abcam

Product datasheet

Human IRF3 knockout HeLa cell lysate ab263784

3 Images

Overview

Product name	Human IRF3 knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon5.
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
ab255454 - Human IRF3 knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Target

Function Mediates interferon-stimulated response element (ISRE) promoter activation. Functions as a molecular switch for antiviral activity. DsRNA generated during the course of an viral infection leads to IRF3 phosphorylation on the C-terminal serine/threonine cluster. This induces a conformational change, leading to its dimerization, nuclear localization and association with CREB binding protein (CREBBP) to form dsRNA-activated factor 1 (DRAF1), a complex which activates the transcription of genes under the control of ISRE. The complex binds to the IE and PRDIII regions on the IFN-alpha and IFN-beta promoters respectively. IRF-3 does not have any transcription activation domains.

Tissue specificity Expressed constitutively in a variety of tissues.

Sequence similarities Belongs to the IRF family.
Contains 1 IRF tryptophan pentad repeat DNA-binding domain.

Post-translational modifications Constitutively phosphorylated on many serines residues. C-terminal serine/threonine cluster is phosphorylated in response of induction by IKBKE and TBK1. Ser-385 and Ser-386 may be specifically phosphorylated in response to induction. An alternate model propose that the five serine/threonine residues between 396 and 405 are phosphorylated in response to a viral infection. Phosphorylation, and subsequent activation of IRF3 is inhibited by vaccinia virus protein E3.
Ubiquitinated; ubiquitination involves RBCK1 leading to proteasomal degradation.
Polyubiquitinated; ubiquitination involves TRIM21 leading to proteasomal degradation.
ISGylated by HERC5 resulting in sustained IRF3 activation and in the inhibition of IRF3 ubiquitination by disrupting PIN1 binding. The phosphorylation state of IRF3 does not alter ISGylation.

Cellular localization Cytoplasm. Nucleus. Shuttles between cytoplasmic and nuclear compartments, with export being the prevailing effect. When activated, IRF3 interaction with CREBBP prevents its export to the cytoplasm.

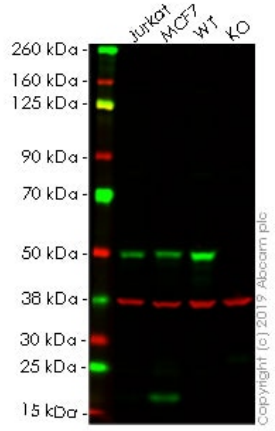
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263784 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.

Images



Western blot - Human IRF3 knockout HeLa cell lysate (ab263784)

Lane 1: Jurkat cell lysate (20 µg)

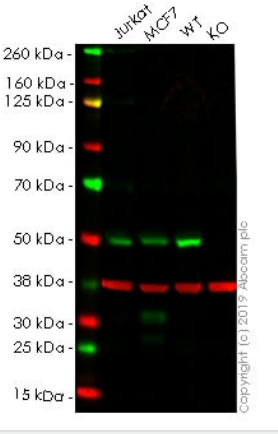
Lane 2: MCF7 cell lysate (20 µg)

Lane 3: Wild-type HeLa cell lysate (20 µg)

Lane 4: IRF3 knockout HeLa cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab68481** observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab68481 was shown to react with IRF3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255345** (knockout cell lysate ab263784) was used. Wild-type and IRF3 knockout samples were subjected to SDS-PAGE. **ab68481** 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.



Western blot - Human IRF3 knockout HeLa cell lysate (ab263784)

Lane 1: Jurkat cell lysate (20 µg)

Lane 2: MCF7 cell lysate (20 µg)

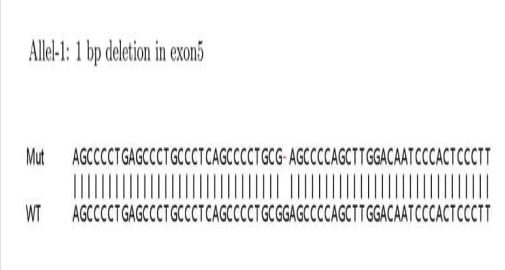
Lane 3: Wild-type HeLa cell lysate (20 µg)

Lane 4: IRF3 knockout HeLa cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - **ab76409** observed at 50 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab76409 was shown to react with IRF3 in wild-type HeLa. Loss of signal was observed when knockout cell line **ab255345** (knockout cell lysate ab263784) was used. Wild-type and IRF3 knockout samples were subjected to SDS-PAGE. **ab76409** 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 IRF3 knockout HeLa cell lysate (ab263784)

Allele-1: 1 bp deletion in exon5

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