


## Product datasheet

### Anti-GAD65 + GAD67 antibody [EPR19366] ab183999

Recombinant RabMAb

★★★★★ [4 Abreviews](#) [8 References](#) [13 Images](#)

#### Overview

<b>Product name</b>	Anti-GAD65 + GAD67 antibody [EPR19366]
<b>Description</b>	Rabbit monoclonal [EPR19366] to GAD65 + GAD67
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> IP, IHC-P, IHC-Fr, WB, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human <b>Predicted to work with:</b> Common marmoset 
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: Mouse GAD67 and GAD65 fragment recombinant proteins; Human, mouse and rat cerebellum lysates; C6 whole cell lysate; Mouse and rat brain lysates. IHC-P: Mouse cerebellum and pancreas tissues; Rat cerebellum and pancreas tissues. IHC-Fr: Mouse hippocampus tissue. ICC/IF: Mouse primary neuron cells
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal

Clone number	EPR19366
Isotype	IgG

## Applications

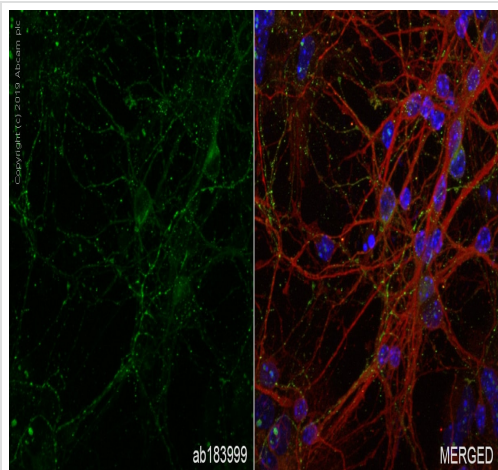
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab183999 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		1/40.
IHC-P	★★★★★ (3)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr	★★★★★ (1)	1/2500.
WB		1/1000. Detects a band of approximately 65, 67 kDa (predicted molecular weight: 65, 67 kDa).
ICC/IF		1/100.

## Target

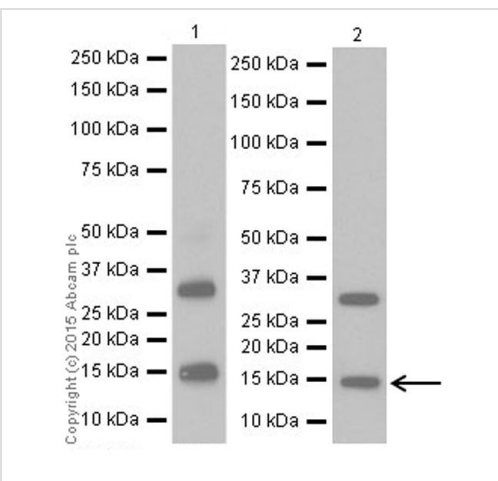
<b>Relevance</b>	This gene encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes. The enzyme encoded is responsible for catalyzing the production of gamma aminobutyric acid from L glutamic acid. A pathogenic role for this enzyme has 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.
<b>Cellular localization</b>	Cytoplasm; cytosol. Associated to cytoplasmic vesicles. In neurons, cytosolic leaflet of Golgi membranes and presynaptic clusters.

## Images



Immunocytochemistry/ Immunofluorescence - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunocytochemistry/ Immunofluorescence analysis of mouse primary neuron cells labeling GAD65 + GAD67 with purified ab183999 at 1/100 (10 µg/mL). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with **ab11267** Anti-MAP2 antibody [HM-2]; **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control. Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.



Western blot - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

**All lanes :** Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999) at 1/1000 dilution

**Lane 1 :** Mouse GAD67 fragment recombinant protein

**Lane 2 :** Mouse GAD65 fragment recombinant protein

Lysates/proteins at 0.01 µg per lane.

#### Secondary

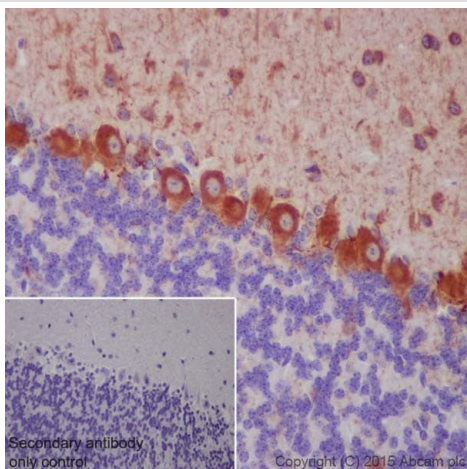
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 65, 67 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 1 second; Lane 2: 15 seconds.

