

PRODUCT DATA SHEET

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Product name(s):	Rabbit polyclonal antibody to glutamic acid decarboxylase (GAD_{65/67})				
Catalogue number:	GC 3008	Batch number:	Z04622	Expiry date:	12 months from receipt

Introduction:

Glutamic acid decarboxylase (GAD; E.C. 4.1.1.15) is the enzyme responsible for the conversion of glutamic acid to γ -aminobutyric acid (GABA) - the major inhibitory transmitter in higher brain regions. Two molecular forms of GAD (GAD₆₅ and GAD₆₇) are known from rat, cat, pig and man, and both forms are expressed in the CNS and in pancreatic islet endocrine cells. A recent Western blotting study has identified that the isoforms are regionally distributed in the brains of rats and mice¹. In rat pancreas, GAD₆₅ and GAD₆₇ appear to be differentially localised, GAD₆₅ primarily in insulin-containing (β) cells and GAD₆₇ in glucagon-containing (A) cells². Evidence from knock-out mice suggests that GABA generated by pancreatic GAD isoforms is not critically involved in normal islet cell development and morphology³. However, β -cell-specific GAD expression has been shown to be required for the development of autoimmune diabetes in non-obese diabetic (NOD) mice⁴.

Product information:

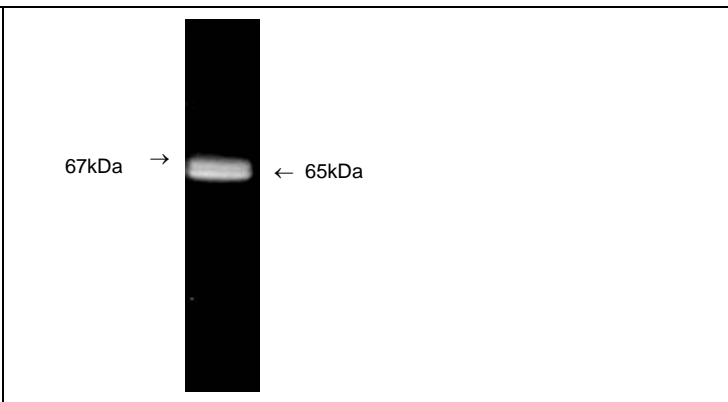
A synthetic peptide with the amino acid sequence: [C]-D-F-L-I-E-E-I-E-R-L-G-Q-D-L was selected from rat glutamate decarboxylase (GAD₆₅; C-terminus residues [Cys]+572-585)⁵. This sequence is conserved in rat GAD₆₇^{6,7} and in human GAD-2⁸. The peptide was conjugated to keyhole limpet haemocyanin *via* the N-terminal cysteine residue using *m*-maleimidobenzoic acid N-hydroxysuccinimide.

The conjugate was used to immunise New Zealand White rabbits and the resultant antiserum (B4;Z00257) has been extensively characterised by ELISA, Western blot and immunohistochemical techniques.

Application data
Western blotting
Test tissues: Human, cat, mouse and rat brain (esp. cerebellum).
Dilution: 1:1000-1:10,000 (using indirect immunoperoxidase with ECL development system (Amersham)).
Protein size: Doublet on SDS-PAGE at approximately 65/68kDa. Total abolition of staining when the antibody is preadsorbed with immunising peptide (<10nmol/mL optimally diluted antibody).
Immunohistochemistry
Test tissues: Human, cat and rat brain (esp. cerebellum).
Fixatives: Several fixative solutions may be used. Aldehyde-combination fixatives (*i.e.* those containing formaldehyde and glutaraldehyde) usually give satisfactory results. Bouin and Susa fixatives containing 0.1-0.2% glutaraldehyde have been used satisfactorily.
Processing: Cryostat and Vibratome[®] sections have been used most commonly. Paraffin wax-embedded tissue has been used successfully. The antiserum has also been used for the ultrastructural detection of GAD using pre-embedding immunocytochemistry⁹.
Dilution range: 1:200-1:5000, using overnight incubation and PAP or ABC-peroxidase procedure¹⁰.
Specificity: Immunostaining may be abolished by pre-incubation with 1-10 μ g peptide *per* mL diluted antibody.



