

Product Datasheet

HSP90 alpha Antibody NB120-2928

Unit Size: 100ug

Store at -20C. Avoid freeze-thaw cycles.

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NB120-2928

HSP90 alpha Antibody

| Product Information | |
|---------------------|--|
| Unit Size | 100ug |
| Concentration | 1 mg/ml |
| Storage | Store at -20C. Avoid freeze-thaw cycles. |
| Clonality | Polyclonal |
| Preservative | 0.05% Sodium Azide |
| Isotype | IgG |
| Purity | Immunogen affinity purified |
| Buffer | PBS with 1 mg/ml BSA |

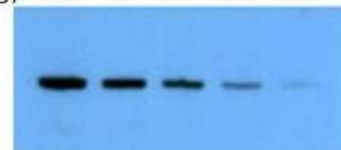
| Product Description | |
|-------------------------|---|
| Description | Novus Biologicals Rabbit HSP90 alpha Antibody (NB120-2928) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and IP. Anti-HSP90 alpha Antibody: Cited in 13 publications. All Novus Biologicals antibodies are covered by our 100% guarantee. |
| Host | Rabbit |
| Gene ID | 3320 |
| Gene Symbol | HSP90AA1 |
| Species | Human, Mouse, Rat, Primate, Rabbit, Sheep |
| Reactivity Notes | Rabbit reactivity reported in scientific literature (PMID: 21129982). |
| Specificity/Sensitivity | Detects Heat Shock Protein 86 (HSP 86). This does not detect HSP 84. |
| Immunogen | Synthetic peptide corresponding to residues P(2) E E T Q T Q D Q P M(12) of mouse HSP86. |

| Product Application Details | |
|-----------------------------|---|
| Applications | Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation |
| Recommended Dilutions | Western Blot 1:500 - 1:2000, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunoprecipitation 2 ug, Immunohistochemistry-Paraffin 5 ug/ml |
| Application Notes | IP: HSP 86 exists primarily as homodimers in HeLa cells. This antibody is capable of precipitating HSP 86 that is complexed with other proteins such as the aryl hydrocarbon (Ah) receptor. |

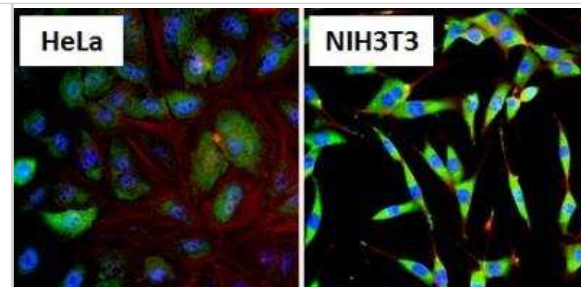
Images

Western Blot: HSP90 alpha Antibody [NB120-2928] - Analysis of 2-fold serial dilutions of HeLa cell lysate, starting at 10 ug, per well.

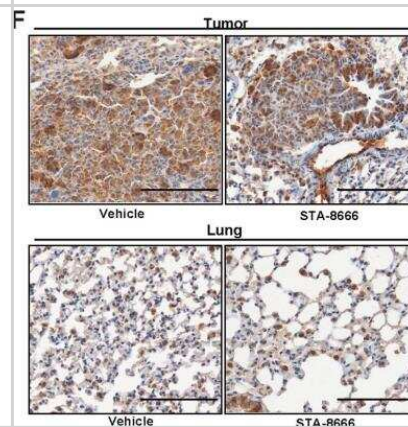
HeLa Lysate (µg): 10 5 2.5 1.3 0.6



Immunocytochemistry/Immunofluorescence: HSP90 alpha Antibody [NB120-2928] - Analysis of Heat Shock Protein 86 (HSP86, green) in HeLa cells and NIH3T3 cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with a HSP86 polyclonal antibody, at a dilution of 1:100 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat-anti-rabbit IgG secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye.



