abcam

Product datasheet

Human EIF3K knockout HEK-293T cell lysate ab258857

2 Images

Overview

Product name Human EIF3K knockout HEK-293T cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HEK293T

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 64 bp deletion in exon 1.

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.

Notes

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.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of

products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH

Authorisation, and any other relevant authorisations, for their intended uses.

This product is subject to limited use licenses from The Broad Institute and ERS Genomics

Limited, and is developed with patented technology. For full details of the limited use licenses and

relevant patents please refer to our limited use license and patent pages.

Tested applications Suitable for: WB

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Properties

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

Components	1 kit
ab260668 - Human EIF3K 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

Target

Function Component of the eukaryotic translation initiation factor 3 (eIF-3) complex, which is required for several steps in the initiation of protein synthesis. The eIF-3 complex associates with the 40S ribosome and facilitates the recruitment of eIF-1, eIF-1A, eIF-2:GTP:methionyl-tRNAi and eIF-5 to form the 43S preinitiation complex (43S PIC). The eIF-3 complex stimulates mRNA recruitment to the 43S PIC and scanning of the mRNA for AUG recognition. The eIF-3 complex is also required for disassembly and recycling of post-termination ribosomal complexes and subsequently prevents premature joining of the 40S and 60S ribosomal subunits prior to initiation. Tissue specificity Ubiquitous, with the highest levels of expression in brain, testis and kidney.

Sequence similarities Belongs to the eIF-3 subunit K family.

Cellular localization Nucleus. Cytoplasm.

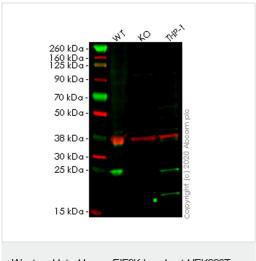
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258857 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 25 kDa.

Images



Western blot - Human EIF3K knockout HEK293T cell lysate (ab258857)

Lane 1:Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate (20 ug)

Lane 2: EIF3K knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate (20 ug)

Lane 3:THP1 (Human monocytic leukemia cell line) whole cell lysate (20 ug)

<u>ab50736</u> was shown to specifically react with eIF3K in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <u>ab266841</u> (knockout cell lysate ab258857) was used. Wild-type and eIF3K knockout samples were subjected to SDS-PAGE. <u>ab50736</u> and Anti-GAPDH antibody[EPR16891] - Loading Control (<u>ab181602</u>) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216777</u>) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216772</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut	TTAGCCTCCAGCCCTGGTTGTGGAAGGCGACAG		
WT	TTAGCCTCCAGCCCTGGTTGTGGAAGGCGACAGAAGTCATGGCGATGTTTGAGCAGATGA		
0-	Commence of the control of the contr		
Sanger Sequencing - Human EIF3K knockout			
HEK293T cell lysate (ab258857)			

Homozygous: 64 bp deletion in exon 1

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