abcam

Product datasheet

Human MTOR knockout HEK-293T cell lysate ab263789

画像数 2

製品の概要

製品名 Human MTOR knockout HEK-293T cell lysate

製品の概要

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HEK293T

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 180 bp insertion in exon 2.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

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.

特記事項

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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アプリケーション **適用あり**: WB

1

製品の特性

保存方法

Store at -80°C. Please refer to protocols.

内容	1 kit
ab255515 - Human MTOR knockout HEK293T cell lysate	1 x 100μg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

ターゲット情報

機能

Kinase subunit of both mTORC1 and mTORC2, which regulates cell growth and survival in response to nutrient and hormonal signals. mTORC1 is activated in response to growth factors or amino-acids. Growth factor-stimulated mTORC1 activation involves AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of mTORC1. Amino-acid-signaling to mTORC1 requires its relocalization to the lysosomes mediated by the Ragulator complex and the Rag GTPases. Activated mTORC1 up-regulates protein synthesis by phosphorylating key regulators of mRNA translation and ribosome synthesis. mTORC1 phosphorylates EIF4EBP1 and releases it from inhibiting the elongation initiation factor 4E (eiF4E). mTORC1 phosphorylates and activates S6K1 at 'Thr-421', which then promotes protein synthesis by phosphorylating PDCD4 and targeting it for degradation. Phosphorylates MAF1 leading to attenuation of its RNA polymerase Ill-repressive function. mTORC2 is also activated by growth. factors, but seems to be nutrientinsensitive. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress-fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PRKCA on 'Ser-657'.

組織特異性

Expressed in numerous tissues, with highest levels in testis.

配列類似性

Belongs to the Pl3/Pl4-kinase family.

Contains 1 FAT domain.
Contains 1 FATC domain.
Contains 7 HEAT repeats.
Contains 1 PI3K/PI4K domain.

翻訳後修飾

Autophosphorylated; when part of mTORC1 or mTORC2.

細胞内局在

Endoplasmic reticulum membrane. Golgi apparatus membrane. Mitochondrion outer membrane. Lysosome. Cytoplasm. Nucleus > PML body. Shuttles between cytoplasm and nucleus.

Accumulates in the nucleus in response to hypoxia (By similarity). Targeting to lysosomes

depends on amino acid availability and RRAGA and RRAGB.

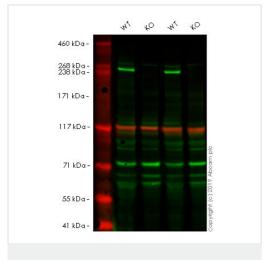
The Abpromise guarantee

Abpromise保証は、次のテスト済みアプリケーションにおけるab263789の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration.

画像



Western blot - Human MTOR knockout HEK293T cell lysate (ab263789)

Lane 1: Wild-type HEK-293 cell lysate (20 µg)

Lane 2: MTOR knockout HEK-293 cell lysate (20 µg)

Lane 3: Wild-type HEK-293T cell lysate (20 µg)

Lane 4: MTOR knockout HEK-293T cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab134903</u> observed at 289 kDa. Red - loading control, <u>ab130007</u> observed at 125 kDa.

ab134903 was shown to react with mTOR in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab255411 (knockout cell lysate ab263789) was used. Wild-type and mTOR knockout samples were subjected to SDS-PAGE. ab134903 and Anti-Vinculin antibody [VIN-54] (ab130007) were incubated overnight at 4°C at 1 in 10000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut	AGCTCCAGCACTATGTCACCATGGA		
WT	AGCTCCAGCACTATGTCACCATGGAACTCCGAGAGGTGCTTCTGGGATCTGGGGCCAGTC		
_			
Sanger Sequencing - Human MTOR knockout			
HE	K293T cell lysate (ab263789)		

Homozygous: 180 bp deletion in exon 2

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