abcam

Product datasheet

Rabbit monoclonal [EPR20578] to GAD67/GAD1 ab213508

Recombinant RabMAb

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Overview

Product name Rabbit monoclonal [EPR20578] to GAD67/GAD1

Rabbit **Host species**

Tested applications Suitable for: ICC/IF, WB, IHC-P, IP

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Common marmoset

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: Human cerebellum and fetal brain lysates; Mouse and rat cerebellum and brain lysates; IHC-

P: Mouse and rat cerebral cortex, cerebellar cortex and pancreas tissues; IP: Human brain lysate;

ICC/IF: Mouse primary neuron and rat midbrain neuron cells.

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

- Animal-free production

For more information see here.

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

Purity Protein A purified

Clonality Monoclonal

Clone number EPR20578

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab213508 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
ICC/IF		1/50.
WB		1/1000. Detects a band of approximately 67 kDa (predicted molecular weight: 67 kDa).
IHC-P	★★★★★ (3)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. We don't recommend this antibody for human in IHC. In our hands human tissues showed weak and non-specific staining.
IP		1/30.

Target

Function Catalyzes the production of GABA.

Tissue specificity Isoform 3 is expressed in pancreatic islets, testis, adrenal cortex, and perhaps other endocrine

tissues, but not in brain.

Involvement in diseaseDefects in GAD1 are the cause of cerebral palsy spastic quadriplegic type 1 (CPSQ1)

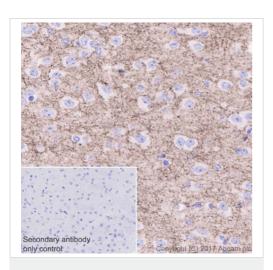
[MIM:603513]. A non-progressive disorder of movement and/or posture resulting from defects in

the developing central nervous system. Affected individuals manifest symmetrical, non-progressive spasticity and no adverse perinatal history or obvious underlying alternative diagnosis. Developmental delay, mental retardation and sometimes epilepsy can be part of the

clinical picture.

Sequence similaritiesBelongs to the group II decarboxylase family.

Images

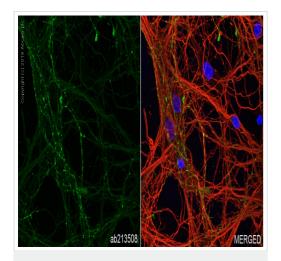


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal
[EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling GAD1/GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse cerebral cortex (PMID: 25904362). Counter stained with Hematoxylin.

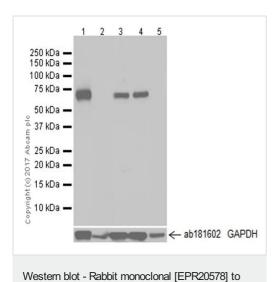
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

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



Immunocytochemistry/ Immunofluorescence - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neuron cells labelling GAD1/GAD67 with ab213508 at 1/50 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing positive staining in mouse primary neuron cell. Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection. ab195889 Anti-alpha Tubulin antibody (Alexa Fluor® 594) was used to counterstain tubulin at 1/1000 dilution (Red). The nuclear counterstain was DAPI (Blue).



GAD67/GAD1 (ab213508)

All lanes : Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508) at 1/1000 dilution

Lane 1: Mouse cerebellum lysate

Lane 2 : Mouse lung lysate

Lane 3: Rat brain lysate

Lane 4: Rat cerebellum lysate

Lane 5: Rat lung 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

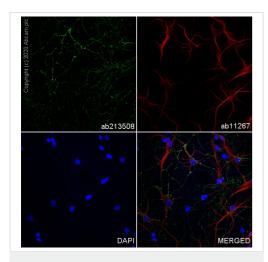
Developed using the ECL technique.

