abcam

Product datasheet

Human HK2 (Hexokinase II) knockout HEK-293 cell lysate ab281353

2 Images

Overview

Product name	Human HK2 (Hexokinase II) knockout HEK-293 cell lysate
Product overview	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Parental Cell Line	HEK-293
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 5 bp deletion; Frameshift: 99%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS)
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 **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
ab281393 - Human HK2 knockout HEK-293 cell lysate	1 x 100µg
ab259780 - Human wild-type HEK-293 cell lysate	1 x 100µg

Cell type epithelial

Gender Female

Target

Tissue specificity Predominant hexokinase isozyme expressed in insulin-responsive tissues such as skeletal muscle.

Pathway Carbohydrate metabolism; hexose metabolism.

Sequence similarities Belongs to the hexokinase family.
Contains 2 hexokinase domains.

Domain The N- and C-terminal halves of this hexokinase show extensive sequence similarity to each other. The catalytic activity is associated with the C-terminus while regulatory function is associated with the N-terminus. Each domain can bind a single glucose and Gluc-6-P molecule.

Cellular localization Mitochondrion outer membrane. Its hydrophobic N-terminal sequence may be involved in membrane binding.

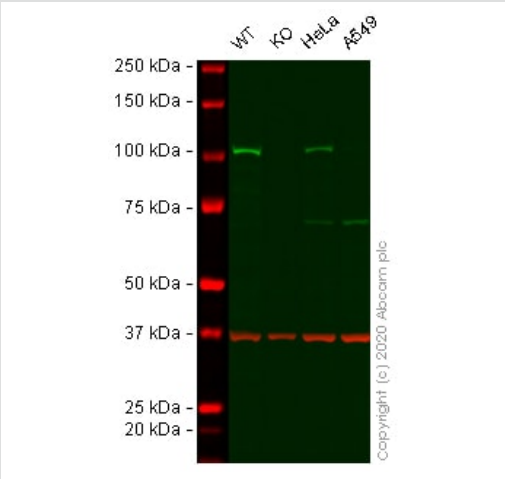
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab281353 in the following tested applications.

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

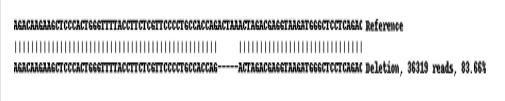
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human HK2 (Hexokinase II) knockout HEK-293 cell lysate (ab281353)

Lane 1: Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate 20 ug
Lane 2: HK2 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate 20 ug
Lane 3: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug
Lane 4: A549 (Human lung carcinoma cell line) whole cell lysate 20 ug
Lanes 1 - 4: Merged signal (red and green). Green - **ab209847** observed at 110 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
ab209847 was shown to bind specifically to HK2. A band was observed at 110 kDa in wild-type cell lysates with no signal at this size in HK2 knockout cell line **ab269485** (knockout cell lysate ab281353). Wild-type and HK2 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab209847** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Next Generation Sequencing - Human HK2 (Hexokinase II) knockout HEK-293 cell lysate (ab281353)

Knockout achieved by CRISPR/Cas9; X = 5 bp deletion; Frameshift: 99%

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