# **Anti-GFAP Antibody [SA03-04]**

### ET1601-23



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IHC-P, IP, IF-Tissue, IHC-Fr, mIHC

Molecular Wt: Predicted band size: 50 kDa

Clone number: SA03-04

Description: Glial fibrillary acidic protein (GFAP) is a protein that is encoded by the GFAP gene in

humans. It is a type III intermediate filament (IF) protein that is expressed by numerous cell types of the central nervous system (CNS), including astrocytes and ependymal cells during development. GFAP has also been found to be expressed in glomeruli and peritubular fibroblasts taken from rat kidneys, Leydig cells of the testis in both hamsters and humans, human keratinocytes, human osteocytes and chondrocytes and stellate cells of the pancreas and liver in rats. GFAP is closely related to the other three non-epithelial type III IF family members, vimentin, desmin and peripherin, which are all involved in the structure and function of the cell's cytoskeleton. GFAP is thought to help to maintain astrocyte mechanical strength as well as the shape of cells, but its exact function remains poorly understood,

despite the number of studies using it as a cell marker.

**Immunogen:** Synthetic peptide within Human GFAP aa 1-50 / 432.

Positive control: Human brain tissue lysate, rat brain tissue lysate, human cerebellum tissue lysate, mouse

brain tissue, rat brain tissue, human brain tissue, human glioblastoma tissue, mouse spinal cord tissue, rat spinal cord tissue, mouse cerebral cortex tissue, rat cerebral cortex tissue, mouse cerebellum tissue, rat cerebellum tissue, mouse hippocampus tissue, rat hippocampus

tissue.

Subcellular location: Cytoplasm

Database links: SwissProt: P14136 Human | P03995 Mouse | P47819 Rat

**Recommended Dilutions:** 

WB 1:5,000 IHC-P 1:50-1:1,000 IF-Tissue 1:50-1:200

**IP** Use at an assay dependent concentration.

**IHC-Fr** 1:100-1:200 **mIHC** 1:1,000-1:10,000

Storage Buffer: 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw

cycles.

**Purity:** Protein A affinity purified.

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#### **Images**

kDaxh A- A- A- Mhair 250-150-150-100-72-55-42-35-25-GAPDH **Fig1:** Western blot analysis of GFAP on different lysates with Rabbit anti-GFAP antibody (ET1601-23) at 1/5,000 dilution.

Lane 1: Human brain tissue lysate Lane 2: Rat brain tissue lysate

Lane 3: Human cerebellum tissue lysate

Lane 4: Mouse brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 50 kDa Observed band size: 50 kDa

Exposure time: 11 seconds;

4-20% SDS-PAGE gel.

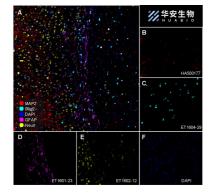


Fig2: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-MAP2 (HA500177, Red), anti-Olig2 (ET1604-29, Cyan), anti-GFAP (ET1601-23, Magenta) and anti-Neun (ET1602-12, Yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA500177 (1/1,000 dilution), ET1604-29 (1/5,000 dilution), ET1601-23 (1/10,000 dilution) and ET1602-12 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.



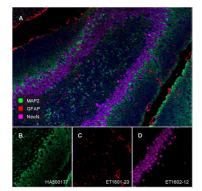


Fig3: Fluorescence multiplex immunohistochemical analysis of mouse hippocampus (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-MAP2 (HA500177, Green), anti-GFAP (ET1601-23, Red) and anti-NeuN (ET1602-12, Magenta) on Mouse hippocampus. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of HA500177 (1/1,000 dilution), ET1601-23 (1/1,000 dilution) and ET1602-12 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

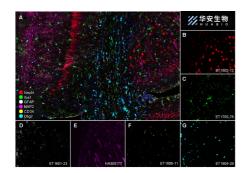
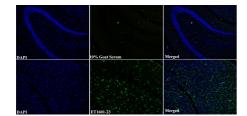


Fig4: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-Iba1 (ET1705-78, green), anti-GFAP (ET1601-23, gray), anti-Olig2 (ET1604-29, cyan), anti-MAP2 (HA500177, magenta) and anti-CD34 (ET1606-11, yellow) on mouse brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of ET1602-12(1/5,000 dilution), ET1705-78 (1/2,000 dilution), ET1601-23 (1/5,000 dilution), ET1604-29 (1/1,000 dilution), HA500177 (1/5,000 dilution) and ET1606-11 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.



