

Product datasheet

Human CCND1 (Cyclin D1) knockout HeLa cell line ab261760

3 Images

Overview

Product name	Human CCND1 (Cyclin D1) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 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. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods.</p>

A guide seeding density of 2×10^4 cells/cm² 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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We will provide viable cells that proliferate on revival.

Properties

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 WWA: 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	Essential for the control of the cell cycle at the G1/S (start) transition.
Involvement in disease	<p>Note=A chromosomal aberration involving CCND1 may be a cause of B-lymphocytic malignancy, particularly mantle-cell lymphoma (MCL). Translocation t(11;14)(q13;q32) with immunoglobulin gene regions. Activation of CCND1 may be oncogenic by directly altering progression through the cell cycle.</p> <p>Note=A chromosomal aberration involving CCND1 may be a cause of parathyroid adenomas. Translocation t(11;11)(q13;p15) with the parathyroid hormone (PTH) enhancer.</p> <p>Defects in CCND1 are a cause of multiple myeloma (MM) [MIM:254500]. MM is a malignant tumor of plasma cells usually arising in the bone marrow and characterized by diffuse involvement of the skeletal system, hyperglobulinemia, Bence-Jones proteinuria and anemia. Complications of multiple myeloma are bone pain, hypercalcemia, renal failure and spinal cord compression. The aberrant antibodies that are produced lead to impaired humoral immunity and patients have a high prevalence of infection. Amyloidosis may develop in some patients. Multiple myeloma is part of a spectrum of diseases ranging from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukemia. Note=A chromosomal aberration involving CCND1 is found in multiple myeloma. Translocation t(11;14)(q13;q32) with the IgH locus.</p>
Sequence similarities	Belongs to the cyclin family. Cyclin D subfamily.
Post-translational modifications	Phosphorylation at Thr-286 by MAP kinases is required for ubiquitination and degradation following DNA damage. It probably plays an essential role for recognition by the FBXO31 component of SCF (SKP1-cullin-F-box) protein ligase complex.

Ubiquitinated, primarily as 'Lys-48'-linked polyubiquitination. Ubiquitinated by a SCF (SKP1-CUL1-F-box protein) ubiquitin-protein ligase complex containing FBXO4 and CRYAB (By similarity). Following DNA damage it is ubiquitinated by some SCF (SKP1-cullin-F-box) protein ligase complex containing FBXO31. Ubiquitination leads to its degradation and G1 arrest. Deubiquitinated by USP2; leading to stabilize it.

Cellular localization

Nucleus.

Applications

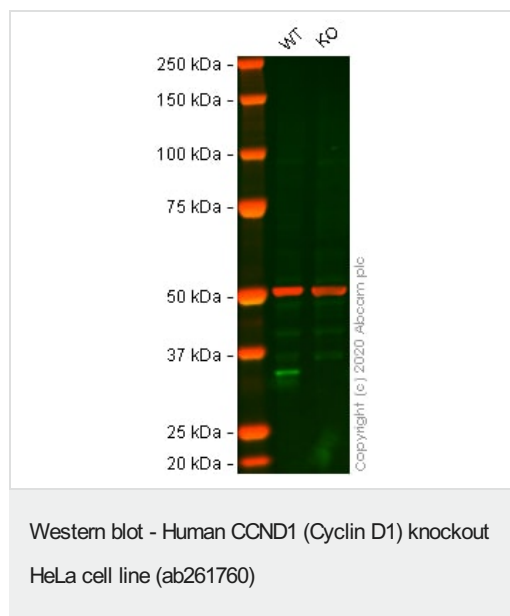
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab261760 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: 34 kDa.

Images



All lanes : Anti-Cyclin D1 antibody [EP272Y] (**ab40754**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CCND1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

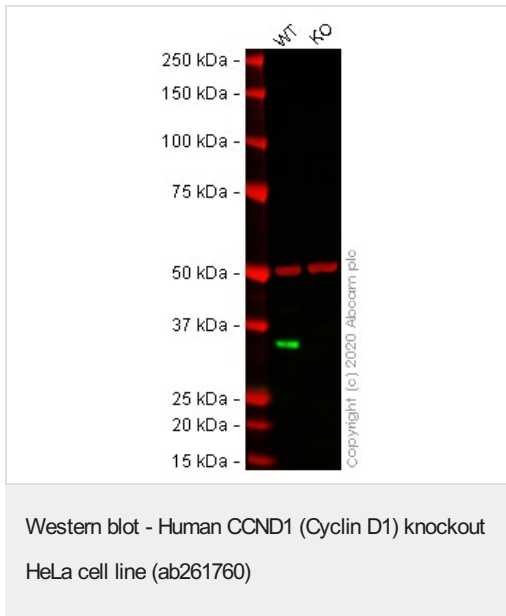
Predicted band size: 34 kDa

Observed band size: 36 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab40754** observed at 36 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab40754 was shown to react with Cyclin D1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261760 (knockout cell lysate **ab256864**) was used. Wild-type HeLa and CCND1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab40754** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**)

overnight at 4°C at a 1 in 1000 dilution and a 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.



All lanes : Anti-Cyclin D1 antibody [SP4] ([ab16663](#)) at 1/200 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CCND1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 34 kDa

Observed band size: 36 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab16663](#) observed at 36 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control ([ab7291](#)) observed at 50 kDa.

[ab16663](#) was shown to react with Cyclin D1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261760 (knockout cell lysate [ab256864](#)) was used. Wild-type HeLa and CCND1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. [ab16663](#) and Anti-alpha Tubulin antibody [DM1A] - Loading Control ([ab7291](#)) overnight at 4°C at a 1 in 200 dilution and a 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.

Mut	CAAATGTGTGCAGAAGGAGG*****Insertion*****TCCTGCCGTCCATGCGGAAG
WT	CAAATGTGTGCAGAAGGAGGTCCTGCCGTCCATGCGGAAG
Sanger Sequencing - Human CCND1 knockout HeLa cell line (ab261760)	

Homozygous: Insertion of the selection cassette in exon 1.

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