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Datasheet for 600-401-981

HSP90 K294 Antibody

Overview

Description:	Anti-Heat Shock Protein HSP 90-alpha acetyl specific K294 (RABBIT) Antibody - 600-401-981
Item No.:	600-401-981
Size:	100 µg
Applications:	ELISA, IHC, WB, IF
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) and is suitable for Cancer, Immunology and Nuclear Signaling research. Hsp90 is a member of the heat shock protein 90 family, the members of which are highly conserved between isoforms and species. Hsp90 functions as a molecular chaperone and has ATPase activity. Hsp90 is a cytoplasmic protein that forms a homodimer in vivo and interacts with TOM34, AHSA1, HDAC6 and SMYD3. Several signal transduction pathways depend on Hsp90 function, including pathways involving erbB2, hypoxia sensitivity (Hif1 alpha), and steroid hormone receptors (for example, androgen, progesterone, glucocorticoid, and aryl-hydrocarbon). Recent reports show that Hsp90 from tumor cells has increased sensitivity to small molecule inhibitors (for example, 17AAG). The mechanism of the differential sensitivity of Hsp90 from normal versus tumor cells is unknown, although mutation has been ruled out. One possible mechanism may be differences in post-translational modification of tumor Hsp90. K294 was found to be acetylated in purified Hsp90 from SkBr3 cells, a breast cancer cell line.
Synonyms:	rabbit anti-Heat Shock Protein HSP 90-alpha acetyl specific K294 antibody, rabbit anti-Heat Shock Protein HSP 90-alpha acetylated Lys294 antibody, HSP 86 antibody, Heat shock 86kDa antibody, Renal carcinoma antigen NY REN 38 antibody, Heat shock 90kDa protein 1 alpha antibody, Lipopolysaccharide-associated protein 2, LAP-2, HSP90A, HSPC1, HSPCA, HSP90AA1, HSP 90-α
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HSP90AA1
Reactivity:	Human
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 amino acids surrounding K294 of human Hsp90.
Purity/Specificity:	This affinity purified antibody is directed against human Hsp90 protein acetylated at K294. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography using acetyl-peptide coupled to agarose beads followed by solid phase adsorption against non-acetyl peptide. While ELISA data show strong reactivity with the acetylated form of the immunizing peptide and minimal reactivity with the non-acetylated form, to date western blotting data are not definitive for acetyl K294 specificity as blots show equivalent staining of lysates from cells either treated or untreated with Trichostatin A (an HDAC inhibitor). A BLAST analysis was used to suggest cross-reactivity with Hsp90 from human, mouse, rat, monkey, chicken and Drosophila based on 100% homology with the immunizing sequence. Reactivity of this antibody with Hsp90 from other species is unknown.
Relevant Links:	• UniProtKB - P07900 • NCBI - NP_001017963.2 • GeneID - 3320

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA, immunohistochemistry and western blot. This antibody reacts strongly with the acetylated form of the immunizing peptide and shows minimal reactivity with the non-acetylated form when tested by ELISA. To date, western blotting shows equivalent staining of lysates either treated or untreated with Trichostatin A (an HDAC inhibitor). Therefore, western blotting results are not definitive for demonstrating the specificity of this reagent. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 90 kDa in size corresponding to Hsp90 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:	1:2,500 - 1:10,000

IHC:	5-10 µg/ml
WB:	1:500 - 1:2,500

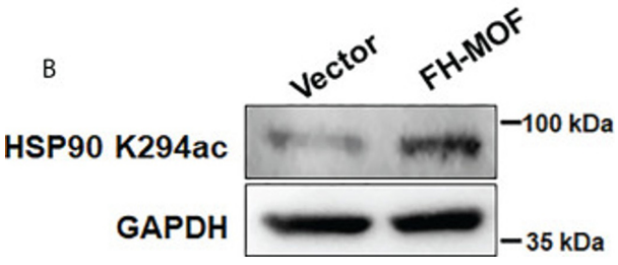
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.06 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

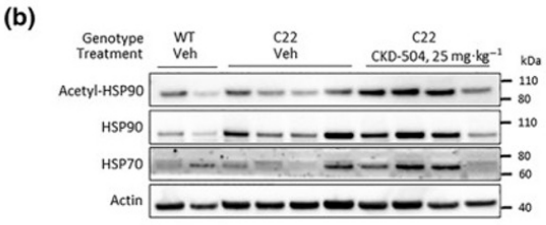
Shipping & Handling

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.

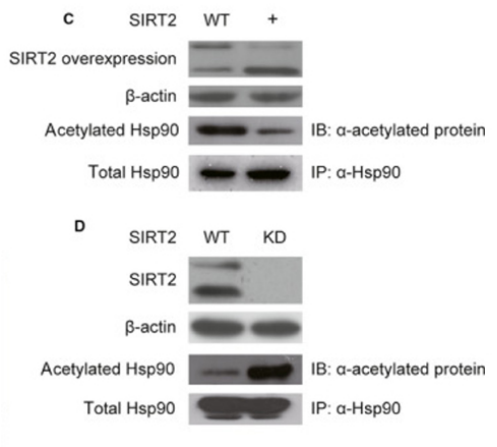
Images



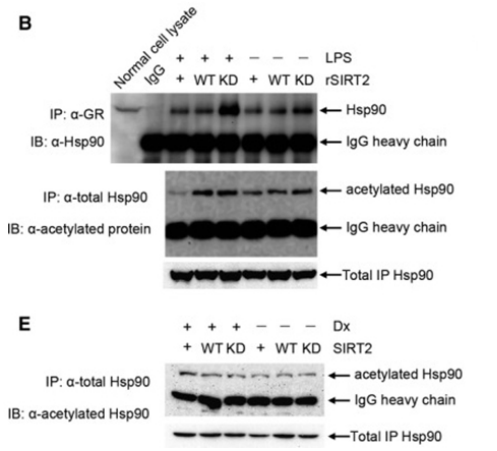
Western Blot
(B) HSP90 K294 acetylation site was identified to be functioning in MOF-induced hyperacetylation of HSP90 by Western blotting assay using specific HSP90 K294ac antibody. Fig 6. PMID: 36212422



