

Rat Glutamate Decarboxylase 65 (GAD65) Antibodies

Cat. # GAD65-AS , Mouse Monoclonal Anti-Rat GAD65 , 100 µl neat serum, Lot # 711M

Cat. # GAD65-A , Mouse Monoclonal Anti-Rat GAD65 (affinity pure), 100 µg, Lot # 711M-A

Cat. # GAD65-P, Rat GAD65 Control peptide, 100 µg/100 µl , Lot # 711P

Source of Antigen and Antibodies

Gamma-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. Glutamic acid decarboxylase (GAD; E.C.4.1.1.15) catalyzes the decarboxylation of glutamate to produce GABA-the rate limiting step in the synthesis of GABA. The two major isoform of GAD, GAD67 (mol. wt 67 kDa) and GAD65 (mol. wt 65 kDa) are derived from distinct genes and differ in their subcellular distribution. GAD67 is 594 aa single chain polypeptide encoded by human chromosome 2 gene, whereas GAD65 is an amphiphilic and membrane anchored protein (585 aa, chromosome 10). GAD isoform shares ~65% homology. GAD is also a major autoantigen associated with SMS (stiff-man syndrome).

An 18-aa peptide sequence (designated as GAD65-P, control peptide) near the amino terminus of mouse/rat GAD65 (1) was synthesized and coupled to KLH. Antibodies were generated in mice. Stable hybridoma secreting anti-GAD65 were propagated in Balb/c mice to generate ascites.

Form & Storage of Antibodies/Peptide Control

Antiserum is supplied as undiluted, neat ascites preserved in 0.02% thimerosal or merthiolate. Affinity pure antibodies have been purified using the peptide-Sepharose columns and supplied as 1 mg/ml soln in PBS, pH 7.4, and 0.1% BSA and 0.02% sodium merthiolate as stabilizers and preservatives. Control peptide is supplied as 1 mg/ml soln. in PBS, pH 7.4 and 0.02% sodium merthiolate. Do not store diluted solutions of antibodies and avoid freeze and thaw. It is recommended to store all products at 4°C for short term use. The lyophilized products should be reconstituted (Add 100 µl water and lightly vortex and mix for 15 min at room temp). Peptide or antibody solution can then be used or aliquoted for storage in small aliquots at -20°C or below. Do not store diluted solutions of peptides or antibodies and avoid freeze and thaw.

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 µg/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 µl of serum or approx. 10-µg-affinity pure antibody (2).

Histochemistry & Immunofluorescence: we recommend the use of affinity purified antibody at 2-20 µg/ml in frozen sections (2).

Specificity & Cross-reactivity

The 18 AA rat GAD65 immunogenic peptide sequences is 100% homologous with pig (18/18 aa), 94% with mouse (17/18 aa), and 83% with human and monkey (16/18 aa). Antibodies have been shown to recognize mouse, rat, and human GAD65 (2). Antibody crossreactivity in various species is not established. Control peptide, because of its low mol size (<3 Kda), is not recommended for Western. It should be used in ELISA, dot blot, and for antibody blocking to confirm specificity of antibodies.

References:

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