

## Product Information

### ANTI-GLUTAMIC ACID DECARBOXYLASE 65 (GAD 65, 5-22) Developed in Rabbit, IgG Fraction of Antiserum

Product Number **G5038**

#### Product Description

Anti-Glutamic Acid Decarboxylase 65 (GAD 65, 5-22), is developed in rabbits using a synthetic peptide GSGFWSFGSEGDGSGDEEN-K corresponding to the N-terminal region of GAD 65 of human origin (amino acids 5-22 with C-terminally added lysine) as immunogen. This sequence is highly conserved in rat and pig GAD 65 (single amino acid substitution), and in mouse and monkey GAD 65 but is not found in GAD 67. The peptide is coupled to KLH with glutaraldehyde. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins. The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide (see MSDS)\* as a preservative.

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

Glutamic Acid Decarboxylase (GAD) catalyzes the conversion of L-glutamate to  $\gamma$ -aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, and a putative paracrine signal molecule in pancreatic islets.<sup>1,3</sup> GAD has a restricted tissue distribution. It is highly expressed in the cytoplasm of GABAergic neurons in the central nervous system (CNS) and pancreatic  $\beta$ -cells. It is also present in other non-neuronal tissues such as testis, oviduct and ovary.<sup>1-5</sup> GAD is also transiently expressed in non-GABAergic cells of the embryonic and adult nervous system, suggesting its involvement in development and plasticity.<sup>6</sup> GAD exists as two isoforms, GAD 65 and GAD 67 (molecular masses of 65 and 67 kD, respectively) that are encoded by two different genes.<sup>2,7,8</sup> GAD65 is an amphiphilic, 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.<sup>9</sup> GAD 65 has been identified as an autoantigen in insulin-dependent diabetes mellitus (IDDM) and stiff-man syndrome (SMS),<sup>10,11</sup> IDDM is an autoimmune disease that results from T cell mediated destruction of pancreatic insulin-secreting  $\beta$ -cells. Islet-reactive T cells and antibodies primarily to GAD 65 (also named  $\beta$ -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.<sup>11</sup> 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.<sup>12,13</sup> 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.<sup>14</sup> 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.

#### Reagents

Anti-Glutamic Acid Decarboxylase 65 (GAD 65, 5-22) reacts specifically with GAD 65 (65 kD), derived from rat brain extract and in 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 ( $\beta$ -cells). Staining of the GAD 65 band (65 kD) in immunoblotting is specifically inhibited with GAD 65 peptide (human, amino acids 5-22 with N-terminally added lysine).

### Precautions and Disclaimer

\* Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

### Product Profile

A minimum working dilution of 1: 4,000 is determined by immunoblotting using a rat brain extract.

A minimum working dilution of 1: 1,000 is determined by indirect peroxidase immunohistochemical 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 to determine optimal working dilutions by titration test.

### References

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