

PRODUCT DATA SHEET

Revised: 19 May 2000
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Product name(s):	Mouse monoclonal antibody to glutamic acid decarboxylase, 65kDa isoform (GAD₆₅)
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Catalogue number:	GC 3208	Batch number:	Z03118	Expiry date:	12 months from receipt
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Introduction:

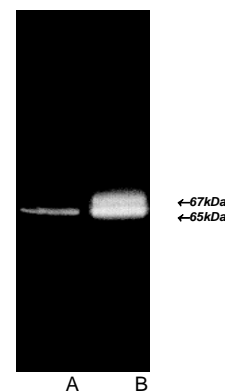
Glutamic acid decarboxylase (GAD; E.C. 4.1.1.15) is the enzyme responsible for the conversion of glutamic acid to γ -aminobutyric acid (GABA) - the major inhibitory transmitter in higher brain regions. Two molecular forms of GAD (GAD₆₅ and GAD₆₇) are known from rat, cat, pig and man, and both forms are 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 islet formation³.

Application data:

The hybridoma secreting the antibody to GAD₆₅ was generated by fusion of splenocytes from a non-obese diabetic (NOD) mouse which had received a single subdiabetogenic injection of streptozotocin⁴. Effectively, GC 3208 (clone 11; IgG₁) is a mouse autoantibody to GAD. The antibody has been extensively characterised by Western blotting and immunohistochemistry, and epitope mapping has shown it to be N-terminally directed.

Antibody GC 3208 (clone 11; IgG₁) recognises a linear epitope at the N-terminus of rat GAD, and is suitable for use in Western blotting⁵ (a single 65kDa band is seen in rat and mouse brain lysates at dilutions up to 1:250,000*) and in immunohistochemistry at dilutions up to 1:5000**. The antibody is suitable for use on de-waxed tissue sections, and gives consistently good labelling of neuronal GAD in human, monkey, rat and mouse CNS. GAD-containing cells in mammalian pancreas (not mouse) are immunostained with this antibody. Full abolition of antibody activity, as determined by Western blotting on whole rat brain lysate, has been achieved using the N-terminal peptide (rat/human GAD₆₅ [4-17]; cat. no. GP 3209) *per* mL of optimally diluted antibody.

NOTES: Optimal dilutions must be determined by experimentation. *Indirect immunoperoxidase procedure with overnight incubation in primary antibody at 4°C. Detection using ECL procedure (Amersham International plc), exposure for 1min. **The antibody has been used successfully on para-formaldehyde-fixed cryostat, Vibratome® and de-paraffinised tissue sections, at dilutions up to 1:5000 when used in combination with sensitive detection methods, such as ABC-peroxidase (Vector-Elite). The antibody is known to react with mouse, rat, monkey and human tissue and is thought to exhibit broad species reactivity.



Luminograph of Western blot of whole rat brain lysate immuno-stained using mouse monoclonal antibodies GC3208 (A) specific to the 65kDa isoform and GC3108 (B) which detects GAD65/67. Antibody dilutions 1:50,000 using ECL procedure (1min exposure).

Vial contents, Storage and Use:

Vial contains an immunoglobulin preparation, partially purified from exhausted supernatant, suspended in phosphate-buffered saline containing 0.01M sodium azide. Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10 μ L) 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.

Dilute to working strength with 50mM Tris-HCl buffer (pH 7.6) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-mouse IgG linker antibody is to be used).

continued

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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. Ziegler, B. <i>et al.</i> Glutamate decarboxylase (GAD) is not detectable on the surface of rat islet cells examined by cytofluorometry and complement-dependent antibody-mediated cytotoxicity of monoclonal GAD antibodies. <i>Horm. Metab. Res.</i>, 28: 11-15, 1996.5. Sasaki, K. <i>et al.</i> Effects of bilobalide on γ-aminobutyric acid levels and glutamic acid decarboxylase in mouse brain. <i>Eur. J. Pharmacol.</i>, 367: 165-173, 1999 [CITATION].