abcam

Product datasheet

# Anti-GFAP antibody [EPR19996] - BSA and Azide free ab223127

Recombinant RabMAb

12 Images

## Overview

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|---|---|
| **Product name** | Anti-GFAP antibody [EPR19996] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR19996] to GFAP - BSA and Azide free |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** ICC/IF, Flow Cyt (Intra), IHC-P, IP, WB, IHC-Fr |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | Flow Cyt: Mouse primary brain cell. ICC/IF: Rat hippocampal mixed glia. |
| **General notes** | ab223127 is the carrier-free version of **ab207165**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.<br><br>Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.<br><br>This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.<br><br>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

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **Storage buffer** | pH: 7.2 |

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|---|---|
| | Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR19996 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab223127 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

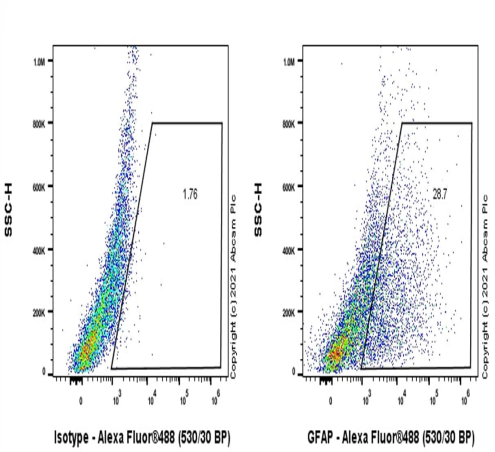
| Application | Abreviews | Notes |
|---|---|---|
| **ICC/IF** | | Use at an assay dependent concentration. |
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **IP** | | Use at an assay dependent concentration. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 40-54 kDa (predicted molecular weight: 49 kDa). |
| **IHC-Fr** | | Use at an assay dependent concentration.<br>Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20) |

## Target

| | |
|---|---|
| **Function** | GFAP, a class-III 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.

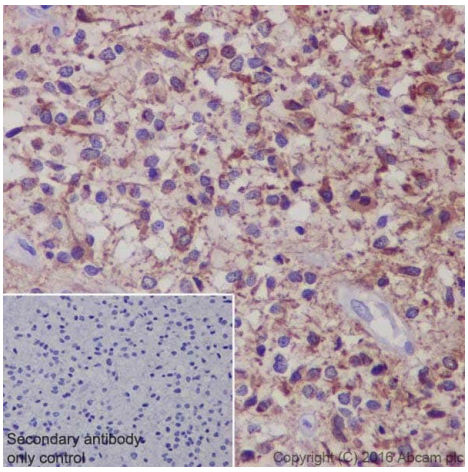
## Images




Flow Cytometry (Intracellular) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

This data was developed using **ab207165**, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Mouse primary brain cells cells labelling GFAP with **ab207165** at 1/500 dilution (0.1ug)/ Right compared with a Rabbit monoclonal IgG (**ab172730**) / Left isotype control. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



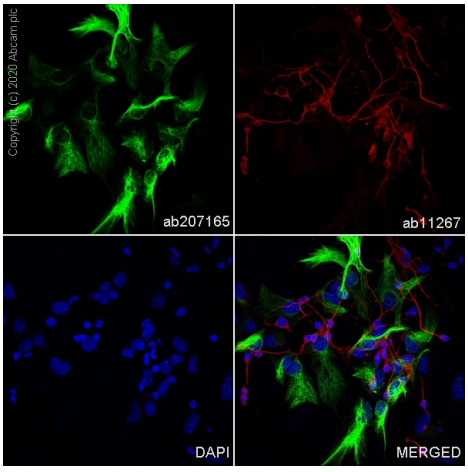

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasmic staining on tumor cells of human glioma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).