Mouse GAD67 fragment recombinant protein contain aa468-592 with a His-Tag®. Mouse GAD65 fragment recombinant protein contain aa460-584 with a His-Tag®. These two fragment recombinant proteins were made in-house. The ~30 kDa band represents doublets of the recombinant fragments.

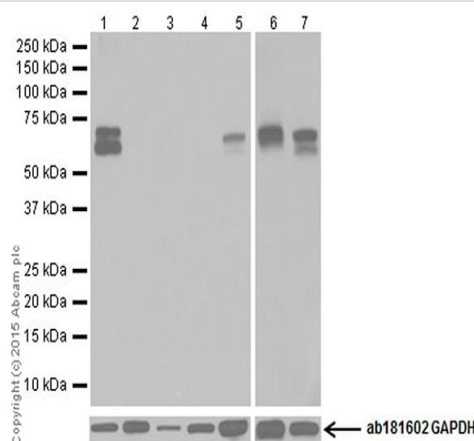


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded Mouse cerebellum tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasm staining on mouse cerebellum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

**All lanes** : Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999) at 1/1000 dilution

**Lane 1** : Human cerebellum lysate

**Lane 2** : Mouse skin lysate

**Lane 3** : Mouse lung lysate

**Lane 4** : Neuro-2a (Mouse neuroblastoma cell line) whole cell lysate

**Lane 5** : C6 (Rat glial tumor cell line) whole cell lysate

**Lane 6** : Mouse cerebellum lysate

**Lane 7** : Rat cerebellum lysate

Lysates/proteins at 20 µg per lane.

### Secondary

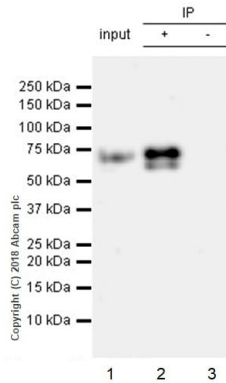
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 65, 67 kDa

**Observed band size:** 65,67 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-5: 3 minutes; Lane 6 and 7: 2 seconds.



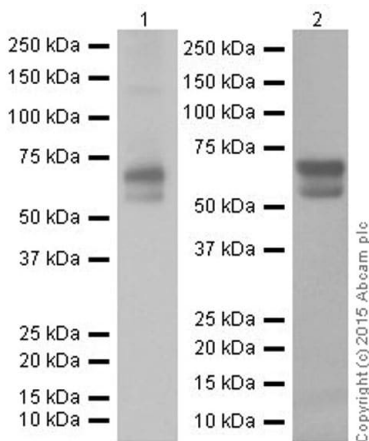
Immunoprecipitation - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Ab183999 Immunoprecipitating GAD65 + GAD67 in Human cerebellum lysate. 10µg of cell lysate was incubated with primary antibody 1/40. For western blotting Ab183999 (1/1000) was used to confirm successful immunoprecipitation. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) was used at 1/5000.

**Lane 1 (Input):** Human cerebellum lysate 10µg

**Lane 2 (+):** Human cerebellum lysate with Ab183999, 1/40

**Lane 3 (-):** Rabbit monoclonal IgG ([ab172730](#)) instead of ab183999 in Human cerebellum lysate



Western blot - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

**Lane 1 :** Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999) at 1/5000 dilution

**Lane 2 :** Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999) at 1/1000 dilution

**Lane 1 :** Mouse brain lysate

**Lane 2 :** Rat brain lysate

Lysates/proteins at 10 µg per lane.

### Secondary

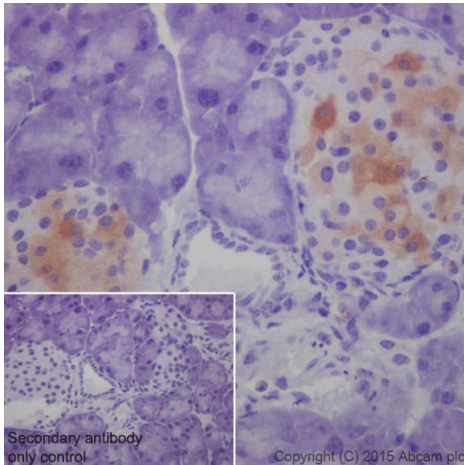
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**Predicted band size:** 65, 67 kDa

