

Human AIFM1 (AIF) knockout HEK-293T cell lysate ab256834

4 图像

概述

产品名称	人AIFM1 (AIF) knockout HEK-293T cell裂解物
产品概述	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 23 bp deletion in exon 2 and 2 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. <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
ab260178 - Human AIFM1 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

靶标

功能 Probable oxidoreductase that has a dual role in controlling cellular life and death; during apoptosis, it is translocated from the mitochondria to the nucleus to function as a proapoptotic factor in a caspase-independent pathway, while in normal mitochondria, it functions as an antiapoptotic factor via its oxidoreductase activity. The soluble form (AIFsol) found in the nucleus induces 'parthanatos' i.e., caspase-independent fragmentation of chromosomal DNA. Interacts with EIF3G, and thereby inhibits the EIF3 machinery and protein synthesis, and activates casapase-7 to amplify apoptosis. Plays a critical role in caspase-independent, pyknotic cell death in hydrogen peroxide-exposed cells. Binds to DNA in a sequence-independent manner.

疾病相关 Defects in AIFM1 are the cause of combined oxidative phosphorylation deficiency type 6 (COXPD6) [MIM:300816]. It is a mitochondrial disease resulting in a neurodegenerative disorder characterized by psychomotor delay, hypotonia, areflexia, muscle weakness and wasting.

序列相似性 Belongs to the FAD-dependent oxidoreductase family.

翻译后修饰 Under normal conditions, a 54-residue N-terminal segment is first proteolytically removed during or just after translocation into the mitochondrial intermembrane space (IMS) by the mitochondrial processing peptidase (MPP) to form the inner-membrane-anchored mature form (AIFmit). During apoptosis, it is further proteolytically processed at amino-acid position 101 leading to the generation of the mature form, which is confined to the mitochondrial IMS in a soluble form (AIFsol). AIFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis in a caspase-independent manner.

细胞定位 Mitochondrion intermembrane space. Mitochondrion inner membrane. Cytoplasm. Nucleus. Cytoplasm > perinuclear region. Proteolytic cleavage during or just after translocation into the mitochondrial intermembrane space (IMS) results in the formation of an inner-membrane-anchored mature form (AIFmit). During apoptosis, further proteolytic processing leads to a mature form, which is confined to the mitochondrial IMS in a soluble form (AIFsol). AIFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis. Colocalizes with EIF3G in the nucleus and perinuclear region.

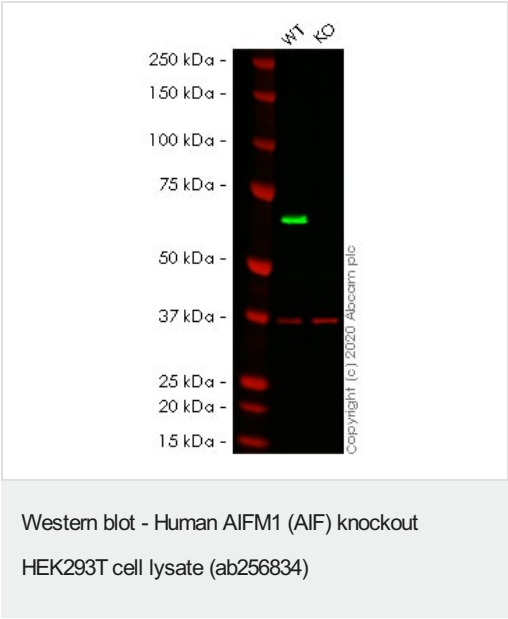
应用

The Abpromise guarantee Abpromise™ 承诺保证使用ab256834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: 67 kDa.

图片

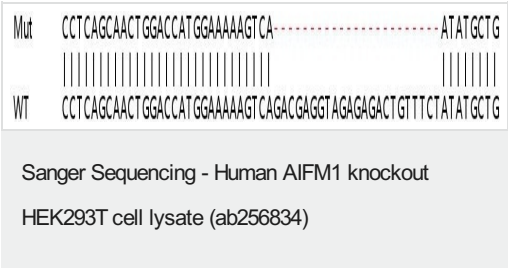


Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: AIFM1 knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - [ab32516](#) observed at 67 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab32516](#) Anti-AIF antibody [E20] - Mitochondrial Marker was shown to specifically react with AIF in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line [ab266347](#) (knockout cell lysate ab256834) was used. Wild-type and AIF 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. [ab32516](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4 °C at 1 in 1000 dilution 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.



Allele-1: 23 bp deletion in exon 2

Mut	CCTCAGCAACTGGACCATGGAAAAAGTCAAGACGAGGTAGAGAGACTGTTTCTATATGCT
WT	CCTCAGCAACTGGACCATGGAAAAAGTCA GACGAGGTAGAGAGACTGTTTCTATATGCT
Sanger Sequencing - Human AIFM1 knockout	
HEK293T cell lysate (ab256834)	

Allele-2: 1 bp insertion in exon 2

Mut	CCTCAGCAACTGGACCATGGAAAAAGTCAAGACGAGGTAGAGAGACTGTTTCTATATGC
WT	CCTCAGCAACTGGACCATGGAAAAAGTCA GACGAGGTAGAGAGACTGTTTCTATATGC
Sanger Sequencing - Human AIFM1 knockout	
HEK293T cell lysate (ab256834)	

Allele-3: 2 bp insertion in exon 2

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