

Certificate of Analysis

Product: Affinity Purified Anti-HMGN pS20/pS24 (Rabbit)

Code: 600-401-898

Lot #: 16789

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.20 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: 0.01% (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 Information: HMGNs are proteins that bind chromatin effectively reducing the compaction of the chromatin fiber and enhancing access to DNA regulatory sequences. Members of this family have a conserved chromatin binding domain which is phosphorylated during mitosis. The sequence immunized is conserved in several species. As such, this reagent is designed as a "universal" reagent for the detection of all phosphorylated HMGN proteins. The High Mobility Group (HMG) proteins were originally isolated from mammalian cells and were named according to their electrophoretic mobility in polyacrylamide gels. HMGs were arbitrarily classed as a specific type of nonhistone proteins based on the observation that they are ubiquitous to mammalian cells, that they share certain physical properties, and that they are associated with isolated chromatin. HMG proteins and are now subdivided into 3 families: the HMGB (formerly HMG-1/-2) family, the HMGN (formerly HMG-14/-17) family, and the HMGA (formerly HMG-I/Y/C) family. Each HMG family has a characteristic functional sequence motif. The functional motif of the HMGB family is called the "HMG-box;" that of the HMGN family, the "nucleosomal binding domain;" and that of the HMGA family, the "AT-hook." The functional motifs characteristic of these canonical HMGs are widespread among nuclear proteins in a variety of organisms. Proteins containing any of these functional motifs embedded in their sequence are known as "HMG motif proteins."

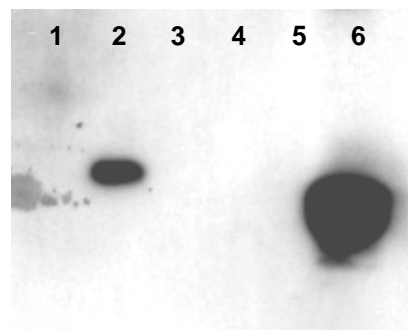


Figure. Western blot using Rockland's Affinity Purified anti-HMNG antibody shows detection of phosphorylated HMGN1 and HMGN2. Recombinant native and mutant HMGN proteins were treated with kinase PKC α to specifically phosphorylate S20 and S24 residues. Lanes contain: 1 - HMGN1, non-phosphorylated, 2 - HMGN1, phosphorylated, 3 - HMGN1 Δ 20E, Δ 24E, non-phosphorylated, 4 - HMGN1, Δ 20E, Δ 24E, phosphorylated, 5 - HMGN2, non-phosphorylated, and 6 - HMGN2, phosphorylated. Molecular weight markers (not shown) confirm the size of each recombinant protein. The primary antibody was diluted 1:1,000 for this experiment. The blot was processed using a 5 sec exposure time. *Personal Communication, Yuri Postnikov, NIH, CCR, Bethesda, MD.*

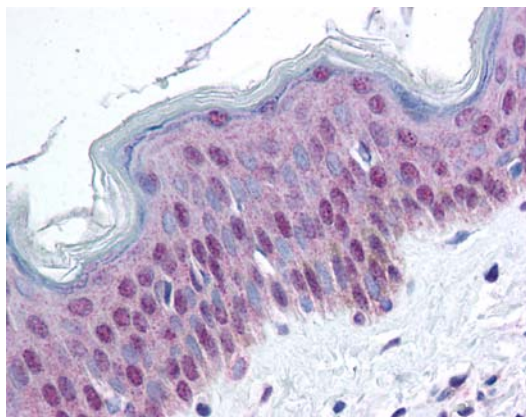


Figure 2. Immunohistochemistry. ROCKLAND's affinity purified anti-HMGN pS20/pS24 antibody was used at 20 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate nuclear and faint cytoplasmic positive staining of epidermal keratinocytes at 40X. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. *Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.*

Application Note(s): This affinity purified antibody has been tested for use in ELISA, immunohistochemistry and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 17 kDa in size corresponding to phosphorylated HMGN proteins by western blotting in the appropriate cell lysate or extract. Less than 0.5% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pS20 and pS24 of HMGN proteins. The antibody was tested against HMGN1 by performing a standard phosphorylation *in vitro* assay using HMGN1 as a substrate and Msk1 as a kinase (known as a main effector kinase of MAPK signaling pathway and *in vivo* kinase for HMGN1). This kinase specifically phosphorylates S20 and S24 of HMGN1 (S24 and S28 of HMGN2). The reaction was resolved on SDS PAGE and immunoblotted, using antibody diluted at 1:1,000 and 1:5,000. The antibody shows strong positive signal for Msk-phosphorylated HMGN1 and no cross-reaction for non-treated HMGN1.

Recommended Dilutions:	ELISA	1:5,000 - 1:25,000
	WESTERN BLOT	1:500 - 1:2,000
	IMMUNOHISTOCHEMISTRY	20 µg/ml
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity-purified antibody is directed against phosphorylated human HMGN protein at pS20 and pS24 residues (other HMGN proteins have this conserved sequence but at other residue positions). The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human HMGN pS20/pS24 proteins and the antibody is specific for the phosphorylated form of the proteins. Reactivity is seen against HMGN1 and HMGN2. HMGN3 is also likely reactive although not tested. Reactivity with non-phosphorylated human HMGNs is minimal by ELISA. A BLAST analysis was used to suggest cross reactivity with HMGN proteins from *Xenopus*, chicken, mouse, bovine, dog, orangutan, rat and swine based on 100% homology with the immunizing sequence. Reactivity against homologues from other sources is not known.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 19-28 of human HMGN protein (see below).

Relevant Links: NCBI [AAA52677](#) Swiss-Prot [P05114](#)

General References:

Landsman, D., McBride, O.W., Soares, N., Crippa, M.P., Srikantha, T. and Bustin, M. (1989) Chromosomal protein HMG-14. Identification, characterization, and chromosome localization of a functional gene from the large human multigene family. *J. Biol. Chem.* **264** (6), 3421-3427.

Landsman, D., McBride, O.W. and Bustin, M. (1989) Human non-histone chromosomal protein HMG-17: identification, characterization, chromosome localization and RFLPs of a functional gene from the large multigene family. *Nucleic Acids Res.* **17** (6), 2301-2314.

Related Products:

#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	MaxTag TM Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

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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