

**Glutamate Decarboxylase 65 (GAD65)**

<b>Cat. GAD65-M</b>	Mouse Monoclonal Anti-Rat GAD65, Ascites	<b>SIZE:</b> 100 ul
<b>Cat. GAD65-P</b>	Rat GAD65 Control/blocking peptide	<b>SIZE:</b> 100 ug

$\gamma$ -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  $\beta$ -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**

<b>Antigen</b>	18-aa peptide of Rat GAD65; <b>Designated (GAD65-P or control peptide)</b> . epitope location ~ N-terminal
<b>Ab Host/type</b>	Mouse, Monoclonal and Aff pure IgG1 (cat #GAD65-M) purified using protein A/G column
<b>2-ab</b>	<b>Goat Anti-mouse IgG-HRP conjugate</b> Cat # 40320 (AP, biotin, FITC conjugates also available)
<b>-ve control</b>	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as -ve control

**Form & Storage of Antibodies/Peptide Control**

**Ascites (unpurified)**  
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, Dirx 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)

**Western Blot recycling kit** (Use the same blot to probe with multiple antibodies GAD65 and GAD67) **recycle blot at room temp in 5-10 min;** No mercaptoethanol or heating required).

GAD65-M-P

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