi-I, NaPi-II, NaPi-III, GTRAP41, GTRAP48

EAT45-S-A-P 71224S

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