# abcam

#### Product datasheet

## Anti-GAD65 antibody [EPR22952-70] ab239372



#### ★★★★★ 2 Abreviews 2 References 9 Images

#### Overview

**Product name** Anti-GAD65 antibody [EPR22952-70]

**Description** Rabbit monoclonal [EPR22952-70] to GAD65

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), IP, IHC-P, WB, IHC-Fr, ICC/IF

Species reactivity Reacts with: Mouse, Rat

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse brain and cerebellum tissue lysates. Rat brain and cerebellum tissue lysates IHC-P:

Rat pancreas and Mouse cerebrum tissues. IHC-Fr: Rat pancreas and Mouse cerebrum tissues.

Flow Cyt (intra): Beta-TC-6IP: Mouse brain lysate

**General notes** This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

For more information see here.

- Animal-free production

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### **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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR22952-70

**Isotype** IgG

#### **Applications**

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab239372 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                                       |
|------------------|------------------|---|
| Flow Cyt (Intra) |                  | 1/60.                                       |
| IP               |                  | 1/30.                                       |
| IHC-P            | <b>★★★★★ (2)</b> | 1/2000.                                     |
| WB               |                  | 1/5000. Predicted molecular weight: 65 kDa. |
| IHC-Fr           |                  | 1/100.                                      |
| ICC/IF           |                  | 1/50.                                       |

#### **Target**

**Function** Catalyzes the production of GABA.

**Sequence similarities**Belongs to the group II decarboxylase family.

Post-translational

Phosphorylated; which does not affect kinetic parameters or subcellular location.

modifications

Palmitoylated; which is required for presynaptic clustering.

**Cellular localization** 

 $\label{lem:cytoplasm} \textbf{Cytoplasmic vesicle. Cell junction > synapse > presynaptic cell membrane.}$ 

Golgi apparatus membrane. Associated to cytoplasmic vesicles. In neurons, cytosolic leaflet of

Golgi membranes and presynaptic clusters.

#### **Images**



Western blot - Anti-GAD65 antibody [EPR22952-70] (ab239372)

**All lanes :** Anti-GAD65 antibody [EPR22952-70] (ab239372) at 1/5000 dilution

Lane 1: Mouse brain tissue lysate

Lane 2: Mouse cerebellum tissue lysate

Lane 3: Rat brain tissue lysate

Lane 4: Rat cerebellum tissue lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

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

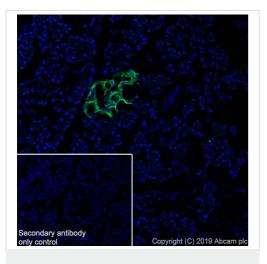
Predicted band size: 65 kDa

Blocking/diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 8 seconds.

Bands above 100kDa are multimers of GAD65.

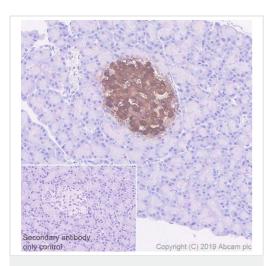
The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 10601283, 21734072).



Immunohistochemistry (Frozen sections) - Anti-GAD65 antibody [EPR22952-70] (ab239372)

Immunohistochemical analysis of 4% PFA fixed 0.2% Triton X-100 permeabilized frozen Rat pancreas tissue labeling GAD65 with ab239372 at 1/100 dilution (Green) followed by <u>ab150077</u> AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2  $\mu$ g/ml) dilution. The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody was <a href="mailto:ab150077"><u>ab150077</u></a> AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/ml) dilution.

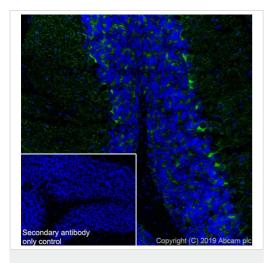


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GAD65 antibody

[EPR22952-70] (ab239372)

Immunohistochemical analysis of paraffin-embedded Rat pancreas tissue labeling GAD65 with ab239372 at 1/2000 (0.323 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Cytoplasmic staining on islet of rat pancreas. The section was incubated with ab239372 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

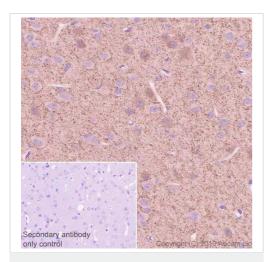
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).



Immunohistochemistry (Frozen sections) - Anti-GAD65 antibody [EPR22952-70] (ab239372)

Immunohistochemical analysis of 4% PFA fixed 0.2% Triton X-100 permeabilized frozen Mouse cerebellum tissue labeling GAD65 with ab239372 at 1/100 dilution (Green) followed by **ab150077**AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/ml) dilution. The nuclear counterstain was DAPI (Blue). Positive staining on mouse cerebellum is observed.

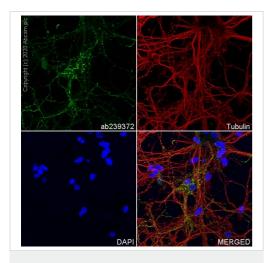
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody was <a href="mailto:ab150077"><u>ab150077</u></a> AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/ml) dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GAD65 antibody
[EPR22952-70] (ab239372)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling GAD65 with ab239372 at 1/2000 (0.323 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse cerebrum is observed. The section was incubated with ab239372 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

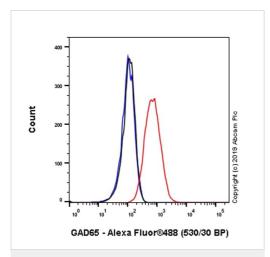
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).



Immunocytochemistry/ Immunofluorescence - Anti-GAD65 antibody [EPR22952-70] (ab239372)

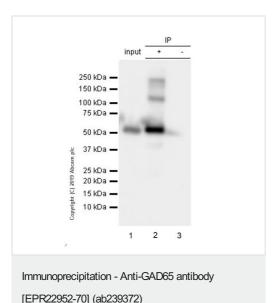
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neuron cells labelling GAD65 with ab239372 at 1/100 dilution, followed by <a href="mailto:ab5150077">ab150077</a> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in mouse primary neurons.is observed. <a href="mailto:ab195889">ab195889</a> Anti-alpha Tubulin antibody (Alexa Fluor® 594) was used to counterstain tubulin at 1/1000 dilution (Red). The Nuclear counterstain was DAPI (Blue). Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <a href="mailto:ab150077"><u>ab150077</u></a> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-GAD65 antibody [EPR22952-70] (ab239372)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Beta-TC-6 (Mouse pancreas insulinoma beta cell) cells labelling GAD65 with ab239372 at 1/60 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



GAD65 was immunoprecipitated from 0.35 mg Mouse brain lysate with ab239372 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab239372 at 1/1000 dilution (0.45  $\mu$ g/ml). VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse brain lysate 10 µg

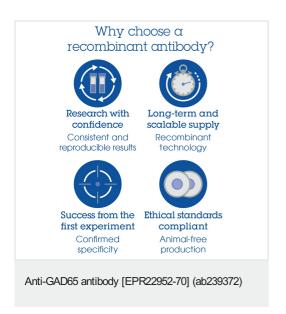
Lane 2: ab239372 IP in Mouse brain lysate

**Lane 3:** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab239372 in Mouse brain lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds

Bands above 100kDa are multimers of GAD65 (PMID: 10601283).



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

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors