abcam

Product datasheet

Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free ab279301

Recombinant

6 Images

Overview

Product name	Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free
Description	Mouse monoclonal [EPR1034Y] to GFAP - Chimeric – BSA and Azide free
Host species	Mouse
Tested applications	**Suitable for:** IHC-Fr, IP, IHC-P, WB, Flow Cyt (Intra), ICC
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 tissue lysate. IHC-Fr: Mouse cerebrum tissue. Flow Cyt (intra): Rat primary neural/glia cells. IP: Rat brain tissue lysate. IHC-P: Human cerebral cortex tissue. ICC: Primary hippocampal rat neurons/glia
General notes	ab279301 is the carrier free version of **ab279289**.

This mouse monoclonal chimeric antibody has been engineered from a RabMAb parent antibody (**ab68428**). By necessity, some rabbit sequence is retained as part of the variable domain. When multiplexing with other rabbit-derived antibodies, using cross absorbed Fc-reactive secondary antibodies are recommended.

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.

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.

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.

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.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1034Y
Isotype	IgG1

Applications

The Abpromise guarantee   Our **Abpromise guarantee** covers the use of ab279301 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-Fr		Use at an assay dependent concentration. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

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

Western blot - Anti-GFAP antibody [EPR1034Y] -
Mouse IgG1 (Chimeric) - BSA and Azide free
(ab279301)
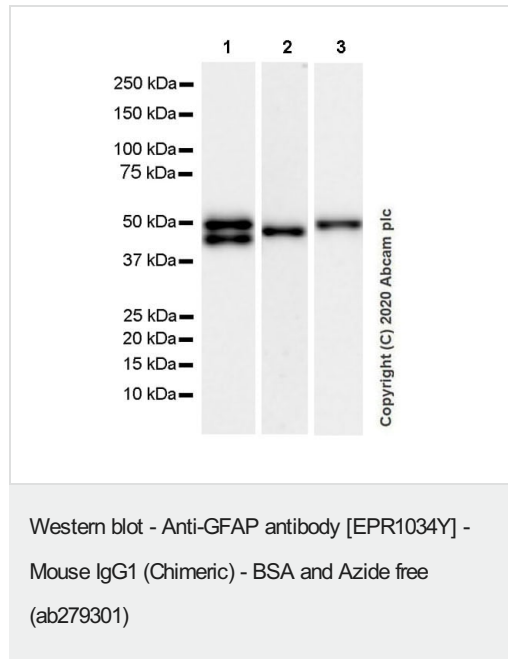
All lanes : Anti-GFAP antibody [EPR1034Y] - Mouse IgG1
(Chimeric) (**ab279289**) at 1/1000 dilution

Lane 1 : Human brain tissue lysate

Lane 2 : Mouse brain tissue lysate

Lane 3 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at
1/5000 dilution

This data was produced using **ab279289**, the same clone in a
different formulation.

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with the literature
(PMID: 824020, 2294, 6340792).

Exposure times: Lane 1: 3.25 seconds; Lane 2: 48 seconds; Lane
3: 10 seconds.
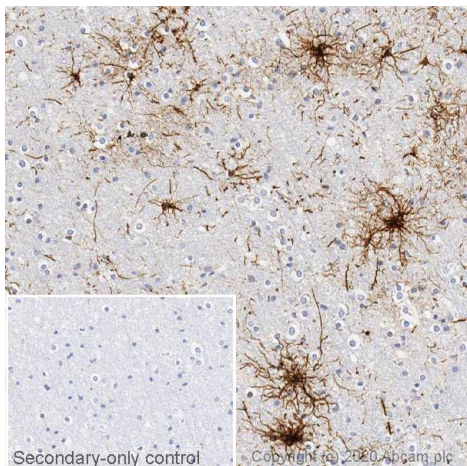
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free (ab279301)
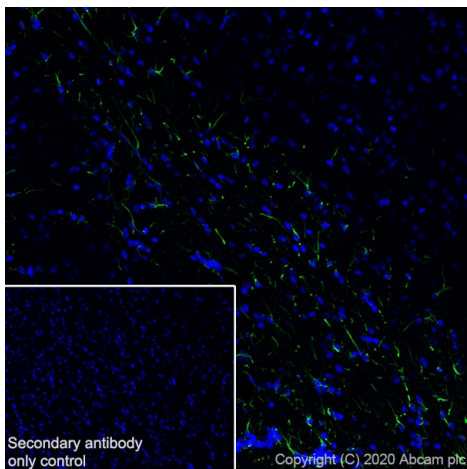
This data was produced using **ab279289**, the same clone in a different formulation.

