HSP90α monoclonal antibody (9D2)

The Hsp90 family of heat shock proteins represents one of the most abundantly expressed and highly conserved families of cellular chaperones whose expression can be upregulated under conditions of cellular stress, and includes cytoplasmic (Hsp90-alpha/beta), ER (grp94), and mitochondrial (TRAP1) localized members. Structurally, Hsp90 is characterized by an N-terminal ATP-binding domain, a medial substrate-binding domain, and a C-terminal dimerization motif. Hsp90 dimers function in cooperation with cochaperones (e.g. Hsp40, Hsp70, Hop, p23) to stabilize a multitude of client protein substrates, including steroid hormone receptors, protein kinases, and transcription factors. The essential binding and hydrolysis of ATP by Hsp90 is inhibited by ansamycin drugs (e.g. geldanamycin, 17-AAG) which occupy the N-terminal Hsp90 nucleotide-binding pocket. Many Hsp90 client proteins such as erbB2/Her-2, c-raf, bcr-abl, p53, and hTERT, are members of well characterized oncogenic pathways, making Hsp90 inhibitors useful anticancer agents.

This antibody is covered by our Worry-Free Guarantee.

Citations: 34 View Online »

Ordering Information

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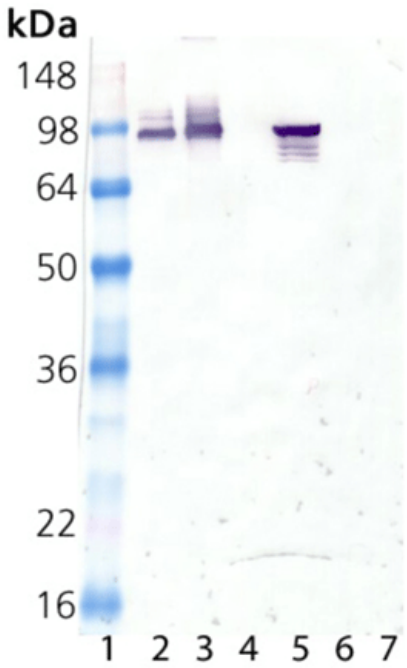
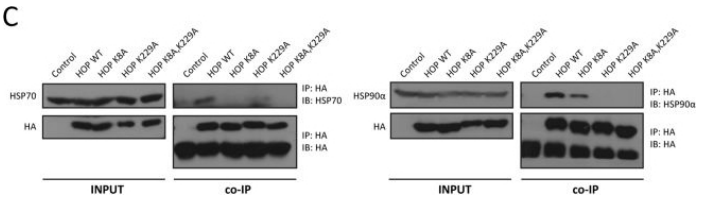
ADI-SPA-840-J	1mg
ADI-SPA-840-D	50µg
ADI-SPA-840-F	200µg

Manuals, SDS & CofA

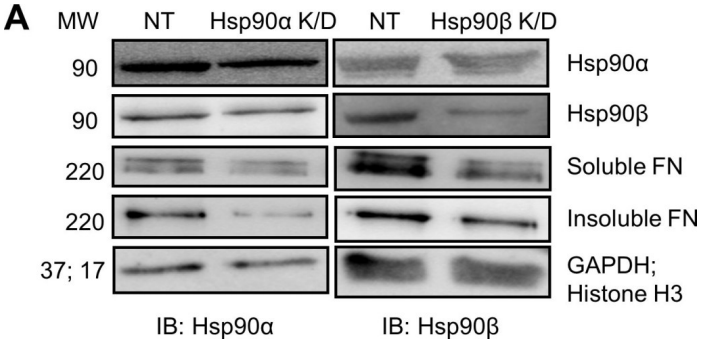
View Online »



Western blot analysis of HSP90α, mAb (9D2) (Prod. No. ADI-SPA-840):
Lane 1: MW marker, Lane 2: HSP90 native protein (Prod. No. ADI-SPP-770),
Lane 3: HSP90 alpha recombinant protein (Prod. No. ADI-SPP-776),
Lane 4: HSP90 beta recombinant protein (Prod. No. ADI-SPP-777),
Lane 5: HeLa Cell Lysate (heat shocked) (Prod. No. ADI-LYC-HL101),
Lane 6: PC-12 Cell Lysate (heat shocked) (Prod. No. ADI-LYC-PC101),
Lane 7: 3T3 Cell Lysate (heat shocked) (Prod. No. ADI-LYC-3T101).