Predicted band size: 67 kDa **Observed band size:** 67 kDa

Exposure time: 10 seconds

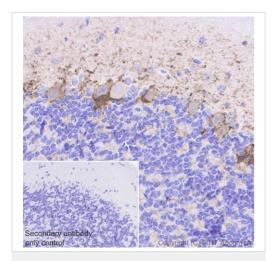
Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control: Mouse lung, rat lung (PMID: 10671565).



Immunocytochemistry/ Immunofluorescence - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized rat midbrain neuron cells labelling GAD1/GAD67 with ab213508 at 1/50 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 μ g/mL) (Green). Confocal image showing positive staining in rat midbrain neuron cell. Confocal scanning Z step was set as 0.3 μ m followed by image processing with maximum Z projection. **ab11267** Anti-MAP2 mouse monoclonal antibody was used to counterstain tubulin at 1/500 dilution (4 μ g/mL) followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution (2 μ g/mL) (Red). The nuclear counterstain was DAPI (Blue).

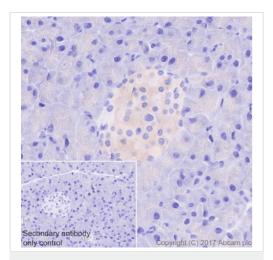


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded mouse cerebellar cortex tissue labeling GAD1/GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse cerebellar cortex (PMID: 17235515). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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

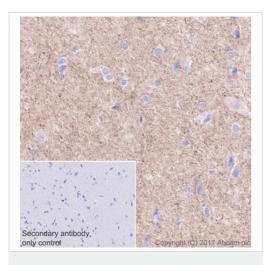


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue labeling GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on islet of mouse pancreas (PMID: 8243826). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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

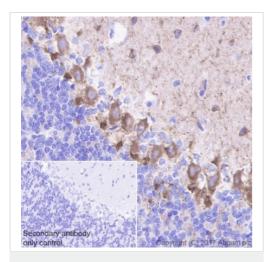


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on rat cerebral cortex (PMID: 25904362). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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

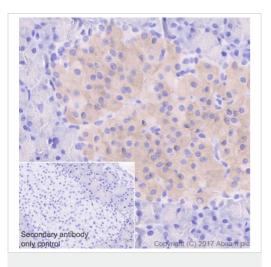


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded rat cerebellar cortex tissue labeling GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on rat cerebellar cortex (PMID: 17235515). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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

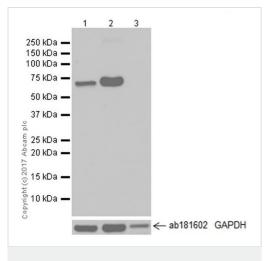


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Immunohistochemical analysis of paraffin-embedded rat pancreas tissue labeling GAD67 with ab213508 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on islet of rat pancreas (PMID: 8243826). Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

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



Western blot - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

All lanes : Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508) at 1/1000 dilution

Lane 1: Human cerebellum lysate

Lane 2: Human fetal brain lysate

Lane 3: Human lung 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

Developed using the ECL technique.

Predicted band size: 67 kDa **Observed band size:** 67 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Negative control: Human lung (PMID: 10671565).

250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 15 kDa — 10 kDa —

Western blot - Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508)

Rabbit monoclonal [EPR20578] to GAD67/GAD1 (ab213508) at 1/1000 dilution + Mouse brain lysate at 10 μg

Secondary

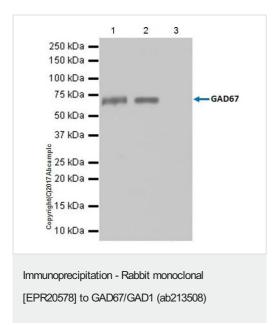
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 67 kDa **Observed band size:** 67 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



GAD67 was immunoprecipitated from 0.35 mg of human brain lysate with ab213508 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab213508 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

Lane 1: Human brain lysate 10 µg (Input).

Lane 2: ab213508 IP in human brain lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab213508 in human brain lysate.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 10 seconds.



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