

Product Information

Monoclonal Anti-Glutamic Acid Decarboxylase 65 Clone GAD-6

produced in mouse, purified immunoglobulin

Catalog Number **G1166**

Synonym: Anti-GAD 65

Product Description

Monoclonal Anti-Glutamic Acid Decarboxylase 65 (mouse IgG2a isotype) is derived from the GAD-6 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with purified rat brain GAD. The antibody is expressed by hybridoma cell line grown in serum-free medium, and is then purified using Protein A chromatography.

Monoclonal Anti-Glutamic Acid Decarboxylase 65 specifically recognizes the lower molecular weight isoform of the two GAD isoforms identified in brain, GAD65. This monoclonal antibody can be used for immunohistochemical localization of GABA secreting neurons.¹ Anti-GAD has also been used to label purified GAD on Western blots.² Species reactivity includes all mammals tested, and some lower vertebrates.

Glutamic Acid Decarboxylase (GAD) catalyzes the conversion of L-glutamate to γ -aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, and a putative paracrine signal molecule in pancreatic islets.^{3,5} GAD has a restricted tissue distribution. It is highly expressed in the cytoplasm of GABAergic neurons in the central nervous system (CNS) and pancreatic β -cells, but is also present in other non-neuronal tissues such as testis, oviduct and ovary.³⁻⁷ GAD is also transiently expressed in non-GABAergic cells of the embryonic and adult nervous system, suggesting its involvement in development and plasticity.⁸ GAD exists as two isoforms, GAD65 and GAD67 (molecular masses of 65 and 67 kDa, respectively) that are encoded by two different genes.^{4,9,10} GAD65 is an amphiphilic, membrane-anchored protein, (585 amino acid residues) and is encoded on human chromosome 10. GAD67 is a cytoplasmic protein (594 amino acid residues) and is encoded on chromosome 2. There is 64% amino acid identity between the two isoforms, with the highest

diversity located at the N-terminus, which in GAD65 is required for targeting the enzyme to GABA-containing secretory vesicles. The two isoforms appear to have distinct intraneuronal distribution in the brain.¹¹ GAD65 has been identified as an autoantigen in insulin-dependent diabetes mellitus (IDDM) and stiff-man syndrome (SMS),^{12,13} IDDM is an autoimmune disease that results from T cell mediated destruction of pancreatic insulin-secreting β -cells. Islet-reactive T cells and antibodies primarily to GAD65 (also named β -cell autoantigen) can be detected in peripheral blood of 80% of recent-onset IDDM patients and in pre-diabetic high-risk subjects before onset of clinical symptoms. This suggests that GAD may be an important marker in the early stages of the disease.¹³ Also, autoantibodies to GAD65 and GAD67 are detected in animal models of IDDM, including the non-obese diabetes (NOD) mouse. In the NOD mouse, T cell reactivity is initially restricted to the C-terminal regions of GAD65, but later spreads to other parts of GAD65.^{14,15} Stiff-man syndrome (SMS), a rare disorder of the CNS, is characterized by progressive rigidity of the body musculature with painful spasms, due to impairment of the GABAergic neurotransmission. High-titer autoantibodies directed against GAD65 and GABAergic neurons (nerve terminals) have been detected in the serum and cerebrospinal fluid (CSF) in 60% of patients with the syndrome.¹⁶ Strikingly, many of the SMS patients also developed late-onset IDDM. Antibodies that react specifically with GAD65 are useful for the study of the differential tissue expression and intracellular localization of this isoform in normal and disease conditions.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, containing 0.04% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: working dilution is 1:1,000-1:5,000

Immunohistochemistry: working dilution is 1:100-1:500

Note: In order to obtain the best results in various techniques and preparations, we recommend determining the optimal working dilution by titration.

References

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