

Datasheet for 600-401-974**AHA1 Antibody****Overview**

Description:	Anti-AHA1 (RABBIT) Antibody - 600-401-974
Item No.:	600-401-974
Size:	100 µg
Applications:	ELISA, WB, IHC, IP
Reactivity:	Human, Monkey
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) and is suitable for Cancer, Immunology and Nuclear Signaling research. Activator of Hsp90 ATPase (AHA1) stimulates the inherent ATPase cycle of Hsp90, which is essential for its chaperone activity in vivo. The activation and/or stability of many of the key regulatory and signaling proteins of the eukaryotic cell depend on their interaction with the Hsp90 molecular chaperone. Hsp90 is assisted and regulated by co-chaperones that participate in an ordered series both to assist client-protein recruitment or release and to modulate progress through the ATPase coupled chaperone cycle. Structural analysis and mutagenesis show that binding of the N-terminal domain of AHA1 to Hsp90 promotes a conformational switch in the middle-segment catalytic loop (aa 370–390) of Hsp90 that exposes the catalytic Arg380 and enables its interaction with ATP in the N-terminal nucleotide-binding domain of the chaperone. Recent studies show that AHA1 modulates Hsp90-dependent stability of the folding of the cystic fibrosis transmembrane conductance regulator (CFTR) in the endoplasmic reticulum (ER). Down-regulation of AHA1 rescues misfolding of CFTR in cystic fibrosis.
Synonyms:	rabbit anti-AHA1 Antibody, Aha-1, Aha 1, Ahsa1 antibody, Activator of Hsp90 ATPase, Activator of 90 kDa heat shock protein ATPase homolog 1 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	AHSA1
Reactivity:	Human, Monkey
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region of human AHA1 protein.
Purity/Specificity:	This affinity purified antibody is directed against human AHA1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography. A BLAST analysis was used to suggest cross-reactivity with AHA1 protein from human and chimpanzee based on 100% homology with the immunizing sequence. Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - O95433 • NCBI - NP_001308370.1 • GenelD - 10598

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC, IP (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 38-40 kDa in size corresponding to AHA1 protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:35,000 - 1:185,000
IHC:	User Optimized
WB:	1 µg/mL

Formulation

Physical State:	Liquid (sterile filtered)
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
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer: None

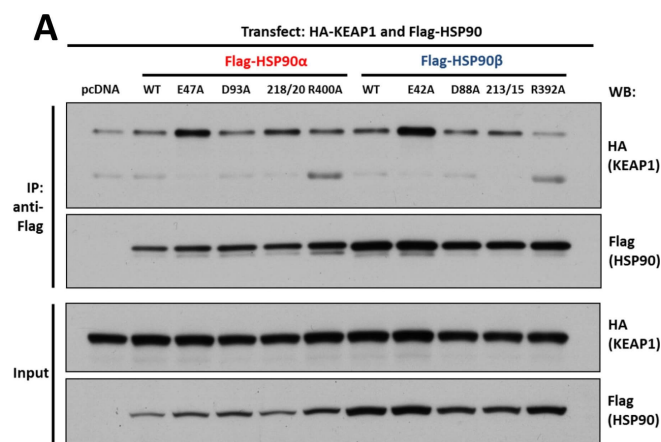
Shipping & Handling

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.

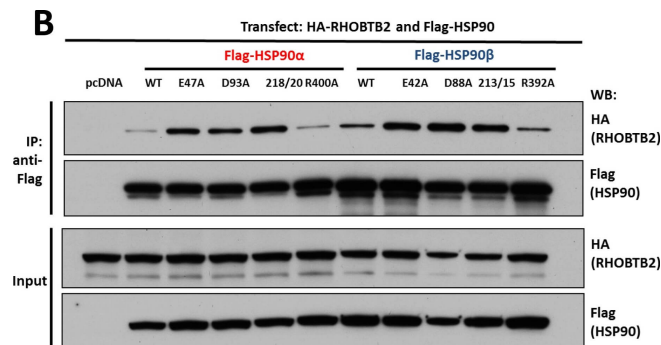
Expiration: Expiration date is one (1) year from date of receipt.

