Datasheet for 600-401-898

HMGN phospho S20/S24 Antibody

Overview

Description:	Anti-HMGN pS20/pS24 (RABBIT) Antibody - 600-401-898
Item No.:	600-401-898
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI). 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 non-histone 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.'' Anti-HMGN pS20-pS24 Antibody is ideal for researchers interested in chromatin binding and Nuclear Signaling research.
Synonyms:	rabbit anti-HMGN pS20/pS24 antibody, Non-histone chromosomal protein HMG-14, High mobility group nucleosome binding domain containing protein 1 antibody, High mobility group protein 14 antibody, High-mobility group nucleosome binding domain 1 antibody, HMG14 antibody, HMGN-1, HMGN 1, HMG 14
Host Species:	Rabbit

Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HMGN1
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic phospho-peptide corresponding to amino acids 19-28 of human HMGN protein.
Purity/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.
Relevant Links:	• UniProtKB - P05114 • NCBI - 386779 • GeneID - 3091

Application Details

Tested Applications:	ELISA, IHC, WB

Application Note:	Anti-HMGN pS20-pS24 affinity purified antibody has been tested for use in ELISA, immunohistochemistry, and 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 immunoblot, 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:25,000
IHC:	20 µg/ml
WB:	1:500 - 1:2,000

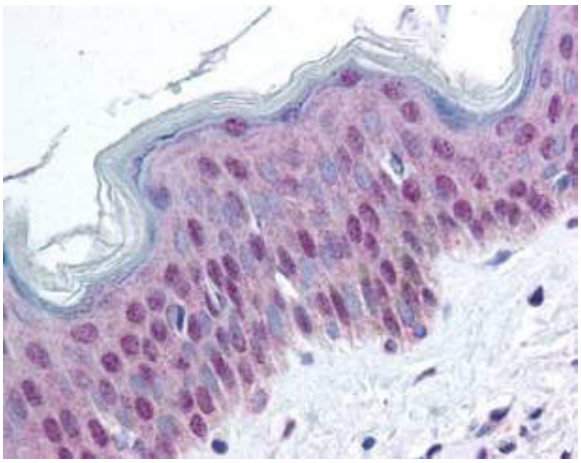
Formulation

Physical State:	Liquid (sterile filtered)
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
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

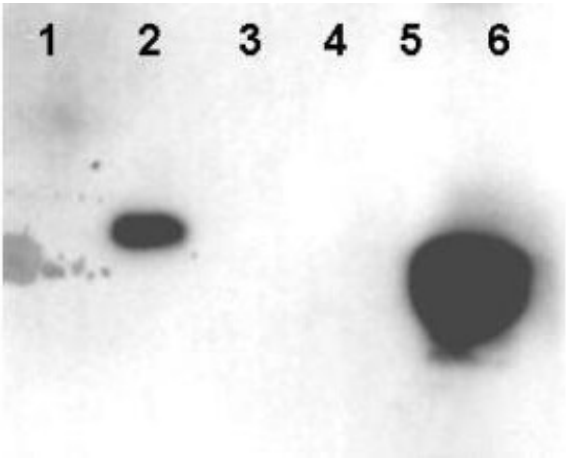
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Immunohistochemistry
Rockland's affinity purified anti-HMGN pS20/pS24 antibody was used at 20 ug/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.



Western Blot
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 PKCalpha to specifically phosphorylate S20 and S24 residues. Lanes contain: 1 - HMGN1, non-phosphorylated, 2 - HMGN1, phosphorylated, 3 - HMGN1 delta20E, delta24E, non-phosphorylated, 4 - HMGN1, delta20E, delta24E, 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.

Disclaimer

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 5199, Limerick, Pennsylvania, USA.