

800-656-7625

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Certificate of Analysis

Product: Affinity Purified Anti-Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) [Rabbit]

Code: 600-401-A33

Size: 100 µg

Lot # 19952

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.02% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD). This is an important energy-yielding step in carbohydrate metabolism. Recent evidence suggests that GAPDH is also involved in a number of cellular processes such as membrane fusion, phosphotransferase activity, DNA replication and repair, and nuclear RNA export. GAPDH has also been implicated in playing a role in different pathologies such as cancer progression, apoptosis, and neuronal diseases such as Alzheimer's and Huntington's disease. GAPDH is constitutively expressed at high levels in almost all tissues and cell lines, making it ideal for use as a loading control marker in immunoblots.



Figure 2. Immunohistochemical staining using Rockland's Affinity Purified anti-GAPDH antibody at 10 μ g/ml shows detection of GAPDH in human liver tissue. Tissue was fixed in 4% paraformaldehyde using a section 4.2 μ m thick.



Figure 1. Western blot using Rockland's Affinity Purified anti-GAPDH antibody shows detection of a single band at ~39 kDa corresponding to GAPDH (arrowhead) in HeLa cell lysates. The predicted MW of GAPDH is 36 kDa. GAPDH was detected using 125 ng/ml (lane A), 250 ng/ml (lane B) and 500 ng/ml (lane C) concentrations of primary antibody.

Application Note(s): This affinity purified antibody has been tested for use in ELISA, immunohistochemistry and western blotting. Specific conditions for reactivity should be optimized by the end user. Paraformaldehyde fixation yields brighter staining than formalin or methanol fixation. Expect a band at ~39 kDa in size corresponding to GAPDH by western blotting in the appropriate cell lysate or extract. GAPDH is constitutively expressed at high levels in almost all tissues and cell lines, making it ideal for use as a loading control marker.

Recommended Dilutions:

ELISA	1:10,000 - 1:40,000
WESTERN BLOT	1:100 - 1:1,000
IF MICROSCOPY	1:100 - 1:1,000
OTHER APPLICATIONS	User Optimized

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Purity and Specificity: This affinity purified antibody is directed against human GAPDH protein. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography. A BLAST analysis was used to suggest that this antibody would react with GAPDH from a wide range of organisms, including most vertebrates and some yeast. Broad reactivity makes this antibody an excellent loading control.

Relevant Link(s): Swiss-Prot P04406

NCBI Link NP_002037

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a region near the amino terminus of human Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH).

General References:

Sirover, M.A. (2005) New nuclear functions of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in mammalian cells. *J. Cell. Biochem.* **95**:45-52.

Glyceraldehyde-3-phosphate dehydrogenase, apoptosis, and neurodegenerative diseases. Annu. Rev. Pharmacol. Toxicol. (2005); 45:269-290.

Raje, C.I., Kumar, S., Harle, A., Nanda, J.S. and Raje, M. (2007) The macrophage cell surface glyceraldehyde-3-phosphate dehydrogenase is a novel transferrin receptor. *J. Biol. Chem.* **282** (5):3252-3261.

Olah, J., Tokesi, N., Vincze, O., Horvath, I., Lehotzky, A., Erdei, A., Szajli, E., Medzihradszky, K.F., Orosz, F., Kovacs, G.G. and Ovadi, J. (2006) Interaction of TPPP/p25 protein with glyceraldehyde-3-phosphate dehydrogenase and their co-localization in Lewy bodies. *FEBS Lett.* **580** (25):5807-5814.

Kolln, J., Ren, H.M., Da, R.R., Zhang, Y., Spillner, E., Olek, M., Hermanowicz, N., Hilgenberg, L.G., Smith, M.A., van den Noort, S. and Qin, Y. (2006) Triosephosphate isomerase- and glyceraldehyde-3-phosphate dehydrogenase-reactive autoantibodies in the cerebrospinal fluid of patients with multiple sclerosis. *J. Immunol.* **177** (8):5652-5658.

Batthyany,C., Schopfer,F.J., Baker,P.R., Duran,R., Baker,L.M., Huang,Y., Cervenansky,C., Branchaud,B.P. and Freeman,B.A. (2006) Reversible post-translational modification of proteins by nitrated fatty acids in vivo. *J. Biol. Chem.* **281** (29):20450-20463.

Related Products:

# <u>600-401-880</u>	Affinity Purified Anti-α-Tubulin [Rabbit]
# <u>600-401-882</u>	Affinity Purified Anti-VDAC/Porin [Rabbit]
# <u>600-401-886</u>	Affinity Purified Antiβ-Actin [Rabbit]
# <u>W09-000-364</u>	HeLa Whole Cell Lysate in SDS-PAGE Sample Buffer
# <u>W09-000-365</u>	293 Whole Cell Lysate in SDS-PAGE Sample Buffer
# <u>W09-001-370</u>	Jurkat Whole Cell Lysate in SDS-PAGE Sample Buffer
# <u>611-703-127</u>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
# <u>611-132-122</u>	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
# <u>MB-070</u>	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	<i>Max</i> Tag [™] Anti-RABBIT IgG Kit for Immunoblotting

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

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