Images



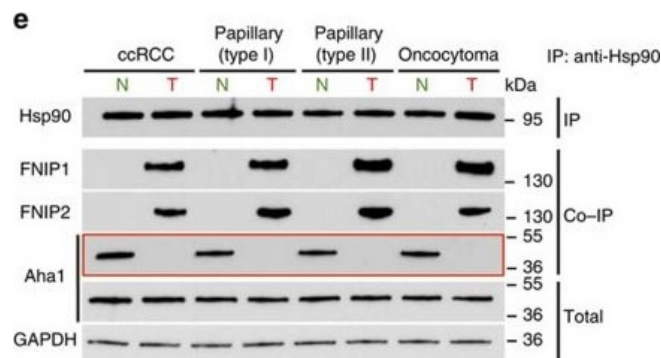
Western Blot

Interaction of KEAP1 and RHOBTB2 with HSP90 isoforms. (A) KEAP1 interaction with HSP90 WT and mutants: HEK293 cells transfected with HA-KEAP1 and each FLAG-HSP90 construct were harvested, immunoprecipitated with anti-FLAG beads and western blotted for HA (p/n 600-401-974). Input lysates were normalized and run as controls. (B) RHOBTB2 interaction with HSP90 WT and mutants: HEK293 cells transfected with HA-RHOBTB2 and each FLAG-HSP90 construct were harvested, immunoprecipitated with anti-FLAG beads and western blotted for HA. Input lysates were normalized and run as controls. (C) Measurement of the relative interaction strength of KEAP1 and RHOBTB2 with each HSP90 isoform by LUMIER: HEK293 cells transfected with KEAP1 or RHOBTB2 and each HSP90 isoform were harvested, applied to a 96-well anti-FLAG plate and assayed for luciferase activity. The difference in relative interaction strength of HSP90 α and HSP90 β for KEAP1 and RHOBTB2 (each approximately 3-fold) was statistically significant ($p < 0.05$) (see Methods). Figure provided by CiteAb. Source: PLoS One, PMID: 26517842. 600-401-974)



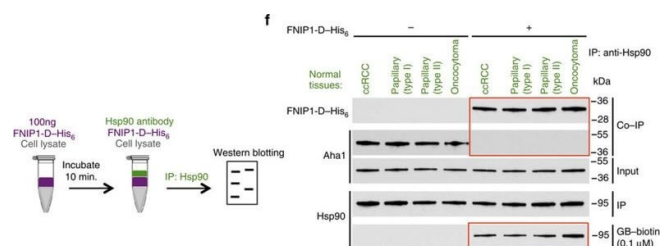
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Western Blot

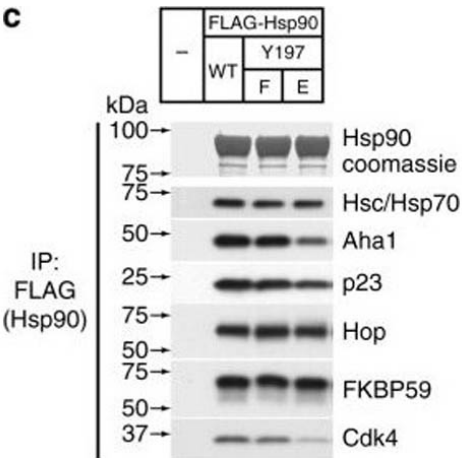
High levels of FNIPs make renal tumours sensitive to Hsp90 inhibitor GB. (a) Clear cell renal cell carcinoma (ccRCC), (b) Papillary type I, (c) Papillary type II, (d) Oncocytoma (Tumours, T) and adjacent normal tissues (Normal, N) were stained with haematoxylin and eosin (H&E). Proteins were also extracted from these tumours and adjacent normal tissues and incubated with indicated amounts of biotinylated GB followed by streptavidin agarose beads. Hsp90 was detected by immunoblotting. Expression of FNIP1 and FNIP2 in these samples was also detected by immunoblotting. (e) Hsp90 immunoprecipitated from tumours (T) and adjacent normal tissues (N) in a–d. Co-IP of FNIPs and Aha1 was examined by immunoblotting. (f) Lysates from normal tissues in a–d were incubated with or without 100 ng of pure FNIP1-D-His6 for 10 min. Hsp90 was immunoprecipitated and co-IP of FNIP1-D-His6 and Aha1 were shown by immunoblotting. Figure provided by CiteAb. Source: Nat Commun, PMID: 27353360.



Western Blot

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C

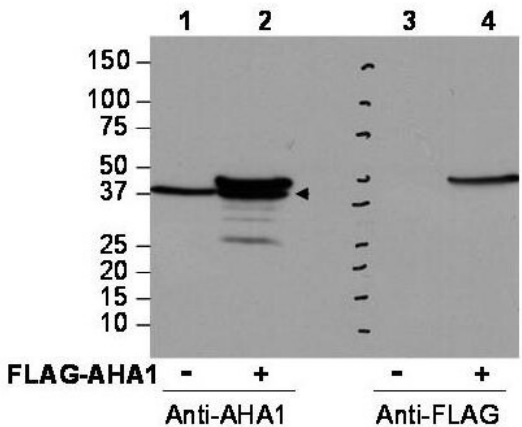


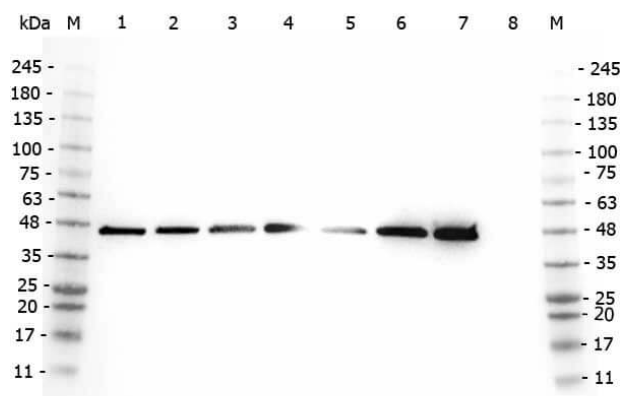
Western Blot

Assembly of complexes of Cdc37 and Hsp90 phosphomimetic variants with clients and cochaperones. a Binary complex formation between Cdc37 variants and bRaf (left), and between Hsp90 β variants and Cdc37 (right), followed by ITC. The corresponding K_d values are displayed in the inset. Error bars in the K_d values correspond to the errors resulted in fitting of the data into a single binding site model. b HEK-293 cells were cotransfected with indicated HA-tagged Cdc37 and FLAG-tagged bRaf plasmids. After cell lysis, proteins were immunoprecipitated with anti-FLAG resin for 1 h at 4 °C with rotation. Bead pellets were washed and analyzed for Cdc37 interaction by SDS-PAGE/western blot, using anti-HA antibody. c HEK-293 cells were transfected with FLAG-tagged Hsp90, Hsp90Y197E, or Hsp90Y197F plasmids. After cell lysis, proteins were immunoprecipitated with anti-FLAG resin for 1 h at 4 °C with rotation. Bead pellets were washed three times before analysis by SDS-PAGE/western blot. Co-precipitating endogenous Hsp70, Aha1, p23, Hop, Fkbp59, and Cdk4 were detected with specific antibodies. d HEK-293 cells were transfected with the indicated Hsp90, androgen receptor (AR), and glucocorticoid receptor (GR) plasmids. Proteins were precipitated with GFP-Trap resin (left) or ANTI-FLAG M2 agarose (right) for 1 h at 4 °C with rotation. Bead pellets were washed three times with lysis buffer before analysis by SDS-PAGE/western blot as indicated. AR was visualized with anti-GFP antibody, GR was visualized with a specific antibody, and Hsp90 was visualized with anti-FLAG antibody. Figure provided by CiteAb. Source: Nat Commun, PMID: 29343704.

Western Blot

Western blot using Rockland's affinity purified anti-AHA1 antibody shows detection of AHA1 in Cos7 cells. For Lanes 2 and 4, Cos7 cells were transfected with pcDNA3-FLAG-AHA1. For Lanes 1 and 3, Cos7 cells were not transfected. Extracts (40 μ g per lane) were electrophoresed and transferred to nitrocellulose. The membrane was probed with anti-AHA1 (lanes 1 and 2, 1:2,000 dilution) or anti-FLAG (lanes 3 and 4). The lower band seen in anti-AHA1 blotting (arrowhead) is endogenous AHA1. Personal Communication, Brad Scroggins, CCR-NCI, Bethesda, MD





Western Blot

Western Blot of Rabbit anti-AHA1 antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-364). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A431 Lysate (p/n W09-000-361). Lane 6: Raji Lysate (p/n W09-001-368). Lane 7: Ramos Lysate (p/n W09-000-GK4). Lane 8: NIH/3T3 Lysate (p/n W10-000-358). Load: 35 µg per lane. Primary antibody: AHA1 antibody at 1:2,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT. Predicted/Observed size: 38 kDa, 38 kDa for AHA1.

References

- Xu W et al. Hsp90 middle domain phosphorylation initiates a complex conformational program to recruit the ATPase-stimulating cochaperone Aha1. *Nat Commun.* (2019)
- Bachman et al. Phosphorylation induced cochaperone unfolding promotes kinase recruitment and client class-specific Hsp90 phosphorylation. *Nature Communications* (2018)
- Woodford et al. The FNIP co-chaperones decelerate the Hsp90 chaperone cycle and enhance drug binding. *Nature Communications* (2016)
- Prince et al. Client Proteins and Small Molecule Inhibitors Display Distinct Binding Preferences for Constitutive and Stress-Induced HSP90 Isoforms and Their Conformationally Restricted Mutants. *PLOS One* (2015)

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.