## abcam

### Product datasheet

# Human HK2 (Hexokinase II) knockout HCT116 cell line ab273721

1 References 2 图像

#### 概述

常规说明

产品名称 人HK2 (Hexokinase II) knockout HCT116 cell line

Parental Cell Line HCT116
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 2

Passage number <20

**Knockout validation** Sanger Sequencing, Western Blot (WB)

经测试应用 适用于: WB

Biosafety level

**Recommended control:** Human wild-type HCT116 cell line (<u>ab273730</u>). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.

**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.

Culture medium: McCoY5a + 10% FBS

**Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.

- 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.
- 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.
- 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10<sup>4</sup> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.
- 4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.

#### Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of  $2x10^4$  cells/cm<sup>2</sup> is recommended.

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A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

#### 性能

Number of cells 1 x 10<sup>6</sup> cells/vial, 1 mL

Adherent /Suspension Adherent

**Tissue** Colon

**Cell type** epithelial

**Disease** Carcinoma

**Gender** Male

Antibiotic resistance Puromycin 1.00µg/ml

Mycoplasma free Yes

**存放说明** Shipped on Dry Ice. Store in liquid nitrogen.

存储溶液 Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

组织**特异性** Predominant hexokinase isozyme expressed in insulin-responsive tissues such as skeletal

muscle.

通路 Carbohydrate metabolism; hexose metabolism.

**序列相似性** Belongs to the hexokinase family.

Contains 2 hexokinase domains.

结**构域** 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.

细胞定位 Mitochondrion outer membrane. Its hydrophobic N-terminal sequence may be involved in

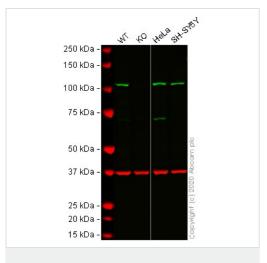
membrane binding.

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab273721于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应 <b>用</b>	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 102 kDa.



Western blot - Human HK2 (Hexokinase II) knockout HCT116 cell line (ab273721)

**All lanes :** Anti-Hexokinase II antibody [EPR20839] (ab209847) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate

Lane 2: HK2 knockout HCT116 cell lysate

Lane 3 : HeLa cell lysate
Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 102 kDa **Observed band size:** 105 kDa

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab209847</u> observed at 105 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab209847 was shown to react with Anti-Hexokinase II in wild-type HCT 116 cells in western blot with loss of signal observed in HK2 knockout cell line ab273721 (HK2 knockout cell lysate ab275250). Wild-type and HK2 knockout HCT 116 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.

WT GCCTCTCTGATGAGACCCTCTT-GGAGATCTCTAAGCGGTTCCG

Mut GCCTCTCTGATGAGACCCTCTTGGGAGATCTCTAAGCGGTTCCG

Sanger Sequencing - Human HK2 (Hexokinase II) knockout HCT116 cell line (ab273721)

Allele-1: 1 bp insertion in exon 2.

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