

Human PAK1 knockout HeLa cell lysate ab257572

4 图像

概述

产品名称	人PAK1 knockout HeLa cell裂解物
产品概述	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	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
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 μ L 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. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

说明

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.](#)

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经测试应用

适用于: WB

性能

存放说明 Store at -80°C. Please refer to protocols.

组件	1 kit
ab260286 - Human PAK1 knockout HeLa cell lysate	1 x 100µg
ab255552 - 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

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.

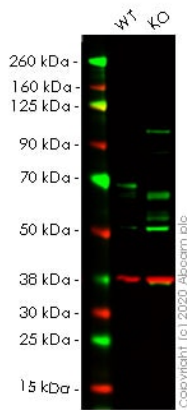
应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab257572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 lysate (ab257572)

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: PAK1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab223849** observed at 65 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab223849 Anti-PAK1 antibody [EPR20048] was shown to specifically react with PAK1 in wild-type HeLa cells in western blot. The band observed in the 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 and PAK1 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. **ab223849** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4 °C at 1 in 1000 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.

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Mut  GCCGGCTCCAATCATAGTGCTGGTA----CATCGGAGGGGCTGGGGTTTGTCTTGAAT
      |||
WT   GCCGGCTCCAATCATAGTGCTGGTATTTCTCATCGGAGGGGCTGGGGTTTGTCTTGAAT
  
```

Sanger Sequencing - Human PAK1 knockout HeLa cell lysate (ab257572)

Allele-1: 5 bp deletion in exon 2

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Mut  GCCGGCTCCAATCATAGTGCTGGTA--TCTCATCGGAGGGGCTGGGGTTTGTCTTGAAT
      |||
WT   GCCGGCTCCAATCATAGTGCTGGTATTTCTCATCGGAGGGGCTGGGGTTTGTCTTGAAT
  
```

Sanger Sequencing - Human PAK1 knockout HeLa cell lysate (ab257572)

Allele-2: 2 bp deletion in exon 2

Mut	ATCATAGTGCTGGTATTTCT*****Insertion*****CATCGGAGGGGCTGGGGGT
WT	ATCATAGTGCTGGTATTTCT CATCGGAGGGGCTGGGGGT

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

Sanger Sequencing - Human PAK1 knockout HeLa
cell lysate (ab257572)

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