abcam

Product datasheet

# Anti-Hsp90 beta antibody [E296] ab32568

KO VALIDATED Recombinant RabMAb

16 References 10 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-Hsp90 beta antibody [E296] |
| **Description** | Rabbit monoclonal [E296] to Hsp90 beta |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** WB, IHC-P, ICC/IF<br>**Unsuitable for:** Flow Cyt |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Synthetic peptide within Human Hsp90 beta aa 1-100 (N terminal). The exact sequence is proprietary. |
| **Positive control** | WB: Saos-2, HL-60, HEK293T, Jurkat, SH-SY5Y, Raji, A431 and HeLa whole cell lysate (**ab150035**); Mouse brain and heart tissue lysates; Rat brain and heart tissue lysates. IHC-P: Stomach and urinary bladder carcinoma tissues. ICC/IF: HepG2 cells. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. |

## Properties

| | |
|---|---|
| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| **Storage buffer** | pH: 7.20<br>Preservative: 0.01% Sodium azide<br>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | E296 |

**Isotype** IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab32568 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **WB** | | 1/100000 - 1/500000. Detects a band of approximately 92 kDa (predicted molecular weight: 83 kDa).<br>**For unpurified, use 1/500.** |
| **IHC-P** | | 1/150. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.<br>See **IHC antigen retrieval protocols**. |
| **ICC/IF** | | 1/100. |

**Application notes** Is unsuitable for Flow Cyt.

## Target

**Function** Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function.

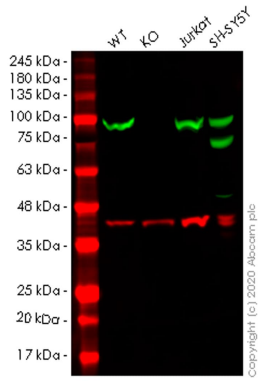
**Sequence similarities** Belongs to the heat shock protein 90 family.

**Domain** The TPR repeat-binding motif mediates interaction with TPR repeat-containing proteins.

**Post-translational modifications** Ubiquitinated in the presence of STUB1-UBE2D1 complex (in vitro).
ISGylated.
S-nitrosylated; negatively regulates the ATPase activity.

**Cellular localization** Cytoplasm. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

## Images




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

**All lanes :** Anti-Hsp90 beta antibody [E296] (ab32568) at 1/1000 dilution

**Lane 1 :** Wild-type HEK293T cell lysate
**Lane 2 :** HSP90AB1 knockout HEK293T cell lysate
**Lane 3 :** Jurkat cell lysate
**Lane 4 :** SH-SY5Y cell lysate

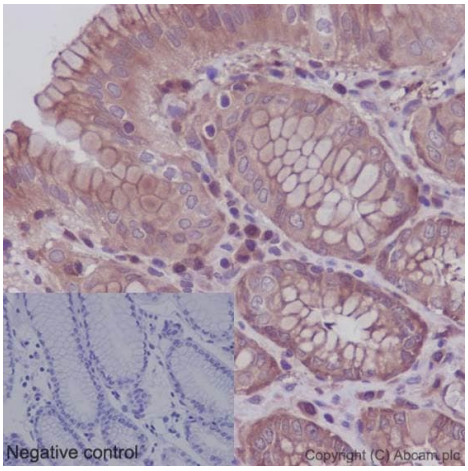
Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

**Predicted band size:** 83 kDa
**Observed band size:** 90 kDa

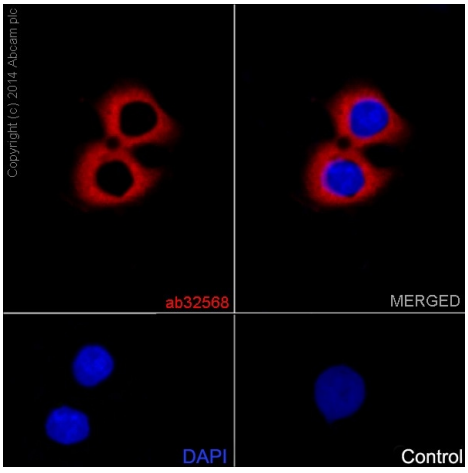
**Lanes 1-4:** Merged signal (red and green). Green - ab32568 observed at 90 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab32568 Anti-Hsp90 beta antibody [E296] was shown to specifically react with Hsp90 beta in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266117** (knockout cell lysate **ab257190**) was used. Wild-type and Hsp90 beta knockout samples were subjected to SDS-PAGE. ab32568 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



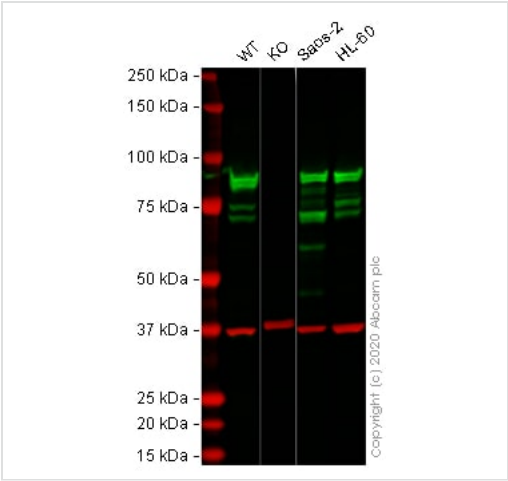

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [E296] (ab32568)

Immunohistochemical staining of paraffin embedded human stomach with purified ab32568 at a working dilution of 1 in 150. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.




