Human/Mouse/Rat GAPDH Antibody

Monoclonal Mouse IgG_1 Clone # 686613

Catalog Number: MAB5718

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat GAPDH/G3PDH in Western blots. In direct ELISAs, 100% cross-reactivity with recombinant mouse GAPDH/G3PDH and no cross-reactivity with recombinant human GAPDH-2 is observed.
Source	Monoclonal Mouse IgG_1 Clone # 686613
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human GAPDH/G3PDH Met1-Ala150 Accession # P04406
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 as a 0.2 μm filtered solution in PBS.

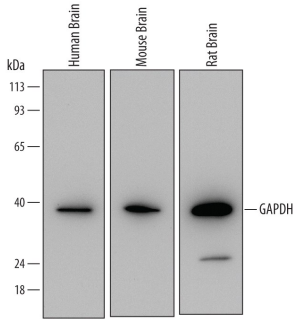
APPLICATIONS

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	0.05 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Simple Western	0.25 µg/mL	See Below

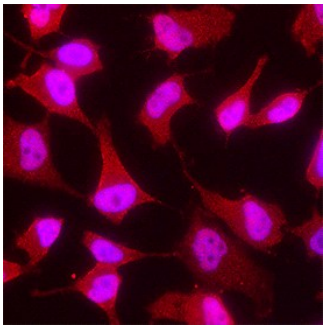
DATA

Western Blot



Detection of Human, Mouse, and Rat GAPDH/G3PDH by Western Blot. Western blot shows lysates of human brain tissue, mouse brain tissue, and rat brain tissue. PVDF Membrane was probed with 0.05 µg/mL of Mouse Anti-Human/Mouse/Rat GAPDH/G3PDH Monoclonal Antibody (Catalog # MAB5718) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for GAPDH/G3PDH at approximately 39 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry

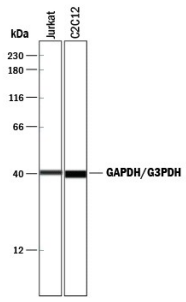


GAPDH in HeLa Human Cell Line. GAPDH was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse/Rat GAPDH Monoclonal Antibody (Catalog # MAB5718) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry

GAPDH/G3PDH in Human Kidney. GAPDH/G3PDH was detected in immersion fixed paraffin-embedded sections of human kidney using Mouse Anti-Human/Mouse/Rat GAPDH/G3PDH Monoclonal Antibody (Catalog # MAB5718) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human and Mouse GAPDH/G3PDH by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line and C2C12 mouse myoblast cell line, loaded at 0.2 mg/mL. A specific band was detected for GAPDH/G3PDH at approximately 41 kDa (as indicated) using 0.25 µg/mL of Mouse Anti-Human/Mouse/Rat GAPDH/G3PDH Monoclonal Antibody (Catalog # MAB5718). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Catalog Number: MAB5718

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
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

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is a 36-40 kDa member of the GAPDH family of enzymes. It is a widely expressed heterotetramer that is found in both the nucleus and cytoplasm. Although GAPDH was initially identified as a glycolytic enzyme that converted G3P into 1,3 diphosphoglycerate, it is now recognized to participate in no less than endocytosis, membrane fusion, vesicular secretory transport, DNA replication and repair, and apoptosis. Human GAPDH is 335 amino acids (aa) in length. There are two NAD binding sites (Asp35 and Asn316) with a catalytic region between aa 151-155. GAPDH contains no fewer that 19 posttranslational modifications, including methylation, deamidation and phosphorylation. One splice variant shows a 10 aa substitution for aa 319-335. Over aa 1-150, human GAPDH shares 92% aa identity with mouse GAPDH.

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