

Product datasheet

Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell lysate ab258472

5 Images

Overview

Product name	Human IRF9 (Interferon regulatory factor 9) 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 insertion in exon2 and 2 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. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

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.](#)

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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.

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Tested applications **Suitable for:** WB

Properties

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

Components	1 kit
ab261252 - Human IRF9 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 WWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

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.

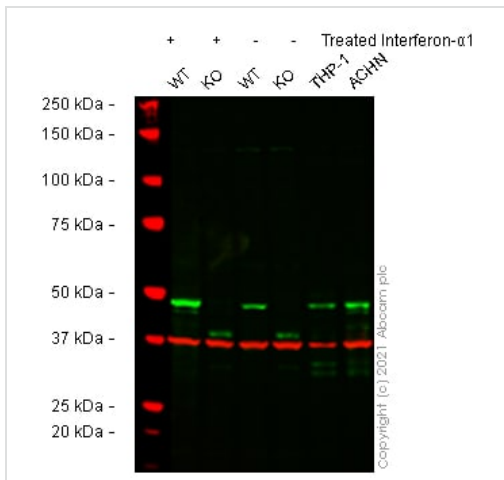
Cellular localization Cytoplasm. Nucleus. Translocated into the nucleus upon activation by IFN-alpha/beta.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258472 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 HeLa cell lysate (ab258472)

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

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

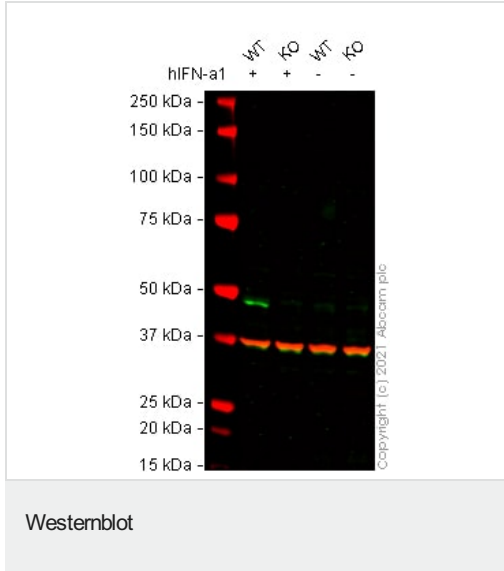
Lane 3: wild-type HeLa Control Interferon-alpha1 (hIFN-alpha1) (0 ng/ml, 16 h) cell lysate 20 ug

Lane 4: IRF9 knockout HeLa vehicle control: 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 HeLa cell lysates with no signal observed at this size in IRF9 knockout cell line [ab266051](#) (knockout cell lysate ab258472). 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 HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 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 IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



Westernblot

Lane 1: Wild-type HeLa treated hIFN-a1 (10 ng/ml, 16 h) cell lysate 20 µg

Lane 2: IRF9 knockout HeLa treated hIFN-a1 (10 ng/ml, 16 h) cell lysate 20 µg

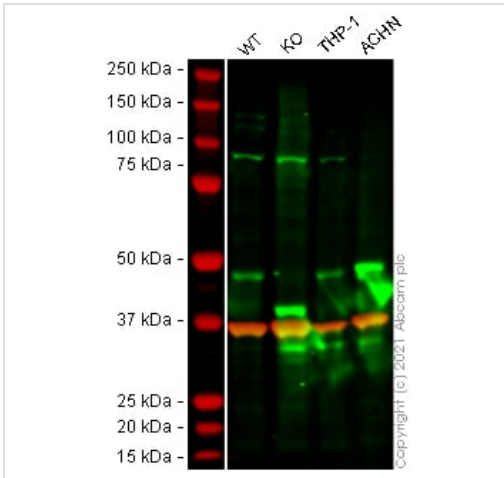
Lane 3: Wild-type HeLa vehicle control hIFN-a1 (0 ng/ml, 16 h) cell lysate 20 µg

Lane 4: IRF9 knockout HeLa vehicle control hIFN-a1 (0 ng/ml, 16 h) cell lysate 20 µg

False colour image of Western blot: Anti-Interferon regulatory factor 9/IRF-9 antibody [14HCLC] staining at 2 µg/ml, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot,

ab277803 was shown to bind specifically to Interferon regulatory factor 9/IRF-9. A band was observed at 48 kDa in treated wild-type HeLa cell lysates with no signal observed at this size in IRF9 knockout cell line **ab266051** (knockout cell lysate ab258472). 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 HeLa 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 IgG H&L (IRDye[®] 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell lysate (ab258472)

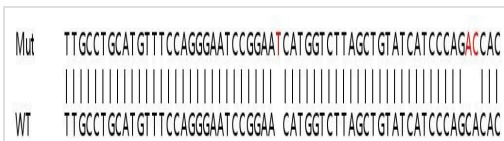
Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: IRF9 knockout HeLa cell lysate 20 µg

Lane 3: THP-1 cell lysate 20 µg

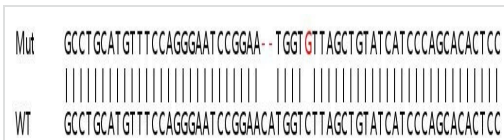
Lane 4: ACHN cell lysate 20 µg

Lanes 1 - 4: Merged signal (red and green). Green - Anti-IRF9 antibody observed at 48 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa. Anti-IRF9 antibody was shown to react with IRF-9 in wild-type HeLa cells in western blot. The bands observed in IRF9 knockout cell line **ab266051** (IRF9 knockout cell lysate ab258472) below 48 kDa may represent truncated forms and cleaved fragments. This has not been investigated further. HeLa wild-type and IRF9 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with anti-IRF9 antibody and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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 h at room temperature before imaging.



Sanger Sequencing - Human IRF9 knockout HeLa cell lysate (ab258472)

Allele-1: 1 bp insertion in exon2



Sanger Sequencing - Human IRF9 knockout HeLa cell lysate (ab258472)

Allele-2: 2 bp deletion in exon2

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