abcam

Product datasheet

Anti-Hsp90 beta antibody [H90-10] ab53497

敲除验证

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概述

产品名称	Anti-Hsp90 beta**抗体**[H90-10]
描述	**小鼠单克隆抗体**[H90-10] to Hsp90 beta
宿主	Mouse
特异性	Detects 90kDa. Detects HSP90 beta in all reactive species except in Chicken, where it detects both alpha and beta isoforms.
经测试应用	**适用于:** Flow Cyt, IHC-P, ICC/IF, WB
种属反应性	**与反应:** Mouse, Human
免疫原	Recombinant full length protein corresponding to Human Hsp90 beta. Database link: **P08238**
阳性对照	WB: HEK-293T, Saos-2 and HL-60 cell lysates; Recombinant human Hsp90 beta protein. IHC-P: Human placenta, backskin and colon carcinoma tissue; mouse colon tissue. ICC/IF: HeLa cells. Flow Cyt: HeLa cells.
常规说明	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.2 Preservative: 0.09% Sodium azide Constituents: 50% Glycerol (glycerin, glycerine), PBS
纯度	Protein G purified
克隆	单克隆

克隆编号	H90-10
同种型	IgG2a

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab53497于以下的经测试应用**

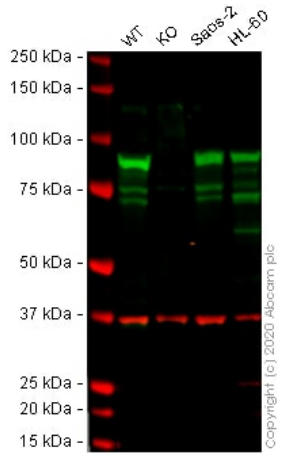
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt		Use 0.5µg for 10^6 cells. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆ (1)	1/100.
ICC/IF		Use at an assay dependent concentration.
WB	★★★★★ (2)	1/2500. Predicted molecular weight: 83 kDa.

靶标

功能	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.
序列相似性	Belongs to the heat shock protein 90 family.
结构域	The TPR repeat-binding motif mediates interaction with TPR repeat-containing proteins.
翻译后修饰	Ubiquitinated in the presence of STUB1-UBE2D1 complex (in vitro). ISGylated. S-nitrosylated; negatively regulates the ATPase activity.
细胞定位	Cytoplasm. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

图片



Western blot - Anti-Hsp90 beta antibody [H90-10] (ab53497)

All lanes : Anti-Hsp90 beta antibody [H90-10] (ab53497) at 1/5000 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

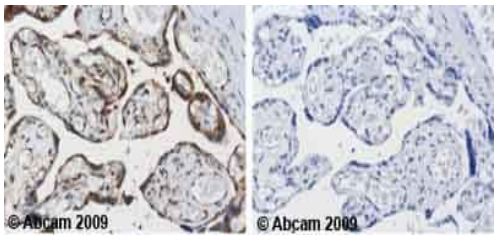
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

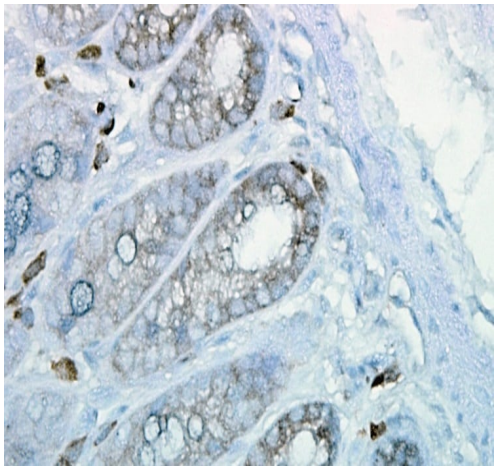
Lanes 1 - 4: Merged signal (red and green). Green - ab53497 observed at 85 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

ab53497 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 ab53497 and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