LRET assays of the interactions of TPR mutants of HOP with HSP70 and HSP90. (A) Sequence alignment of the relevant portions of the TPR of CHIP and of TPR1 and TPR2A of HOP; K30 of CHIP, which is known to be important for binding HSP70 and HSP9032,33, is highlighted with a blue arrow. (B) Immunoblot analysis of TPR point mutants; HA-tagged constructs were transiently expressed in HEK293T cells and revealed using both anti-HOP and anti-HA antibodies as indicated with GAPDH as loading control. (C) Co-immunoprecipitation experiments to check the association between HOP mutants and endogenous HSP70 and HSP90; IP, immunoprecipitation; co-IP, coimmunoprecipitation; IB, immunoblot with indicated antibody. The uncropped original images of the immunoblots shown in panels B and C are presented in Supplementary Fig. S2. (D) Luminescence patterns of Tb3+ bound wild-type (LBT-TPR2A WT) and point mutant (LBT-TPR2A K229A). (E) Intrinsic EGFP fluorescence and LRET profiles for wild-type (WT) and point mutant TPR2A. (F) LRET titration experiment comparing the binding of wild-type and mutant TPR2A to HSP90. TPR2A WT and K229A (10 μM) loaded with equimolar Tb3+ were titrated with increasing concentrations of EGFP-C90 (0–6 μM). The Scatchard plot of the normalized LRET from three independent experiments represents means ± SEM. (G) Intrinsic TagRFP fluorescence and LRET profiles for wild-type (WT) and point mutant TPR1. In some panels, the position of the LRET signal is indicated.



Knockdown of Hsp90α or Hsp90β with siRNA decreased the proportion of extracellular FN matrix.(A) Hs578T cells were transfected with a pool of siRNA against either Hsp90α (Hsp90α K/D) or Hsp90β (Hsp90β K/D). Scrambled non-targeting (NT) siRNA served as a negative control. After 48 hours, levels of Hsp90α and Hsp90β were analysed by immunoblotting with mouse anti human Hsp90α or Hsp90β primary antibodies respectively. Transfected cells were harvested and DOC-soluble and DOC-insoluble fractions separated. Levels of DOC-soluble and DOC-insoluble FN were analyzed by immunoblotting with mouse anti human FN primary antibody. Levels of GAPDH or Histone H3 were used to ensure equal sample loading (B) Relative densitometry of expression levels of Hsp90α and Hsp90β of NT and Hsp90 knockdown (K/D) transfected Hs578T cells, determined by Image J 1.43m. (C) Relative densitometry of levels of soluble and insoluble FN of NT and Hsp90 K/D transfected Hs578T cells, determined by Image J. (D) Transfected Hs578T cells were fixed and stained with mouse anti human FN followed by donkey anti mouse DyLight® 488 secondary antibody. Nuclei were stained with Hoechst 33342 (1 μg.ml−1). Images were captured using a Zeiss LSM 510 Meta laser scanning confocal microscope and analyzed using AxiovisionLE 4.7.1, Zeiss, Germany. Confocal microscopy images for each treatment were captured in triplicate and values in white represent the mean grey values (± standard deviation) for each treatment. Scale bars are equivalent to 20 μm. The data shown are from triplicate experiments. (E) Exogenous Hsp90β partially recovered the FN phenotype in Hsp90β knockdown cells. Adherent Hs578T cells were treated with siRNA against Hsp90β and then remained untreated or were treated with Hsp90β (100 ng.ml−1). Cells were fixed and stained for FN as described previously. Scale bars are equivalent to 100 μm. The average mean gray values and standard deviation from 5 different fields of view are indicated on the images in white text. Statistical analysis was performed by comparing siRNA treated cells to cells treated with siRNA and exogenous Hsp90β, using one way ANOVA with Bonferroni post-test. Images shown

Handling & Storage

Handling	Avoid freeze/thaw cycles.
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	HSP86, Heat shock protein 90α
Application	Flow Cytometry, ICC, IHC, IP, WB
Application Notes	Detects a band of ~90kDa by Western blot.
Clone	9D2
Formulation	Liquid. In PBS, pH 7.2, containing 50% glycerol and 0.09% sodium azide.
Host	Rat
Immunogen	Purified Hsp90 isolated from human therapeutic orchiectomy specimens.
Isotype	IgG2a
Purity Detail	Protein G affinity purified.
Recommendation Dilutions/Conditions	Flow Cytometry (1:100)Western Blot (1:1,000, colorimetric)Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.
Source	Purified from rat ascites.
Species Reactivity	Chicken, Human
UniProt ID	P07900

Worry-free Guarantee

This antibody is covered by our Worry-Free Guarantee .

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