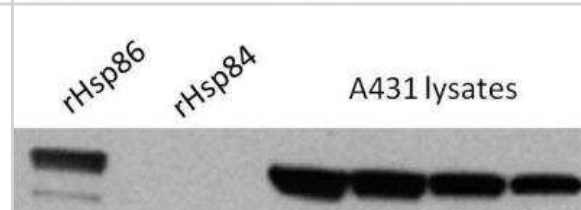
Immunohistochemistry-Paraffin: HSP90 alpha Antibody [NB120-2928] - Signaling consequences of STA-8666 treatment. HSP90 alpha. Magnification: 20x. Scale bars: 75um, except for vimentin, where scale bar is 40um. Image collected and cropped by CiteAb from the following publication (<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0176747>) licensed under a CC-BY license.



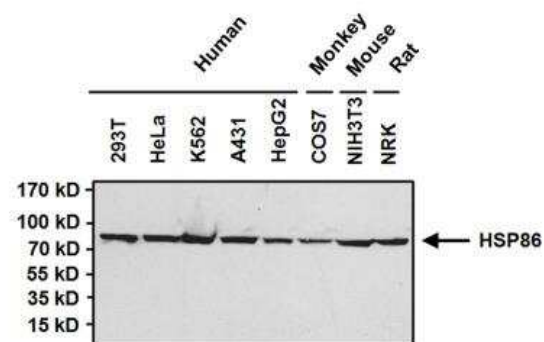
Western Blot: HSP90 alpha Antibody [NB120-2928] - Figure 1 shows a Western blot of mouse HSP84 and HSP86 using NB120-2928 and NB120-2927 respectively.



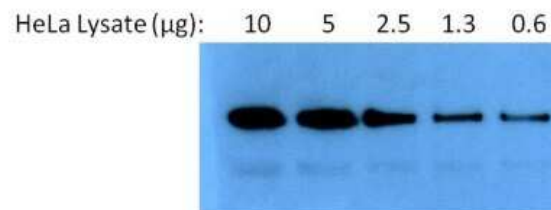
Western Blot: HSP90 alpha Antibody [NB120-2928] - Analysis of epidermoid carcinoma (A431) cells using Heat Shock Protein 86 Polyclonal Antibody. Decreasing amounts of A431 whole cell lysates were probed with PA3-013 at a dilution of 1:2000 followed by an anti-rabbit IgG secondary antibody and SuperSignal West Pico Chemiluminescent Substrate. Lanes 1 and 2 are control samples of purified, recombinant Hsp86 (rHsp86) and Hsp84 (rHsp84), respectively. Data courtesy of the Innovators Program.



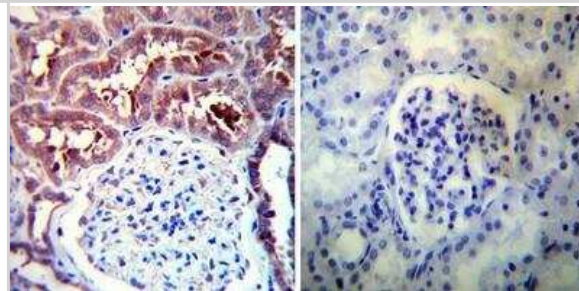
Western Blot: HSP90 alpha Antibody [NB120-2928] - Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a HSP86 polyclonal antibody at a dilution of 1:1000 overnight at 4C on a rocking platform, washed in TBS-0.1%Tween 20, and probed with a goat anti-rabbit IgG HRP secondary antibody at a dilution of 1:20,000 for at least 1 hour.



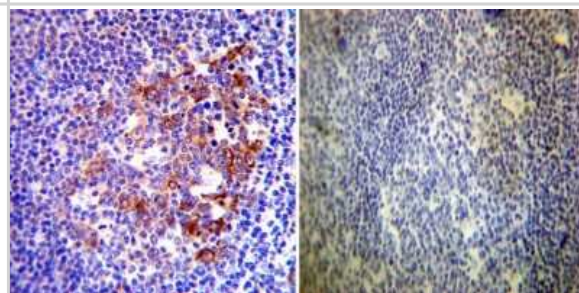
Western Blot: HSP90 alpha Antibody [NB120-2928] - Analysis of 2-fold serial dilutions of HeLa cell lysate, starting at 10 μ g, per well.



