# abcam

### Product datasheet

## Anti-GFAP antibody ab7260

\*\*\*\* 103 Abreviews 1012 References 14 Images

Overview

Product name Anti-GFAP antibody

**Description** Rabbit polyclonal to GFAP

Host species Rabbit

**Specificity** Specifically recognizes mammalian GFAP on western blots and immunocytochemically. Detects

a band of 55kDa corresponding to GFAP and also a GFAP derived 48kDa band. Some

customers have successfully used ab7260 on Zebrafish lysates; however we have conflicting data

to suggest that not all batches will be suitable for work on Zebrafish. For further information,

please contact Abcam Scientific Support.

Tested applications Suitable for: IHC (PFA fixed), ICC/IF, IP, WB, IHC-P

Species reactivity Reacts with: Mouse, Rat

Predicted to work with: Horse, Cow, Human, Pig, Mammals

Immunogen Recombinant full length protein corresponding to Human GFAP. Isotype 1 expressed in and

purified from E. coli.

**General notes** In some cases, the antibody may appear red in color. This is due to small amounts of hemolysis,

and does not affect antibody performance.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

**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 or -

80°C. Avoid freeze / thaw cycle.

**Storage buffer** Preservative: 0.03% Sodium azide

**Purity** Whole antiserum

**Clonality** Polyclonal

1

**Isotype** IgG

### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab7260 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
IHC (PFA fixed)		Use at an assay dependent concentration.
ICC/IF	<b>★★★★★ (22)</b>	1/5000.
IP		1/30.
WB	★★★★☆ (13)	1/10000. Detects a band of approximately 55,48 kDa. This lower 48kDa band is thought to be a degradation product.
IHC-P	<b>★★★★★</b> (26)	1/1000 - 1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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**Function** 

GFAP, a class-Ill intermediate filament, is a cell-specific marker that, during the development of the central nervous system, distinguishes astrocytes from other glial cells.

Tissue specificity

Expressed in cells lacking fibronectin.

Involvement in disease

Defects in GFAP are a cause of Alexander disease (ALEXD) [MIM:203450]. Alexander disease is a rare disorder of the central nervous system. It is a progressive leukoencephalopathy whose hallmark is the widespread accumulation of Rosenthal fibers which are cytoplasmic inclusions in astrocytes. The most common form affects infants and young children, and is characterized by progressive failure of central myelination, usually leading to death usually within the first decade. Infants with Alexander disease develop a leukoencephalopathy with macrocephaly, seizures, and psychomotor retardation. Patients with juvenile or adult forms typically experience ataxia, bulbar signs and spasticity, and a more slowly progressive course.

Sequence similarities

Belongs to the intermediate filament family.

Post-translational modifications

Phosphorylated by PKN1.

Cellular localization

Cytoplasm. Associated with intermediate filaments.

### **Images**

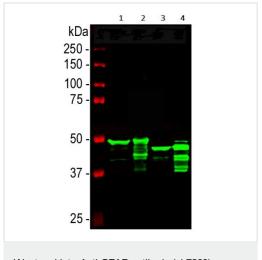


Immunohistochemistry (PFA fixed) - Anti-GFAP antibody (ab7260)

GFAP antibody ab7260 was used with Tissue Clearing Kit <a href="mailto:ab243298"><u>ab243298</u></a> to penetrate, stain and clear a 1 mm coronal section of mouse brain. Blue: DAPI, Green: GFAP.

Learn more about <u>tissue clearing kits, reagents, and</u>
<u>protocols</u> designed to make it easier to stain thick tissue sections and get more data from each valuable tissue section.

To use this antibody with tissue clearing, use Tissue Clearing Kit **ab243298**. For 1 mm brain sections, we recommend a starting dilution of 1:1000, and also using Goat Anti-Rabbit lgG H&L Alexa Fluor<sup>®</sup> 488 (**ab150077**) at a dilution of 1:400.



Western blot - Anti-GFAP antibody (ab7260)

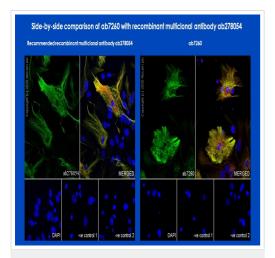
All lanes: Anti-GFAP antibody (ab7260) at 1/5000 dilution

Lane 1: Rat brain lysate

Lane 2: Rat spinal cord lysate

Lane 3: Mouse brain lysate

Lane 4: Mouse spinal cord lysate



Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody (ab7260) ICC/IF side-by-side comparison with the recombinant multiclonal antibody **ab278054** 

This ICC/IF image is a comparison between ab7260 and the alternative recombinant multiclonal antibody <a href="mailto:ab278054">ab278054</a>.

