Overview

Product name: Anti-GAD67 antibody [K-87]
Description: Mouse monoclonal [K-87] to GAD67
Host species: Mouse
Tested applications: Suitable for: ICC/IF, Flow Cyt, IHC-P, WB, IHC-Fr
Species reactivity: Reacts with: Mouse, Rat, Human
Immunogen: Synthetic peptide:
RFRRTETDFSNLFARDLLPA
, corresponding to amino acids 87-106 of Human GAD67

Positive control: Recombinant Human GAD67 protein (ab114255) can be used as a positive control in WB. In WB, this antibody gave a positive signal in Mouse and Rat Brain Tissue Lysates. GAD67 has been thought to be primarily located in the nerve cell body, but using this new K-87 mAb, GAD67 can now also be detected in dendrites and axons.

General notes: ab26116 mouse monoclonal [K-87] to GAD67 specically recognizes GAD67 and has no cross reactivity with GAD65; this has been shown on western blots of mouse brain or purified recombinant GAD67 and GAD65. In immunohistochemical analysis of brain sections, the K-87 monoclonal recognizes GAD67 in nerve cell bodies and has an enhanced ability to detect GAD67 in dendrites and axon buttons compared to the original anti-GAD67 K-2 polyclonal antibody (see Kaufman et al, 1991).

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer: pH: 7.40
Preservative: 0.01% Sodium azide
Constituent: 0.0268% PBS
Purity: Protein A purified
Primary antibody notes: ab26116 mouse monoclonal [K-87] to GAD67 specically recognizes GAD67 and has no cross
reactivity with GAD65; this has been shown on western blots of mouse brain or purified recombinant GAD67 and GAD65. In immunohistochemical analysis of brain sections, the K-87 monoclonal recognizes GAD67 in nerve cell bodies and has an enhanced ability to detect GAD67 in dendrites and axon buttons compared to the original anti-GAD67 K-2 polyclonal antibody (see Kaufman et al, 1991).

Clonality
Monoclonal

Clone number
K-87

Isotype
IgG1

Light chain type
kappa

Applications

Our Abpromise guarantee covers the use of ab26116 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>ICC/IF</td>
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<td>Use a concentration of 10 µg/ml.</td>
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Flow Cyt
Use 1µg for 10^6 cells. (methanol fixed cells)
ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

IHC-P
1/1000 - 1/10000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. An antigen retrieval step is necessary (e.g. incubating the sections at 90 degrees C in a 50mM citrate buffer). Cell body labeling is optimized when Triton is omitted from the tissue processing. Axon terminal labeling is substantially increased (and cell body labeling decreased) when Triton is included (see Soghomonian et al, 1998).

WB
1/1000 - 1/20000. Detects a band of approximately 67 kDa (predicted molecular weight: 67 kDa).

IHC-Fr

Target

Function
Catalyzes the production of GABA.

Tissue specificity
Isoform 3 is expressed in pancreatic islets, testis, adrenal cortex, and perhaps other endocrine tissues, but not in brain.

Involvement in disease
Defects in GAD1 are the cause of cerebral palsy spastic quadriplegic type 1 (CPSQ1) [MIM:603513]. A non-progressive disorder of movement and/or posture resulting from defects in the developing central nervous system. Affected individuals manifest symmetrical, non-progressive spasticity and no adverse perinatal history or obvious underlying alternative diagnosis. Developmental delay, mental retardation and sometimes epilepsy can be part of the clinical picture.

Sequence similarities
Belongs to the group II decarboxylase family.
ab26116, at a 1/2000 dilution, staining of fixed mouse cerebellum sections that underwent an antigen retrieval step.

Anti-GAD67 antibody [K-87] (ab26116) at 1/2000 dilution + mouse cerebellum at 0.75 µg

Secondary
HRP horse anti-mouse IgG at 1/5000 dilution

Predicted band size: 67 kDa

Immunohistochemical staining of mouse pancreatic islet beta cells after antigen retrieval with ab26116 at a dilution of 1/1000.
Immunocytochemistry/ Immunofluorescence - Anti-GAD67 antibody [K-87] (ab26116)

ICC/IF image of ab26116 stained PC12 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab26116, 10µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

Flow Cytometry - Anti-GAD67 antibody [K-87] (ab26116)

Overlay histogram showing PC12 cells stained with ab26116 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab26116, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a slightly decreased signal in PC12 cells fixed with 4% paraformaldehyde (10 min) used under the same conditions.

Please note that Abcam does not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.

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