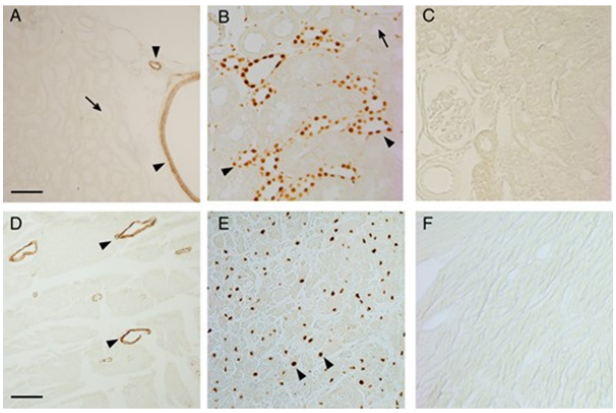
Western Blot
(b) The acetylation of HSP90 and expression of HSP70 were analysed (WT-DW: N = 2; C22-DW: N = 4; C22-504: N = 4). Fig 6. PMID: 33460073



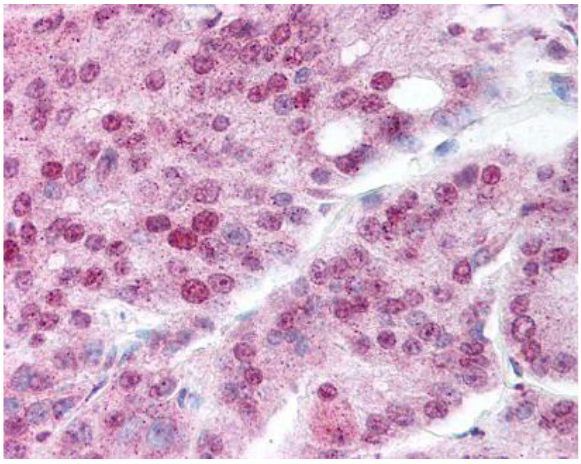
Immunoprecipitation
Co-IP with anti-Hsp90 in B104 cells overexpressing rSIRT2 (+) showed a decrease in the acetylation levels in the protein band corresponding to Hsp90 compared with control cells (WT) (C), whereas SIRT2 knock-down 36 showed an increase in the acetylation levels of the protein band corresponding to Hsp90 compared with control cells (WT) (D). Taken together, these results suggest that Hsp90 is a direct target of SIRT2 deacetylation. Hsp90 co-IPs (C and D) were repeated three separate times, and a representative immunoblots are shown. Fig 3. PMID: 32515550



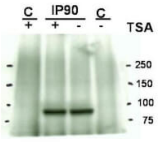
Immunoprecipitation
SIRT2 overexpression resulted in decreased interaction between Hsp90 and GR compared with SIRT2 knock-down. Co-IP assays were also performed with anti-GR in SIRT2 overexpression, SIRT2 knocked-down or control cells, and Hsp90 was detected in the IP complex by immunoblot (B; top panel). Co-IP with anti-Hsp90 under the same conditions, followed by detection of acetylated Hsp90 (B; bottom panel), showed that the interaction between Hsp90 and GR corresponds to the acetylation levels of Hsp90. (E) The B104 cell or with SIRT2 overexpression or with SIRT2 knocked-down were treated with 10 µmol/L of Dx for 12 h. The cell lysate was incubated with anti-Hsp90 antibody to immunoprecipitate total Hsp90 protein. Subsequently, the acetylated Hsp90 was detected in the complex through Western blot. Fig 4. PMID: 32515550



Immunohistochemistry
Immunohistochemical analysis of Hsp90 acetylation in rat kidney and heart shows predominant labeling in vascular smooth muscle cells. A, Representative micrograph of a paraffin-embedded section of rat kidney cortex stained with anti-Hsp90-acetyl-K295 antibody. Staining was restricted to renal arteries and arterioles (arrowheads). Renal tubules were negative for anti-Hsp90-acetyl-K295 staining (arrow). B, Rat kidney cortex stained with 12A7 anti-MR antibody. Staining is prominent in distal nephron (arrowheads) but negative in proximal tubules (arrow). C, Negative control of rat kidney cortex obtained omitting the primary anti-Hsp90-acetyl-K295 antibody. D, Representative micrograph of rat heart stained with anti-Hsp90-acetyl-K295 antibody. Staining was restricted to coronary arteries and arterioles (arrowheads). E, Rat heart stained with anti-MR antibody. Cardiomyocyte nuclei are clearly stained (arrowheads). F, Negative control of rat heart obtained omitting the primary anti-Hsp90-acetyl-K295 antibody. Bars in panels A and D, 50 µm. Fig 9. PMID: 27100623



Immunohistochemistry
Rockland's affinity purified anti-Hsp90 acetyl K294 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 granular cytoplasmic positive staining in human prostate carcinoma 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-Hsp90 acetyl K294 antibody shows detection of a band at ~90 kDa corresponding to Hsp90 in an SkBr3 cell lysate (p/n W09-001 -MP4). Western blotting results do not definitively demonstrate the acetyl K294 specificity of this reagent because similar staining is seen in the control lane with no treatment of TSA - Trichostatin A (an HDAC inhibitor). Immunoprecipitation with anti-Hsp90 was performed prior to western blotting with anti-Hsp90 acetyl K294.

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