

Product Specification Sheet

Glutamate Decarboxylase 65/67 (GAD65/67) Antibodies

Cat. GAD6567-A	Rabbit Anti- Rat GAD6567 IgG (aff pure)	SIZE: 100 ug
Cat. GAD6567-P	Rat GAD6567 Control peptide	SIZE: 100 ug

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 4.1.1.15). In the CNS GABA 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	16-aa peptide from GAD65/67 of human (1); Designation (GAD6567-P, control peptide) Conjugated to KLH; Epitope location ~C-terminus
Antibody host/type	Rabbit, Polyclonal IgG (Cat # GAD6567-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.

Form & Storage of Antibodies/Peptide Control

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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 for less than a week at 4oC.

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 (1-10 ug/ml for affinity pure using Chemiluminescence technique). Antibodies recognize both 65 and 67 kDa bands of GAD.

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

Histochemistry & Immunofluorescence: not tested. we recommend the use of affinity purified antibody at 2-20 ug/ml in frozen sections (2).

Specificity & Cross-reactivity

The 16 AA rat GAD6567-P peptide sequences 100% conserved in both GAD65 and GAD67 from mouse, rat, and human. It is also highly conserved in other species GAD such gold fish, chicken, cat, pig, and zebra fish. 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).

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) Ewur. 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; Solimena M (unpublised results on file).

*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)

GAD6567-A-P 71214J