Anti-GAD67 Antibody [PSH08-30] - BSA and Azide free

HA610216

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IHC-P, IHC-Fr
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, mouse cerebellum tissue, rat cerebellum tissue.

Subcellular location: Cytoplasm, Plasma Membrane.

Database links: SwissProt: P48318 Mouse | P18088 Rat

Recommended Dilutions:

WB	1:10,000
IHC-P	1:500
IHC-Fr	1:500

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

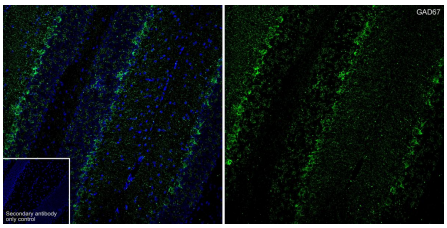

Fig1: Immunofluorescence analysis of frozen mouse cerebellum tissue with Mouse anti-GAD67 antibody (HA610216) at 1/500 dilution.

Important Notice: The section was pre-treated using 1% SDS buffer (in PBS, pH 7.4) for 5 minutes at room temperature.

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 (HA610216, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).

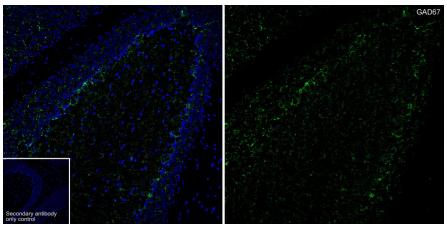

Fig2: Immunofluorescence analysis of frozen rat cerebellum tissue with Mouse anti-GAD67 antibody (HA610216) at 1/500 dilution.

Important Notice: The section was pre-treated using 1% SDS buffer (in PBS, pH 7.4) for 5 minutes at room temperature.

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 (HA610216, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/500 dilution. Nuclei were counterstained with DAPI (blue).

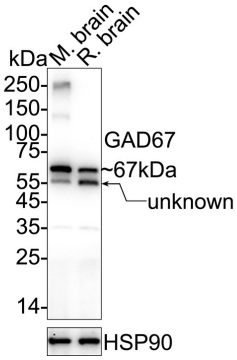

Fig3: Western blot analysis of GAD67 on different lysates with Mouse anti-GAD67 antibody (HA610216) at 1/10,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 6 seconds; ECL: K1801;

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 (HA610216) at 1/10,000 dilution was used in primary antibody dilution at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

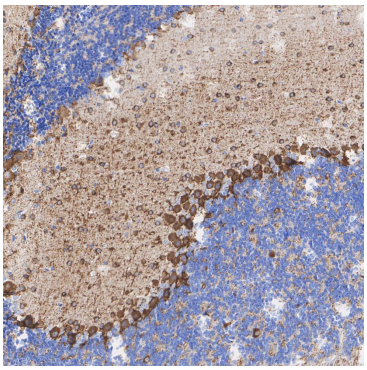

Fig4: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Mouse anti-GAD67 antibody (HA610216) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA610216) 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.

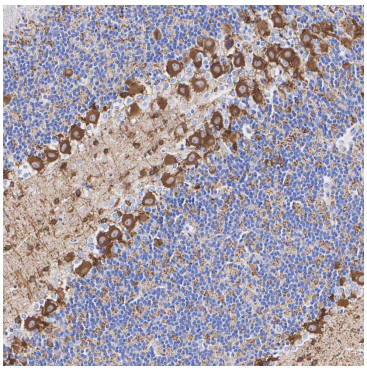

Fig5: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Mouse anti-GAD67 antibody (HA610216) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (HA610216) 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.

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).