Anti-GFAP Antibody [1-D4]

EM140707

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, FC
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	1-D4

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. There are multiple disorders associated with improper GFAP regulation, and injury can cause glial cells to react in detrimental ways. Glial scarring is a consequence of several neurodegenerative conditions, as well as injury that severs neural material. Another condition directly related to GFAP is Alexander disease, a rare genetic disorder. Notably, the expression of some GFAP isoforms have been reported to decrease in response to acute infection or neurodegeneration. Additionally, reduction in GFAP expression has also been reported in Wernicke's encephalopathy.

Immunogen: Synthetic peptide within C-terminal human GFAP.

Positive control: Human brain tissue lysate, rat brain tissue lysate, human cerebellum tissue lysate, rat cerebellum tissue lysate, A172, N2A, rat brain tissue, rat cerebellum tissue, Hela, rat hippocampus tissue.

Subcellular location: Cytoplasm.

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

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:500
IF-Tissue	1:50-1:500
IHC-P	1:500-1:1,000
FC	1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

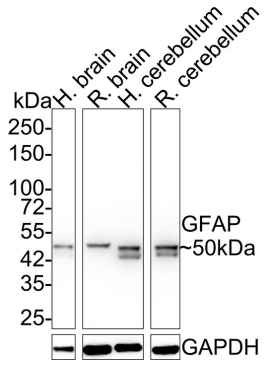

Fig1: Western blot analysis of GFAP on different lysates with Mouse anti-GFAP antibody (EM140707) at 1/5,000 dilution.

Lane 1: Human brain tissue lysate
Lane 2: Rat brain tissue lysate
Lane 3: Human cerebellum tissue lysate
Lane 4: Rat cerebellum tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 2 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM140707) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:50,000 dilution was used for 1 hour at room temperature.

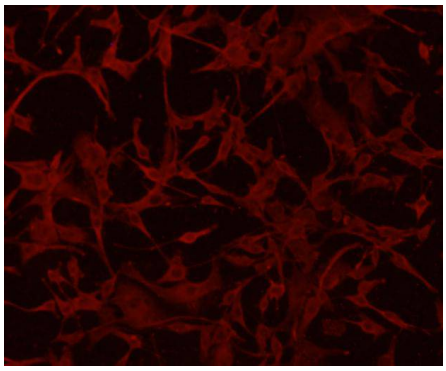

Fig2: ICC staining of GFAP in A172 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (EM140707, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Mouse IgG was used as the secondary antibody at 1/1,000 dilution.

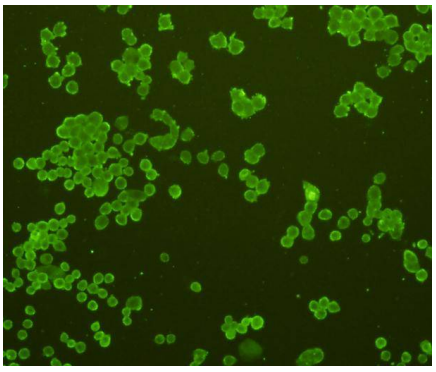

Fig3: ICC staining of GFAP in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (EM140707, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Mouse IgG was used as the secondary antibody at 1/1,000 dilution.

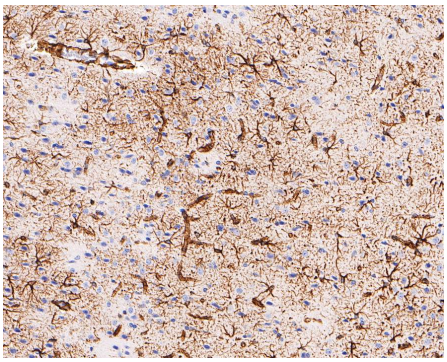

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-GFAP antibody (EM140707) at 1/600 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) at 1/600 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.

