abcam

Product datasheet

Human UBE2C knockout HeLa cell line ab265032

3 Images

Overview

Product name Human UBE2C knockout HeLa cell line

Parental Cell Line HeLa
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp deletion in exon 2

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level

General notesRecommended control: Human wild-type HeLa cell line (<u>ab255448</u>). 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 2x10⁴ cells/cm². 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% CO₂. 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/cm² is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

Properties

Number of cells 1 x 10⁶ cells/vial, 1 mL

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% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function

Accepts ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins. In vitro catalyzes 'Lys-11'- and 'Lys-48'-linked polyubiquitination. Acts as an essential factor of the anaphase promoting complex/cyclosome (APC/C), a cell cycle-regulated ubiquitin ligase that

controls progression through mitosis. Acts by initiating 'Lys-11'-linked polyubiquitin chains on APC/C substrates, leading to the degradation of APC/C substrates by the proteasome and

promoting mitotic exit.

Pathway Protein modification; protein ubiquitination.

Sequence similaritiesBelongs to the ubiquitin-conjugating enzyme family.

Post-translational Autoubiquitinated by the APC/C complex, leading to its degradation by the proteasome. Its

modifications degradation plays a central role in APC/C regulation, allowing cyclin-A accumulation before S phase entry. APC/C substrates inhibit the autoubiquitination of UBE2C/UBCH10 but not its E2

function, hence APC/C remaining active until its substrates have been destroyed.

Applications

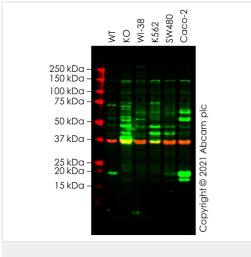
The Abpromise guarantee Our Abpromise guarantee covers the use of ab265032 in the following tested applications.

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: 20 kDa.

Application	Abreviews	Notes

Images



Western blot - Human UBE2C knockout HeLa cell line (ab265032)

All lanes: Anti-UBE2C antibody (ab12290) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: UBE2C knockout HeLa cell lysate

Lane 3: WI-38 cell lysate

Lane 4: K-562 (Human chronic myelogenous leukemia

lymphoblast cell line) whole cell lysate

Lane 5 : SW480 cell lysate

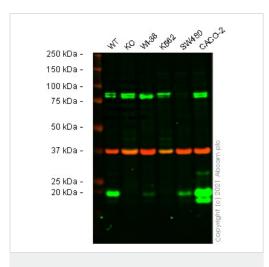
Lane 6 : CACO2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 20 kDa Observed band size: 20 kDa

False colour image of Western blot: Anti-UBE2C antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab12290 was shown to bind specifically to UBE2C. A band was observed at 20 kDa in wild-type HeLa cell lysates with no signal observed at this size in UBE2C knockout cell line ab265032 (knockout cell lysate ab257775). To generate this image, wild-type and UBE2C knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Human UBE2C knockout HeLa cell line (ab265032)

All lanes : Anti-UBE2C antibody [EPR23165-31] (**ab252940**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: UBE2C knockout HeLa cell lysate

Lane 3: WI-38 cell lysate

Lane 4: K-562 (Human chronic myelogenous leukemia

lymphoblast cell line) whole cell lysate

Lane 5 : SW480 cell lysate

Lane 6 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 20 kDa Observed band size: 20 kDa

False colour image of Western blot: Anti-UBE2C antibody [EPR23165-31] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab252940 was shown to bind specifically to UBE2C. A band was observed at 20 kDa in wild-type HeLa cell lysates with no signal observed at this size in UBE2C knockout cell line ab265032 (knockout cell lysate ab257775). To generate this image, wild-type and UBE2C knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

Mut	GGTTCTGGGCACTTAATCACTCACCATGAGG-TCATCAGCTCCTGCTGTAGCCTGAAAAA			
WT	GGTTCTGGGCACTTAATCACTCACCATGAGGGTCATCAGCTCCTGCTGTAGCCTGAAAAA			
Sa	Sanger Sequencing - Human UBE2C knockout HeLa			

cell line (ab265032)

Homozygous: 1 bp deletion in exon 2.

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