

Anti-Glial Fibrillary Acidic Protein

Polyclonal Antibody

Cat. # AB5541

Lot # LV1450178

pack size: 50 µL

Store at -20°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



Certificate of Analysis

page 1 of 3

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IC, IH(P)	H, M, R, B, Po	N/A	N/A	Ch	55 kDa	NP_002046

Background

Glial fibrillary acidic protein (GFAP) is a class-III intermediate filament. Type III intermediate filaments contain three domains, the most conserved of which is the rod domain. The specific sequence for this region of the protein may differ between the different intermediate filament genes for type III proteins, but the structure of the protein is highly conserved. GFAP is the main constituent of intermediate filaments in astrocytes and serves as a cell specific marker that distinguishes differentiated astrocytes from other glial cells during the development of the central nervous system. GFAP is also found in the lens epithelium, Kupffer cells of the liver, in some cells in salivary tumors and has been reported in erythrocytes.

Presentation

Purified chicken polyclonal in buffer containing PBS containing 5 mM sodium azide.

Specificity

Glial fibrillary acidic protein (GFAP). Reacts with both native and recombinant protein.

Species Cross-reactivity

Human, rat, and mouse. Expected to cross-react with bovine and porcine. Other species have not yet been tested.

Immunogen

Purified bovine GFAP.

Molecular Weight

55 kDa

Method of Purification

Immunoaffinity Purified

Storage and Handling

Stable for 1 year at -20°C in undiluted aliquots from date of receipt. Do not store in a self-defrosting freezer.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

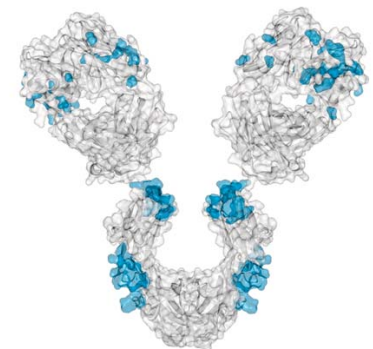
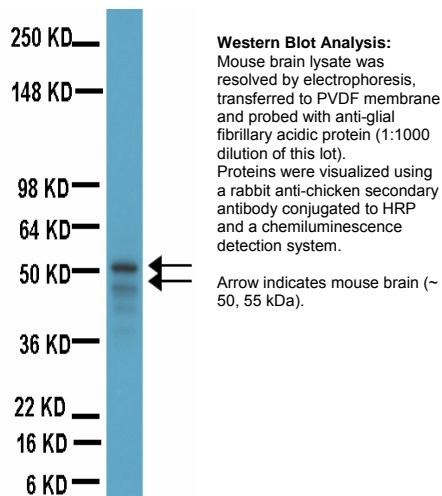
Control

Astrocytes, astrocytomas, neural stem cells.

Quality Control Testing

Routinely evaluated by Western Blot on **Mouse Brain** lysates.

Western Blot Analysis: 1:1000 dilution of this lot detected **glial fibrillary acidic protein** on 10 µg of **Mouse Brain** lysates.



Additional Research Applications

Immunohistochemistry(paraffin):

Representative images from a previous lot. **Pictures of Optimal Staining With Citrate Buffer Epitope Retrieval: Human Brain.**

(See page 2).

Immunocytochemistry: 1:200-1:1,000 on cells in tissue culture fixed for one minute in 3.7% formalin and one minute in -20°C methanol.

Optimal working dilutions must be determined by the end user.

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH(P) Immunohistochemistry (Paraffin)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates Bo Bovine Po Porcine

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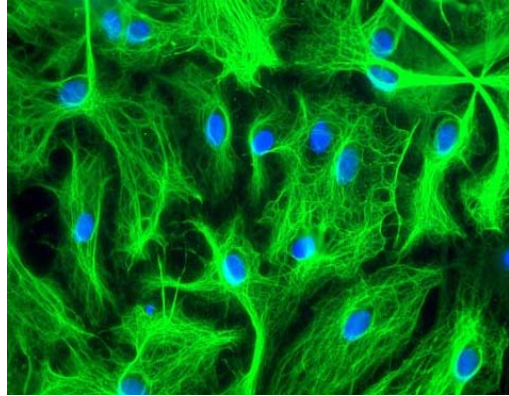
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Additional Research Applications

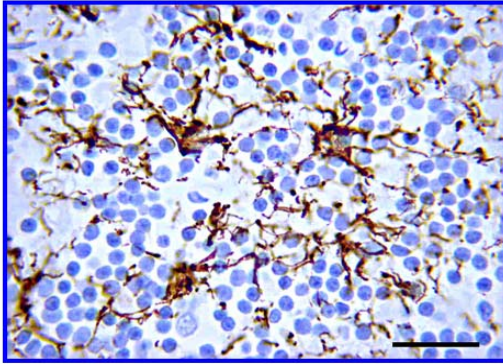
Immunocytochemistry:

Representative image from a previous lot.

