abcam

Product datasheet

Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free ab288450



16 Images

Overview

Product name Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free

Description Rabbit monoclonal [EPR25149-62] to EAAT3 - BSA and Azide free

Host species Rabbit

Specificity ICC application does not react with Rat species

Tested applications Suitable for: WB, IHC-Fr, IHC-P, ICC/IF, IP

Unsuitable for: Flow Cyt (Intra)

Species reactivity Reacts with: Mouse, Rat

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse kidney, Mouse brain, Rat hippocampus and Rat brain lysates. IHC-P: Mouse kidney,

> Mouse hippocampus, Rat kidney and Rat cerebrum tissues. IHC-Fr: Mouse cerebellum, Rat hippocampus, Mouse kidney and Rat kidney tissues. ICC: Mouse primary neuron cells. IP: Mouse

hippocampus and Rat hippocampus cells.

General notes Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of

BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-

based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP,

biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the

need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C.

Storage buffer pH: 7.2

Constituent: 100% PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR25149-62

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab288450 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 57 kDa.
IHC-Fr		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt (Intra).

Target

Function Transports L-glutamate and also L- and D-aspartate. Essential for terminating the postsynaptic

action of glutamate by rapidly removing released glutamate from the synaptic cleft. Acts as a

symport by cotransporting sodium. Negatively regulated by ARL6IP5.

Tissue specificity Expressed in all tissues tested including liver, muscle, testis, ovary, retinoblastoma cell line,

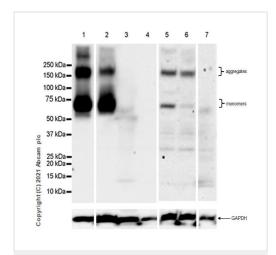
neurons and brain (in which there was dense expression in substantia nigra, red nucleus,

hippocampus and in cerebral cortical layers).

Sequence similarities Belongs to the sodium:dicarboxylate (SDF) symporter (TC 2.A.23) family. SLC1A1 subfamily.

Post-translational Glycosylated.

Images



Western blot - Anti-EAAT3 antibody [EPR25149-62]
- BSA and Azide free (ab288450)

All lanes : Anti-EAAT3 antibody [EPR25149-62] (**ab288441**) at 1/1000 dilution

Lane 1: Mouse kidney tissue lysate

Lane 2: Mouse brain tissue lysate

Lane 3: Mouse spleen tissue lysate

Lane 4: Mouse testis tissue lysate

Lane 5: Rat hippocampus tissue lysate

Lane 6: Rat brain tissue lysate

Lane 7: Rat testis tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 57 kDa

Observed band size: 150-250,50-75 kDa

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

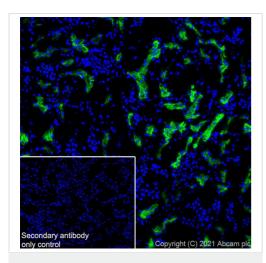
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: Lanes 1-4: 26 seconds; Lanes 5-7: 3 minutes

Monomers: 50-75 kda; Aggregates: 150-250 kda

The MW and expression pattern is consistent to the literature

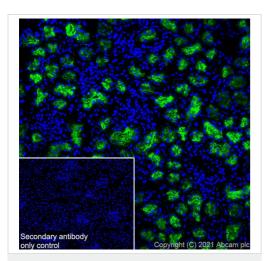
(PMID: 30840898)



Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat kidney (fresh) tissue labeling EAAT3 with **ab288441** at 1/500 (0.982 ug/ml) dilution followed by **ab150081** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green). Positive staining on the proximal convoluted tubule of rat kidney is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution.

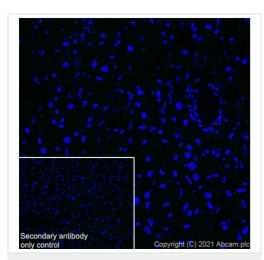


Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

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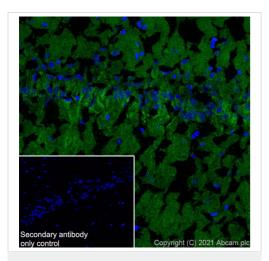
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Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat liver (fresh) (-) tissue labeling EAAT3 with ab288441 at 1/50 (9.82 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green). No staining on rat liver (Negative control: PMID 11242046) is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution.

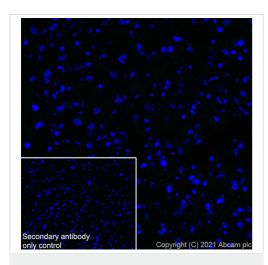


Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat hippocampus (fresh) tissue labeling EAAT3 with <u>ab288441</u> at 1/50 (9.82 ug/ml) dilution followed by <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green). Positive staining on rat hippocampus is observed. The nuclear counterstain was DAPI (Blue).

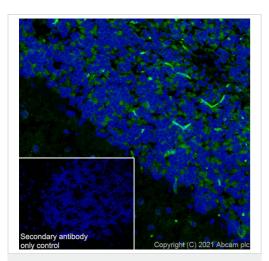
Secondary antibody control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

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Secondary antibody control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution.

