abcam

Product datasheet

Human IRF9 (Interferon regulatory factor 9) knockout A549 cell lysate ab258473

3 Images

Overview

Product name Human IRF9 (Interferon regulatory factor 9) 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, 5 bp deletion in exon2 and 5 bp insertion in exon2.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Reconstitution notesTo 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

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licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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Properties

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

Components	1 kit
ab262426 - Human IRF9 knockout A549 cell lysate	1 x 100µg
ab255554 - Human wild-type A549 cell lysate	1 x 100μg

Cell typeepithelialDiseaseCarcinoma

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 Transcription regulatory factor that mediates signaling by type I IFNs (IFN-alpha and IFN-beta).

Following type I IFN binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are $\,$

activated, leading to tyrosine phosphorylation of STAT1 and STAT2. The phosphorylated STATs dimerize, associate with IRF9/ISGF3G to form a complex termed ISGF3 transcription factor, that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the

transcription of interferon stimulated genes, which drive the cell in an antiviral state.

Sequence similarities Belongs to the IRF family.

Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

Cellular localization Cytoplasm. Nucleus. Translocated into the nucleus upon activation by IFN-alpha/beta.

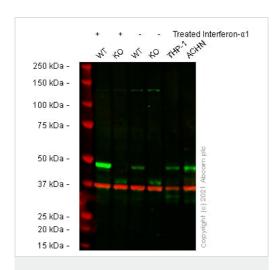
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab258473 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: 44 kDa.

Images



Western blot - Human IRF9 (Interferon regulatory factor 9) knockout A549 cell lysate (ab258473)

Lane 1: wild-type A549 Treated Interferon-alpha1 (hlFN-alpha1) (10 ng/ml, 16 h) cell lysate 20 ug

Lane 2: IRF9 knockout A549 Treated Interferon-alpha1 (hIFN-alpha1) (10 ng/ml, 16 h) cell lysate 20 ug

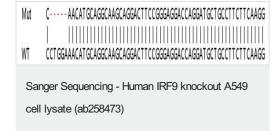
Lane 3: wild-type A549 Control Interferon-alpha1 (hlFN-alpha1) (0 ng/ml, 16 h) cell lysate 20 ug

Lane 4: IRF9 knockout A549 Control Interferon-alpha1 (hIFN-alpha1) (0 ng/ml, 16 h) cell lysate 20 ug

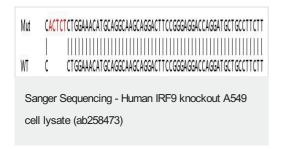
Lane 5: THP-1 cell lysate 20 ug

Lane 6: ACHN cell lysate 20 ug

False colour image of Western blot: Anti-Interferon regulatory factor 9/IRF-9 antibody [EPR24260-55] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab271043 was shown to bind specifically to Interferon regulatory factor 9/IRF-9. A band was observed at 48 kDa in treated wild-type A549 cell lysates with no signal observed at this size in IRF9 knockout cell line ab267119 (knockout cell lysate ab258473). The band observed in the knockout lysate lane below 48 kDa is likely to represent a truncated form of Interferon regulatory factor 9/IRF-9. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and IRF9 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Allele-1: 5 bp deletion in exon2



Allele-2: 5 bp insertion in exon2

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