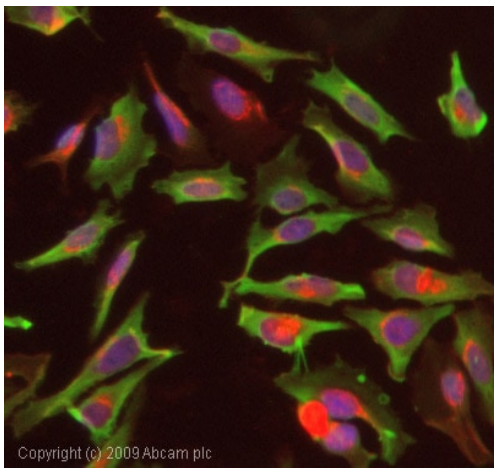
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

Ab53497 staining Human normal placenta. Staining is localized to cytoplasmic compartment.
Left panel: with primary antibody at 2 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffers EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



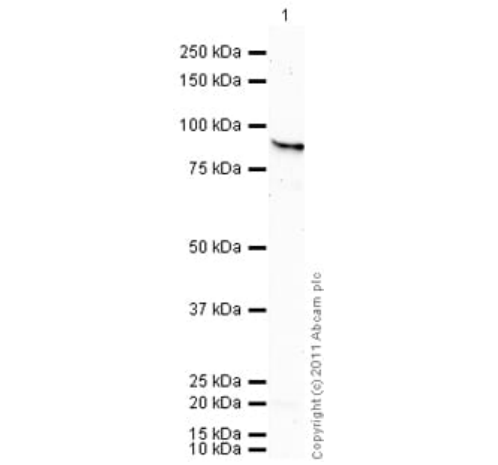
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

Immunohistochemistry analysis of formalin-fixed paraffin-embedded inflamed mouse colon using ab53497 at 1/10000. Primary antibody was incubated for 12 hours at 4°C. Secondary Antibody was a Biotin Goat Anti-Mouse at 1/2000 dilution incubated for 1 hour at room temperature. Counterstain was Mayer Hematoxylin (purple/blue) nuclear stain at 200 µl for 2 minutes at room temperature. Magnification: 40x.



Immunocytochemistry/ Immunofluorescence - Anti-Hsp90 beta antibody [H90-10] (ab53497)

ICC/IF image of ab53497 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab53497, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-Hsp90 beta antibody [H90-10] (ab53497)

Anti-Hsp90 beta antibody [H90-10] (ab53497) at 1 µg/ml + Recombinant human Hsp90 beta protein (Active) (**ab80033**) at 0.1 µg

Secondary

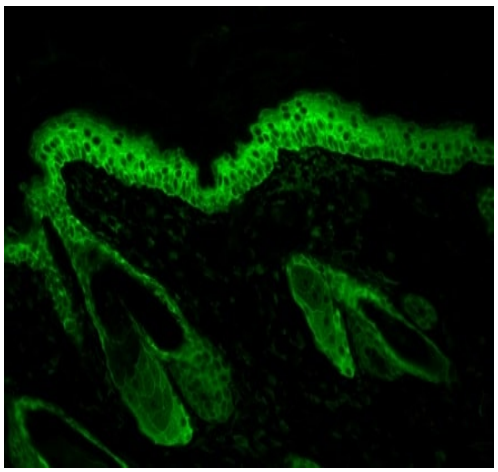
Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

