ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

Product: Affinity Purified Anti-HDAC-1 [Rabbit]

Code: 600-401-879

Lot #: 16248

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.33 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: HDAC1 (also known as HD1, histone deacetylase 1, RPD3, RPD3L1) belongs to the histone deacetylase/acuc/apha family and is a component of the histone deacetylase complex. Histone acetylation and deacetylation, catalyzed by multisubunit complexes, play a key role in the regulation of eukaryotic gene expression. It also interacts with retinoblastoma tumor-suppressor protein and this complex is a key element in the control of cell proliferation and differentiation. Together with metastasis-associated protein-2, it deacetylates p53 and modulates its effect on cell growth and apoptosis.

Application Note(s): This 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. Specific nuclear staining is observed by IHC. Expect bands at 65 kDa in size corresponding to HDAC-1 by western blotting in the appropriate cell lysate or extract.

Figure 1. Western blot using Rockland's Affinity Purified anti-HDAC-1 antibody shows detection of a band at ~65 kDa corresponding to human HDAC1 present in a 293 whole cell lysate (arrowhead). Approximately 35 µg of lysate was separated on a 4-20% Tris-HEPES gel by SDS-PAGE and transferred onto nitrocellulose. After blocking the membrane was probed with the primary antibody diluted to 1:1,350. Reaction occurred 2 h at room temperature followed by washes and reaction with a 1:10,000 dilution of IRDye™800 conjugated Rb-a-Goat IgG [H&L] MXHu (605-432-013) for 45 min at room temperature. IRDye™800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

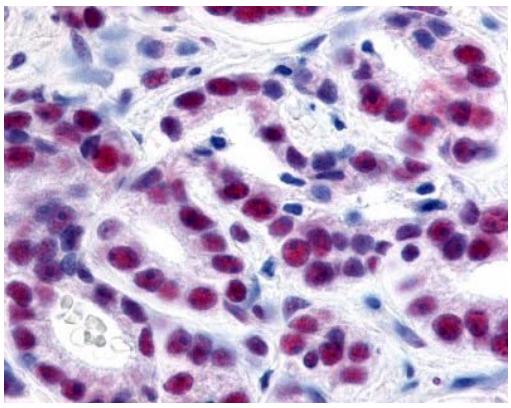

Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-HDAC-1 antibody was used at a 1:500 dilution to detect HDAC-1 by immunohistochemistry in human prostate cancer tissue. Tissue was formalin-fixed and paraffin embedded. *Personal Communication, Alan Yen, LifeSpanBiosciences, Seattle, WA.*

Purity and Specificity: This affinity purified antibody is directed against human HDAC-1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with this protein from human, mouse, rat and chimpanzee sources based on 100% homology for the immunogen sequence. Cross reactivity may occur with HDAC-1 from bovine (82% homology) and chicken (80% homology) sources. Cross reactivity with HDAC-1 homologues from other sources has not been determined.

Recommended Dilutions:

ELISA	1:10,000 - 1:50,000
WESTERN BLOT	1:1,000 - 1:5,000
IMMUNOHISTOCHEMISTRY	1:200 - 1:1,000
OTHER APPLICATIONS	User Optimized

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Relevant Link(s): Swis-Prot Q13547 NCBI Link NP_004955

Protein Sequence: Human HDAC1, 428 aa, predicted MW 55.1 kDa

1	maqtqgtrrk	vcyyydgdvg	nyyygqghpm	kphrirmthn	lllnyglyrk	meiyrphkan
61	aeemtkyhsd	dyikflrsir	pdnmseyskq	mqrfnvgedc	pvfdglfefc	qlstggsvas
121	avklnkqqtd	iavnwagglh	hakkseasgf	cyvndivlai	lellkyhqrv	lyididihhg
181	dgveeafytt	drvmtvsfhk	ygeyfpgtgd	lrdigagkgk	yyavnyplrd	giddesyeai
241	fkpvmskvme	mfqpsavvlq	cgsdslsgdr	lgcfnltikg	hakcvefvks	fnlpmlmlgg
301	ggytirnvar	cwtyetaval	dteipnelpy	ndyfeyfgpd	fklhispsnm	tnqntneyle
361	kikqrlfenl	rmlphapgvq	mqaipedaip	eesgdededd	pdkrisicss	dkriaceeef
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481	**la**					

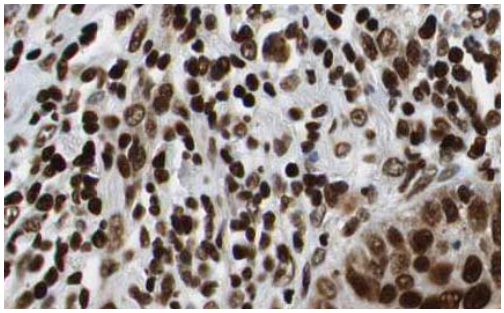

Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-HDAC1 antibody shows strong nuclear staining of tumor cells in human lung tissue. Tissue was formalin-fixed and paraffin embedded. Brown color indicates presence of protein, blue color shows cell nuclei. *Personal Communication, Kenneth Wester, www.proteinatlas.org, Upsala, Sweden.*

Immunogen: This affinity-purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 666-482 of Human HDAC-1.

General References:

Unoki,M., Nishidate,T. and Nakamura,Y. (2004) ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* **23** (46), 7601-7610.

Zhao,Q., Cumming,H., Cerruti,L., Cunningham,J.M. and Jane,S.M. (2004) Site-specific acetylation of the fetal globin activator NF-E4 prevents its ubiquitination and regulates its interaction with the histone deacetylase, HDAC1. *J. Biol. Chem*. **279** (40), 41477-41486.

Ammanamanchi,S. and Brattain,M.G. (2004) Restoration of transforming growth factor-beta signaling through receptor RI induction by histone deacetylase activity inhibition in breast cancer cells. *J. Biol. Chem*. **279** (31), 32620-32625.

Waltregny,D., North,B., Van Mellaert,F., de Leval,J., Verdin,E. and Castronovo,V. (2004) Screening of histone deacetylases (HDAC) expression in human prostate cancer reveals distinct class I HDAC profiles between epithelial and stromal cells. *J. Biol. Chem*. **48** (3), 273-290.

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