abcam

Product datasheet

Anti-GFAP antibody [RM1003] ab278054

重组 RabMAb

12 图像

概述

产品名称	Anti-GFAP抗体[RM1003]
描述	兔重组multiclonal [RM1003] to GFAP
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, IHC-Fr, IP, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Human, mouse and rat brain tissue lysates; U-87 MG, C6 whole cell lysates. IHC-P: Human, mouse and rat cerebrum tissue. IHC-Fr: Mouse cerebrum tissue. Flow Cyt: Mouse primary brain cells. IP: Mouse brain lysate. ICC/IF: Rat hippocampal mixed glia and mouse primary neural/glia cells.
常规说明	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	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.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
纯度	Protein A purified
克隆	Recombinant Multiclonal
克隆编号	RM1003
同种型	IgG

应用

The Abpromise guarantee	**Abpromise™承诺保证使用ab278054于以下的经测试应用**

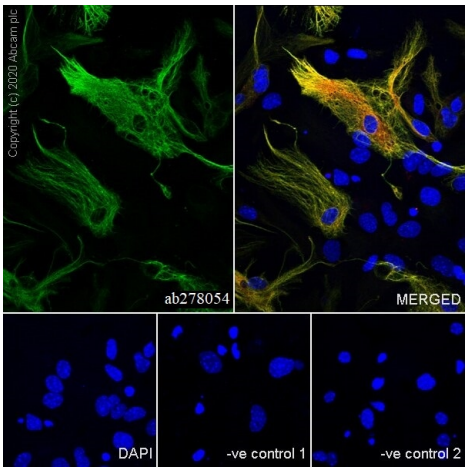
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 40-54 kDa (predicted molecular weight: 49 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		1/700. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		1/30.
ICC/IF		Use a concentration of 0.1 - 1 µg/ml.

靶标

功能	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.
组织特异性	Expressed in cells lacking fibronectin.
疾病相关	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.
序列相似性	Belongs to the intermediate filament family.
翻译后修饰	Phosphorylated by PKN1.
细胞定位	Cytoplasm. Associated with intermediate filaments.

图片



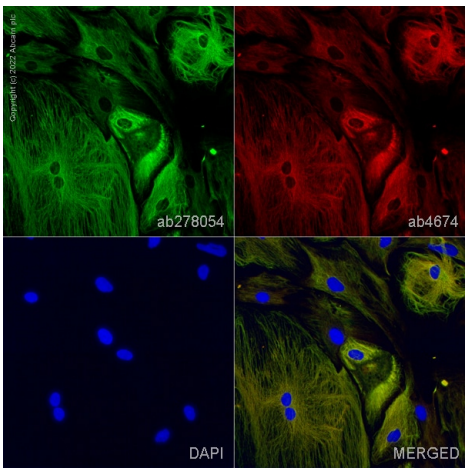
Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [RM1003] (ab278054)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neural/glia cells labelling GFAP with ab278054 at 1/500 (0.938 µg/ml) dilution, followed by **ab150077** 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: ab278054 at a 1/500 dilution followed by **ab150120** at a 1/500 dilution.

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

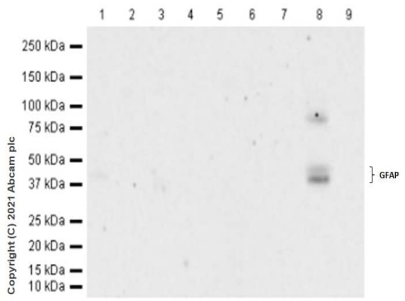


Immunocytochemistry/ Immunofluorescence - Anti-GFAP antibody [RM1003] (ab278054)

Immunofluorescence staining of GFAP using ab278054 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 ab278054 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 ab278054 gave comparable results using 4%

formaldehyde fixation (10 min).



