

# Anti-Glial Fibrillary Acidic Protein, clone GA5

Monoclonal Antibody

Cat. # MAB3402

Lot # LV1467441

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

pack size: 40 µg

Store at 2-8°C  
DO NOT FREEZE



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IC, IH	H, R, M, Po, Ch, B, Rb	IgG1	N/A	M	50 kDa	NP_002046

### Background

Glial fibrillary acidic protein is a class-III intermediate filament. 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.

### Presentation

Purified mouse monoclonal IgG1 in buffer containing 0.02 M phosphate buffer, 0.25 M NaCl with 0.1% sodium azide, pH 7.6.

### Concentration

1 mg/mL

### Specificity

The antibody reacts with GFAP from human, pig, chicken and rat. In tissue sections this antibody stains astrocytes and Bergman glia cells (Debus, E., 1983).

### Species Cross-reactivity

Human, mouse, and rat. Expected to cross-react with porcine, chicken, bovine, and rabbit.

### Immunogen

Purified glial filament (Debus, E., 1983).

### Molecular Weight

50 kDa

### Method of Purification

Protein A Purified

### Storage and Handling

Store the reconstituted antibody at 2-8°C for up to 6 months after date of receipt.

**DO NOT FREEZE.**

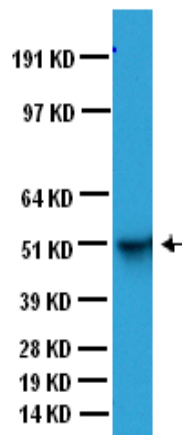
### Control

Mouse brain tissue, Astrocyte culture.

### Quality Control Testing

Routinely evaluated by Western Blot on Mouse brain lysate.

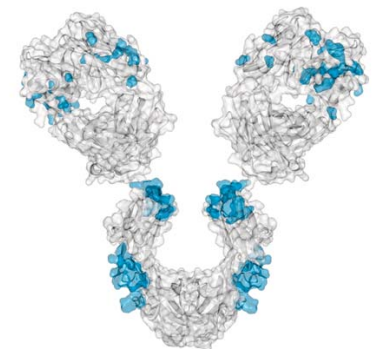
Western Blot Analysis: 1:1000 dilution of this lot detected Glial Fibrillary Acidic Protein on 10 µg of Mouse Brain lysate.



### Western Blot Analysis:

Mouse Brain lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-Glial Fibrillary Acidic Protein (1:1000 dilution of this lot). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein Glial Fibrillary Acidic Protein (~51 kDa).



### References

1. Ravizza, T., and Vezzani, A., *et al.* (2006). *Neuroscience*. 137:301-308.
2. Hua-Qing Liu, *et al.* (2006). *British J. Pharmacol.* :1-12.
3. Hagemann, Tracy L., *et al.* (2006). *J Neurosci*. 26:11162-11173.
4. Liu, J., *et al.* (2006). *Brain Res*. 1089:162-170.
5. Bronger, H., *et al.* (2005). *Cancer Res*. 65:11419-28.
6. Debus, E., *et al.* (1983) *Differentiation*. 25, 193-203.

### Additional Research Applications

Immunocytochemistry: 5 µg/mL of a previous lot was used.

Optimal working dilutions must be determined by end user.

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

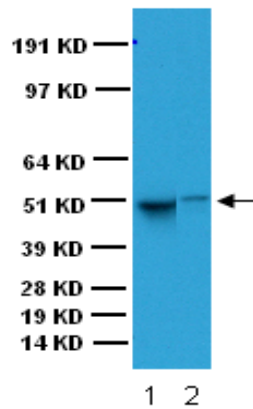
**SPECIES LEGEND:** B Bovine Ch Chicken H Human M Mouse Po Porcine (Pig) R Rat Rb Rabbit

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## Additional Research Applications



### Western Blot Analysis:

Representative image from a previous lot.

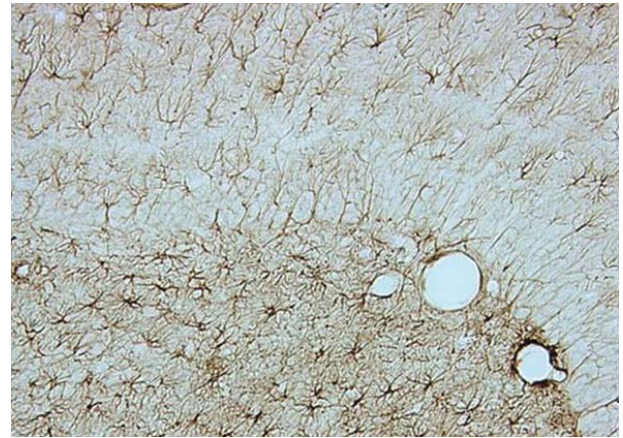
Mouse Brain lysate (Lane 1) and Mouse brain membrane lysate (Lane 2) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-Glial Fibrillary Acidic Protein (1:1000 dilution of a previous lot).

Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein Glial Fibrillary Acidic Protein (~51 kDa).

Immunohistochemistry: Representative image from a previous lot.

5 µg/mL of a previous lot was used. Optimal working dilutions must be determined by end user.



## 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% milk with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-Glial Fibrillary Acidic Protein diluted in freshly prepared 5% milk 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 goat anti-mouse HRP conjugated IgG, Catalog # AP124P 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.

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**Immunohisto-/cytochemistry**

Ideal specimens are obtained from frozen sections from shock-frozen tissue samples. The frozen sections are dried in the air and then fixed with acetone at -20°C for 10 min. Excess acetone is allowed to evaporate at 15-25°C. Material fixed in alcohol and embedded in paraffin can also be used (2). The antibody appears to react with tissue fixed in formaldehyde for a short time (10 min) (1). Other fixation conditions must be first tested by the investigator.

It is advantageous to block unspecific binding sites by overlaying the sections with fetal calf serum (FCS) for 20–30 min at 15-25°C. Excess of FCS is removed by decanting before application of the antibody solution.

Cytocentrifuge preparations of single cells or cell smears are also fixed in acetone. These preparations should, however not be dried in the air. Instead, the excess acetone is removed by briefly washing in phosphate-buffered saline (PBS).

Further treatment is as follows:

- Overlay the preparation with 10–20 µL anti-Glial Fibrillary Acidic Protein solution and incubate in a humid chamber at 37°C for 1 h.
- Dip the slide briefly in PBS and then wash 3 x in PBS for 3 min (using a fresh PBS bath in each case). Wipe the margins of the preparation dry and overlay the preparation with 10–20 µL of a solution of anti-mouse Ig-FITC or anti-mouse IgG-peroxidase solution and allow to incubate for 1 h at 37°C in a humid chamber.
- Wash the slide as described above.

The preparation must not be allowed to dry out during any of the steps. If using an indirect immunofluorescence technique, the preparation should be overlaid with a suitable embedding medium (e.g. Moviol, Hoechst) and examined under the fluorescence microscope. If a POD conjugate has been used as the secondary antibody, the preparation should be overlaid with a substrate solution (see below) and incubated at 15-25°C until a clearly visible red-brown color develops. A negative control (e.g. only the secondary antibody) should remain unchanged in color during this incubation period. Subsequently, the substrate is washed off with PBS and the preparation is stained, if desired, with hemalum stain for about 1 min. The hemalum solution is washed off with PBS; the preparation is embedded and examined.

**Substrate solutions:**

Aminoethyl-carbazole: Dissolve 2 mg 3-amino-9-ethylcarbazole with 1.2 mL dimethylsulfoxide and add 28.8 mL 50 mM Tris-HCl, pH 7.3, and 20 µL 3% H<sub>2</sub>O<sub>2</sub> (w/v). Prepare solution freshly each day.

Diaminobenzidine: Dissolve 25 mg 3,3'-diaminobenzidine with 50 mL 50 mM Tris-HCl, pH 7.3, and add 40 µL 3% H<sub>2</sub>O<sub>2</sub> (w/v). Prepare solution freshly each day.

**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
AB5541	■ Anti-Glial Fibrillary Acidic Protein
AB5804	■ Anti-Glial Fibrillary Acidic Protein
AB1540	■ Anti-Glial Fibrillary Acidic Protein
MAB5628	■ 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
IHC2078-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted
IHC2079-6	■ IHC Select® Anti-Glial Fibrillary Acidic Protein, prediluted, clone GA5
AB9598	■ Anti-Glial fibrillary acidic protein delta
07-650	■ Anti-GFAP+1
AP124P	■ Goat anti-Mouse IgG, Peroxidase Conjugated, H+L
AP192F	■ Donkey anti-Mouse IgG, FITC Conjugated

**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
<a href="#">DAB150-MS</a>	■ <a href="#">IHC SELECT® DAB KIT SINGLE SPECIES-MOUSE 150 TESTS; 1 kit</a>

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