



800-656-7625

fax 610-367-7825

Certificate of Analysis

Product: Affinity Purified Anti-NAG-1/GDF15 (D-variant) [Rabbit] TRIAL SIZE

Code: 600-401-B09S

Lot #: 23401

Size: 25 μ l

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.69 mg/ml (by UV absorbance at 280 nm)

Stabilizer: None

Preservative: 0.1% (w/v) Sodium Azide

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

Storage Conditions for Trial Size: Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 μ l). To minimize loss of volume dilute 1:10 by adding 225 μ l of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20° C or below after dilution. Avoid cycles of freezing and thawing. Expiration date is three (3) months from date of opening.

Background Information: The non-steroidal anti-inflammatory drug (NSAID) activated gene (NAG-1) is a member of the transforming growth factor-beta (TGF-beta) superfamily. NAG-1 is also known as Macrophage Inhibitory Cytokine-1 (MIC-1), Growth Differentiation Factor 15 (GDF15), Placental Bone Morphogenetic Protein (PLAB), or Prostate Derived Factor (PDF). NAG-1 is expressed in human placenta, prostate and colon. It possesses antitumorigenic and proapoptotic activities. NAG-1 expression is dramatically increased in inflammation, injury and malignancy. Increase of NAG-1 expression is a feature of many cancers including breast, colon, pancreas and prostate. In a number of studies, NAG-1 expression was increased by a number of NSAIDs. This increase in expression may correlate with the chemopreventive effect NSAIDs seem to have with certain cancers. NAG-1 expression is also induced by PPAR gamma ligands and by several dietary compounds such as conjugated linoleic acids (CLAs), naturally occurring fatty acids in ruminant food products, indoles, epicatechin gallate, and genistein. The induced expression of NAG-1 results in a stimulation of apoptosis and inhibition of cell growth. Inhibition of NAG-1 induced expression by small interference RNA (siRNA) results in repression of induced apoptosis. NAG-1 expression is regulated by a numbers of transcription factors such as ERG-1 and Sp1. EGR-1 may be necessary for NSAID-induced NAG-1 expression. The study of expression of NAG-1 proteins, including variants, is important to define their potential role as serum biomarkers for cancer diagnosis, treatment monitoring, epidemiology study, and nutrition surveys.

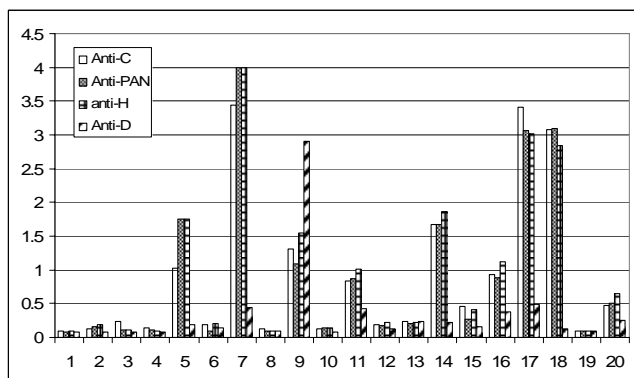


Figure. In this sandwich ELISA, NAG-1 was captured from human serum using the following antibodies (see Related Products below): anti-NAG-1/GDF15 (C terminal specific), anti-NAG-1/GDF15 (N terminal specific (PAN)), anti-NAG-1/GDF15 (H-variant) and anti-NAG-1/GDF15 (D-variant) polyclonal antibodies. Micro titer plates were coated with capture antibody at 1 μ g/mL. Control plates received PBS only (data not shown). After overnight incubation and blocking, independent experiments using 20 random normal human sera were performed. Neat normal sera were applied and incubated for 1 h at 37° C. After washing, HRP conjugated anti-NAG-1/GDF15 (C terminal specific) antibody was added for detection at 100 μ L per well at 1 μ g/mL. Following further incubation for 1 hr at 37° C, the plates were washed and TMBE was added as an HRP substrate for 30 min. The reaction was stopped by 1 M H_2SO_4 and values were measured at 450nm.

Application Note(s): This affinity purified antibody is suitable for use in ELISA and Western blotting (data not shown) assays. This reagent is particularly useful to differentiate variant specific NAG-1 protein present in human serum samples. This antibody is useful in dual antibody immunometric assays, such as sandwich ELISA formats (see figure above). Other NAG-1 specific antibodies (see Related Products below) are required for capture and detection. Specific conditions for reactivity should be optimized by the end user.

Recommended Dilutions:	ELISA	1:400
	WESTERN BLOT	1,000-2,000
	IMMUNOHISTOCHEMISTRY	User Optimized
	IF MICROSCOPY	User Optimized
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This product was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody specifically reacts with a D variant sequence of human NAG-1 protein (see General References below) from human tissues. A BLAST analysis was used to suggest reactivity with variant NAG-1 from human based on a 100% homology, with chimpanzee and macaque based on a 92% homology, and with rat and mouse based on a 90% homology with the immunizing sequence. Cross-reactivity with NAG-1 from other sources has not been determined.

Immunogen: This affinity purified antibody was prepared by repeated immunizations with a synthetic peptide corresponding to a D-variant sequence located at the amino terminus of mature NAG-1 protein. A residue of cysteine was added to facilitate coupling.

Relevant Links: Swiss-Prot [Q99988](#)

Related Product(s):

# 600-401-B07	Affinity Purified Anti-NAG-1/GDF15 (C-terminal specific) [Rabbit]
# 600-401-B08	Affinity Purified Anti-NAG-1/GDF15 (H variant specific) [Rabbit]
# 600-401-B09	Affinity Purified Anti-NAG-1/GDF15 (D variant specific) [Rabbit]
# 600-401-B10	Affinity Purified Anti-NAG-1/GDF15 (N-terminal specific) [Rabbit]
# 611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
# 611-132-122	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
# 611-144-122	DyLight™ 680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
# B501-0500	BLOTTO (500 g)
# BSA-30	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
# B304	NORMAL GOAT SERUM (NGS) (10 ml)
# KIA-003	MaxTag™ Anti-RABBIT IgG Kit for Immunoblotting
# MB-070	Blocking Buffer for Fluorescent Western Blotting

General References:

Baek, S.J., Eling, T.E. (2006) Changes in gene expression contribute to cancer prevention by COX inhibitors. *Prog Lipid Res.* **45**(1):1-16.

Lindmark, F., Zheng, S.L., Wiklund, F., Bensen, J., Balter, K.A., Chang, B., Hedelin, M., Clark, J., Stattin, P., Meyers, D.A., Adami, H-O., Isaacs, W., Gronberg, H. and Xu, J. (2004) H6D Polymorphism in Macrophage-Inhibitory Cytokine-1 Gene Associated With Prostate Cancer *J Natl Cancer Inst.* **96**(16): 1248-1254.

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. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.