

# GAD-65 (GAD-6): sc-32270

## BACKGROUND

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 ( $\gamma$ -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.

## REFERENCES

1. Chessler, S.D., et al. 2002. Immune reactivity to GAD-25 in type 1 diabetes mellitus. *Autoimmunity* 35: 335-341.
2. Kanter, I.C., et al. 2007. Cyclophosphamide for anti-GAD antibody-positive refractory status epilepticus. *Epilepsia* 49: 914-920.

## CHROMOSOMAL LOCATION

Genetic locus: GAD2 (human) mapping to 10p12.1; Gad2 (mouse) mapping to 2 A3.

## SOURCE

GAD-65 (GAD-6) is a mouse monoclonal antibody raised against purified rat brain GAD (glutamic acid decarboxylase).

## PRODUCT

Each vial contains 200  $\mu$ g IgG<sub>2a</sub> kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

## APPLICATIONS

GAD-65 (GAD-6) is recommended for detection of GAD-65 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2  $\mu$ g per 100-500  $\mu$ g of total protein (1 ml of cell lysate)] and immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

Suitable for use as control antibody for GAD-65 siRNA (h): sc-41964, GAD-65 siRNA (m): sc-41965, GAD-65 siRNA (r): sc-61888, GAD-65 shRNA Plasmid (h): sc-41964-SH, GAD-65 shRNA Plasmid (m): sc-41965-SH, GAD-65 shRNA Plasmid (r): sc-61888-SH, GAD-65 shRNA (h) Lentiviral Particles: sc-41964-V, GAD-65 shRNA (m) Lentiviral Particles: sc-41965-V and GAD-65 shRNA (r) Lentiviral Particles: sc-61888-V.

Molecular Weight of GAD-65: 65 kDa.

Positive Controls: rat cerebellum extract: sc-2398 or rat brain extract: sc-2392.

## RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## SELECT PRODUCT CITATIONS

1. Shao, J., et al. 2012. Phosphatidylcholine-specific phospholipase C/heat shock protein 70 (Hsp70)/transcription factor B-cell translocation gene 2 signaling in rat bone marrow stromal cell differentiation to cholinergic neuron-like cells. *Int. J. Biochem. Cell Biol.* 44: 2253-2260.
2. Yang, J.M., et al. 2013. Development of GABA circuitry of fast-spiking basket interneurons in the medial prefrontal cortex of erb4-mutant mice. *J. Neurosci.* 33: 19724-19733.
3. Zhang, Y., et al. 2016. Loss of MeCP2 in cholinergic neurons causes part of RTT-like phenotypes via  $\alpha 7$  receptor in hippocampus. *Cell Res.* 26: 728-742.
4. González-González, M.A., et al. 2017. Identification of novel cellular clusters define a specialized area in the cerebellar periventricular zone. *Sci. Rep.* 7: 40768.
5. Gonzalez-Reyes, L.E., et al. 2019. Sonic hedgehog is expressed by hilar mossy cells and regulates cellular survival and neurogenesis in the adult hippocampus. *Sci. Rep.* 9: 17402.
6. Kato, Y., et al. 2021. Involvement of MCH-oxytocin neural relay within the hypothalamus in murine nursing behavior. *Sci. Rep.* 11: 3348.

## STORAGE

Store at 4° C, \*\*DO NOT FREEZE\*\*. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

## CONJUGATES

See **GAD-65 (A-3): sc-377145** for GAD-65 antibody conjugates, including AC, HRP, FITC, PE, and Alexa Fluor® 488, 546, 594, 647, 680 and 790.