GAD67 Recombinant Antibody [PSH08-30] - Rabbit IgG (Chimeric)

HA724362

Product Type:	Recombinant Chimeric, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, mIHC
Molecular Wt:	Predicted band size: 67 kDa
Clone number:	PSH08-30

Description: There are two forms of glutamic acid decarboxylases (GADs) that are found in the brain: GAD-65 (also known as GAD2) and GAD-67 (also known as GAD1, GAD or SCP). GAD-65 and GAD-67 are members of the group II decarboxylase family of proteins and are responsible for catalyzing the rate limiting step in the production of GABA (g-aminobutyric acid) from L-glutamic acid. Although both GADs are found in the brain, GAD-65 localizes to synaptic vesicle membranes in nerve terminals, while GAD-67 is distributed throughout the cell. GAD-67 is responsible for the basal levels of GABA synthesis. In the case of a heightened demand for GABA in neurotransmission, GAD-65 will transiently activate to assist in GABA production. The loss of GAD-65 is detrimental and can impair GABA neurotransmission, however the loss of GAD-67 is lethal. Due to alternative splicing, two isoforms exist for GAD-67, the predominant GAD-67 form and the minor GAD-25 form. GAD-25 is not expressed in brain but can be found in a variety of endocrine tissues.

Immunogen: Recombinant protein within human GAD67 aa 1-594.

Positive control: Mouse brain tissue lysate, Rat brain tissue lysate.

Subcellular location: Cytoplasm, Plasma Membrane.

Database links: SwissProt: Q99259 Human | P48318 Mouse | P18088 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:1,000
IF-Tissue	1:500
mIHC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

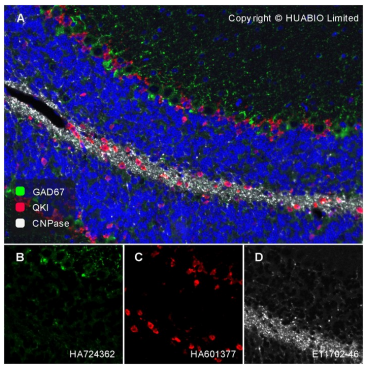


Fig1: mIHC analysis of mouse cerebellum tissue (paraffin-embedded) with GAD67 (HA724362, 1/1,000), QKI (HA601377, 1/500) and CNPase (ET1702-46, 1/2,000).

The immunostaining was performed with the IRISKitCmTSA Kit (900802). DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

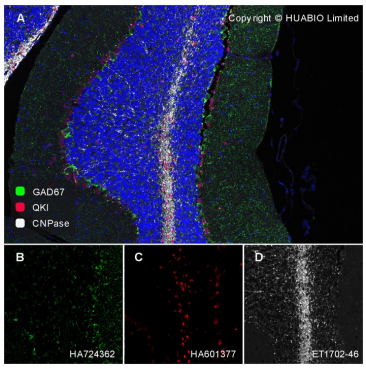


Fig2: mIHC analysis of rat cerebellum tissue (paraffin-embedded) with GAD67 (HA724362, 1/1,000), QKI (HA601377, 1/500) and CNPase (ET1702-46, 1/2,000).

The immunostaining was performed with the IRISKitCmTSA Kit (900802). DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

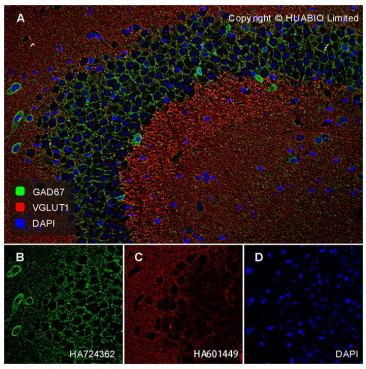


Fig3: Application: Immunofluorescence (IF-tissue)

Species: Mouse
Tissue: Hippocampus
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× PBST
Endogenous peroxidase blocking: 3% H2O2, 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: GAD67, 1/500 (HA724362, Rabbit, Green), VGLUT1, 1/500 (HA601449, Guinea pig, Red), overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), Goat anti-Guinea pig IgG (iFluor™ 594, HA1143), 1.5 hours at room temperature.