Localisation of GABAergic neurones within lamina I of cat dorsal horn using rabbit polyclonal antiserum to glutamic acid decarboxylase (GAD_{65/67}). Photomicrograph courtesy of Dr TFC Batten, Institute for Cardiovascular Research, University of Leeds, UK.



Western blot luminograph of whole rat brain lysate immunostained using the rabbit polyclonal antiserum to GAD_{65/67} showing both isoforms. Antiserum titre 1:1000; indirect immunoperoxidase procedure; 1 min exposure (ECL).

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Vial contents, Storage and Use:
<p>Vial contains an immunoglobulin preparation partially purified from serum (from rabbit GADB4;Z00257) by precipitation with caprylic acid and ammonium sulphate. The antibody is supplied in one of two forms:</p> <ol style="list-style-type: none">Liquid. The antibody is suspended in PBS-BSA and contains 0.1% w/v sodium azide as a preservative.Lyophilised solid. The antibody has been freeze-dried from the solution described in (a). Re-suspend the antibody with sterile distilled water (volume stated on label). <p>Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10µL) prior to freezing if appropriate. The use of high quality 'antiserum-grade' plastic or glass vials is recommended. Store diluted antibody at 2-4°C (do not freeze) and use within 1 month.</p> <p>Dilute to working strength with 50mM phosphate buffered saline (pH 7.2) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-rabbit IgG linker antibody is to be used).</p>

References:
<ol style="list-style-type: none">1. Sheikh, S.N., Martin, S.B. and Martin D.L. Regional distribution and relative amounts of glutamate decarboxylase isoforms in rat and mouse brain. <i>Neurochem. Int.</i>, 35: 73-80, 1999.2. Li, L. <i>et al.</i> Differential detection of rat islet and brain glutamic acid decarboxylase (GAD) isoforms with sequence-specific peptide antibodies. <i>J. Histochem. Cytochem.</i>, 43: 53-59, 1995.3. Kash, S.F., Condie, B.G. and Baekkeskov, S. Glutamate decarboxylase and GABA in pancreatic islets: Lessons from knock-out mice. <i>Horm. Metab. Res.</i>, 31: 340-344, 1999.4. Yoon, J.-W. <i>et al.</i> Control of autoimmune diabetes in NOD mice by GAD expression or suppression in β cells. <i>Science</i>, 284: 1183-1187, 1999.5. Erlander, M.G. <i>et al.</i> Two genes encode distinct glutamate decarboxylases. <i>Neuron</i>, 7: 91-100, 1991.6. Julien, J.F., Samama, P and Mallet, J. Rat brain glutamic acid decarboxylase sequence deduced from a cloned cDNA. <i>J. Neurochem.</i>, 54: 703-705, 1990.7. Wyborski, R.J., Bond, R.W. and Gottlieb, D.I. Characterization of a cDNA coding for rat glutamic acid decarboxylase. <i>Mol. Brain Res.</i>, 8: 193-198, 1990.8. Karlsen, A.E. <i>et al.</i> Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. <i>Proc. Natl. Acad. Sci. USA</i>, 88: 8337-8341, 1991.9. Castel, M. and Morris, J.F. Morphological heterogeneity of the GABAergic network in the suprachiasmatic nucleus, the brain's circadian pacemaker. <i>J. Anat.</i>, 196: 1-13, 2000. [CITATION]10. Leao, R.M., Mellor, J.R. and Randall, A.D. Tonic benzodiazepine-sensitive GABAergic inhibition in cultured rodent cerebellar granule cells. <i>Neuropharmacology</i>, 39: 990-1003, 2000. [CITATION]