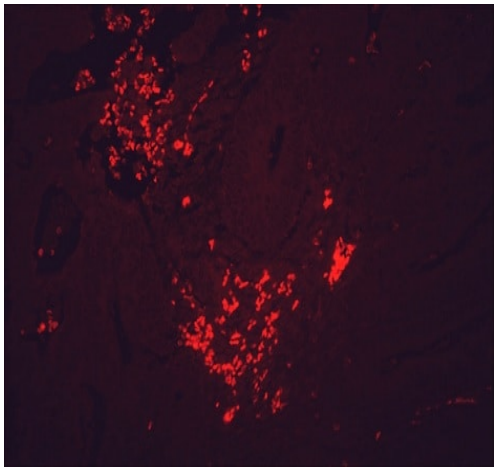
Predicted band size: 83 kDa

Exposure time: 8 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

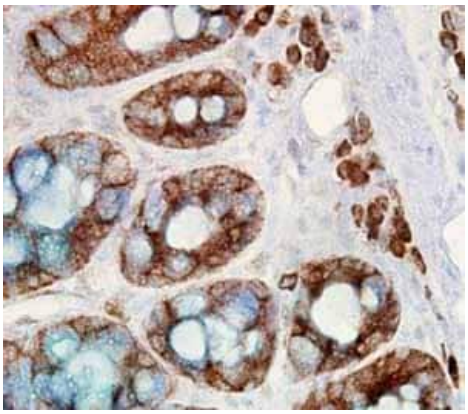
Paraffin-embedded mouse backskin (epidermis) tissue fixed with Bouin's fixative, stained for Hsp90 beta using ab53497 at 1/100 dilution in immunohistochemical analysis. Primary antibody was incubated for 1 hour at room temperature. Secondary antibody was a FITC-conjugated goat anti-mouse (green) at 1/50 dilution ncubated for 1 hour at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

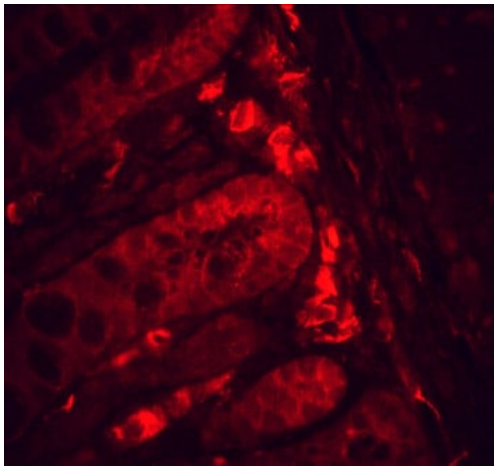
Formalin-fixed, paraffin-embedded human colon carcinoma tissue stained for Hsp90 beta using ab53497 at 1/10000 in immunohistochemical analysis. Primary antibody was incubated for 12 hours at 4°C. Secondary antibody was an Alexa Fluor® 555 goat anti-mouse (red) at 1/5000 dilution incubated for 1 hour at room temperature.

40x magnification.



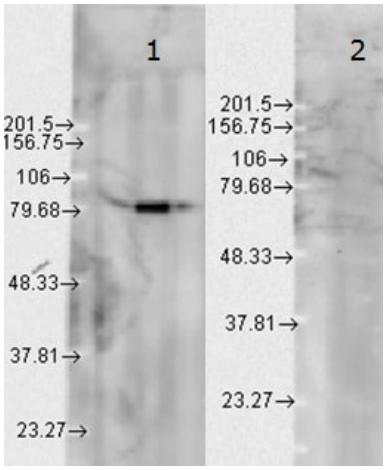
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

ab53497 at 100,000 dilution staining Hsp90 in human colon cancer tissue section by immunohistochemistry (Formalin/ PFA fixed paraffin-embedded tissue sections). A antibody amplifier™ system was used for staining. A HRP-conjugated secondary antibody was used at 1/10 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Hsp90 beta antibody [H90-10] (ab53497)

ab53497 at 100,000 dilution staining Hsp90 beta in mouse colon tissue section by immunohistochemistry (Formalin/ PFA fixed paraffin-embedded tissue sections). A antibody amplifier™ system was used for staining. An Alexa Fluor® 568 conjugated secondary antibody was used at 1/10 dilution.



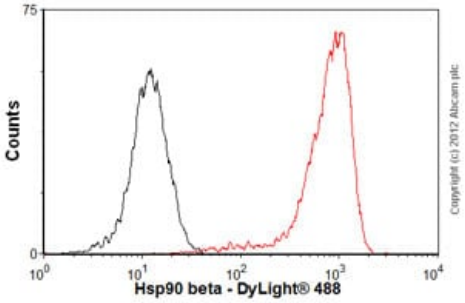
Western blot - Anti-Hsp90 beta antibody [H90-10] (ab53497)

All lanes : Anti-Hsp90 beta antibody [H90-10] (ab53497)

Lane 1 : Hsp90 beta protein
Lane 2 : Hsp90 alpha protein

Lysates/proteins at 2 µg per lane.

Predicted band size: 83 kDa



Flow Cytometry - Anti-Hsp90 beta antibody [H90-10] (ab53497)

Overlay histogram showing HeLa cells stained with ab53497 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab53497, 0.5µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

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