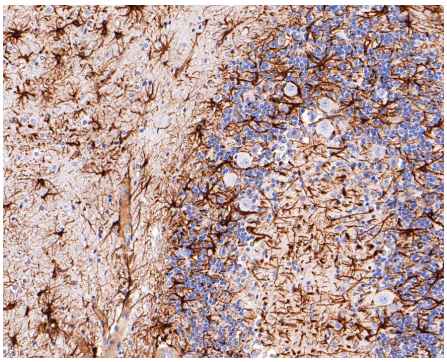

Fig5: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Mouse anti-GFAP antibody (EM140707) at 1/600 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) at 1/600 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.

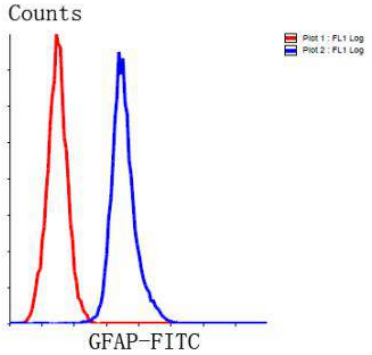

Fig6: Flow cytometric analysis of GFAP was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (EM140707, 1/100) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

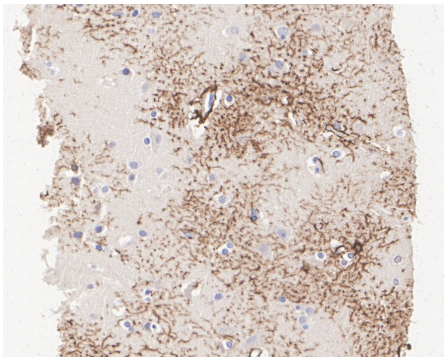

Fig7: Immunohistochemical analysis of paraffin-embedded human brain tissue with Mouse anti-GFAP antibody (EM140707) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) 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.

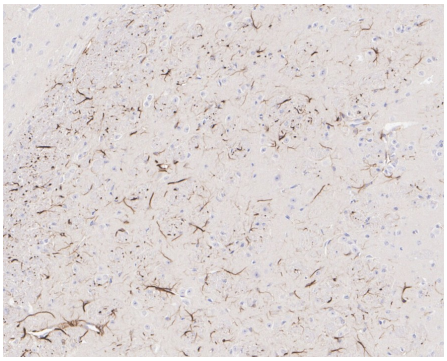

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Mouse anti-GFAP antibody (EM140707) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) 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.

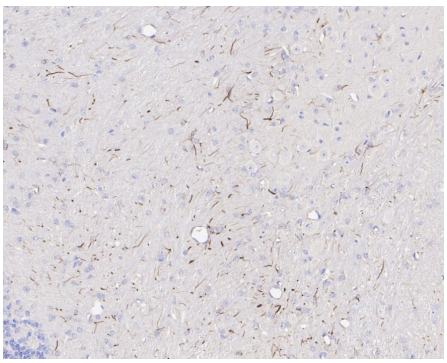

Fig9: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Mouse anti-GFAP antibody (EM140707) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) 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.

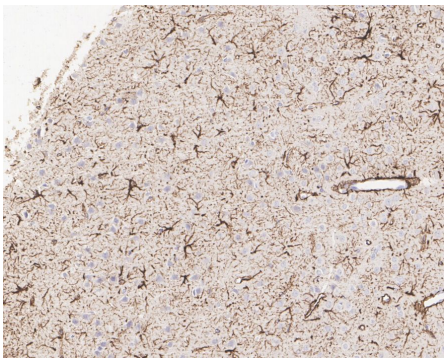

Fig10: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Mouse anti-GFAP antibody (EM140707) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (EM140707) 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.

Background References

1. "A new splice variant of glial fibrillary acidic protein GFAP epsilon, interacts with the presenilin proteins." Nielsen A.L., Holm I.E., Johansen M., Bonven B., Jorgensen P., Jorgensen A.L. J. Biol. Chem. 277:29983-29991(2002)
2. "Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease." Brenner M., Johnson A.B., Boespflug-Tanguy O., Rodriguez D., Goldman J.E., Messing A. Nat. Genet. 27:117-120(2001)