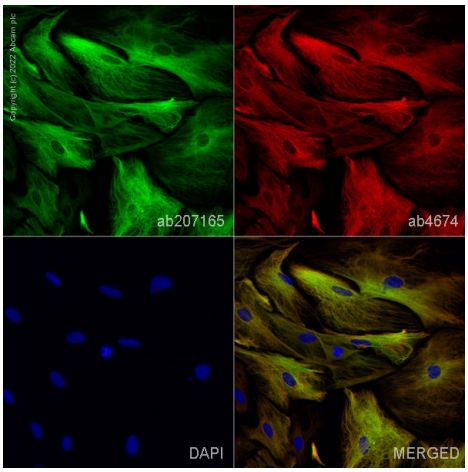
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.




Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neural/glia cells labelling GFAP with **ab207165** at 1/1000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (2 µg/mL) (Green). Confocal image showing cytoplasmic staining in mouse primary astrocyte. Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection. **ab11267** Anti-MAP2 mouse monoclonal antibody was used to counterstain tubulin at 1/500 dilution (4 µg/mL) followed by **ab150120** Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) at 1/1000 dilution (2 µg/mL) (Red). The nuclear counterstain was DAPI (Blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).



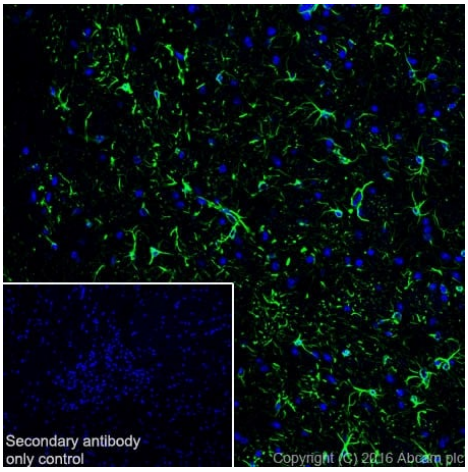

Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunofluorescence staining of GFAP using **ab207165** in primary rat hippocampal mixed glia, (prepared from P2 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDPHP4m), DIV4. The cells were fixed with 100% MeOH (5 min), permeabilized with 0.1% Triton-X-100 (in PBS) for 5 mins 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 **ab207165** at 0.1 µg/ml and **ab4674**, Anti-GFAP antibody, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150176**, Goat Anti-Chicken IgY H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown. The antibody **ab207165** gave comparable results using 4% formaldehyde fixation (10 min).

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).



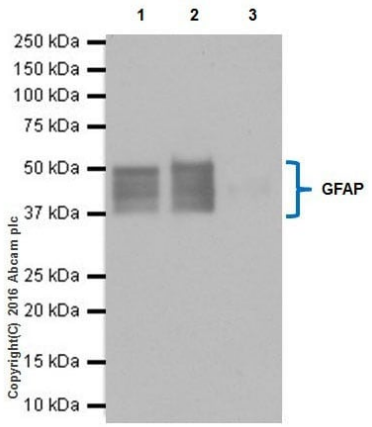

Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebrum tissue labeling GFAP with **ab207165** at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Cytoplasmic and membrane staining on glial cells of mouse cerebrum is observed.

The nuclear counterstain is DAPI (blue).

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




Immunoprecipitation - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

GFAP was immunoprecipitated from 0.35 mg of human brain lysate with **ab207165** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab207165** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

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

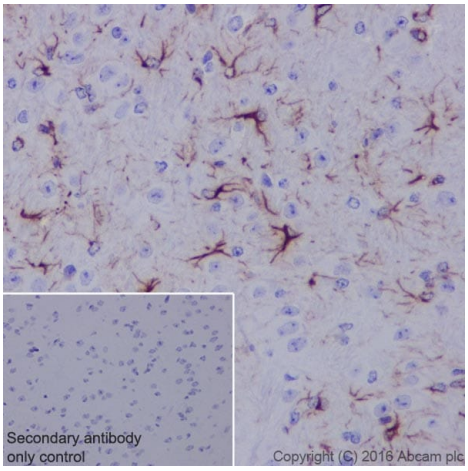
Lane 2: **ab207165** IP in human brain lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of **ab207165** in human brain lysate.

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

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).



