abcam

Product datasheet

Human UCHL1 (PGP9.5) knockout HEK-293T cell line ab255443

2 Images

Overview

Product name	Human UCHL1 (PGP9.5) knockout HEK-293T cell line
Description	UCHL1 KO HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 45 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (ab255593). 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: 11, 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 15, 20 TH01: 7, 9.3 TPOX: 11, 12 CSF1PO: 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	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. This enzyme is a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. Also binds to free monoubiquitin and may prevent its degradation in lysosomes. The homodimer may have ATP-independent ubiquitin ligase activity.
Tissue specificity	Found in neuronal cell bodies and processes throughout the neocortex (at protein level). Expressed in neurons and cells of the diffuse neuroendocrine system and their tumors. Weakly expressed in ovary. Down-regulated in brains from Parkinson disease and Alzheimer disease patients.
Involvement in disease	Parkinson disease 5 Neurodegeneration with optic atrophy, childhood-onset
Sequence similarities	Belongs to the peptidase C12 family.
Post-translational modifications	O-glycosylated.
Cellular localization	Cytoplasm. Endoplasmic reticulum membrane. About 30% of total UCHL1 is associated with membranes in brain.

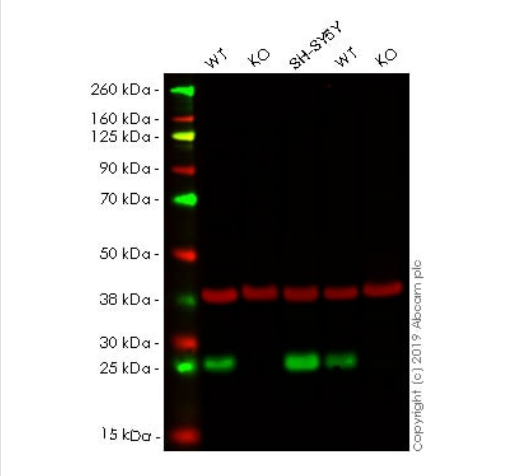
Applications

The Abpromise guarantee	Our Abpromise guarantee covers the use of ab255443 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: 24 kDa.

Images



Western blot - Human UCHL1 knockout HEK293T cell line (ab255443)

All lanes : Anti-PGP9.5 antibody [EPR4118] (ab108986) at 1/1000 dilution

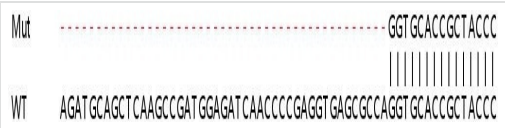
Lane 1 : Wild-type Hap1 cell lysate at 20 µg
Lane 2 : UCHL1 knockout Hap1 cell lysate at 20 µg
Lane 3 : SH-SY5Y cell lysate at 20 µg
Lane 4 : Wild-type HEK-293T cell lysate at 20 µg
Lane 5 : UCHL1 knockout HEK-293T cell lysate

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Predicted band size: 24 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 5: Merged signal (red and green). Green - ab108986 observed at 25 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab108986 was shown to react with PGP9.5 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab255443 (knockout cell lysate ab263773) was used. Wild-type and PGP9.5 knockout samples were subjected to SDS-PAGE. ab108986 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 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human UCHL1 knockout HEK293T cell line (ab255443)

Homozygous: 45 bp deletion in exon 1.

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