

Anti-GAD65 Polyclonal Antibody 3

Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse,Rat (predicted:Chicken,Dog,Pig,Cow)
Molecular Weight:	Theoretical: 65 kDa.
Purification:	Protein A purified

Applications

1. Blank control (Black line): Molt4 (Black).
Primary Antibody (green line): Rabbit Anti-GAD65 antibody (TMAB-06265)
Dilution: 3 μ g /10⁶ cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647
Dilution: 3 μ g /test.

Protocol

The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with PBST for 20 min at room temperature. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

2. SHSY5Y cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (GAD65) polyclonal Antibody, Unconjugated (TMAB-06265) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nuclei.

Verified Activity:

3. Molt-4 cells were fixed with 4% PFA for 10 min at room temperature, permeabilized with 0.1% PBST for 20 min at room temperature, and incubated in 5% BSA blocking buffer for 30 min at room temperature. Cells were then stained with GAD65 antibody (TMAB-06265) at 1:100 dilution in blocking buffer and incubated for 30 min at room temperature, washed twice with 2%BSA in PBS, followed by secondary antibody incubation for 40 min at room temperature. Acquisitions of 20,000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).

4. Sample:

- Lane 1: Mouse Cerebrum tissue lysates
 - Lane 2: Mouse Cerebellum tissue lysates
 - Lane 3: Mouse Hippocampus tissue lysates
 - Lane 4: Rat Cerebrum tissue lysates
 - Lane 5: Rat Cerebellum tissue lysates
 - Lane 6: Rat Hippocampus tissue lysates
 - Lane 7: Human SH-SY5Y cell lysates
 - Lane 8: Human U87MG cell lysates
 - Lane 9: Human HeLa cell lysates
 - Lane 10: Human Jurkat cell lysates
- Primary: Anti-GAD65 (TMAB-06265) at 1/1000 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 65 kDa

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Observed band size: 62 kDa

Application: WB,ICC/IF,FCM

Recommended WB: 1:500-2000; ICC/IF: 1:100-500; FCM: 1µg/Test

Properties

Stability & Storage: Store at 2°C-8°C for 1 month. Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

Antigen Details

Immunogen: KLH conjugated synthetic peptide: human GAD65

Antigen Species: Human

Gene ID: 2572

Uniprot ID: Q05329

Research Background

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. [provided by RefSeq, Oct 2008]

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Tel:781-999-4286 E_mail:info@targetmol.com Address:34 Washington Street,Wellesley Hills,MA 02481
