abcam

Product datasheet

Human UBE2C knockout HeLa cell line ab265032

画像数 3

製品の概要

製品名	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)
アプリケーション	**適用あり:** WB
Biosafety level	2

特記事項

Recommended control: Human wild-type HeLa cell line (**ab255448**). 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 and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant 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	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
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	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.
パスウェイ	Protein modification; protein ubiquitination.
配列類似性	Belongs to the ubiquitin-conjugating enzyme family.
翻訳後修飾	Autoubiquitinated by the APC/C complex, leading to its degradation by the proteasome. Its 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.

アプリケーション

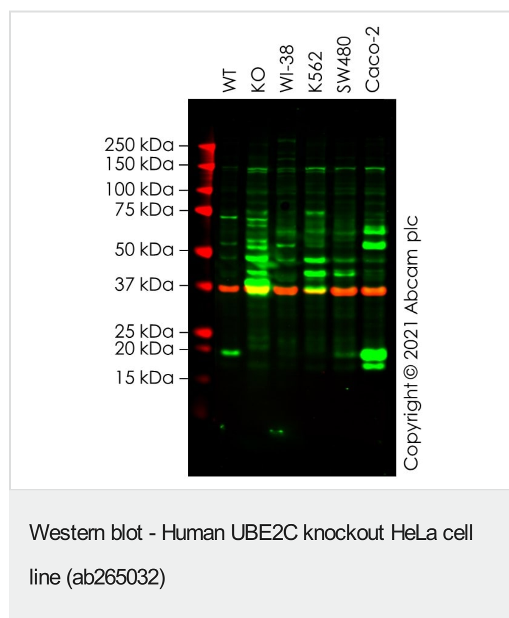
The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab265032の使用に適用されます

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: 20 kDa.

アプリケーション	Abreviews	特記事項

画像



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

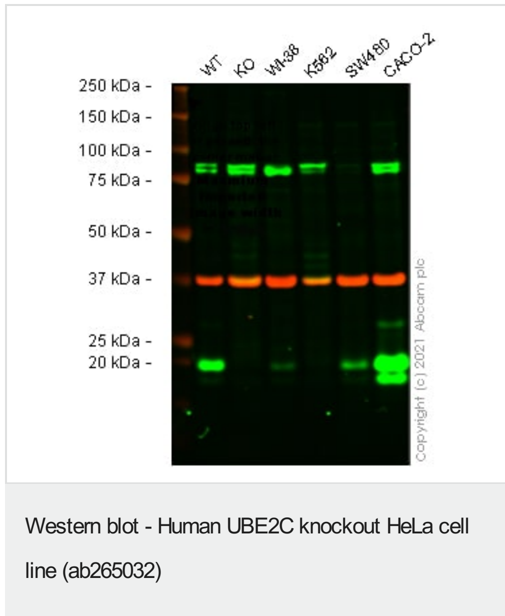
Lysates/proteins at 20 µg per lane.

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 Â°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$^{Â®}$ 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye$^{Â®}$ 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 IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

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Mut  GGTTCTGGGCACTTAATCACTCACCATGAGG-TCATCAGCTCCTGCTGTAGCCTGAAAAA
     ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
WT   GGTTCTGGGCACTTAATCACTCACCATGAGGGTCATCAGCTCCTGCTGTAGCCTGAAAAA
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Sanger Sequencing - Human UBE2C knockout HeLa cell line (ab265032)

Homozygous: 1 bp deletion in exon 2.

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