abcam

Product datasheet

Human DDX58 (RIG-I) knockout A549 cell lysate ab257917

4 Images

Overview

Product name	Human DDX58 (RIG-I) knockout A549 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 16 bp deletion in exon2 and 5 bp deletion in exon2 and 8 bp deletion 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 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

Properties

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

Components	1 kit
ab263599 - Human DDX58 knockout A549 cell lysate	1 x 100µg
ab255554 - Human wild-type A549 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

STR Analysis Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 vWA: 14 TH01: 8,9.3 TPOX: 8,11 CSF1PO: 10, 12

Target

Function Involved in innate immune defense against viruses. Upon interaction with intracellular dsRNA produced during viral replication, triggers a transduction cascade involving MAVS/IPS1, which results in the activation of NF-kappa-B, IRF3 and IRF7 and the induction of the expression of antiviral cytokines such as IFN-beta and RANTES (CCL5). Detects dsRNA produced from non-self dsDNA by RNA polymerase III, such as Epstein-Barr virus-encoded RNAs (EBERs). Essential for the production of interferons in response to RNA viruses including paramyxoviruses, influenza viruses, Japanese encephalitis virus and HCV.

Tissue specificity Present in vascular smooth cells (at protein level).

Sequence similarities Belongs to the helicase family.
Contains 2 CARD domains.
Contains 1 helicase ATP-binding domain.
Contains 1 helicase C-terminal domain.

Domain The repressor domain controls homomultimerization and interaction with MAVS.
The helicase domain is responsible for dsRNA recognition.
The 2 CARD domains are responsible for interaction with and signaling through MAVS.
The second CARD domain is the primary site for 'Lys-63'-linked ubiquitination.

Post-translational modifications Isgylated. Conjugated to ubiquitin-like protein ISG15 upon IFN-beta stimulation.
Ubiquitinated. Undergoes 'Lys-63'-linked ubiquitination. Lys-172 is the critical site for TRIM25-mediated ubiquitination, for MAVS binding and to induce anti-viral signal transduction. Lys-154, Lys-164 and Lys-172 are critical sites for RNF135-mediated ubiquitination. Deubiquitinated by CYLD, a protease that selectively cleaves 'Lys-63'-linked ubiquitin chains.

Cellular localization Cytoplasm. Colocalized with TRIM25 at cytoplasmic perinuclear bodies.

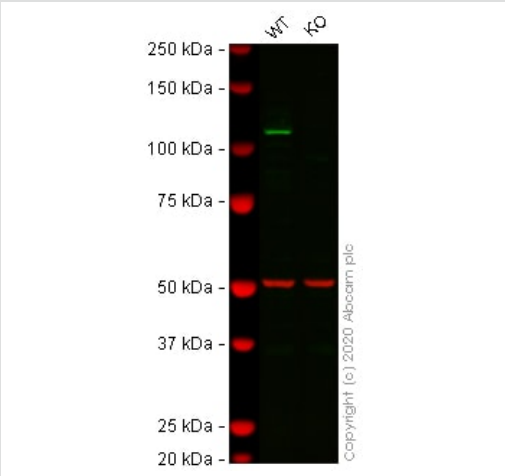
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257917 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: 107 kDa.

Images

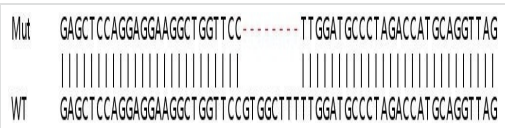


Western blot - Human DDX58 (RIG-I) knockout A549 cell lysate (ab257917)

Lane 1: Wild-type A549 cell lysate 20 ug
Lane 2: DDX58 knockout A549 cell lysate 20 ug
Lanes 1 - 2: Merged signal (red and green). Green - **ab180675** observed at 107 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.
ab180675 was shown to react with DDX58 in wild-type A549 cells in western blot with loss of signal observed in DDX58 knockout cell line **ab267117** (DDX58 knockout cell lysate ab257917). Wild-type and DDX58 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab180675** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively.. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human DDX58 knockout A549 cell lysate (ab257917)

Allele-1: 16 bp deletion in exon2



Sanger Sequencing - Human DDX58 knockout A549 cell lysate (ab257917)

Allele-2: 8 bp deletion in exon2

Sanger Sequencing - Human DDX58 knockout A549 cell lysate (ab257917)

Allele-3: 5 bp deletion in exon2

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