abcam

Product datasheet

Human USP7 (HAUSP) knockout HEK-293T cell line ab266535

画像数 5

製品の概要

製品名	Human USP7 (HAUSP) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods.

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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

製品の特性

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
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Hydrolase that deubiquitinates target proteins such as FOXO4, p53/TP53, MDM2, ERCC6, DNMT1, UHRF1, PTEN and DAXX (PubMed:11923872, PubMed:15053880, PubMed:16964248, PubMed:18716620, PubMed:25283148). Together with DAXX, prevents MDM2 self-ubiquitination and enhances the E3 ligase activity of MDM2 towards p53/TP53, thereby promoting p53/TP53 ubiquitination and proteasomal degradation. Deubiquitinates p53/TP53, preventing degradation of p53/TP53, and enhances p53/TP53-dependent transcription regulation, cell growth repression and apoptosis (PubMed:25283148). Deubiquitinates p53/TP53 and MDM2 and strongly stabilizes p53/TP53 even in the presence of excess MDM2, and also induces p53/TP53-dependent cell growth repression and apoptosis. Deubiquitination of FOXO4 in presence of hydrogen peroxide is not dependent on p53/TP53 and inhibits FOXO4-induced transcriptional activity. In association with DAXX, is involved in the deubiquitination and translocation of PTEN from the nucleus to the cytoplasm, both processes that are counteracted by PML. Involved in cell proliferation during early embryonic development. Involved in transcription-coupled nucleotide excision repair (TC-NER) in response to UV damage: recruited to DNA damage sites following interaction with KIAA1530/UVSSA and promotes deubiquitination of ERCC6, preventing UV-induced degradation of ERCC6. Contributes to the overall stabilization and trans-activation capability of the herpesvirus 1 trans-acting transcriptional protein ICP0/VMW110 during HSV-1 infection. Involved in maintenance of DNA methylation via its interaction with UHRF1 and DNMT1: acts by mediating deubiquitination of UHRF1 and DNMT1, preventing their degradation and promoting DNA methylation by DNMT1 (PubMed:21745816). Exhibits a preference towards 'Lys-48'-linked ubiquitin chains. Increases regulatory T-cells (Treg) suppressive capacity by deubiquitinating and stabilizing the transcription factor FOXP3 which is crucial for Treg cell function (PubMed:23973222).

組織特異性	Widely expressed. Overexpressed in prostate cancer.
配列類似性	Belongs to the peptidase C19 family. Contains 1 MATH domain. Contains 1 USP domain.
ドメイン	The C-terminus plays a role in its oligomerization.
翻訳後修飾	Isoform 1: Phosphorylated. Isoform 1 is phosphorylated at positions Ser-18 and Ser-963. Isoform 2: Not phosphorylated. Isoform 1: Polyneddylated. Isoform 2: Not Polyneddylated. Isoform 1 and isoform 2: Not sumoylated. Isoform 1 and isoform 2: Polyubiquitinated by herpesvirus 1 trans-acting transcriptional protein ICP0/VMW110; leading to its subsequent proteasomal degradation. Isoform 1: Ubiquitinated at Lys-869.
細胞内局在	Nucleus. Cytoplasm. Nucleus, PML body. Present in a minority of ND10 nuclear bodies. Association with ICP0/VMW110 at early times of infection leads to an increased proportion of USP7-containing ND10. Colocalizes with ATXN1 in the nucleus. Colocalized with DAXX in speckled structures. Colocalized with PML and PTEN in promyelocytic leukemia protein (PML) nuclear bodies.

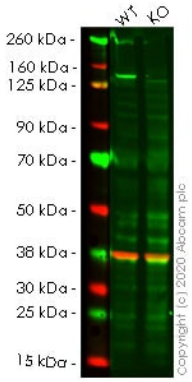
アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab266535の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 128 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

画像



Western blot - Human USP7 (HAUSP) knockout HEK293T cell line (ab266535)

All lanes : Anti-HAUSP / USP7 antibody [EPR4254] (**ab109109**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : TRIM24 knockout HEK293T cell lysate

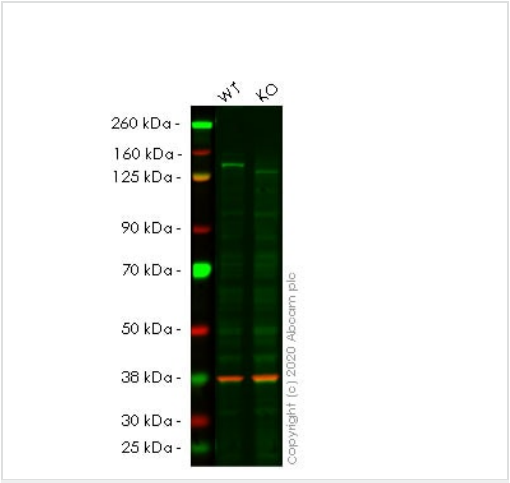
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 128 kDa
Observed band size: 128 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab109109** observed at 128 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109109 was shown to react with HAUSP / USP7 in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266535 (knockout cell lysate **ab257284**) lane below 128kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and USP7 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab109109** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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 USP7 (HAUSP) knockout HEK293T cell line (ab266535)

All lanes : Anti-HAUSP / USP7 antibody [EPR4253] (**ab108931**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : USP7 knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

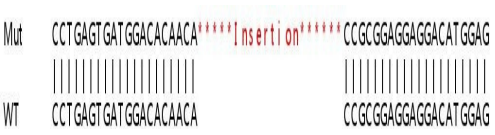
Predicted band size: 128 kDa
Observed band size: 128 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab108931** observed at 128 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab108931 was shown to react with HAUSP / USP7 in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266535 (knockout cell lysate **ab257284**) lane below 128kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and USP7 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab108931** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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.

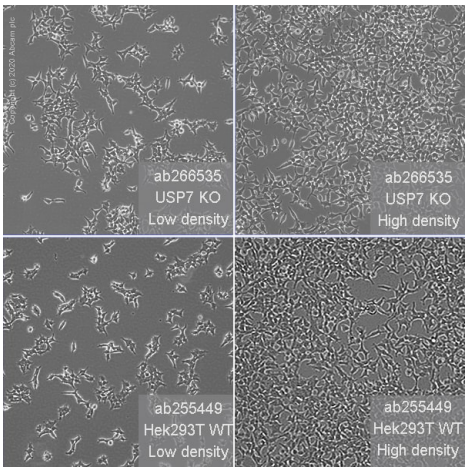
Sanger Sequencing - Human USP7 knockout HEK293T cell line (ab266535)

Allele-1: 1 bp insertion in exon2



Sanger Sequencing - Human USP7 knockout HEK293T cell line (ab266535)

Allele-2: Insertion of the selection cassette in exon 2.



Cell Culture - Human USP7 (HAUSP) knockout HEK293T cell line (ab266535)

Representative images of USP7 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.co.jp/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors