abcam

Product datasheet

# Human HSP90AB1 (Hsp90 beta) knockout HEK-293T cell line ab266117

8 图像

## 概述

| | |
|---|---|
| **产品名称** | 人HSP90AB1 (Hsp90 beta) knockout HEK-293T cell line |
| **Parental Cell Line** | HEK293T |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 3 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing, Western Blot (WB) |
| **经测试应用** | **适用于:** WB |
| **Biosafety level** | 2 |
| **常规说明** | **Recommended control:** Human wild-type HEK293T cell line (**ab255449**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.<br>3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/$cm^2$. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended. |

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

## 性能

| | |
|---|---|
| **Number of cells** | 1 x $10^6$ cells/vial, 1 mL |
| **Adherent /Suspension** | Adherent |
| **Tissue** | Kidney |
| **Cell type** | epithelial |
| **STR Analysis** | Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12 |
| **Antibiotic resistance** | Puromycin 1.00µg/ml |
| **Mycoplasma free** | Yes |
| **存放说明** | Shipped on Dry Ice. Store in liquid nitrogen. |
| **存储溶液** | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether |

## 靶标

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| **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).<br>ISGylated.<br>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. |

## 应用

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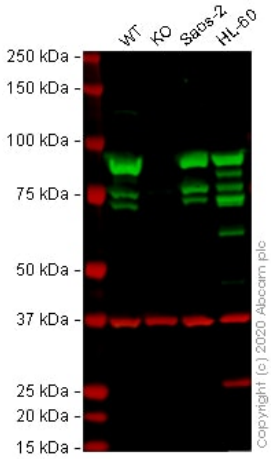
|---|---|
| **The Abpromise guarantee** | **Abpromise™承诺保证使用**ab266117**于以下的**经测试应用 |

“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 |
|---|---|---|
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 83 kDa. |

## 图片




Western blot - Human HSP90AB1 (Hsp90 beta) knockout HEK293T cell line (ab266117)

**All lanes :** Anti-Hsp90 antibody [H90-10] (**ab58950**) at 0.5 µg/ml

**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

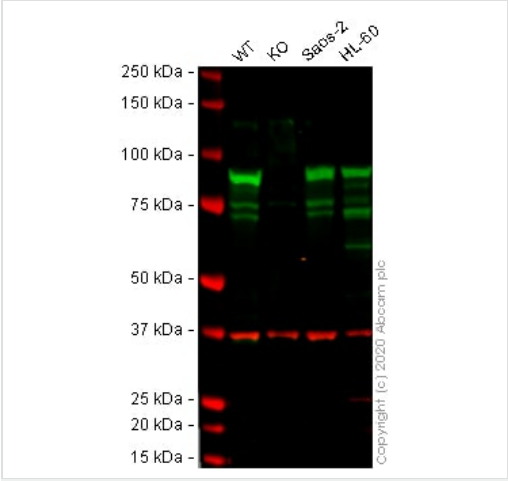
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1 - 4:** Merged signal (red and green). Green - **ab58950** observed at 85 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

**ab58950** was shown to react with Hsp90 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 **ab58950** and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at 0.5 µg/ml 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.




Western blot - Human HSP90AB1 (Hsp90 beta) knockout HEK293T cell line (ab266117)

**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

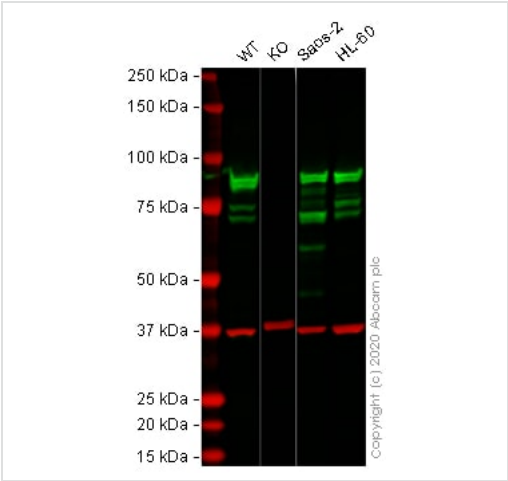
Lysates/proteins at 20 µg per lane.

