Anti-GABA Transporter 1 / GAT 1 Antibody [JE34-61]

HA721496

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 67 kDa
Clone number:	JE34-61

Description: GABA transporter 1 (GAT1) also known as sodium- and chloride-dependent GABA transporter 1 is a protein that in humans is encoded by the SLC6A1 gene and belongs to the solute carrier 6 (SLC6) family of transporters. It mediates gamma-aminobutyric acid's translocation from the extracellular to intracellular spaces within brain tissue and the central nervous system as a whole. GAT1 is a gamma-aminobutyric acid (GABA) transporter, which removes GABA from the synaptic cleft by shuttling it to presynaptic neurons (where GABA can be recycled) and astrocytes (where GABA can be broken down). GABA Transporter 1 uses energy from the dissipation of a Na+ gradient, aided by the presence of a Cl− gradient, to translocate GABA across CNS neuronal membranes. The stoichiometry for GABA Transporter 1 is 2 Na+: 1 Cl−: 1 GABA. The presence of a Cl−/Cl− exchange is also proposed because the Cl− transported across the membrane does not affect the net charge. GABA is also the primary inhibitory neurotransmitter in the cerebral cortex and has the highest level of expression within it. The GABA affinity (Km) of the mouse isoform of GAT1 is 8 μM. In the brain of a mature mammal, glutamate is converted to GABA by the enzyme glutamate decarboxylase (GAD) along with the addition of vitamin B6. GABA is then packed and released into the post-synaptic terminals of neurons after synthesis. GABA can also be used to form succinate, which is involved in the citric acid cycle. Vesicle uptake has been shown to prioritize newly synthesized GABA over preformed GABA, though the reasoning behind this mechanism is currently not completely understood. The regulation of the modular functioning of GATs is highly dependent on a multitude of second messengers and synaptic proteins.

Immunogen: Synthetic peptide within Human GABA Transporter 1/ GAT 1 aa 1-52.

Positive control: Mouse cerebellum tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, human brain tissue, human liver tissue, mouse cerebellum tissue, mouse retina tissue, rat brain tissue, rat retina tissue.

Subcellular location: Cell membrane, Presynapse.

Database links: SwissProt: P30531 Human | P31648 Mouse | P23978 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:500-1:2,000
IHC-Fr	1:500
IF-Tissue	1:500

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

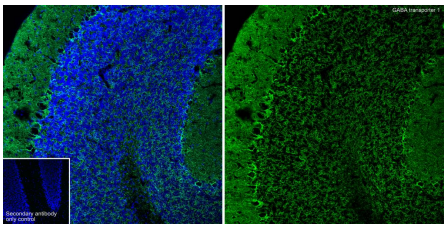

Fig1: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721496, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

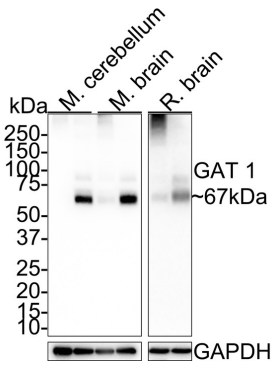

Fig2: Western blot analysis of GABA Transporter 1 / GAT 1 on different lysates with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/1,000 dilution.

Lane 1: Mouse cerebellum tissue lysate
Lane 2: Mouse cerebellum tissue lysate (70℃ heat)
Lane 3: Mouse brain tissue lysate
Lane 4: Mouse brain tissue lysate (70℃ heat)
Lane 5: Rat brain tissue lysate
Lane 6: Rat brain tissue lysate (70℃ heat)

Lysates/proteins at 30 µg/Lane.

Predicted band size: 67 kDa
Observed band size: 67 kDa

Exposure time: Lane 1-4: 1 minute 40 seconds; Lane 5-6: 5 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721496) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

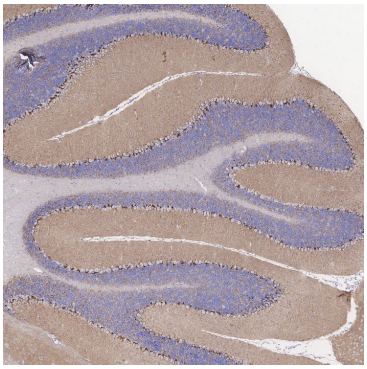

Fig3: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

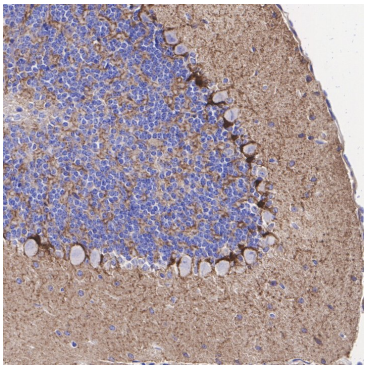

Fig4: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

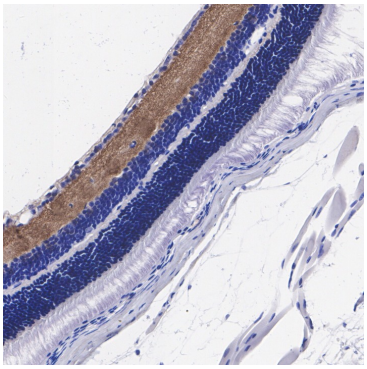

Fig5: Immunohistochemical analysis of paraffin-embedded mouse retina tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

