Human GFAP Antibody

Monoclonal Mouse IgG_{2B} Clone # 987268

Catalog Number: MAB25941

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human GFAP in direct ELISAs.
Source	Monoclonal Mouse IgG_{2B} Clone # 987268
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic peptide containing human GFAP Accession # P14136
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.25 µg/mL	See Below
Immunocytochemistry	1-25 µg/mL	See Below
Multiplex Immunofluorescence	0.25 µg/mL	Immersion fixed paraffin-embedded sections of human Brain Cortex
Immunohistochemistry	0.2-25 µg/mL	See Below
Simple Western	10 µg/mL	Human cerebellum
Knockout Validated	GFAP was detected in immersion fixed U937 human histiocytic lymphoma cell line but is not detected in GFAP knockout (KO) U937 Human cell line	

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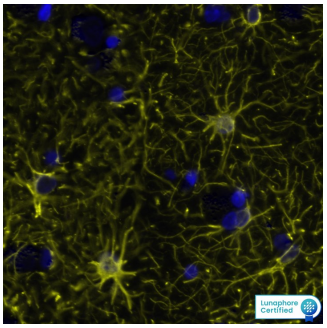
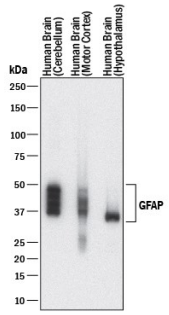
DATA

Multiplex Immunofluorescence



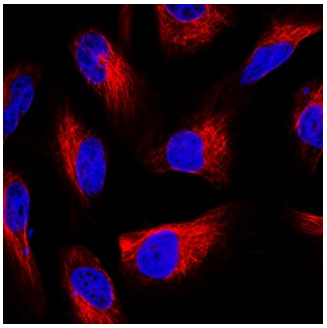
Detection of GFAP in Human Brain Cortex via seqIF™ staining on COMET™ GFAP Antibody was detected in immersion fixed paraffin-embedded sections of human Brain Cortex using Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # Catalog # MAB25941) at 0.25ug/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 555 Goat anti-Mouse IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm. Protocol available in COMET™ Panel Builder.

Western Blot



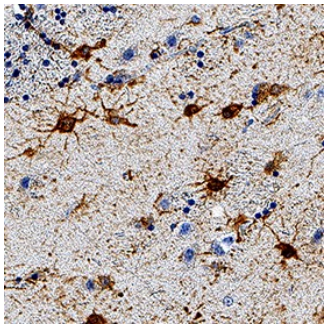
Detection of Human GFAP by Western Blot. Western blot shows lysates of human brain (cerebellum) tissue, human brain (motor cortex) tissue, and human brain (hypothalamus) tissue. PVDF membrane was probed with 0.25 µg/mL of Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # MAB25941) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # Catalog # HAF018). Specific bands were detected for GFAP at approximately 35-50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



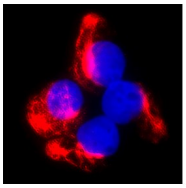
GFAP in U-251MG Human Cell Line. GFAP was detected in immersion fixed U-251MG human glioblastoma cell line using Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # MAB25941) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry

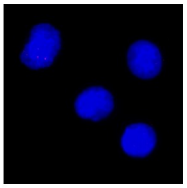


GFAP in Human Brain. GFAP was detected in immersion fixed paraffin-embedded sections of human brain (caudate nucleus) using Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # MAB25941) at 0.2 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to glial cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Knockout Validated



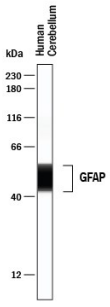
Positive (U937 cells)



Negative (U937 KO cells)

GFAP Specificity is Shown by Immunocytochemistry in Knockout Cell Line. GFAP was detected in immersion fixed U937 human histiocytic lymphoma cell line but is not detected in GFAP knockout (KO) U937 Human cell line using Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # MAB25941) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. Staining was performed using our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Simple Western



Detection of Human GFAP by Simple Western™. Simple Western lane view shows lysates of human cerebellum, loaded at 0.2 mg/mL. A specific band was detected for GFAP at approximately 45-55 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human GFAP Monoclonal Antibody (Catalog # MAB25941) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Glial Fibrillary Acidic Protein (GFAP) is the predominant component of astrocyte intermediate filaments in the central nervous system. It has also been detected in the glial cells of the enteric nervous system and some Schwann cells in the peripheral nervous systems.

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