abcam

Product datasheet

Anti-VGluTl antibody [EPR22269] ab227805



★★★★★ 7 Abreviews 4 References 16 Images

Overview

Product name Anti-VGluT1 antibody [EPR22269]

Rabbit monoclonal [EPR22269] to VGluT1 **Description**

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Human, mouse and rat brain lysates. IHC-P: Mouse cerebrum tissue; Rat and human

cerebral cortex tissues. IHC-Fr: Mouse and rat hippocampus tissues. Human cerebellum and

hippocampus tissue. IP: 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

- 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, 49% Glycerol (glycerin, glycerine), PBS

Purity Protein A purified

Clonality Monoclonal Clone number EPR22269

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab227805 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	★★★★ (5)	Use a concentration of 1 µg/ml.
WB		1/1000. Detects a band of approximately 62 kDa (predicted molecular weight: 62 kDa).
IHC-P	**** <u>(2)</u>	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		Use a concentration of 0.1 µg/ml.
IP		1/30.

larget		
Function	Mediates the uptake of glutamate into synaptic vesicles at presynaptic nerve terminals of excitatory neural cells. May also mediate the transport of inorganic phosphate.	
Tissue specificity	Expressed in several regions of the brain including amygdala, cerebellum, cerebral cortex, hippocampus, frontal lobe, medulla, occipital lobe, putamen and temporal lobe.	
Sequence similarities	Belongs to the major facilitator superfamily. Sodium/anion cotransporter family. VGLUT subfamily.	
Cellular localization	Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Membrane. Cell junction, synapse, synaptosome.	

Images



Immunohistochemistry (Frozen sections) - Anti-VGluT1 antibody [EPR22269] (ab227805)



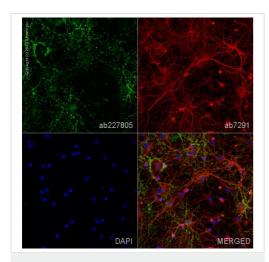
Immunohistochemistry (Frozen sections) - Anti-VGluT1 antibody [EPR22269] (ab227805)

IHC image of vGluT1 staining in a section of frozen normal human cerebellum performed on a Leica BONDTM system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab227805, 0.1ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

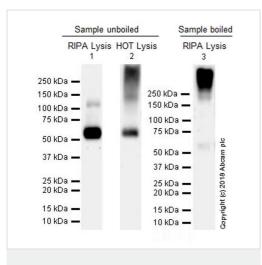
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

IHC image of vGluT1 staining in a section of frozen normal human cerebellum performed on a Leica BONDTM system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab227805, 0.1ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-VGluT1 antibody [EPR22269] (ab227805)



Western blot - Anti-VGluT1 antibody [EPR22269] (ab227805)

ab227805 staining VGluT1 in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab227805 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS,
Perkin Elmer) and a maximum intensity projection of confocal
sections is shown.

All lanes : Anti-VGluT1 antibody [EPR22269] (ab227805) at 1/1000 dilution

All lanes: Mouse brain lysate

Lysates/proteins at 20 µg/ml per lane.

Secondary

Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000

Lanes 2-3 : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

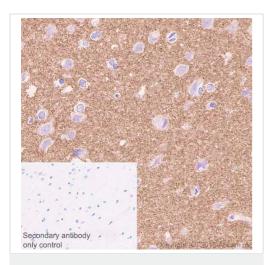
Predicted band size: 62 kDa Observed band size: 62 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Different lysate preparation and boiling methods resulted in different banding patterns observed. The lysates in lanes 1 and 3 were prepared by RIPA lysis method with and without boiling. The lysate in lane 2 was prepared by 1% SDS Hot lysis method. For

WB sample preparation, we recommend using RIPA lysis buffer and without boiling.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody

[EPR22269] (ab227805)

Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on human cerebral cortex (PMID: 29532891). Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

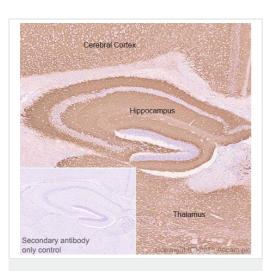


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody
[EPR22269] (ab227805)

Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Cytoplasmic staining on rat cerebral cortex (PMID: 29532891) is observed. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