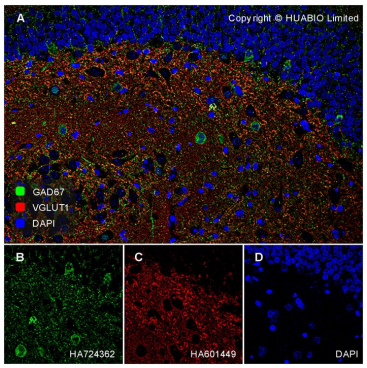


Fig4: Application: Immunofluorescence (IF-tissue)

Species: Rat
Tissue: Hippocampus
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× PBST
Endogenous peroxidase blocking: 3% H2O2, 10 minutes.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: GAD67, 1/500 (HA724362, Rabbit, Green), VGLUT1, 1/500 (HA601449, Guinea pig, Red), overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), Goat anti-Guinea pig IgG (iFluor™ 594, HA1143), 1.5 hours at room temperature.

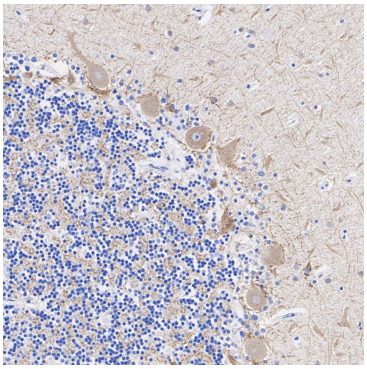


Fig5: Application: Immunohistochemistry (IHC-P)

Species: Human
Tissue: Cerebellum
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H O , 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724362, 1/1,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

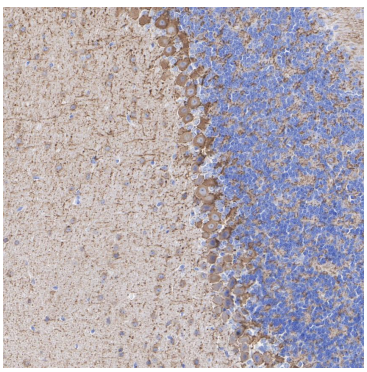


Fig6: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Cerebellum
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H O , 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

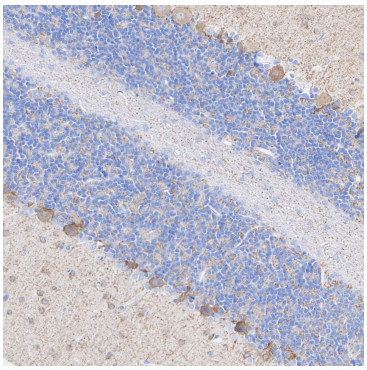


Fig7: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Cerebellum
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724362, 1/1,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

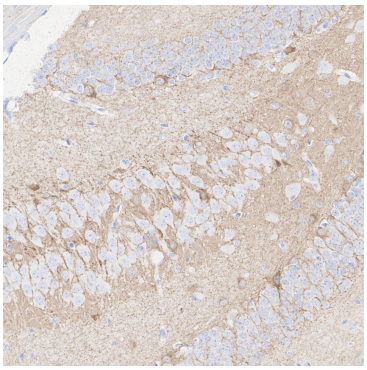


Fig8: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Hippocampus
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724362, 1/1,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

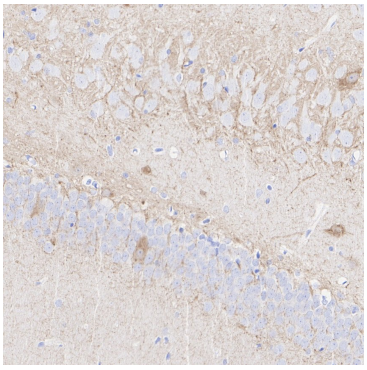
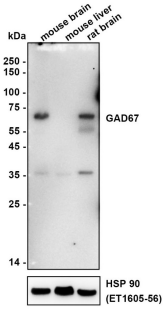


Fig9: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Hippocampus
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724362, 1/1,000, 1 hour at room



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Fig10: Western blot analysis of GAD67 on different lysates with Rabbit anti-GAD67 antibody (HA724362) at 1/5,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Mouse liver tissue lysate (negative)
Lane 3: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.
Exposure time: 3 minutes; ECL: K1802

Mouse liver is a negative control (PMID: 33968199).

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724362, 1/5,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Li JT et al. Repeated Blockade of NMDA Receptors During Adolescence Impairs Reversal Learning and Disrupts GABAergic Interneurons in Rat Medial Prefrontal Cortex. Front Mol Neurosci 9:17 (2016).
2. Fu Q et al. MHC-I promotes apoptosis of GABAergic interneurons in the spinal dorsal horn and contributes to cancer induced bone pain. Exp Neurol 286:12-20 (2016).