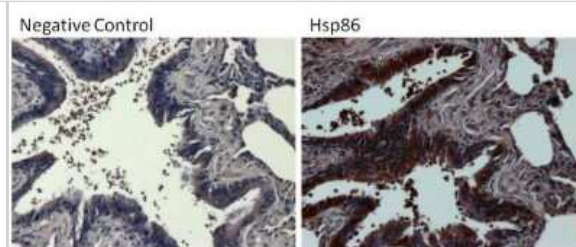
Immunohistochemistry-Paraffin: HSP90 alpha Antibody [NB120-2928] - Both normal and cancer biopsies of deparaffinized Human kidney tissues.



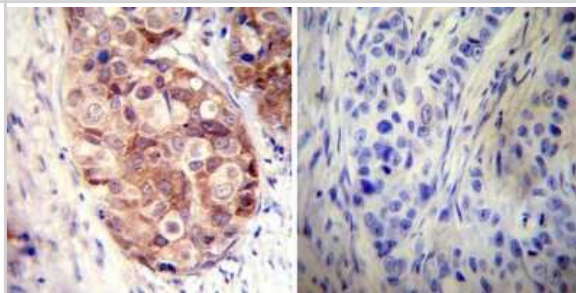
Immunohistochemistry-Paraffin: HSP90 alpha Antibody [NB120-2928] - Both normal and cancer biopsies of deparaffinized Human tonsil tissues.



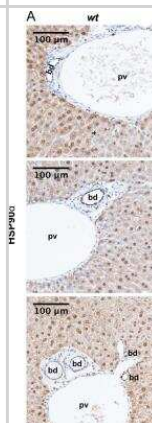
Immunohistochemistry-Paraffin: HSP90 alpha Antibody [NB120-2928] - Heat-induced epitope retrieval was performed using 10mM sodium citrate (pH 6.0) buffer for 20 mins at 95C. Following antigen retrieval, tissues blocked in 3% BSA in PBST for 30 mins at RT & then probed with Heat Shock Protein 86 (Hsp86) polyclonal antibody at 1:100 for 1 hr in a humidified chamber (right panel). As a -ve control, primary antibody eliminated from the staining procedure (left panel). Tissues washed with PBS/0.025% Tween-20 & endogenous peroxidase activity quenched with Peroxidase Suppressor for 30 mins at RT. Detection performed using an HRP-conjugated goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:250 followed by colorimetric detection using Metal Enhanced DAB Substrate Kit. Tissues were counterstained with hematoxylin & prepped for mounting. Images were taken on a Zeiss Axiovision microscope at 40X magnification (x1.6 Optovar).



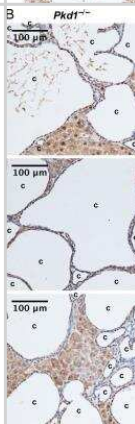
Immunohistochemistry-Paraffin: HSP90 alpha Antibody [NB120-2928] - Both normal and cancer biopsies of deparaffinized Human breast carcinoma tissues.



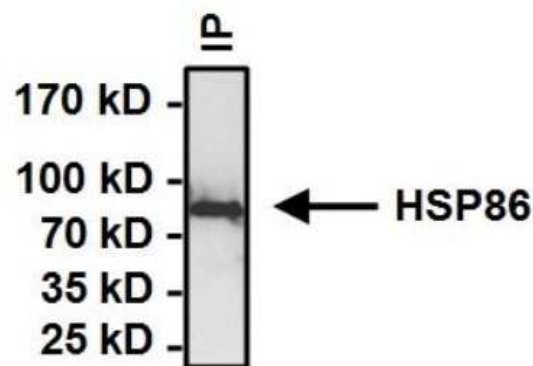
Immunohistochemistry: HSP90 alpha Antibody [NB120-2928] - HSP90 alpha is upregulated in epithelial cells lining liver cysts.(A, B) Representative hematoxylin stained liver sections with immunohistochemical detection of HSP90 alpha (brown) from three (A) wild type (wt) and (B) *Pkd1*^{-/-} mice. bd = bile duct, pv = portal vein, c = cyst. Scale bars = 100 μ m; magnification, 20x. Image collected and cropped by CiteAb from the following publication (<https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0114403>) licensed under a CC-BY license.



