OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26579AF-N

GAD67 (GAD1) (1-585) Mouse Monoclonal Antibody [Clone ID: 9A6]

Product data:

Product Type:	Primary Antibodies
Clone Name:	9A6
Applications:	IHC, WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. Immunohistochemistry on paraffin sections: 1-10 µg/ml.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant GST-human brain GAD 65 fusion protein corresponding to full-length amino acids (1-585 a.a.)
Specificity:	This antibody reacts with GAD 65 and GAD 67 on Western blotting and Immunohistochemistry in murine and rat samples. It detects 65 kDa and 67 kDa GAD by Western blotting using mouse and rat total brain cell lysate.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	glutamate decarboxylase 1
Database Link:	Entrez Gene 2571 Human Q99259

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Background: Glutamic acid decarboxylase (GAD) catalyzes formation of γ -Aminobutyric acid (GABA), which is an inhibitory neurotransmitter, and is present in brain as well as several tissues outside the central nervous system. Biological functions of GAD and GABA extend beyond regulation of neurotransmission to include effects on the immune system as well as modulation of cell proliferation, protein synthesis, and metabolism. Two isoforms of GAD, GAD 65 and GAD 67 , each derived from a single separate gene, were isolated from human fetal brain λ gt11 cDNA library.

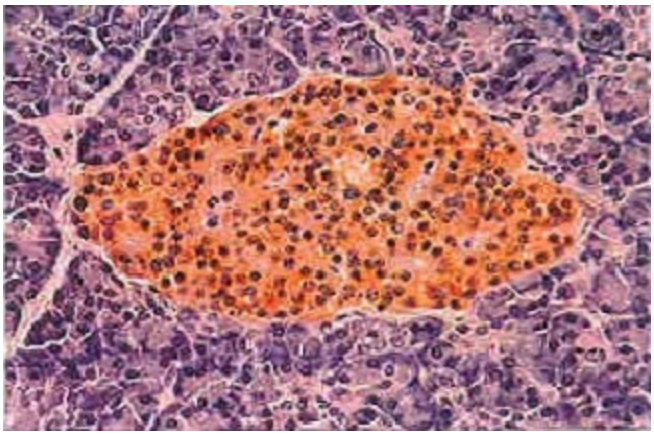
Synonyms: Glutamate decarboxylase 1, GAD-67

Note: This product was originally produced by MBL International.

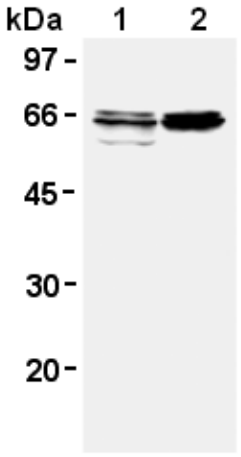
Protocol:
SDS-PAGE & Western Blotting
1) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
3) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 o C.
4) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with the 1:5,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Wipe excess buffer from the membrane, then incubate it with appropriate chemilumines cence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
9) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The conditions for exposure and development may vary.
(Positive controls for Western blotting; mouse or rat brain)
Immunohistochemical staini ng for paraffin- embedded sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H 2 O 2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.

6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the APPLICATIONS .
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody Incubate for 10 minutes at room temperature. Wash as in step 8.
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 8.
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µ L of 30% H 2 O 2 in 150 mL PBS.
* DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

Product images:



Immunohistochemical detection of GAD on formalin fixed paraffin embedded section of human pancreas with AM26579AF-N.



Western blot analysis of GAD expression in mouse (1) and rat (2) brain using AM26579AF-N.