**Observed band size:** 65,67 kDa

**Exposure time:** 2 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

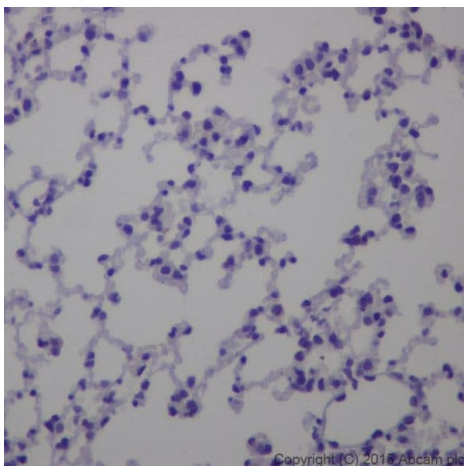


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded Mouse pancreas tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution. Cytoplasm staining on mouse pancreas islets is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab97051](#) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

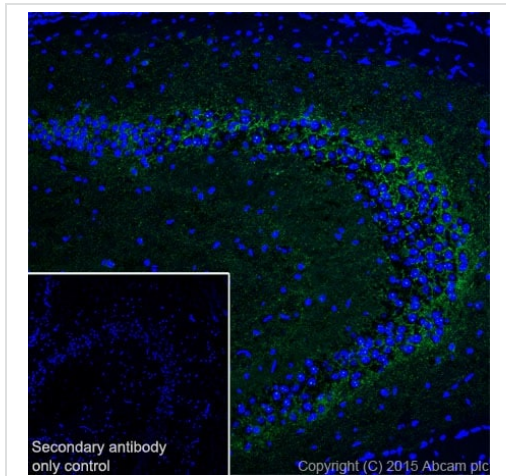


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution. Negative on mouse lung. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab97051](#) at 1/500 dilution.

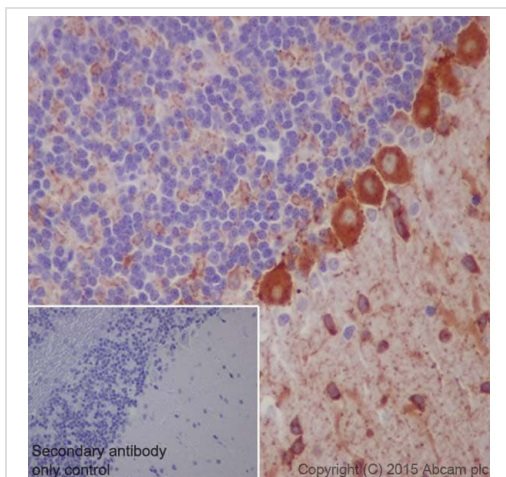
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Frozen sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of 4% paraformaldehyde fixed, 0.2% Triton X-100 permeabilized frozen section of Mouse hippocampus tissue labeling GAD65 + GAD67 with ab183999 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) secondary ([ab150077](#)) at 1/1000 dilution (green). The result showed mainly cytoplasmic staining on mouse hippocampus. The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab150077](#) at 1/1000 dilution.

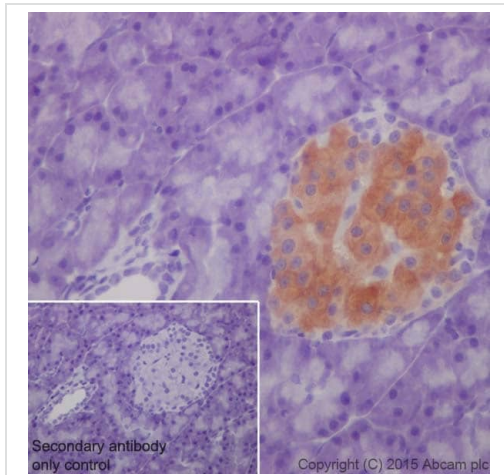


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded Rat cerebellum tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution. Cytoplasm staining on rat cerebellum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab97051](#) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

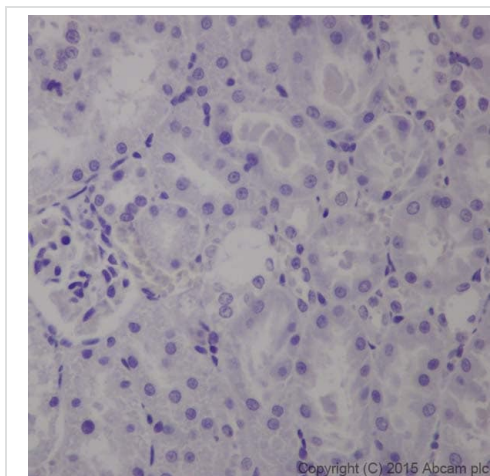


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded Rat pancreas tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution. Cytoplasm staining on rat pancreas islet is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab97051](#) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GAD65 + GAD67 antibody [EPR19366] (ab183999)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling GAD65 + GAD67 with ab183999 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution. Negative on rat kidney. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is [ab97051](#) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-GAD65 + GAD67 antibody [EPR19366]  
(ab183999)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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