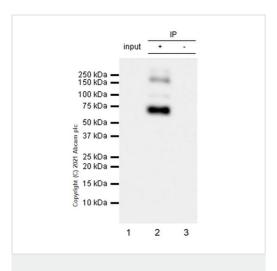


Immunohistochemistry (Frozen sections) - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

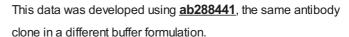
This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse cerebellum (fresh) tissue labeling EAAT3 with ab288441 at 1/50 (9.82 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/mL) dilution (Green). Positive staining on mouse cerebellum is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) preadsorbed at 1/1000 (2 ug/mL) dilution.



Immunoprecipitation - Anti-EAAT3 antibody
[EPR25149-62] - BSA and Azide free (ab288450)



EAAT3 was immunoprecipitated from 0.35 mg Rat hippocampus tissue lysate 10 ug with <u>ab288441</u> at 1/30 dilution (2ug in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using <u>ab288441</u> at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

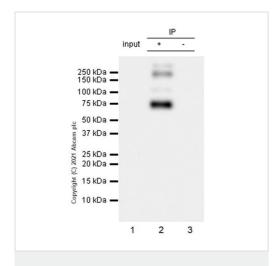
Lane 1: Rat hippocampus tissue lysate 10 ug

Lane 2: ab288441 IP in Rat hippocampus tissue lysate

Lane 3: Rabbit monoclonal $\lg G \left(\underline{ab172730} \right)$ instead of $\underline{ab288441}$ in rat hippocampus tissue lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 8 seconds



Immunoprecipitation - Anti-EAAT3 antibody
[EPR25149-62] - BSA and Azide free (ab288450)

This data was developed using the same antibody clone in a different buffer formulation.

EAAT3 was immunoprecipitated from 0.35 mg Mouse hippocampus tissue lysate 10 ug with <u>ab288441</u> at 1/30 dilution (2 ug in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using <u>ab288441</u> at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

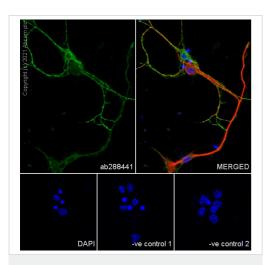
Lane 1: Mouse hippocampus tissue lysate 10 ug

Lane 2: ab288441 IP in Mouse hippocampus tissue lysate

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab288441}$ in mouse hippocampus tissue lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 8 seconds



Immunocytochemistry/ Immunofluorescence - Anti-EAAT3 antibody [EPR25149-62] - BSA and Azide free (ab288450)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EAAT3 antibody

[EPR25149-62] - BSA and Azide free (ab288450)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neuron cells labelling EAAT3 with ab288441 at 1/100 (4.91 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2ug/ml) dilution (Green). Confocal image showing cytoplasmic staining in mouse primary neuron cells. Confocal scanning Z step was set as 0.3 um followed by image processing with maximum Z projection. ab11267 Anti-MAP2 mouse monoclonal antibody was used to counterstain neuron-specific cytoskeletal protein at 1/500 (4ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

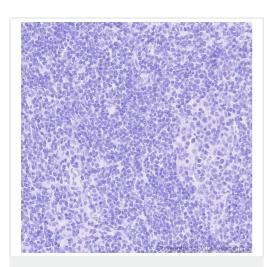
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2ug/ml) dilution.

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labelling EAAT3 with <u>ab288441</u> at 1/1000 (0.491 ug/ml) followed by a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used. Negative control: no staining on rat testis.The section was incubated with <u>ab288441</u> for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (BOND $^{\text{TM}}$, Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



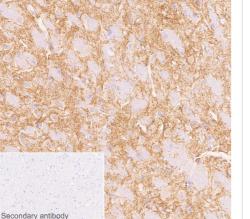
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EAAT3 antibody

[EPR25149-62] - BSA and Azide free (ab288450)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labelling EAAT3 with <u>ab288441</u> at 1/1000 (0.491 ug/ml) followed by a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used. Negative control: no staining on mouse spleen.The section was incubated with <u>ab288441</u> for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EAAT3 antibody

[EPR25149-62] - BSA and Azide free (ab288450)

only control

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labelling EAAT3 with <u>ab288441</u> at 1/1000 (0.491 ug/ml) followed by a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used. Cytoplasmic staining on rat cerebrum (PMID: 11242046). The section was incubated with <u>ab288441</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EAAT3 antibody

[EPR25149-62] - BSA and Azide free (ab288450)

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labelling EAAT3 with <u>ab288441</u> at 1/1000 (0.491 ug/ml) followed by a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used. Apical staining on proximal convoluted tubules in rat kidney (PMID: 9435692).The section was incubated with <u>ab288441</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EAAT3 antibody

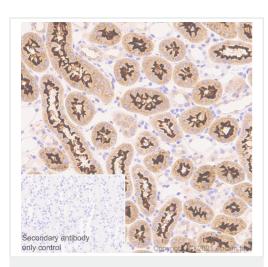
[EPR25149-62] - BSA and Azide free (ab288450)

This data was developed using <u>ab288441</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse hippocampus tissue labelling EAAT3 with <u>ab288441</u> at 1/1000 (0.491 ug/ml) followed by a ready to use Leica DS9800 (BOND™, Polymer Refine Detection) was used. Cytoplasmic staining on mouse hippocampus (PMID: 11242046).The section was incubated with <u>ab288441</u> for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

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