abcam

Product datasheet

Human CLOCK (KAT13D) knockout HeLa cell lysate ab258365

3 Images

Overview

Product name	Human CLOCK (KAT13D) 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 exon11.
Passage number	<20
Knockout validation	Sanger Sequencing
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
ab261218 - Human CLOCK 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 ARNTL/2-CLOCK heterodimers activate E-box element (3'-CACGTG-5') transcription of a number of proteins of the circadian clock. Activates transcription of PER1 and PER2. This transcription is inhibited in a feedback loop by PER and CRY proteins. Has intrinsic histone acetyltransferase activity and this enzymatic function contributes to chromatin-remodeling events implicated in circadian control of gene expression (By similarity). Acetylates primarily histones H3 and H4 (By similarity). Acetylates also a non-histone substrate: ARNTL.

Tissue specificity Expressed in all tissues examined including spleen, thymus, prostate, testis, ovary, small intestine, colon, leukocytes, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Highest levels in testis and skeletal muscle. Low levels in thymus, lung and liver. Expressed in all brain regions with highest levels in cerebellum. Highly expressed in the suprachiasmatic nucleus (SCN).

Sequence similarities Contains 1 basic helix-loop-helix (bHLH) domain.
Contains 1 PAC (PAS-associated C-terminal) domain.
Contains 2 PAS (PER-ARNT-SIM) domains.

Post-translational modifications Phosphorylation is dependent on CLOCK-ARNTL heterodimer formation.

Cellular localization Cytoplasm. Nucleus. Shuffling between the cytoplasm and the nucleus is under circadian regulation and is ARNTL-dependent. Phosphorylated form located in the nucleus.

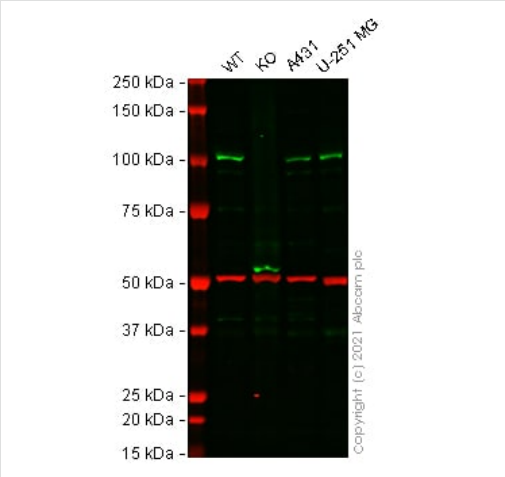
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258365 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 CLOCK (KAT13D) knockout HeLa cell lysate (ab258365)

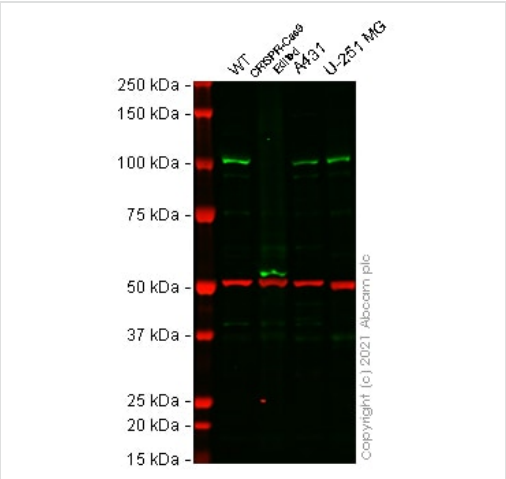
Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: CLOCK knockout HeLa cell lysate 20 µg

Lane 3: A431 cell lysate 20 µg

Lane 4: U-251 MG cell lysate 20 µg

False colour image of Western blot: Anti-KAT13D / CLOCK antibody staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab93804** was shown to bind specifically to KAT13D / CLOCK. A band was observed at 100 kDa in wild-type HeLa cell lysates with no signal observed at this size in CLOCK knockout cell line **ab266054** (knockout cell lysate ab258365). The band observed in the knockout lysate lane below 100 kDa is likely to represent a truncated form of KAT13D / CLOCK. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and CLOCK 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 5 % 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 CLOCK (KAT13D) knockout HeLa cell lysate (ab258365)

Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: CLOCK CRISPR-Cas9 edited HeLa cell lysate 20 µg

Lane 3: A431 cell lysate 20 µg

Lane 4: U-251 MG cell lysate 20 µg

False colour image of Western blot: Anti-KAT13D / CLOCK antibody staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab93804** was shown to bind specifically to KAT13D / CLOCK. A band was observed at 100 kDa in wild-type HeLa cell lysates with no signal observed at this size in CLOCK CRISPR-Cas9 edited cell line **ab266054** (CRISPR-Cas9 edited cell lysate ab258365). The band observed in the CRISPR-Cas9 edited lysate lane below 100 kDa is likely to represent a truncated form of KAT13D / CLOCK. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and CLOCK CRISPR-Cas9 edited HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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.

Sanger Sequencing - Human CLOCK knockout HeLa cell lysate (ab258365)

Homozygous: 1 bp insertion in exon11

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

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