10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-GAD65/GAD2 Antibody Picoband™ (monoclonal, 7G2)

Catalog # ABO14966

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q05329
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-GAD65/GAD2 Antibody Picoband™ (monoclonal, 7G2) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	2572
Other Names	Glutamate decarboxylase 2, 4.1.1.15, 65 kDa glutamic acid decarboxylase, GAD-65, Glutamate decarboxylase 65 kDa isoform, GAD2 (HGNC:4093), GAD65
Calculated MW	65411
Application Details	Western blot, 0.1-0.5 □g/ml, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human Immunocytochemistry/Immunofluorescence, 4 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg NaN3.
Clone Names	Clone: 7G2
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.
Purification	Immunogen affinity purified.
Storage	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.

Protein Information

Name	GAD2 (HGNC:4093)
Synonyms	GAD65
Function	Catalyzes the production of GABA.
Cellular Location	Cytoplasm, cytosol. Cytoplasmic vesicle. Presynaptic cell membrane; Lipid-anchor. Golgi apparatus membrane; Peripheral membrane protein; Cytoplasmic side. Note=Associated to cytoplasmic vesicles In neurons, cytosolic leaflet of Golgi membranes and presynaptic clusters

Background

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.

Images

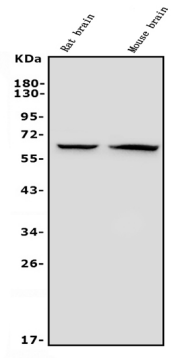
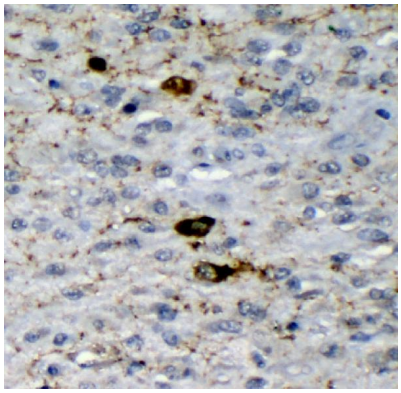


Figure 1. Western blot analysis of GAD65/GAD2 using anti-GAD65/GAD2 antibody (M03142).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat brain 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 µg/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.

Figure 2. 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 1 µg/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.

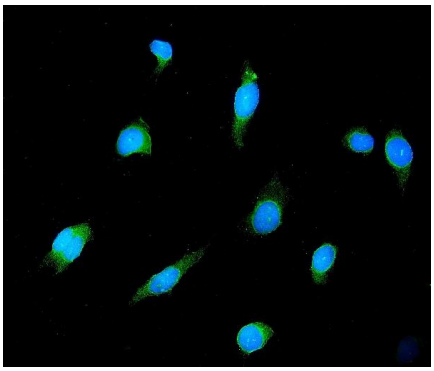


Figure 3. IF analysis of GAD65/GAD2 using anti-GAD65/GAD2 antibody (M03142).
GAD65/GAD2 was detected in immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 4 µg/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.

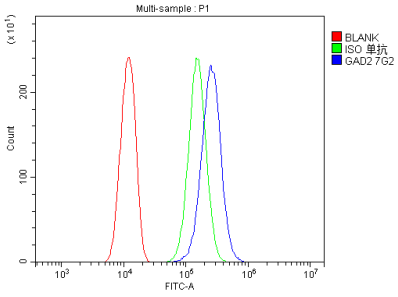


Figure 4. Flow Cytometry analysis of U20S cells using anti-GAD65/GAD2 antibody (M03142).
Overlay histogram showing U20S cells stained with M03142 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GAD65/GAD2 Antibody (M03142, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

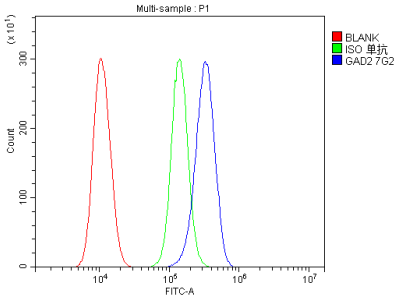


Figure 5. Flow Cytometry analysis of 293T cells using anti-GAD65/GAD2 antibody (M03142).
Overlay histogram showing 293T cells stained with M03142 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GAD65/GAD2 Antibody (M03142, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.