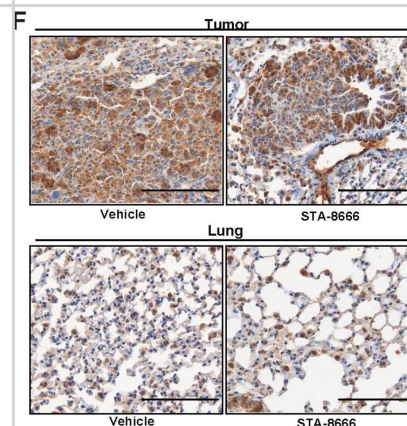
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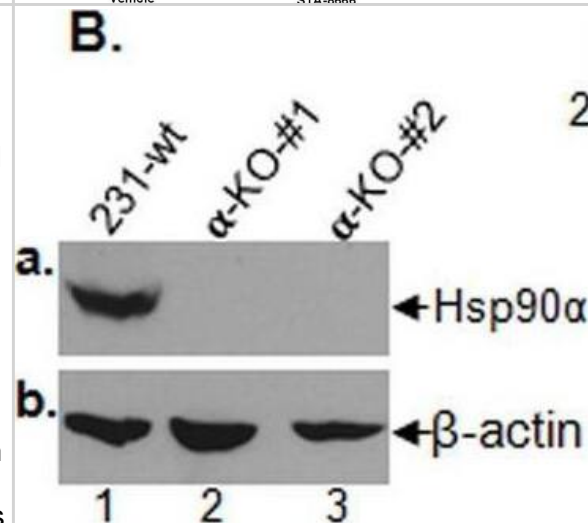
Immunoprecipitation: HSP90 alpha Antibody [NB120-2928] - Analysis of Heat Shock Protein (HSP86) was performed on HeLa cells. Antigen-antibody complexes formed by incubating 500ug whole cell lysate with 2ug of HSP86 polyclonal antibody overnight on a rocking platform at 4C. The immune complexes were captured on 50ul Protein A/G Plus Agarose, washed extensively, and eluted with Lane Marker Reducing Sample Buffer. Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a HSP86 polyclonal antibody at a dilution of 1:1000 overnight rotating at 4C. The membrane was washed in TBST, and probed with Clean-Blot IP detection Reagent at a dilution of 1:1000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura.



Signaling consequences of STA-8666 treatment. A.-F. Left, Representative images and right, quantification of IHC or immunofluorescence for Ki-67 (A), cleaved caspase 3 (B), pS824-KAP1 (C), pT202/Y204-ERK1/2 (D), vimentin (light red) and DAPI (blue) (E) and HSP90 (F). Magnification: 20x. Scale bars: 75 μ m, except for vimentin, where scale bar is 40 μ m.



The "eHsp90 α > LRP-1 autocrine loop" only accounts for 50% of the constitutive motility of the tumour cells. (A) A schematic illustration of the "eHsp90 α > LRP-1 autocrine loop" that promotes cell motility. (B) Western blot shows 231-wt and two clones of 231-Hsp90 α -knockout (231- α -KO) cells (panel a). (C) Anti-Hsp90 α antibody blotting of secreted Hsp90 α in serum-free conditioned medium (CM, 10X) from 231-wt, 231- α -KO (panel c) or 231-LRP-1-KD (panel d) cells. (D) Western blot analysis shows the total Hsp90 α level in 231-wt or 231-LRP-1-KD cells under normoxia or hypoxia. (E) Western blot analysis shows the relative levels of LRP-1 in indicated cell lines. (F) CM from 231-wt, but not 231- α -KO, cells stimulated B16 melanoma cell migration (panels k and l vs. panel i), with human recombinant (hr) Hsp90 α protein (panel j) as positive control. (G) Computer-assisted quantitation of the B16 cell migration shown in panel F as Migration Index (%) ("Methods"). CM from 231- α -KO cells completely lost pro-motility activity (panel l). Asterisks indicate significance of the induced migration by indicated stimuli (panels j, k) versus the serum-free control (panel i). (H) The indicated cells were simultaneously compared for their motility under serum-free conditions. Neither total α -KO (panels o and s) nor extracellular blockade of secreted Hsp90 α function by mAb1G7-D7 (panels p, t) re-produced the degree of inhibition of cell motility by LRP-1-KD (panels n and u), in reference to 231-wt cells (panels m and q). (L) Computer-assisted quantitation of the cell migration data with indicated panels. Asterisks indicate the significance of indicated cell motility over that of the LRP-1-KD cells. All experiments were repeated multiple times to reach the final conclusion. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/35835845>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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Publications