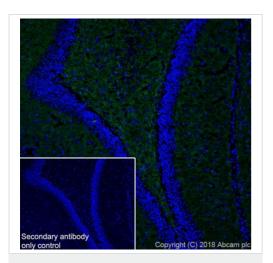
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody
[EPR22269] (ab227805)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Specific cytoplasmic staining on mouse hippocampus, positive staining was also observed on mouse cerebral cortex and thalamus (PMID: 29532891). Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

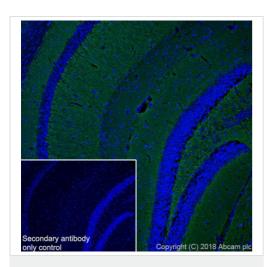


Immunohistochemistry (Frozen sections) - Anti-VGluT1 antibody [EPR22269] (ab227805)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen rat hippocampus tissue labeling VGluT1 with ab227805 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining on rat hippocampus (PMID: 29532891) is observed.

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunohistochemistry (Frozen sections) - Anti-VGluT1 antibody [EPR22269] (ab227805)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse hippocampus tissue labeling VGluT1 with ab227805 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Cytoplasmic staining on mouse hippocampus (PMID: 29532891) is observed.

The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-VGluT1 antibody [EPR22269] (ab227805)

VGluT1 was immunoprecipitated from 0.35 mg of mouse brain lysate with ab227805 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab227805 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

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

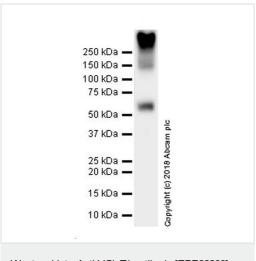
Lane 2: ab227805 IP in mouse brain lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab227805 in mouse brain lysate.

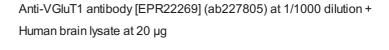
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

The pattern of oligomeric/dimeric forms observed is consistent with what has been described in the literature (PMID: 15192755).



Western blot - Anti-VGluT1 antibody [EPR22269] (ab227805)



Secondary

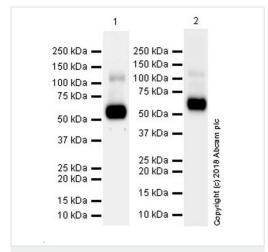
VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Predicted band size: 62 kDa **Observed band size:** 62 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Oligomeric forms observed in the human brain lysate were due to the 1% SDS Hot lysate preparation method, consistent with what has been described in the literature (PMID: 15192755).



Western blot - Anti-VGluT1 antibody [EPR22269] (ab227805)

All lanes : Anti-VGluT1 antibody [EPR22269] (ab227805) at 1/1000 dilution

Lane 1: Mouse brain lysate

Lane 2: Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Lane 2 : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

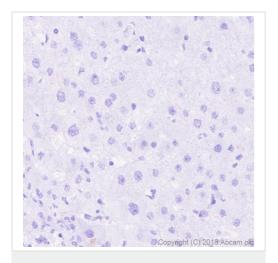
Predicted band size: 62 kDa Observed band size: 62 kDa Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

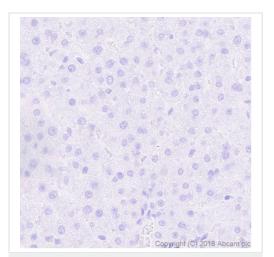
Brain lysates were prepared with RIPA lysis buffer. The pattern of oligomers/dimers observed is consistent with what has been described in the literature (PMID: 15192755).

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Negative control: No staining on human liver is observed.



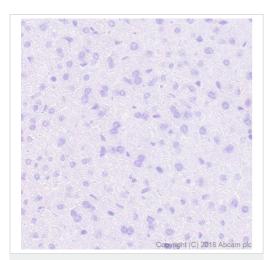
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody
[EPR22269] (ab227805)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody
[EPR22269] (ab227805)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

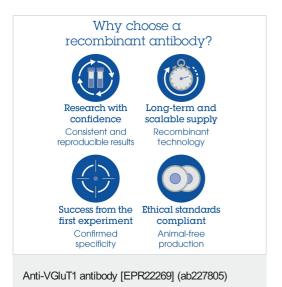
Negative control: No staining on rat liver is observed.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VGluT1 antibody
[EPR22269] (ab227805)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling VGluT1 with ab227805 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Negative control: No staining on mouse liver.



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