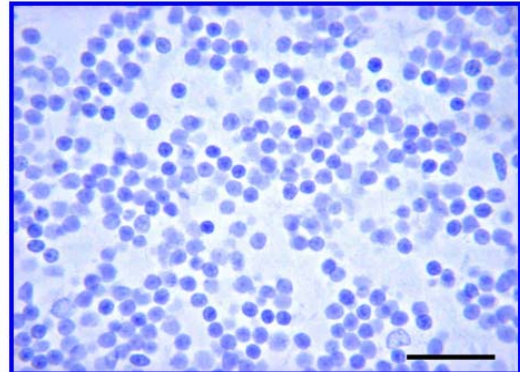
Chicken anti-GFAP (Catalog Number AB5541). Localization of GFAP in cultured rat neonatal forebrain cells grown in tissue culture for 15 days. The blue staining is Hoechst DNA stain.



Immunohistochemistry (Paraffin):



GFAP (IHC2078) staining of Human Brain. Tissue was pretreated with Citrate Buffer, pH 6.0. Pre-diluted polyclonal antibody, using IHC-Select® Detection with HRP-DAB. Glial cells stain strongly (brown). Bar = 25 µm.



Negative Control staining of Human Brain. Tissue pretreated with Citrate Buffer, pH 6.0. Negative Control Reagent, IHC-Select® Detection with HRP-DAB. No background staining is detected. Bar = 25 µm.

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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PROTOCOL

Western Blotting

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-GFAP diluted in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a rabbit anti-chicken HRP conjugated IgG, Catalog # 12-341 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

Immunohistochemistry

1. Use standard deparaffinization techniques on tissue specimens.
2. Pretreat tissues using a citrate buffer, pH 6.0 and high heat epitope retrieval techniques.
Note: Do not allow tissues to dry out during the staining procedure.

The following steps are taken from the product manual for IHC Select® HRP/DAB Detection Kit (Cat. No. DAB050)

3. Apply the blocking reagent to the tissue specimen and incubate in an enclosed chamber for 5 minutes.
4. While holding the slide at a 45° angle, gently rinse the specimen with 1X Rinse Buffer for a minimum of 15 seconds. Tap the end of the slide onto a paper towel to remove excess Rinse Buffer.
5. Apply a 1:50-1:500 dilution of primary antibody over the entire tissue specimen and incubate in an enclosed chamber at room temperature for 60 minutes.
6. Rinse specimen as performed in Step 3.
7. Apply the biotinylated secondary antibody to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
8. Rinse specimen as performed in Step 3.
9. Apply the Streptavidin-HRP solution to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
10. Rinse specimen as performed in Step 3.
11. Apply the DAB (chromogen reagent) to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
12. Rinse specimen as performed in Step 3.
13. Apply the Hematoxylin counterstain solution to the tissue specimen and incubate in an enclosed chamber for 1 minute.
14. Rinse specimen as performed in Step 3.
15. Place the tissue slides directly into a container filled with deionized water until mounting.
16. Mount a coverslip using an aqueous-based mounting media or for permanent mounting, dehydrate tissue through a graded series of alcohols, immerse in xylene, then apply a xylene-based mounting media (e.g. Permount) and coverslip.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

RELATED PRODUCTS (specific)

cat #	description
AB5804	■ Anti-Glial Fibrillary Acidic Protein
AB1540	■ Anti-Glial Fibrillary Acidic Protein
MAB5268	■ Anti-Glial Fibrillary Acidic Protein
MAB360	■ Anti-Glial Fibrillary Acidic Protein, clone GA5
MAB3402	■ Anti-Glial Fibrillary Acidic Protein, clone GA5
MAB3402X	■ Anti-Glial Fibrillary Acidic Protein, Clone GA5, AlexaFluor® 488 Conjugated
CBL411	■ Anti-Glial Fibrillary Acidic Protein, clone GF12-24
AB1540-100UG	■ Glial Fibrillary Acidic Protein
IHCR2078-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted
IHCR2079-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted, clone GA5
AG230	■ Glial Fibrillary Acidic Protein, porcine
IHC2076-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted
IHC2079-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted, clone GA5
12-341	■ Rabbit Anti-Chicken IgY, HRP conjugate

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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