Chang C, Tang X, Li W A Modified Differential Centrifugation Protocol for Isolation and Quantitation of Extracellular Heat Shock Protein 90 (eHsp90) *Methods in molecular biology* (Clifton, N.J.) 2023-08-04 [PMID: 37540440]

M Bzowska, A Nogie?, K Bania, M Zygmunt, M Zar?bski, J Dobrucki, K Guzik Involvement of cell surface 90 kDa heat shock protein (HSP90) in pattern recognition by human monocyte-derived macrophages *J. Leukoc. Biol.*, 2017-05-26;0(0):. 2017-05-26 [PMID: 28550115]

Chang C, Tang X, Mosallaei D et al. LRP-1 receptor combines EGFR signalling and eHsp90 alpha autocrine to support constitutive breast cancer cell motility in absence of blood supply *Scientific reports* 2022-07-14 [PMID: 35835845]

Tang X, Chang C, Mosallaei D et al. Heterogeneous Responses and Isoform Compensation Dim Therapeutic Window of Hsp90 ATP-Binding Inhibitors in Cancer *Molecular and cellular biology* 2021-12-06 [PMID: 34871064]

He X, Smith SE, Chen S et al. Tumor-initiating stem cell shapes its microenvironment into an immunosuppressive barrier and pro-tumorigenic niche *Cell reports* 2021-09-07 [PMID: 34496236]

Tang X, Chang C, Hao M et al. Heat shock protein-90alpha (Hsp90 alpha) stabilizes hypoxia-inducible factor-1alpha (HIF-1alpha) in support of spermatogenesis and tumorigenesis *Cancer gene therapy* 2021-03-04 [PMID: 33664459]

Deneka AY, Haber L, Kopp MC et al. Tumor-targeted SN38 inhibits growth of early stage non-small cell lung cancer (NSCLC) in a KRas/p53 transgenic mouse model. *PLoS ONE* 2017-04-28 [PMID: 28453558] (IHC-P, Mouse)

Smithline Zachary B, Nikonova Anna S, Hensley Harvey H et al. Inhibiting heat shock protein 90 (HSP90) limits the formation of liver cysts induced by conditional deletion of Pkd1 in mice. *PLoS One*. 2014-12-04 [PMID: 25474361] (IF/IHC, Mouse)

Seeger-Nukpezah T, Proia DA, Egleston BL et al. Inhibiting the HSP90 chaperone slows cyst growth in a mouse model of autosomal dominant polycystic kidney disease. *Proc Natl Acad Sci U S A* 2013-07-30 [PMID: 23858461] (IHC-P, Mouse)

Shelton SN, Shawgo ME, Matthews SB et al. KU135, a novel novobiocin-derived C-terminal inhibitor of the 90-kDa heat shock protein, exerts potent antiproliferative effects in human leukemic cells. *Mol Pharmacol*. 2009-12-01 [PMID: 19741006]

De Nardo D, Masendycz P, Ho S et al. A central role for the Hsp90.Cdc37 molecular chaperone module in interleukin-1 receptor-associated-kinase-dependent signaling by toll-like receptors. *J Biol Chem*. 2005-03-01 [PMID: 15647277]

Peterson JK, Moran F, Conley AJ et al. Zonal expression of endothelial nitric oxide synthase in sheep and rhesus adrenal cortex. *Endocrinology*. 2001-12-01 [PMID: 11713235]

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| | |
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| HAF008 | Goat anti-Rabbit IgG Secondary Antibody [HRP] |
| NB7160 | Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP] |
| NBP2-24891 | Rabbit IgG Isotype Control |

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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