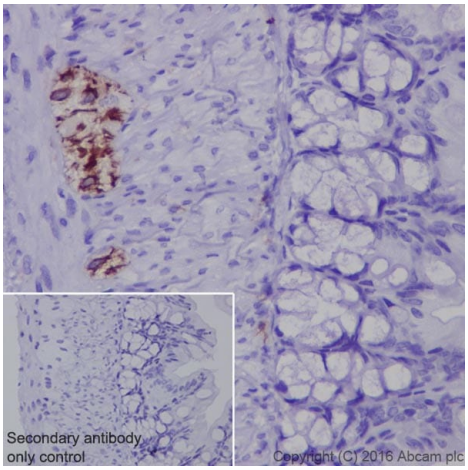

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasmic and membrane staining on glial cells of mouse cerebrum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).

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-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Specific cytoplasmic staining on myenteric nerve plexus, and negative on epithelial cells and smooth muscle cells of mouse colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).

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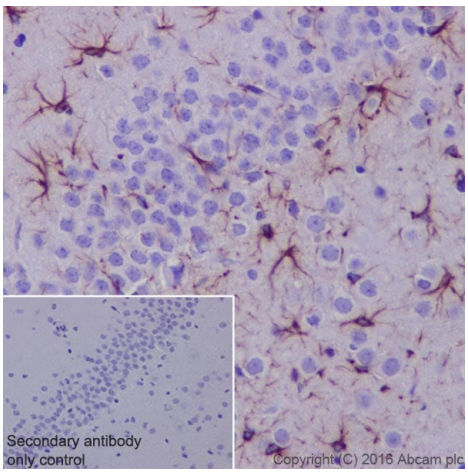


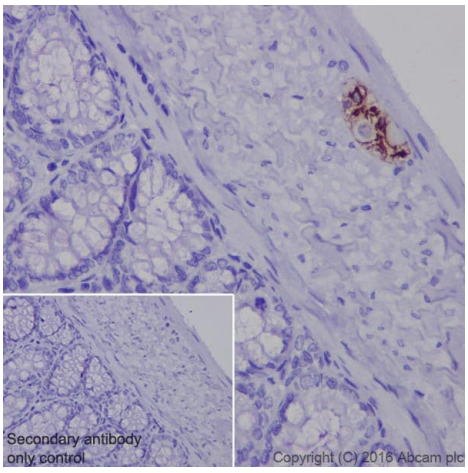

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasmic and membrane staining on glial cells of rat hippocampus is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab207165**).

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-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Specific cytoplasmic staining on myenteric nerve plexus, and negative on epithelial cells and smooth muscle cells of rat colon is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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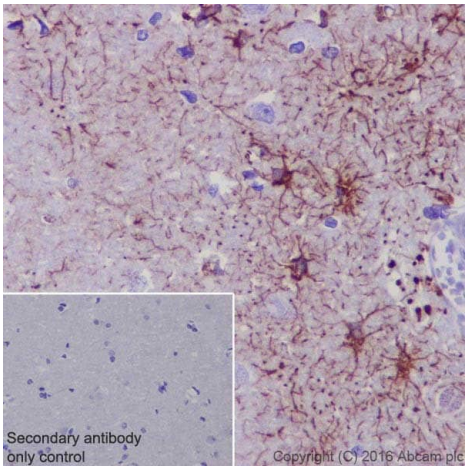
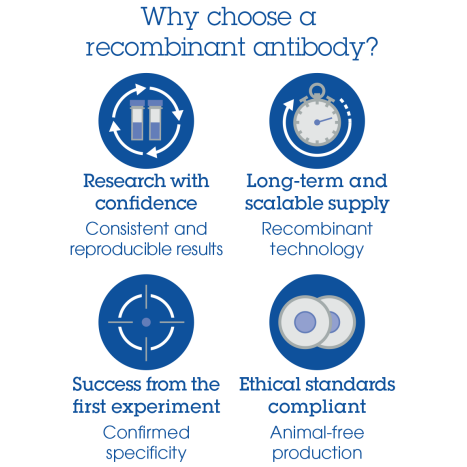



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling GFAP with **ab207165** at 1/400 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Cytoplasmic and membrane staining on glial cells of human cerebrum [PMID: 15378652] is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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




Anti-GFAP antibody [EPR19996] - BSA and Azide free (ab223127)



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