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Product Information

Anti-Glutamic Acid Decarboxylase 65/67
produced in rabbit, IgG fraction of antiserum

Catalog Number **G5163**

Synonym: Anti-GAD 65/67

Product Description
Anti-Glutamic Acid Decarboxylase 65/67 is produced in rabbits using a synthetic peptide KDIDFLIEEIER-LGQDL corresponding to the C-terminal region of GAD 67 of human origin (amino acids 579-594 with N-terminally added lysine) as immunogen. This sequence is identical in human GAD 65 (amino acids 570-585), in rat, mouse and pig GAD 65 and in pig, rat and cat GAD 67 and is highly conserved in GAD 67 of mouse origin (single amino acid substitution). The peptide is coupled to KLH with glutaraldehyde. Whole antiserum is purified to provide an IgG fraction of antiserum.

Anti-GAD 65/67 may be used for the detection and localization of GAD 65/67 isoforms by immunoblotting using a rat brain extract and by immunohistochemical staining of formalin-fixed, paraffin-embedded sections of rat pancreas (β-cells).

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.[1,3] 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. It is also present in other non-neuronal tissues such as testis, oviduct, and ovary.[1-5] GAD is also transiently expressed in non-GABAergic cells of the embryonic and adult nervous system, suggesting its involvement in development and plasticity.[6] GAD exists as two isoforms, GAD 65 and GAD 67 (molecular masses of 65 and 67 kDa, respectively) that are encoded by two different genes.[2,7,8] GAD65 is an ampiphilic, membrane-anchored protein, (585 amino acid residues) and is encoded on human chromosome 10. GAD 67 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 GAD 65 is required for targeting the enzyme to GABA-containing secretory vesicles. The two isoforms appear to have distinct intraneuronal distribution in the brain.[9] GAD 65 has been identified as an autoantigen in insulin-dependent diabetes mellitus (IDDM) and stiff-man syndrome (SMS),[10,11] 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 GAD 65 (also named β-cell autoantigen) can be detected in peripheral blood of 80% of recent-onset IDD 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.[11] Also, autoantibodies to GAD 65 and GAD 67 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 GAD 65, but later spreads to other parts of GAD 65.[12,13] 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 GAD 65 and GABAergic neurons (nerve terminals) have been detected in the serum and cerebrospinal fluid (CSF) in 60% of patients with the syndrome.[14] Strikingly, many of the SMS patients also developed late-onset IDDM. Antibodies that react specifically with GAD 65 are useful for the study of the differential tissue expression and intracellular localization of this isoform in normal and disease conditions.

Anti-GAD 65/67 reacts specifically with GAD 65 and 67 isoforms (65-67 kDa, usually observed as a doublet) derived from rat brain extract by immunoblotting and

with rat pancreas by immunohistochemical staining. The antibody may be used for immunoblotting of rat brain extract and for immunohistochemical staining of formalin-fixed, paraffin-embedded sections of rat pancreas (β-cells). Staining of the GAD 65/67 bands (65-67 kDa) in immunoblotting is specifically inhibited with GAD 65/67 peptide (human, amino acids 579-594 with N-terminally added lysine).

Reagents
The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

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.

Product Profile
Immunoblotting: a minimum working dilution of 1:10,000 is determined using a rat brain extract.

Immunohistochemistry: a minimum working dilution of 1:1,000 is determined by indirect peroxidase staining of formalin-fixed, paraffin-embedded sections of rat pancreas (β-cells).

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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MG, KAA,PHC 09/08-1