abcam

Product datasheet

Human CCND1 (Cyclin D1) knockout HeLa cell lysate ab263808

3 Images

Overview

Product name Human CCND1 (Cyclin D1) knockout HeLa cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HeLa

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 1.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Reconstitution notes To use as WB control, resuspend the lyophilizate in 50 μL of LDS* Sample Buffer to have a final

concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M

DTT.

*Usage of SDS sample buffer is not recommended with these lyophilized lysates.

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease

inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are

prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -

20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

See here for more information on knockout cell lysates.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of

products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH

Authorisation, and any other relevant authorisations, for their intended uses.

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 <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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Properties

Storage instructions

Store at -80°C. Please refer to protocols.

Components	1 kit
ab255457 - Human CCND1 knockout HeLa cell lysate	1 x 100μg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

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

Target

Function

Essential for the control of the cell cycle at the G1/S (start) transition.

Involvement in disease

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.

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.

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 lgH locus.

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.

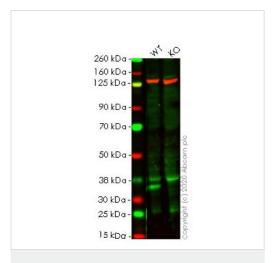
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab263808 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.

Images



Western blot - Human CCND1 (Cyclin D1) knockout HeLa cell lysate (ab263808) Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: CCND1 knockout HeLa cell lysate (20µg)

Lanes 1-2: Merged signal (red and green). Green - <u>ab40754</u> observed at 36 kDa. Red - loading control <u>ab130007</u> observed at 124 kDa.

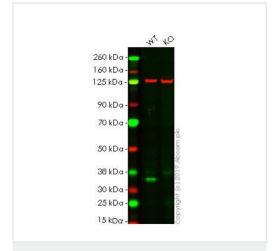
ab40754 Recombinant Anti-Cyclin D1 antibody [EP272Y] was shown to specifically react with CCND1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255348 (knockout cell lysate ab263808) was used. Wild-type and CCND1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab40754 and Anti-Vinculin antibody [VIN-54] 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.



Lane 2: CCND1 knockout HeLa cell lysate (20µg)

Lanes 1-2: Merged signal (red and green). Green - <u>ab16663</u> observed at 36 kDa. Red - loading control <u>ab130007</u> observed at 124 kDa.

ab16663 Recombinant Anti-Cyclin D1 antibody [SP4] was shown to specifically react with CCND1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255348 (knockout cell lysate ab263808) was used. Wild-type and CCND1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1%



Western blot - Human CCND1 (Cyclin D1) knockout HeLa cell lysate (ab263808) TBST with 3% non-fat dried milk. <u>ab16663</u> and Anti-Vinculin antibody [VIN-54] were incubated overnight at 4°C at 1 in 200 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut AAATGTGTGCAGAAGGAGGTCCTGCCGTCC-----GAAGATCGTCGCCACCTGGATGCTG

WT AAATGTGTGCAGAAGGAGGTCCTGCCGTCCATGCGGAAGATCGTCGCCACCTGGATGCTG

Sanger Sequencing - Human CCND1 knockout HeLa

cell lysate (ab263808)

Homozygous: 5 bp deletion in exon 1

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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