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.




Western blot - Human HSP90AB1 (Hsp90 beta) knockout HEK293T cell line (ab266117)

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

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

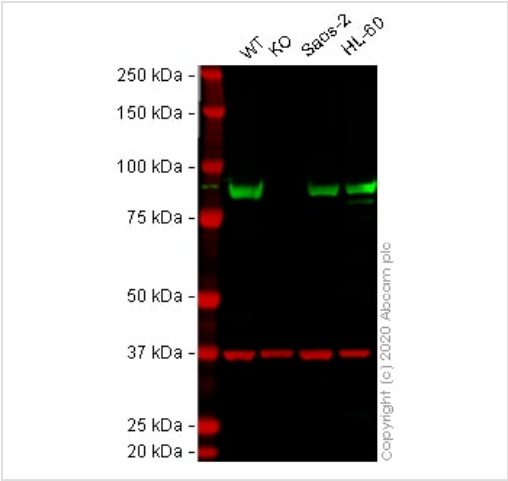
Lysates/proteins at 20 µg per lane.

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 - Human HSP90AB1 (Hsp90 beta) knockout HEK293T cell line (ab266117)

**All lanes :** Anti-Hsp90 beta antibody [EPR16621] (**ab203085**) 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

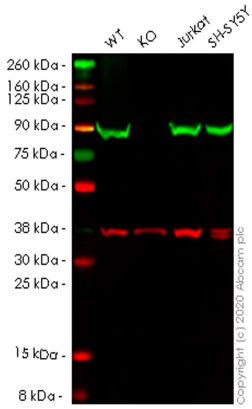
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

**Lanes 1 - 4:** Merged signal (red and green). Green - **ab203085** observed at 85 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

**ab203085** 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 **ab203085** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 5000 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 - Human HSP90AB1 knockout HEK293T cell line (ab266117)

**All lanes :** Anti-Hsp90 beta antibody [EPR16621] (**ab203085**) 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

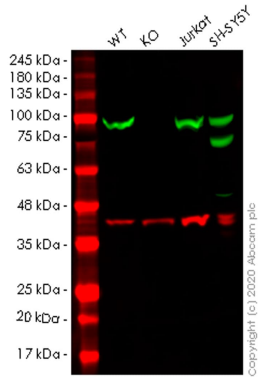
Lysates/proteins at 20 µg per lane.

**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 - **ab203085** observed at 90 kDa. Red - loading control **ab8245** observed at 36 kDa.

**ab203085** Anti-Hsp90 beta antibody [EPR16621] 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. **ab203085** 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.




Western blot - Human HSP90AB1 knockout HEK293T cell line (ab266117)

**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

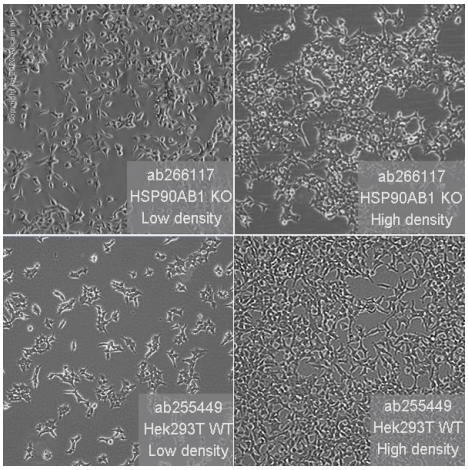
**Predicted band size:** 83 kDa
**Observed band size:** 90 kDa

**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.


Homozygous: 5 bp deletion in exon3

Sanger Sequencing - Human HSP90AB1 knockout HEK293T cell line (ab266117)




Human HSP90AB1 (Hsp90 beta) knockout HEK293T cell line (ab266117)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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