abcam

Product datasheet

Human UBE2K (HIP2/LIG) knockout HeLa cell line ab266031

2 Images

Overview

Product name	Human UBE2K (HIP2/LIG) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 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 HeLa cell line (ab255928). 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 -80ºC. Storage at -80ºC may result in loss of viability. 1. Thaw the vial in 37ºC water bath 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 (Click here to view haemocytometer protocol) 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. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37ºC 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 for confluency (80-90% confluence) within 48 hours.
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.
Click here to view the Mammalian cell tissue culture protocol

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Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~90%
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

Target

Function	Accepts ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins. In vitro, in the presence or in the absence of BRCA1-BARD1 E3 ubiquitin-protein ligase complex, catalyzes the synthesis of 'Lys-48'-linked polyubiquitin chains. Does not transfer ubiquitin directly to but elongates monoubiquitinated substrate protein. Mediates the selective degradation of short-lived and abnormal proteins, such as the endoplasmic reticulum-associated degradation (ERAD) of misfolded lumenal proteins. Ubiquitinates huntingtin. May mediate foam cell formation by the suppression of apoptosis of lipid-bearing macrophages through ubiquitination and subsequence degradation of p53/TP53. Proposed to be involved in ubiquitination and proteolytic processing of NF-kappa-B; in vitro supports ubiquitination of NFKB1. In case of infection by cytomegaloviruses may be involved in the US11-dependent degradation of MHC class I heavy chains following their export from the ER to the cytosol. In case of viral infections may be involved in the HPV E7 protein-dependent degradation of RB1.
Tissue specificity	Expressed in all tissues tested, including spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes, T-lymphocytes, monocytes, granulocytes and bone marrow mononuclear cells. Highly expressed in brain, with highest levels found in cortex and striatum and at lower levels in cerebellum and brainstem.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Belongs to the ubiquitin-conjugating enzyme family.

	Contains 1 UBA domain.
Post-translational modifications	Sumoylation at Lys-14 impairs catalytic activity.
Cellular localization	Cytoplasm.

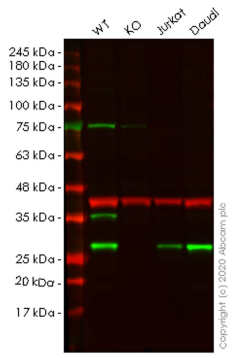
Applications

Our Abpromise guarantee covers the use of **ab266031** 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: 22 kDa.

Images



Western blot - Human UBE2K knockout HeLa cell line (ab266031)

All lanes : Anti-HIP2/LIG antibody [EP1145Y] (ab52930) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : UBE2K knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

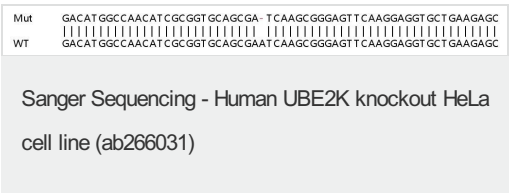
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 22 kDa
Observed band size: 26 kDa
why is the actual band size different from the predicted?

Lanes 1-4: Merged signal (red and green). Green - ab52930 observed at 26 kDa. Red - loading control ab8245 observed at 36 kDa.

ab52930 Anti-HIP2/LIG antibody [EP1145Y] was shown to specifically react with HIP2/LIG in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab266031 (knockout cell lysate ab257778) was used. Wild-type and HIP2/LIG knockout

samples were subjected to SDS-PAGE. ab52930 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 UBE2K knockout HeLa cell line (ab266031)

Homozygous: 1 bp deletion in exon1

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