Left side - Recombinant multiclonal to GFAP - <a href="mailto:ab278054">ab278054</a>
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1%

TritonX-100 permeabilized mouse primary neural/glia cells labelling GFAP with <a href="mailto:ab278054">ab278054</a> at 1/500 (0.938 µg/ml) dilution, followed by <a href="mailto:ab278054">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 astrocytes. Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.

**ab10062** Anti-GFAP mouse monoclonal antibody at 1/200 dilution, followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at a 1/500 dilution (Red) to counterstain. The nuclear counterstain was DAPI (Blue).

Negative control 1: <u>ab278054</u> at a 1/500 dilution followed by <u>ab150120</u> at a 1/500 dilution.

Negative control 2: <u>ab10062</u> at a 1/500 dilution followed by ab150077 at a 1/1000 dilution.

Right side - Polyclonal antibody to [GFAP] - ab7260 Same testing conditions as **ab278054**.

Why choose a recombinant antibody?

Research with confidence - consistent and reproducible results with every batch

Long-term and scalable supply - powered by recombinant technology for fast production

Success from the first experiment - confirmed specificity through extensive validation

Ethical standards compliant - production is animal-free



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

IHC-P side-by-side comparison with the recombinant multiclonal antibody **ab278054** 

This IHC-P image is a comparison between ab7260 and the alternative recombinant multiclonal antibody <u>ab278054</u>.

Left side - Recombinant multiclonal to GFAP - <u>ab278054</u>

IHC image of GFAP staining in a formalin fixed, paraffin embedded normal rat brain tissue section, performed on a Leica Bond™ system using the standard protocol F.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with <u>ab278054</u> at 1/2000 dilution 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 hematoxylin and mounted with DPX.

Right side - Polyclonal antibody to [GFAP] - ab7260 Same testing conditions as **ab278054**.

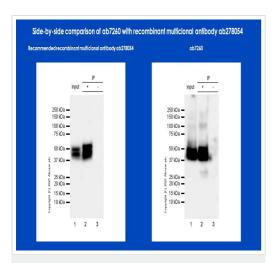
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Immunoprecipitation - Anti-GFAP antibody (ab7260)

Immunoprecipitation side-by-side comparison with the recombinant multiclonal antibody <u>ab278054</u>

This immunoprecipitation image is a comparison between ab7260 and the alternative recombinant multiclonal antibody <u>ab278054</u>. Left side - Recombinant multiclonal to GFAP - <u>ab278054</u> GFAP was immunoprecipitated from 0.35 mg mouse brain lysate with <u>ab278054</u> at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab278054</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: Mouse brain lysate 10 µg.

Lane 2: <u>ab278054</u> IP in mouse brain lysate.

Lane 3: Rabbit monoclonal  $\lg G(\underline{ab172730})$  instead of  $\underline{ab278054}$  in mouse brain lysate.

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

Exposure time: 3 seconds.

Right side - Polyclonal antibody to [GFAP] - ab7260

Same testing conditions as ab278054.

Why choose a recombinant antibody?

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All lanes: Anti-GFAP antibody (ab7260) at 1/5000 dilution

**Lanes 1-3:** Rat thoracotomy, spinal cord homogenate

Lanes 4-5: Rat thoracotomy sham, spinal cord homogenate

Lanes 6-7: Rat nerve transect sham, spinal cord homogenate

Lanes 8-9: Rat nerve transect, spinal cord homogenate

Lysates/proteins at 30 µg per lane.



All lanes: HRP conjugated goat anti-rabbit at 1/3000 dilution

Developed using the ECL technique.

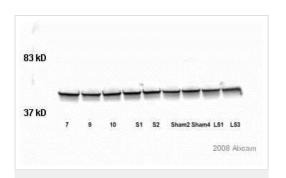
Observed band size: 53 kDa

Exposure time: 1 minute

IHC image of GFAP staining in a formalin fixed, paraffin embedded normal mouse brain tissue section, performed on a Leica Bond  $^{\text{TM}}$  system using the standard protocol B.

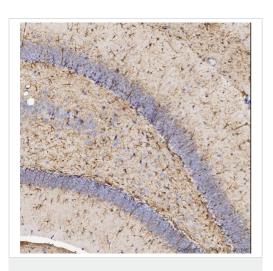
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7260 at 1/2000 dilution for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with hematoxylin and mounted with DPX.

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



Western blot - Anti-GFAP antibody (ab7260)

This image is courtesy of an anonymous Abreview



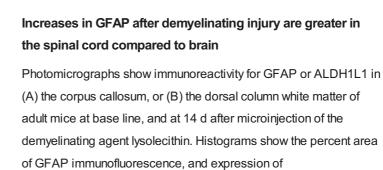
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

incubation times.

# Brain Control 14d Control 14d Control 14d Control 14d Control 14d Control 14d

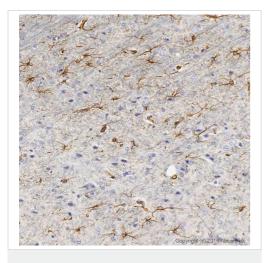
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

Yoon, H. et al PLoS One. 2017 Jul 10;12(7):e0180697. doi: 10.1371/journal.pone.0180697. eCollection 2017 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



GFAP was detected using ab7260.

(From Figure 4A and 4B of Hoon et al)



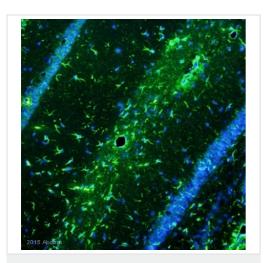
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

IHC image of GFAP staining in a formalin fixed, paraffin embedded normal rat brain tissue section, performed on a Leica Bond™ system using the standard protocol F.

GFAP/ALDH1L1+ astrocyte, was significantly greater in the spinal cord compared to the corpus callosum 14d post-lysolecithin lesion.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7260 at 1/2000 dilution 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 hematoxylin and mounted with DPX.

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.

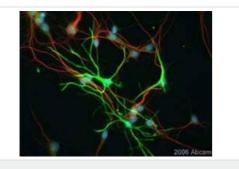


Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody (ab7260)

This image is courtesy of an Abreview submitted by Chia-Li Lin.

ab7260 staining GFAP in cells from mouse brain tissue sections by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with paraformaldehyde, permeabilized with 0.1% Tween 20 in PBS and blocked with 1% BSA for 40 minutes at 25°C. Samples were incubated with primary antibody (1/1200 in TBS) for 24 hours at 4°C. Goat Anti-Rabbit IgG H&L (DyLight® 488) (ab96883) was used as the secondary antibody at a dilution of 1/200.

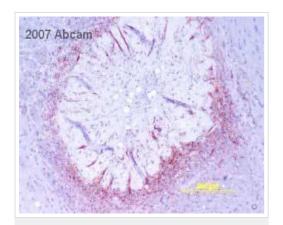


Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody (ab7260)

ab7260 at 1/10000 dilution staining mouse cortical astrocytes by Immunocytochemistry.

The cells were permeabilized with Triton/HEPES buffer prior to primary application. The antibody was incubated with the cells for 18 hours and then bound antibody was detected with an Alexa Fluor <sup>®</sup> 488 conjugated goat anti-rabbit antibody.

This image is courtesy of an Abreview submitted by **Charmaine Noonan.** 

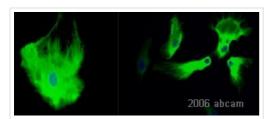


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

This image is courtesy of an Abreview submitted by Mr Osama Mohsen

ab7260 staining rat brain tissue sections by IHC-P.

Sections were fixed in formaldehyde and blocked with a commercialy available blocking agent prior to incubating with ab7260, diluted 1/5000 for 20 hours at 4°C. An HRP conjugated mouse polyclonal (universal HRP polymer detection) antibody was used as the secondary.

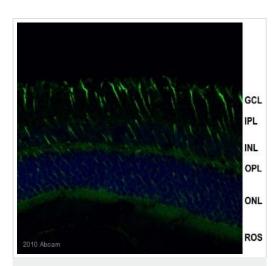


Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody (ab7260)

This image is courtesy of an Abreview submitted by Ms Nancy Nutile-McMenemy

ab7260 staining rat pup cortical preps by ICC/IF.

The preps were grown for 14 days in culture and plated onto coverslips. The preps were acid/alcohol fixed and blocked prior to incubation with ab7260. Bound antibody was detected using an Alexa Fluor <sup>®</sup>488 conjugated goat polyclonal antibody. Nuclei were visualized using DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GFAP antibody (ab7260)

This image was kindly supplied by Dr Vladimir Mlenkovic by Abreview

ab7260 staining GFAP in mouse eye tissue sections by Immunohistochemistry (paraffin embedded sections).

Tissue was fixed with paraformaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer pH 6.0. Samples were then permeabilized using 0.5% Triton X-100 and blocked with 5% serum for 20 minutes at 25°C; followed by incubation with the primary antibody, at a 1/500 dilution, for 16 hours at 4°C. The secondary antibody used was a goat anti-rabbit lgG conjugated to Alexa Fluor<sup>®</sup> 488 used at a 1/5000 dilution.

The retinal layers are: ganglion cells layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and photoreceptor outer segments (ROS). Nuclei were counterstained with DAPI.

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