ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Glutamate Decarboxylase 67 (GAD67/Gad1) Antibodies

Cat. GAD672-M	Monoclonal Anti-Human Glutamic acid decarboxylase (GAD67/GAD1) IgG	**SIZE**: 100 ug
Cat. GAD672-C	Recombinant human GAD67/GAD1 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 GAB is entirely restricted to GABAergic neurons. GAD is also present in the b-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	Recombinant human GAD1 fusion protein (GAD1-100 aa) (1); epitope location ~ N-terminus
Antibody host/type	Mouse, monoclonal IgG1 (Cat # **GAD672**-M);
Secondary Ab	**Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)
Negative Control Ab	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Human GAD1 protein (full length; gene accession # NM_000817) 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 # GAD672-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **GAD672**-C for good visibility with antibody Cat # **GAD672**-M. 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 **GAD672**-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

Affinity pure IgG

100 ug/100ul solution lyophilized powder

Supplied in **Buffer**: PBS+1% Trehalose

Reconstitute powder in PBS at 1mg/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 (1-5 ug/ml) 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 2-10 ug/ml in formaldehyde fixed, paraffin-embedded tissues (1).

Specificity & Cross-reactivity

Antibody cross-reactivity in various species has not been studied. Recombinant human GAD1 protein #GAD672-C) can be used as positive control.

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

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

Hacker J, 2006 Neurosci. In press, IHC mice brain

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified, undiluted)

*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)

GAD672-M-C 80630A

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