BOSTER BIOLOGICAL TECHNOLOGY
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Anti-GAD65/GAD2 Antibody Picoband® (monoclonal, 7G2)

Catalog Number: M03142

About GAD2

Glutamate decarboxylase 2, also known as GAD65, is an enzyme that in humans is encoded by the GAD2 gene. This gene encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes. The enzyme encoded is responsible for catalyzing the production of gamma-aminobutyric acid from L-glutamic acid. A pathogenic role for this enzyme has been identified in the human pancreas since it has been identified as an autoantibody and an autoreactive T cell target in insulin-dependent diabetes. This gene may also play a role in the stiff man syndrome. Alternative splicing results in multiple transcript variants that encode the same protein.

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Overview

Product Name	Anti-GAD65/GAD2 Antibody Picoband® (monoclonal, 7G2)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-GAD65/GAD2 Antibody Picoband® (monoclonal, 7G2) catalog # M03142. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 7G2
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Mouse
Uniprot ID	Q05329

Technical Details

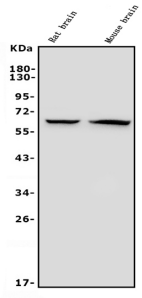
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human GAD65, different from the related mouse and rat sequences by one amino acid.

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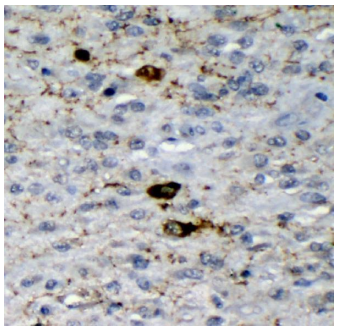
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2a
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human Immunocytochemistry/Immunofluorescence, 4ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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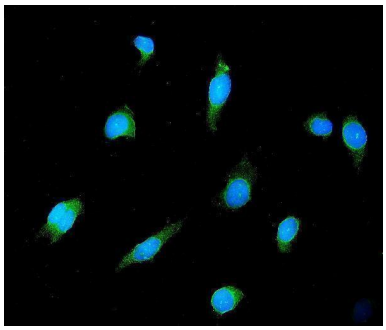
Anti-GAD65/GAD2 Antibody Picoband® (monoclonal, 7G2) (M03142) Images



Western blot analysis of GAD65/GAD2 using anti-GAD65/GAD2 antibody (M03142). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: rat brain tissue lysates, Lane 2: mouse brain tissue lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-GAD65/GAD2 antigen affinity purified monoclonal antibody (Catalog # M03142) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for GAD65/GAD2 at approximately 62KD. The expected band size for GAD65/GAD2 is at 62KD.



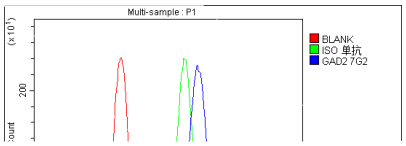
IHC analysis of GAD65/GAD2 using anti-GAD65/GAD2 antibody (M03142). GAD65/GAD2 was detected in paraffin-embedded section of human glioma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-GAD65/GAD2 Antibody (M03142) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.



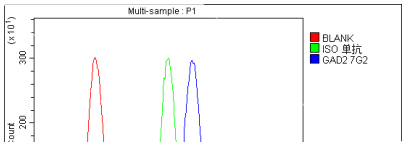
IF analysis of GAD65/GAD2 using anti-GAD65/GAD2 antibody (M03142). GAD65/GAD2 was detected in immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 4ug/mL mouse anti-GAD65/GAD2 Antibody (M03142) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Flow Cytometry analysis of U20S cells using anti-GAD65/GAD2 antibody (M03142). Overlay histogram showing U20S cells stained with M03142 (Blue line). To facilitate intracellular staining, cells were fixed with 4%

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paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GAD65/GAD2 Antibody (M03142, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of 293T cells using anti-GAD65/GAD2 antibody (M03142). Overlay histogram showing 293T cells stained with M03142 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GAD65/GAD2 Antibody (M03142, 1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

1 Publications Citing This Product

1. PubMed ID: 30068915, Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis Zehua Li, et al. Lab Invest. 2019 Mar;99(3):346-357. doi: 10.1038/s41374-018-0091-y. Epub 2018 Aug 1.

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