abcam

Product datasheet

Human CDK6 knockout HeLa cell lysate ab257088

5 Images

Overview

Product name	Human CDK6 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, 1 bp insertion in exon2 and Insertion of the selection cassette in exon2.
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
ab260923 - Human CDK6 knockout HeLa cell lysate	1 x 100µg
ab255929 - 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 Probably involved in the control of the cell cycle. Interacts with D-type G1 cyclins.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily.
Contains 1 protein kinase domain.

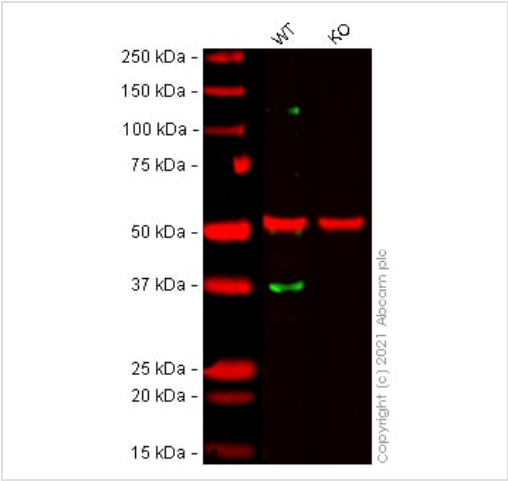
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257088 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

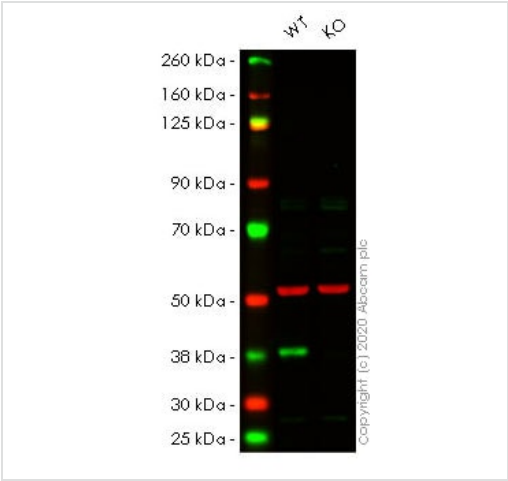
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

Images



Western blot - Human CDK6 knockout HeLa cell lysate (ab257088)

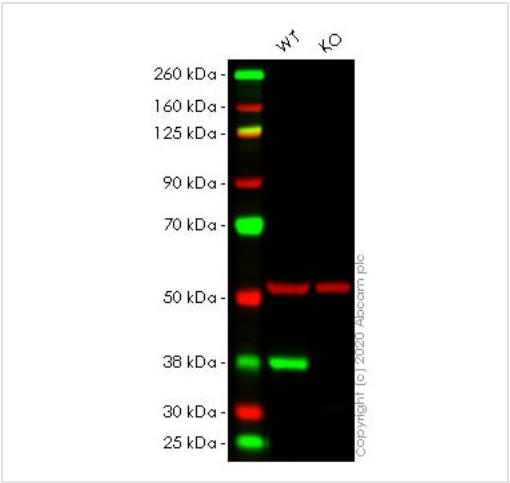
Lane 1: Wild-type HeLa cell lysate 20 ug
Lane 2: CDK6 knockout HeLa cell lysate 20 ug
Lanes 1 - 2: Merged signal (red and green). Green - **ab241554** observed at 38 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.
ab241554 was shown to react with Cdk6 in wild-type HeLa cells in Western blot with loss of signal observed in CDK6 knockout cell line **ab266059** (CDK6 knockout cell lysate ab257088). Wild-type HeLa and CDK6 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab241554** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 ˚C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human CDK6 knockout HeLa cell lysate (ab257088)

Lane 1: Wild-type HeLa cell lysate (20µg)
Lane 2: CDK6 knockout HeLa cell lysate (20µg)
Lanes 1- 2: Merged signal (red and green). Green - **ab54576** observed at 37 kDa. Red - loading control **ab52866** observed at 50 kDa.
ab54576 Anti-Cdk6 antibody was shown to specifically react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab266059** (knockout cell lysate ab257088) was used. Wild-type and Cdk6 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. **ab54576** and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) were incubated overnight at 4˚C at 1 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed

(**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human CDK6 knockout HeLa cell lysate (ab257088)

Lane 1: Wild-type HeLa cell lysate (20µg)
Lane 2: CDK6 knockout HeLa cell lysate (20µg)
Lanes 1- 2: Merged signal (red and green). Green - **ab124821** observed at 37 kDa. Red - loading control **ab7291** observed at 50 kDa.
ab124821 Anti-Cdk6 antibody [EPR4515] was shown to specifically react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab266059** (knockout cell lysate ab257088) was used. Wild-type and Cdk6 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. **ab124821** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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.

Sanger Sequencing - Human CDK6 knockout HeLa cell lysate (ab257088)

Allele-1: 1 bp insertion in exon2

Sanger Sequencing - Human CDK6 knockout HeLa cell lysate (ab257088)

Allele-2: Insertion of the selection cassette in exon2

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