

Product Specification Sheet

Glutamate Decarboxylase 67 (GAD67/GAD1) Antibodies

Cat. GAD67-S	Rabbit Anti-Rat GAD67 antiserum	SIZE: 100 ul
Cat. GAD67-A	Rabbit Anti- Rat GAD67 IgG (aff pure)	SIZE: 100 ug
Cat. GAD67-P	Rat GAD67 Control/blocking peptide	SIZE: 100 ug
Cat. GAD671-C	Recombinant mouse GAD67 protein control for WB	SIZE: 100 ul

γ -Aminobutyric acid (GABA) is the major known inhibitory neurotransmitter. The rate-limiting step in the synthesis of GABA is the decarboxylation of glutamate by glutamate decarboxylase (GAD; L-glutamate 1-carboxy-lyase, EC 4.1.115). In the CNS GAD is entirely restricted to GABAergic neurons. GAD is also present in the β -cells of the pancreas and autoantibodies to various GAD polypeptides are detected in insulin-dependent diabetes mellitus. Cloning of GAD genes have identified two subtypes: GAD65 (65 kDa; human 585 AA chromosome 10) and GAD67 (67 kDa; human 594 AA, chromosome 2) share approx. 65% amino acid homology. The N-terminus is the most divergent while the C-terminus is highly conserved. Although both GAD isoforms catalyzes the conversion of GABA but interact differently with the co-factor pyridoxal 5'-phosphate suggesting their activities are differentially regulated. GAD67 is cytosolic, while GAD65 is membrane associated. GAD65 is a major autoantigen in diabetes mellitus and stiff-man syndrome, a rare disease of the brain.

Source of Antigen and Antibodies

Antigen	18-aa peptide from rat/mouse GAD67 (1) ; Designation (GAD67-P, control peptide) epitope location ~ N-terminus
Antibody host/type	Rabbit, Polyclonal unpurified antiserum (Cat # GAD67-S); Rabbit, Polyclonal IgG (Cat # GAD67-A), purified over antigen-Agarose
Secondary Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
Negative Control Ab	Non-immune rabbit IgG (Cat # 20009-1) to be used as -ve control for ELISA, WB, IHC etc.

Mouse GAD67/GAD1 protein (full length; gene accession # BC027059) was expressed as fusion protein (His tag-GAD1) in E.coli and purified (>95% with major band at ~67kDa). For Western blot +ve control (**Cat # GAD671-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **GAD671-C** for good visibility with antibody Cat # **GAD67-A**. Store at -20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **GAD671-C** solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer:** PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20oC and powder at 4oC or -20oC..

Long-term: at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence. We recommend the use of affinity purified antibody at 10-30 ug/ml in formaldehyde fixed, paraffin-embedded tissues (1).

Specificity & Cross-reactivity

The 18 AA rat GAD67 peptide sequences 100% conserved in mouse, 88% conserved in human and 61% in monkey GAD67. Antibody cross-reactivity in various species has not been studied. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at the web site). Recombinant purified mouse GAD67 protein (#GAD671-C) is also available.

General References: (1) Wyborski RJ et al (1990) Mol. Brain., Res. 8, 193-198; Julien JF et al (1990) J Neurochem. 54, 703; Katarove Z et al (1990) Eur. J. Neurosci. 2, 190; Michelson BK et al (1992) Diabetes 41, 1182; Faulkner-Jones BE (1993) Endocrinol. 133, 2962; (2) Dirxxx R et al (1995) JBC 270, 2241;

(2) Citations of ADI's Antibodies (see web site for updated list)

Hacker J, 2006 Neurosci. In press, IHC mice brain
*This product is for in vitro research use only.

Related material available from ADI
Anti-GAD65; Anti-GABA, -Glutamate transporter; Anti-Vesicular GABA transporter (VGAT)
GAD67-S-A-P 80630A

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi - 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com