abcam

Product datasheet

Human ADAR (ADAR1) knockout HEK-293T cell lysate ab257131

3 Images

Overview

Product name	Human ADAR (ADAR1) 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, 17 bp deletion in exon2 and 1 bp insertion in exon2.
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, 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**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab260197 - Human ADAR 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 Converts multiple adenosines to inosines and creates I/U mismatched base pairs in double-helical RNA substrates without apparent sequence specificity. Has been found to modify more frequently adenosines in AU-rich regions, probably due to the relative ease of melting A/U base pairs as compared to G/C pairs. Functions to modify viral RNA genomes and may be responsible for hypermutation of certain negative-stranded viruses. Edits the messenger RNAs for glutamate receptor (GLUR) subunits by site-selective adenosine deamination. Produces low-level editing at the GLUR-B Q/R site, but edits efficiently at the R/G site and HOTSPOT1. Binds to short interfering RNAs (siRNA) without editing them and suppresses siRNA-mediated RNA interference. Binds to ILF3/NF90 and up-regulates ILF3-mediated gene expression.

Tissue specificity Ubiquitously expressed, highest levels were found in brain and lung.

Involvement in disease Defects in ADAR are a cause of dyschromatosis symmetrical hereditaria (DSH) [MIM:127400]; also known as reticulate acropigmentation of Dohi. DSH is a pigmentary genodermatosis of autosomal dominant inheritance characterized by a mixture of hyperpigmented and hypopigmented macules distributed on the dorsal parts of the hands and feet.

Sequence similarities Contains 1 A to I editase domain.
Contains 2 DRADA repeats.
Contains 3 DRBM (double-stranded RNA-binding) domains.

Post-translational modifications Sumoylation reduces RNA-editing activity.

Cellular localization Cytoplasm. Nucleus > nucleolus. Isoform 1 is found predominantly in cytoplasm but appears to shuttle between the cytoplasm and nucleus. Isoform 5 is found exclusively in the nucleolus.

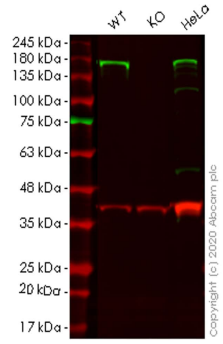
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257131 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: 136 kDa.

Images



Western blot - Human ADAR knockout HEK293T cell lysate (ab257131)

Lane 1: Wild-type HEK293T cell lysate (20 ug)
Lane 2: ADAR knockout HEK293T cell lysate (20 ug)
Lane 3: HeLa cell lysate (20 ug)

ab126745 was shown to specifically react with ADAR1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266846** (knockout cell lysate ab257131) was used. Wild-type and ADAR1 knockout samples were subjected to SDS-PAGE. **ab126745** 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.

Sanger Sequencing - Human ADAR knockout HEK293T cell lysate (ab257131)

Allele-1: 17 bp deletion in exon2

Sanger Sequencing - Human ADAR knockout HEK293T cell lysate (ab257131)

Allele-2: 1 bp insertion in exon2

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