ALPHA DIAGNOSTIC INTERNATIONAL

LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

Cat. GAD651-M	Mouse Monoclonal Anti-Rat GAD65 IgG, aff pure	**SIZE**: 100 ul
Cat. GAD65-P	Rat GAD65 Control/blocking peptide	**SIZE**: 100 ug
Cat. GAD651-C	Recombinant purified **Mouse** GAD65 protein +ve 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	Rat GAD65 peptide
Ab Host/type	Mouse, Monoclonal , IgG1, Protein A/G Aff pure IgG (**cat #** GAD652**-M**) in PBS, pH 7.5 containing 0.05% azide,
2-ab	Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Mouse GAD65/GAD2 protein (protein accession # BC018380) was expressed as His-tag fusion protein in E. coli and purified (>95%). For WB +ve control mouse GAD65 (**#GAD651-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **GAD651**-C for good visibility with antibody Cat # **GAD65**-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 **GAD651**-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

Aff pure IgG
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

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.

Recommended Usage

Western Blotting (1:1K-5K for ascites and 1-10 ug/ml for affinity pure using Chemiluminescence technique). Antibodies made to this epitope have been shown to recognize the 65 kDa mol. wt. Band in rat brain (2).

ELISA (1:10K-1:100K; using 50-100 ng UCP2 control peptide/well).
Immunoprecipitations use 10 ul of ascites or approx. 10-ug-affinity pure antibody (2).
Histochemistry & Immunofluorescence: we recommend the use of affinity purified antibody at 2-20 ug/ml in frozen sections (2).

Specificity & Cross-reactivity

Rat GAD65-P immunogenic peptide sequences is 100% homologous with pig, 94% with mouse, and 83% with human and monkey. Antibodies have been shown to recognize mouse, rat, and human GAD65 (2). Antibody crossreactivity with other species is not established. 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).

General References: Erlanger MG et al (1991) Neuron 7, 91-100, Dirkx R et al (1995) J Biol. Chem. 270, 2241; Solimena, M (unpublished results on file).

Shipping: 4oC for solutions and room temp for powder

*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)

ELISA kits to detect autoimmune antibodies to GAD65/67 in mouse, rat, human and rabbits.

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