abcam

Product datasheet

Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate ab257875

4 Images

Overview

Product name	Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 2 bp deletion in exon1 and 8 bp insertion in exon1.
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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab262242 - Human CCND2 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

Target

Function Regulatory component of the cyclin D2-CDK4 (DC) complex that phosphorylates and inhibits members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G(1)/S transition. Phosphorylation of RB1 allows dissociation of the transcription factor E2F from the RB/E2F complex and the subsequent transcription of E2F target genes which are responsible for the progression through the G(1) phase. Hypophosphorylates RB1 in early G(1) phase. Cyclin D-CDK4 complexes are major integrators of various mitogenenic and antimitogenic signals. Also substrate for SMAD3, phosphorylating SMAD3 in a cell-cycle-dependent manner and repressing its transcriptional activity. Component of the ternary complex, cyclin D2/CDK4/CDKN1B, required for nuclear translocation and activity of the cyclin D-CDK4 complex.

Sequence similarities Belongs to the cyclin family. Cyclin D subfamily.
Contains 1 cyclin N-terminal domain.

Cellular localization Nucleus. Cytoplasm. Membrane. Cyclin D-CDK4 complexes accumulate at the nuclear membrane and are then translocated into the nucleus through interaction with KIP/CIP family members.

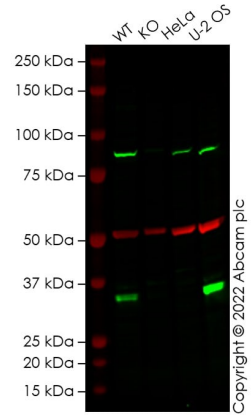
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257875 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: 33 kDa.

Images



Western blot - Anti-Cyclin D2 antibody [EPR19659] (

ab207604

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All lanes : Anti-Cyclin D2 antibody [EPR19659] (**ab207604**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : Human CCND2 (Cyclin D2) knockout HEK-293T cell lysate (ab257875)
Lane 3 : HeLa cell lysate
Lane 4 : U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

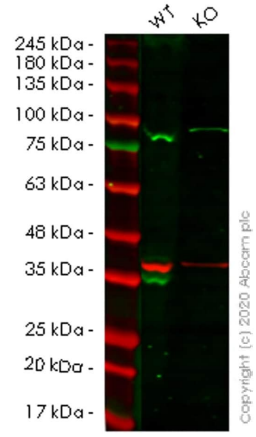
Predicted band size: 33 kDa
Observed band size: 33 kDa

False colour image of Western blot: Anti-Cyclin D2 antibody [EPR19659] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab207604** was shown to bind specifically to Cyclin D2.

A band was observed at 33 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in Ccnd2 knockout cell line **ab267318** (knockout cell lysate ab257875). To generate this image, wild-type and Ccnd2 knockout HEK-293T 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.



Western blot - Human CCND2 knockout HEK293T cell lysate

Lane 1: Wild-type HEK293T cell lysate (20 µg)

Lane 2: CCND2 knockout HEK293T cell lysate (20 µg)

Lanes 1-2: Merged signal (red and green). Green - **ab207604** observed at 36 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab207604 Anti-Cyclin D2 antibody [EPR19659] was shown to specifically react with Cyclin D2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab267318** (knockout cell lysate ab257875) was used. Wild-type and Cyclin D2 knockout samples were subjected to SDS-PAGE. **ab207604** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours 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 CCND2 knockout HEK293T cell lysate (ab257875)

Allele-1: 2 bp deletion in exon1

Sanger Sequencing - Human CCND2 knockout HEK293T cell lysate (ab257875)

Allele-2: 8 bp insertion in exon1

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