abcam

Product datasheet

Human OTUB1 knockout HEK-293T cell line ab266551

7 Images

Overview

Product name	Human OTUB1 knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 19 bp deletion in exon 1 and Insertion of the selection cassette in exon 1
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, ICC
Biosafety level	2
General notes	**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.
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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Hydrolase that can remove conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation. Regulator of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to antigen rechallenge and no longer respond to their cognate antigen. Acts via its interaction with RNF128/GRAIL, a crucial inductor of CD4 T-cell anergy. Isoform 1 destabilizes RNF128, leading to prevent anergy. In contrast, isoform 2 stabilizes RNF128 and promotes anergy. Surprisingly, it regulates RNF128-mediated ubiquitination, but does not deubiquitinate polyubiquitinated RNF128. Deubiquitinates estrogen receptor alpha (ESR1). Mediates deubiquitination of 'Lys-48'-linked polyubiquitin chains, but not 'Lys-63'-linked polyubiquitin chains. Not able to cleave di-ubiquitin. Also capable of removing NEDD8 from NEDD8 conjugates, but with a nuch lower preference compared to 'Lys-48'-linked ubiquitin.
Tissue specificity	Isoform 1 is ubiquitous. Isoform 2 is expressed only in lymphoid tissues such as tonsils, lymph nodes and spleen, as well as peripheral blood mononuclear cells.
Sequence similarities	Belongs to the peptidase C65 family. Contains 1 OTU domain.
Domain	In addition to ubiquitin-binding at the Cys-91 active site, a proximal ubiquitin-binding site is also present at Cys-23 Occupancy of the active site is needed to enable tight binding to the second site. Distinct binding sites for the ubiquitins may allow to discriminate among different isopeptide linkages (i.e. 'Lys-48'-, 'Lys-63'-linked polyubiquitin) in polyubiquitin substrates and achieve linkage-specific deubiquitination.
Cellular localization	Cytoplasm.

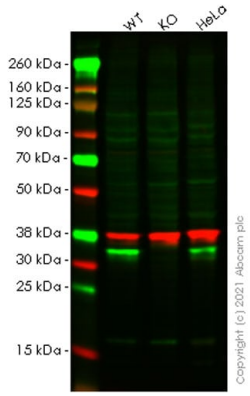
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266551 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		Use at an assay dependent concentration. Predicted molecular weight: 31 kDa.
ICC		Use at an assay dependent concentration.

Images



Western blot - Human OTUB1 knockout HEK-293T cell line (ab266551)

All lanes : Anti-OTUB1 antibody [EPR24917-75] (**ab270959**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : OTUB1 knockout HEK293T (ab266551) whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

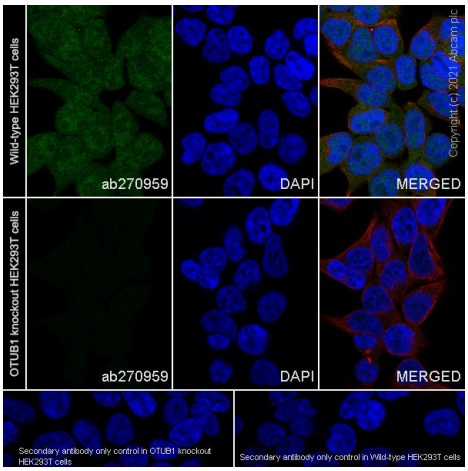
Predicted band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Lanes 1-3: Merged signal (red and green). Green -**ab270959** observed at 31kDa. Red - loading control **ab8245** observed at 36 kDa. **ab270959** Anti-OTUB1 antibody [EPR24917-75] was shown to specifically react with OTUB1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266551 (knockout cell lysate **ab257569**) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE.

ab270959 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat

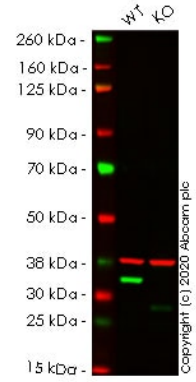
anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry - Human OTUB1 knockout HEK-293T cell line (ab266551)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized OTUB1 KO HEK293T (ab266551) cells labelling OTUB1 with **ab270959** at 1/250 (2.204 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing no staining in OTUB1 KO HEK293T cell line and nuclear and cytoplasmic staining in Parental HEK293T. is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Western blot - Human OTUB1 knockout HEK293T cell line (ab266551)

All lanes : Anti-OTUB1 antibody [EPR13028(B)] (**ab175200**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : OTUB1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

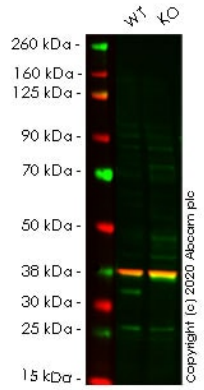
Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 31 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab175200** observed at 31 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab175200 Anti-OTUB1 antibody [EPR13028(B)] was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266551 (knockout cell lysate **ab257569**) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. **ab175200** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated

overnight at 4°CC 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 OTUB1 knockout HEK293T cell line (ab266551)

All lanes : Anti-OTUB1 antibody (**ab101471**) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : OTUB1 knockout HEK-293T cell lysate

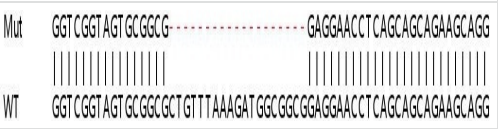
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 31 kDa
Observed band size: 130 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab101471** observed at 130 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab101471 Anti-OTUB1 antibody was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266551 (knockout cell lysate **ab257569**) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. **ab101471** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 ug/ml 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.

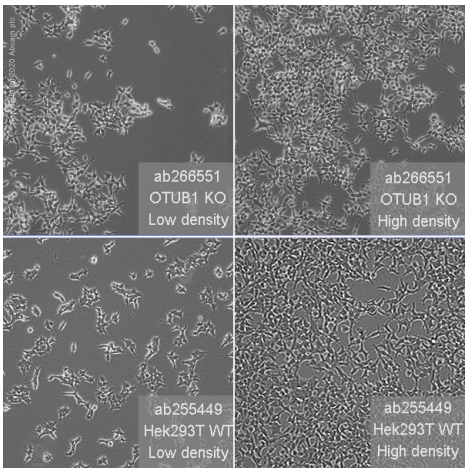


Sanger Sequencing - Human OTUB1 knockout HEK293T cell line (ab266551)

Allele-1: 19 bp deletion in exon 1

Sanger Sequencing - Human OTUB1 knockout HEK293T cell line (ab266551)

Allele-2: Insertion of the selection cassette in exon 1.



Cell Culture - Human OTUB1 knockout HEK293T cell line (ab266551)

Representative images of OTUB1 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 a EVOS XL Core microscope.

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