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Datasheet for ABIN233816 anti-AHSA1 antibody (Internal Region)

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Overview

Quantity:	100 µg
Target:	AHSA1
Binding Specificity:	Internal Region
Reactivity:	Human, Monkey
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC)

Product Details

Purpose:	AHA1 Antibody
Immunogen:	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. Immunogen Type: Conjugated Peptide
lsotype:	lgG
Cross-Reactivity (Details):	This affinity purified antibody is directed against human AHA1 protein.
Characteristics:	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
Purification:	The product was affinity purified from monospecific antiserum by immunoaffinity chromatography.
Sterility:	Sterile filtered

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Target Details					
Target:	AHSA1				
Alternative Name:	AHSA1 (AHSA1 Products)				
Background:	Background: This antibody is designed, produced, and validated as part of a collaboration with				
	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.				
Gene ID:	10598				
NCBI Accession:	NP_001308370				
UniProt:	095433				
Application Details					

Application Notes:	Immunohistochemistry Dilution: User Optimized				
	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.				
	Western Blot Dilution: 1 µg/mL				
	ELISA Dilution: 1:35,000 - 1:185,000				
	Other: User Optimized				
Restrictions:	For Research Use only				

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Format:	Liquid					
Concentration:	1.08 mg/mL					
Buffer:	Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2					
	Stabilizer: None					
	Preservative: 0.01 % (w/v) Sodium Azide					
Preservative:	Sodium azide					
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which					
	should be handled by trained staff only.					
Storage:	4 °C,-20 °C					
Storage Comment:	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.					
Expiry Date:	12 months					
Publications						
Product cited in:	Xu, Beebe, Chavez, Boysen, Lu, Zuehlke, Keramisanou, Trepel, Prodromou, Mayer, Bruce, Gelis,					
	Neckers: "Hsp90 middle domain phosphorylation initiates a complex conformational program					
	to recruit the ATPase-stimulating cochaperone Aha1." in: Nature communications, Vol. 10,					
	Issue 1, pp. 2574, (2019) (PubMed).					
	Woodford, Dunn, Blanden, Capriotti, Loiselle, Prodromou, Panaretou, Hughes, Smith, Ackerman,					
	Haystead, Loh, Bourboulia, Schmidt, Marston Linehan, Bratslavsky, Mollapour: "The FNIP co-					
	chaperones decelerate the Hsp90 chaperone cycle and enhance drug binding." in: Nature					
	communications, Vol. 7, pp. 12037, (2018) (PubMed).					
	Bachman Keramisanou Xu Beehe Moses Vasantha Kumar Gray Noor van der Vaart					
	Neckers: Gelis: "Phosphorylation induced cochanerone unfolding promotes kingse requitment					
	and client class-specific Hsn00 phosphorylation " in: Nature communications Vol. 9. Issue 1					
	nn 265 (2018) (PubMed)					
	Prince, Kijima, Tatokoro, Lee, Tsutsumi, Yim, Rivas, Alarcon, Schwartz, Khamit-Kush, Scroggins,					
	Beebe, Trepel, Neckers: "Client Proteins and Small Molecule Inhibitors Display Distinct Binding					
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Preferences for Constitutive and Stress-Induced HSP90 Isoforms and Their Conformationally Restricted Mutants." in: **PLoS ONE**, Vol. 10, Issue 10, pp. e0141786, (2016) (PubMed).

Images



Western Blotting

Image 1. 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 Hsp90β variants and Cdc37 (right), followed by ITC. The corresponding Kd values are displayed in the inset. Error bars in the Kd 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 1h 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 1h 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 1h 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



kDa	М	1	2	3	4	5	6	7	8	М
245 -										- 245
180 -										- 180
135 -	-									- 135
100 - 75 -	-									- 100 - 75
63 -	-									- 63
48 -	-	-	-	-	-		-	-		- 48
35 -	-							-		
25 - 20 -	-									- 25
17 -	**									- 17
11 -	inter .									- 11

- figure provided by CiteAb. Source: PMID29343704

Western Blotting

Image 2. Western blot using 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 µg 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 Blotting

Image 3. Western Blot of Rabbit anti-AHA1 antibody. Marker: Opal Pre-stained ladder . Lane 1: HEK293 lysate . Lane 2: HeLa Lysate . Lane 3: MCF-7 Lysate . Lane 4: Jurkat Lysate . Lane 5: A431 Lysate . Lane 6: Raji Lsyate . Lane 7: Ramos Lysate . Lane 8: NIH/3T3 Lysate . Load: 35 µg per lane. Primary antibody: AHA1 antibody at 1:2,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:30,000 for 60 min at RT. Blocking 1% Casein-TTBS Buffer: for 30 min at RT. Predicted/Observed size: 38 kDa, 38 kDa for AHA1. Other band(s): N/A.

Please check the product details page for more images. Overall 8 images are available for ABIN233816.