**Fig5:** Immunofluorescence analysis of frozen rat brain tissue labelling GFAP (ET1601-23).

Upper Side - Negative Control (10% Goat Serum)

Lower Side - Recombinant Rabbit monoclonal to GFAP (ET1601-23)

The rat brain tissue section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes, blocked in 10% goat serum, and then incubated with ET1601-23 at 1/100 dilution , followed by iFluor <sup>TM</sup> 488 Conjugated Goat antirabbit IgG at 1:1,000 dilution.

Confocal images shows specific cytoplasmic staining of GFAP in rat brain tissue.

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BATT 10% Goat Serum Merged

Table 23 Merged

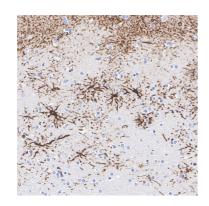
**Fig6:** Immunofluorescence analysis of frozen mouse brain tissue labelling GFAP (ET1601-23).

Upper Side - Negative Control (10% Goat Serum)

Lower Side - Recombinant Rabbit monoclonal to GFAP (ET1601-23)

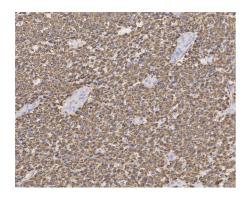
The mouse brain tissue section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes, blocked in 10% goat serum, and then incubated with ET1601-23 at 1/100 dilution , followed by iFluor 488 Conjugated Goat anti-rabbit IgG at 1:1,000 dilution.

Confocal images shows specific cytoplasmic staining of GFAP in mouse brain tissue.



**Fig7:** Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig8:** Immunohistochemical analysis of paraffin-embedded human glioblastoma tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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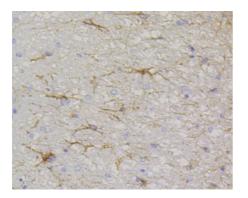


Fig9: Immunohistochemical analysis of paraffin-embedded mouse spinal cord tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

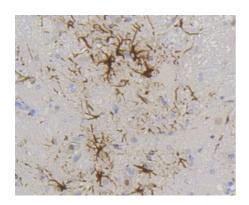


Fig10: Immunohistochemical analysis of paraffin-embedded rat spinal cord tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

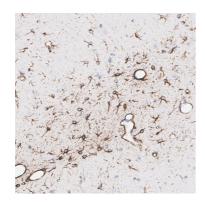


Fig11: Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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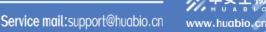




Fig12: Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

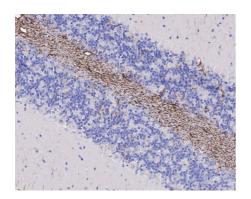


Fig13: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig14:** Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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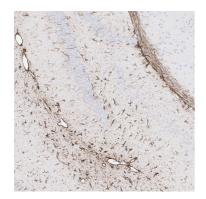


Fig15: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

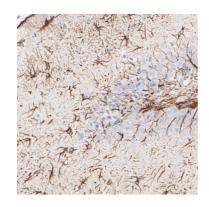


Fig16: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1601-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

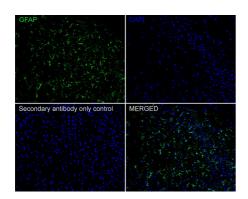


Fig17: Immunofluorescence analysis of frozen mouse cerebral cortex (fresh) tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/200 dilution.

The section was not undergone antigen retrieval. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-23, green) at 1/200 dilution overnight at 4  $^{\circ}\mathrm{C}$ , washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

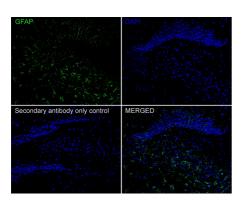


Fig18: Immunofluorescence analysis frozen mouse hippocampus (fresh) tissue with Rabbit anti-GFAP antibody (ET1601-23) at 1/200 dilution.

The section was not undergone antigen retrieval. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1601-23, green) at 1/200 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with NAPI (hlue)

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Zhang N et al. A self-assembly peptide nanofibrous scaffold reduces inflammatory response and promotes functional recovery in a mouse model of intracerebral hemorrhage. Nanomedicine N/A:N/A (2016).
- 2. Green AL et al. Preclinical antitumor efficacy of selective exportin 1 inhibitors in glioblastoma. Neuro Oncol 17:697-707 (2015).