Western blot - Anti-GFAP antibody [RM1003] (ab278054)

All lanes : Anti-GFAP antibody [RM1003] (ab278054) at 1/1000 dilution

Lane 1 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 2 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate
Lane 3 : SK-N-BE (2) (Human neuroblastoma neuroblast) whole cell lysate
Lane 4 : IMR-32 (Human lung fibroblast) whole cell lysate
Lane 5 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 6 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysate
Lane 7 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 8 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 9 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/20000 dilution

Predicted band size: 49 kDa
Observed band size: 40-54 kDa

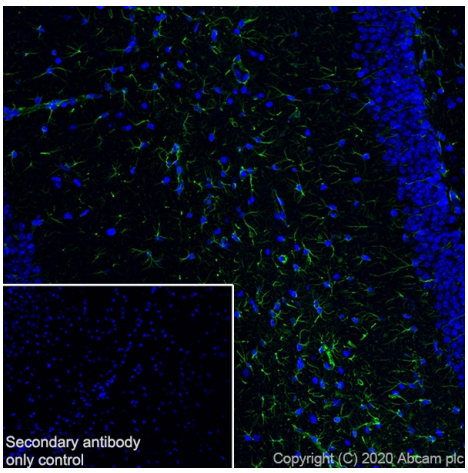
Exposure time: 180 seconds

Blocking and Diltuing buffer and concentration: 5% NFDM/TBST

GFAP is expressed primarily by astrocytes in the CNS as astrocytes marker. We have no suitable astrocyte for western blot testing.
U-87 MG and C6 were reported to express low level of GFAP (PMID: 23839947, PMID: 22096544, PMID: 20669222).

NIH/3T3, IMR-32, HeLa and PC-12 were reported as negative cell lines for GFAP (PMID: 824020; PMID:2294; PMID: 6340792; PMID:9466565, PMID:7895062, PMID: 28700643, PMID: 19272755).

No literature was found to support the expression in SH-SY5Y, Neuro-2a and SK-N-BE (2) cell.

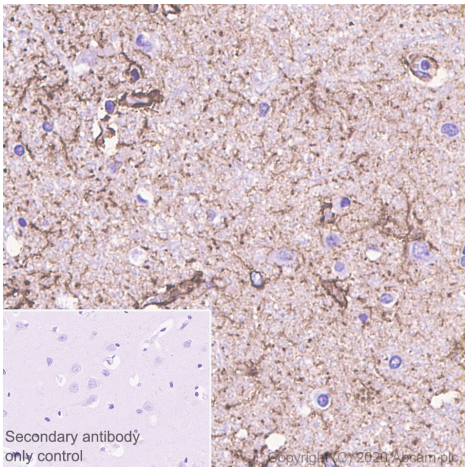


Immunohistochemistry (Frozen sections) - Anti-GFAP antibody [RP1003] (ab278054)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized mouse cerebrum tissue labeling GFAP with ab278054 at 1/700 (0.938 µg/ml) dilution followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at a 1/1000 dilution. Nuclear counterstain is DAPI. Positive staining on mouse cerebrum.

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) used at a 1/1000 dilution.

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

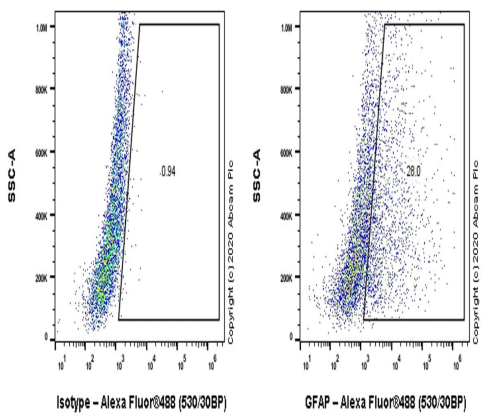


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [RP1003] (ab278054)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling GFAP with ab278054 at 1/2000 (0.235 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human cerebrum. The section was incubated with ab278054 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

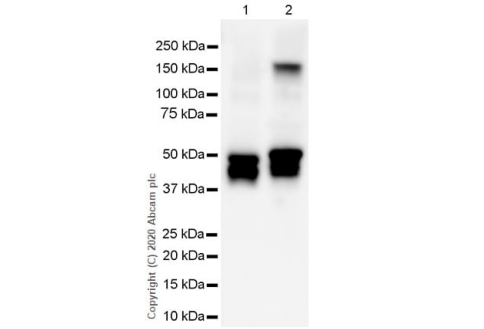
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-GFAP antibody [RP1003] (ab278054)

Flow Cytometry analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized mouse primary brain cells labeling GFAP using ab278054 at a 1/500 dilution (0.1 µg) (Right panel) compared to Rabbit monoclonal IgG (**ab172730**) (Left panel).

Secondary antibody is Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) used at a 1/2000 dilution.