Immunocytochemistry/ Immunofluorescence - Anti-Hsp90 beta antibody [E296] (ab32568)

Immunofluorescence staining of HepG2 cells with purified ab32568 at a working dilution of 1 in 100, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 555 goat anti rabbit (**ab150082**), used at a dilution of 1 in 400. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, purified ab32568 was used at a dilution of 1/200 followed by an Alexa Fluor® 488 goat anti-mouse antibody at a dilution of 1/500.




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

**All lanes :** Anti-Hsp90 beta antibody [E296] (ab32568) at 1/200000 dilution

**Lane 1 :** Wild-type HEK-293T cell lysate
**Lane 2 :** HSP90AB1 knockout HEK-293T cell lysate
**Lane 3 :** Saos-2 cell lysate
**Lane 4 :** HL-60 cell lysate

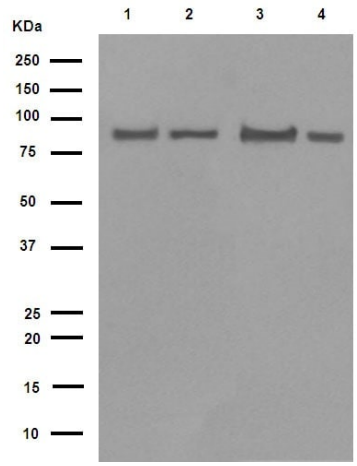
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 83 kDa
**Observed band size:** 85 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab32568 observed at 85 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab32568 was shown to react with Hsp90 beta in wild-type HEK-293T cells in western blot with loss of signal observed in HSP90AB1 knockout cell line **ab266117** (HSP90AB1 knockout cell lysate **ab257190**). Wild-type and HSP90AB1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab32568 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 200000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

**All lanes :** Anti-Hsp90 beta antibody [E296] (ab32568) at 1/100000 dilution (purified)

**Lane 1 :** Mouse brain tissue lysate
**Lane 2 :** Mouse heart tissue lysate
**Lane 3 :** Rat brain tissue lysate
**Lane 4 :** Rat heart tissue lysate

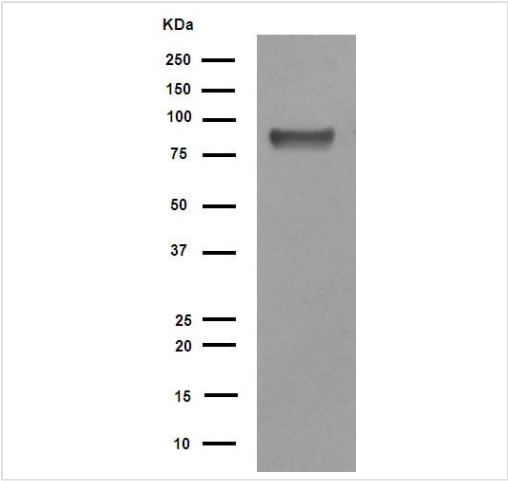
Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 83 kDa
**Observed band size:** 90 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

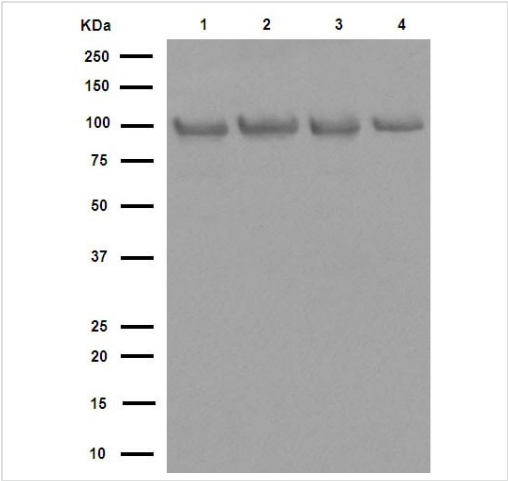
Anti-Hsp90 beta antibody [E296] (ab32568) at 1/100000 dilution (purified) + SH-SH5Y cell lysate at 20 µg

**Secondary**

HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 83 kDa

**Observed band size:** 90 kDa




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

**All lanes :** Anti-Hsp90 beta antibody [E296] (ab32568) at 1/100000 dilution (purified)

**Lane 1 :** HeLa cell lysate

**Lane 2 :** Jurkat cell lysate

**Lane 3 :** Raji cell lysate

**Lane 4 :** A431 cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

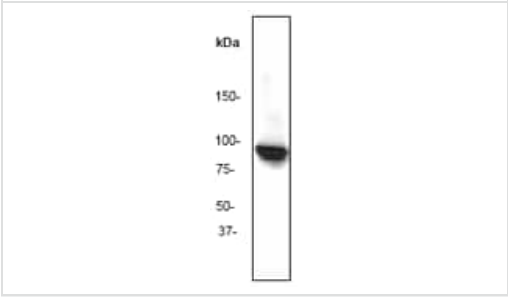
**All lanes :** HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 83 kDa

**Observed band size:** 90 kDa

Blocking buffer: 5% NFDM/TBST

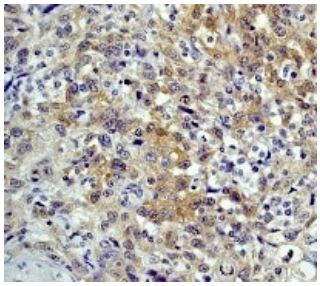
Dilution buffer: 5% NFDM/TBST




Western blot - Anti-Hsp90 beta antibody [E296] (ab32568)

Anti-Hsp90 beta antibody [E296] (ab32568) at 1/500 dilution (unpurified) + Hela cell lysate

**Predicted band size:** 83 kDa
**Observed band size:** 92 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [E296] (ab32568)

Immunohistochemical analysis of paraffin-embedded human urinary bladder carcinoma using unpurified ab32568 at 1/50 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.


Anti-Hsp90 beta antibody [E296] (ab32568)



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