IHC image of GFAP staining in a section of formalin-fixed paraffin-embedded normal human cerebral cortex performed on a Leica BOND^{TM} system using the standard protocol F.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab279289**, 1µg/ml, for 15 mins at room temperature. A rabbit anti-mouse IgG1, **ab125913**, was added for 8 mins at room temperature and detected using an HRP conjugated goat anti-rabbit 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.
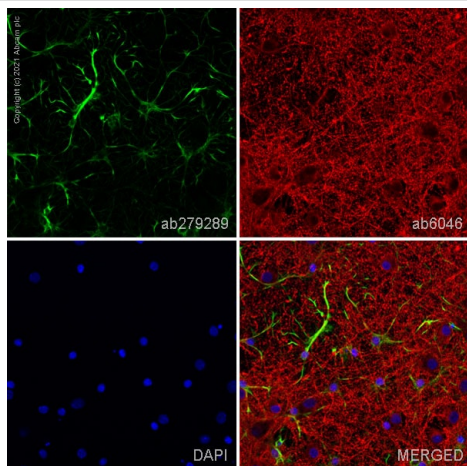

Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free (ab279301)

This data was produced using **ab279289**, the same clone in a different formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebrum tissue labeling GFAP with **ab279289** at /500 (1.968 µg/ml) dilution followed by **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). Positive staining on mouse cerebrum is observed. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is **ab150113** Goat Anti-Mouse IgG H&L (Alexa Fluor®488)at 1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Immunocytochemistry - Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free (ab279301)

This data was produced using **ab279289**, the same clone in a different formulation.

ab279289 staining GFAP in primary hippocampal rat neurons/glia (obtained from Neuromics, cat. no. PC35101), DIV14. The cells were fixed with 4% paraformaldehyde (10 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 **ab279289** at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150084**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). The antibody **ab279289** gave comparable results using MeOH fixation (100%, 5 min).
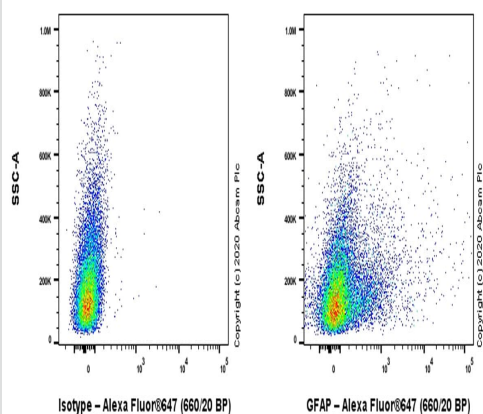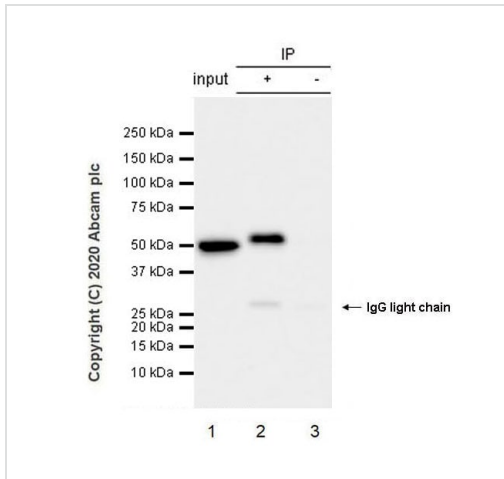

Flow Cytometry (Intracellular) - Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free (ab279301)

This data was produced using **ab279289**, the same clone in a different formulation.

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized rat primary neural/glia cells labelling GFAP with **ab279289** at 1/1000 dilution (0.1µg)/ Right compared with a Mouse monoclonal IgG isotype control/ Left.

Goat Anti-Mouse IgG (Alexa Fluor® 647, **ab150119**) at 1/2000 dilution was used as the secondary antibody.

Immunoprecipitation - Anti-GFAP antibody [EPR1034Y] - Mouse IgG1 (Chimeric) - BSA and Azide free (ab279301)

This data was produced using **ab279289**, the same clone in a different formulation.

GFAP was immunoprecipitated from 0.35 mg rat brain tissue lysate 10 µg with **ab279289** at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab279289** at 1/1000 dilution. mouse IgG for IP (HRP) (**ab131368**) was used at 1/5000 dilution.

Lane 1: Rat brain tissue lysate 10µg.

Lane 2: **ab279289** IP in rat brain tissue lysate.

Lane 3: Mouse monoclonal IgG1 (**ab18443**) instead of **ab279289** in rat brain tissue lysate.

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

Exposure time: 23 seconds.

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