Western blot - Anti-GFAP antibody [RP1003] (ab278054)

All lanes : Anti-GFAP antibody [RM1003] (ab278054) at 1/1000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

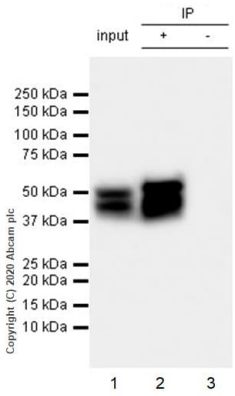
Predicted band size: 49 kDa
Observed band size: 40-54 kDa

Exposure time: 3 seconds

The molecular weight observed is consistent with the literature

(PMID: 25975510).

Blocking/Dilution buffer: 5% NFDM/TBST.

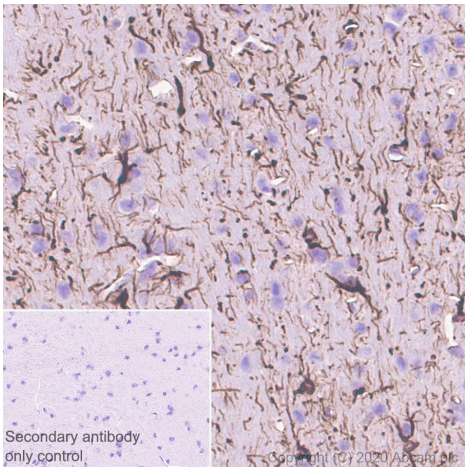


Immunoprecipitation - Anti-GFAP antibody [RP1003] (ab278054)

GFAP was immunoprecipitated from 0.35 mg mouse brain lysate with ab278054 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab278054 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: ab278054 IP in mouse brain lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab278054 in mouse brain lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 seconds.
The molecular weight observed is consistent with the literature (PMID: 824020; PMID:2294; PMID: 6340792).

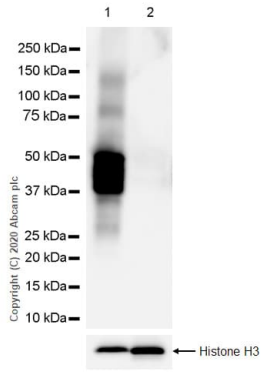


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [RP1003] (ab278054)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling GFAP with ab278054 at 1/2000 (0.235 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on mouse cerebrum. The section was incubated with ab278054 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-GFAP antibody [RP1003] (ab278054)

All lanes : Anti-GFAP antibody [RM1003] (ab278054) at 1/1000 dilution

Lane 1 : Human brain lysate
Lane 2 : Human liver lysate

Lysates/proteins at 20 µg per lane.

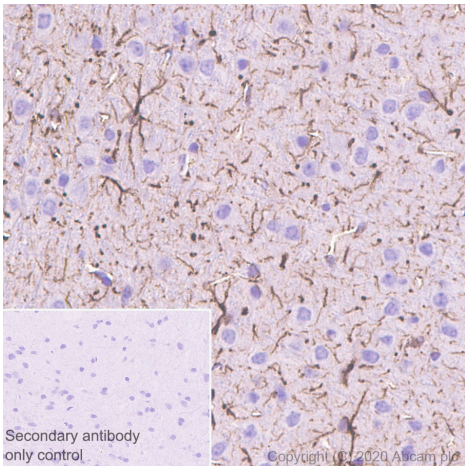
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 49 kDa
Observed band size: 40-54 kDa

Exposure time: 3 seconds

Negative control: Human liver. (PMID: 25975510).
The molecular weight observed is consistent with the literature (PMID: 25975510).

Blocking/Diluting buffer: 5% NFDM/TBST.

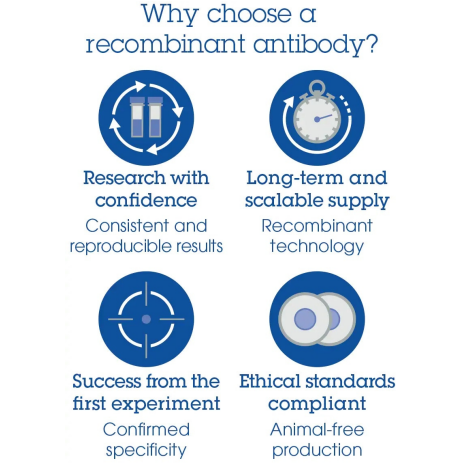


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GFAP antibody [RP1003] (ab278054)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue labeling GFAP with ab278054 at 1/2000 (0.235 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on rat cerebrum. The section was incubated with ab278054 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Anti-GFAP antibody [RP1003] (ab278054)

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