abcam

Product datasheet

Human PAK1 knockout HeLa cell line ab264889

4 图像

概述

产品名称	人PAK1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 2 bp deletion in exon 2 and 5 bp deletion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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:** DMEM (High Glucose) + 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 $2x10^4$ cells/cm^2. 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_2. 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^2 is recommended.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
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**.

We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
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
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

功能	The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links the Rho-related GTPases to the JNK MAP kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB. Activity is inhibited in cells undergoing apoptosis, potentially due to binding of CDC2L1 and CDC2L2.
序列相似性	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. STE20 subfamily. Contains 1 CRIB domain. Contains 1 protein kinase domain.
翻译后修饰	Autophosphorylated when activated by CDC42/p21 and RAC1.
细胞定位	Cytoplasm. Cell junction > focal adhesion. Recruited to focal adhesions upon activation.

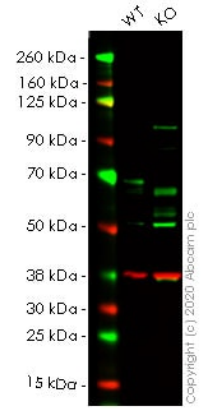
应用

The Abpromise guarantee	**Abpromise™承诺保证使用**ab264889**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。**

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 61 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

图片



Western blot - Human PAK1 knockout HeLa cell line (ab264889)

All lanes : Anti-PAK1 antibody [EPR20048] (**ab223849**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PAK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 65 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab223849** observed at 65 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab223849 was shown to react with PAK1 in wild-type HeLa cells in western blot. The band observed in knockout cell line ab264889 (knockout cell lysate **ab257572**) lane below 65kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and PAK1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab223849** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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 PAK1 knockout HeLa cell line (ab264889)

Allele-1: 5 bp deletion in exon 2.

Sanger Sequencing - Human PAK1 knockout HeLa cell line (ab264889)

Allele-2: 2 bp deletion in exon 2.

Sanger Sequencing - Human PAK1 knockout HeLa cell line (ab264889)

Allele-3: Insertion of the selection cassette in exon 2.

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