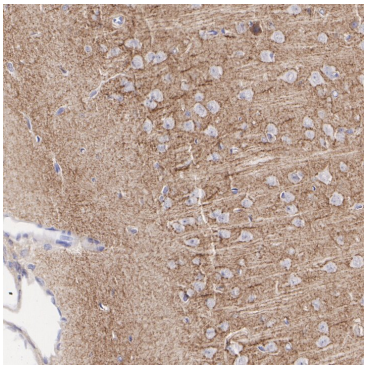

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

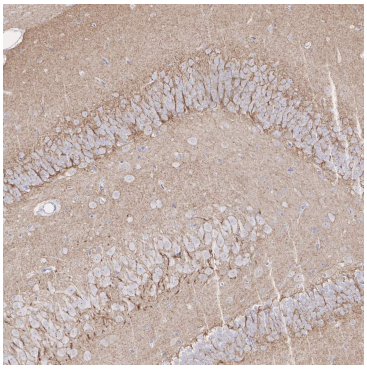

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

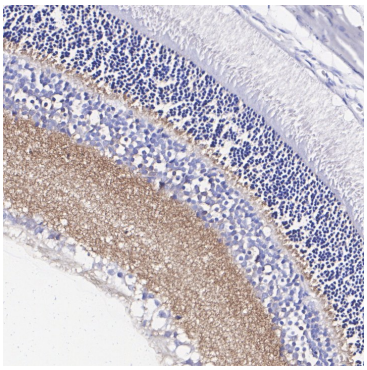

Fig8: Immunohistochemical analysis of paraffin-embedded rat retina tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

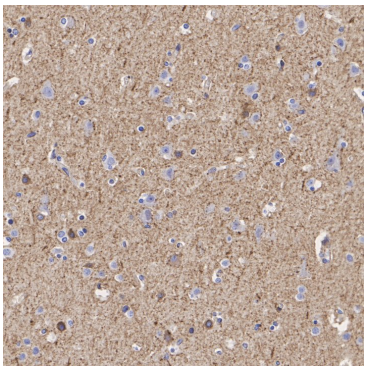

Fig9: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

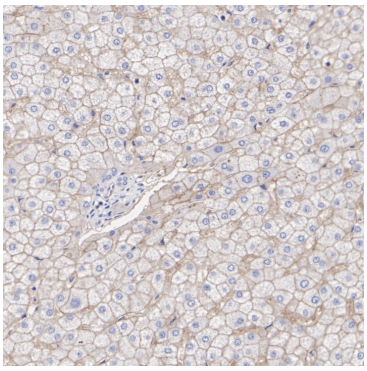

Fig10: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721496) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

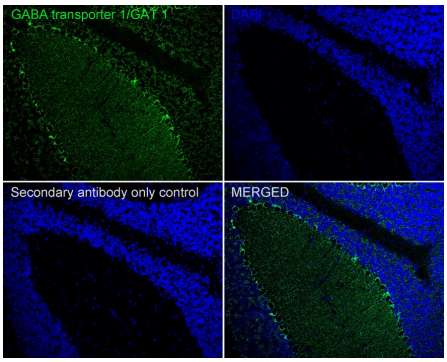

Fig11: Immunofluorescence analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721496, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

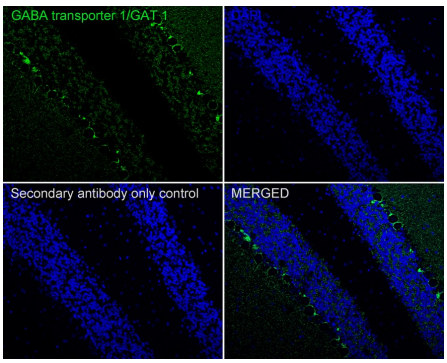

Fig12: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721496, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

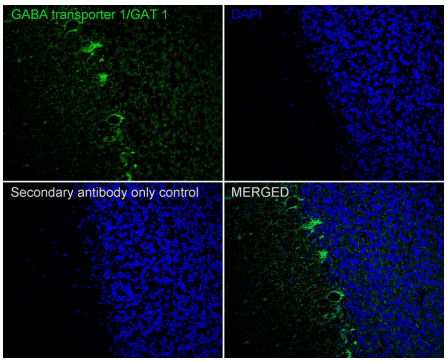

Fig13: Immunofluorescence analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721496, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Fattorini G et al. A Reappraisal of GAT-1 Localization in Neocortex. Front Cell Neurosci. 2020 Feb
2. Fattorini G et al. Microglial expression of GAT-1 in the cerebral cortex. Glia. 2020 Mar