abcam

Product datasheet

# Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell line ab266051

6 Images

## Overview

| | |
|---|---|
| **Product name** | Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell line |
| **Parental Cell Line** | HeLa |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and 2 bp deletion in exon 2 |
| **Passage number** | <20 |
| **Knockout validation** | Immunocytochemistry (ICC), Sanger Sequencing |
| **Tested applications** | **Suitable for:** ICC, WB |
| **Biosafety level** | 2 |
| **General notes** | **Recommended control:** Human wild-type HeLa cell line (**ab255928**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.<br>3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/$cm^2$. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended. |

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
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We will provide viable cells that proliferate on revival.

## Properties

| | |
|---|---|
| **Number of cells** | 1 x $10^6$ cells/vial, 1 mL |
| **Adherent /Suspension** | Adherent |
| **Tissue** | Cervix |
| **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 |
| **Mycoplasma free** | Yes |
| **Storage instructions** | Shipped on Dry Ice. Store in liquid nitrogen. |
| **Storage buffer** | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether |

## Target

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|---|---|
| **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.<br>Contains 1 IRF tryptophan pentad repeat DNA-binding domain. |
| **Cellular localization** | Cytoplasm. Nucleus. Translocated into the nucleus upon activation by IFN-alpha/beta. |

## Applications

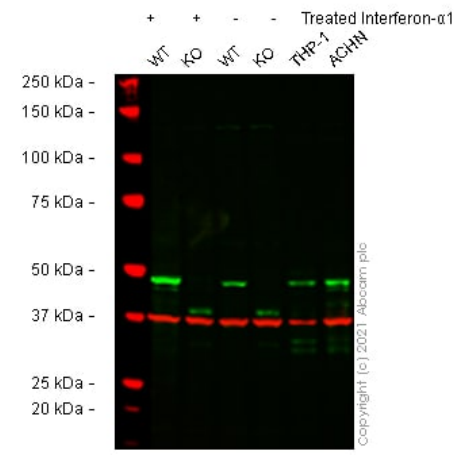
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab266051 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **ICC** | | Use at an assay dependent concentration. |

| 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 line (ab266051)

**All lanes :** Anti-Interferon regulatory factor 9/IRF-9 antibody [EPR24260-55] (**ab271043**) at 1/1000 dilution

**Lane 1 :** wild-type HeLa Treated Interferon-alpha1 (hIFN-a1) (10 ng/ml, 16 h) cell lysate
**Lane 2 :** IRF9 knockout HeLa treated: hIFN-a1 (10 ng/ml, 16 h) cell lysate
**Lane 3 :** wild-type HeLa Control Interferon-alpha1 (hIFN-a1) (0 ng/ml, 16 h) cell lysate
**Lane 4 :** IRF9 knockout HeLa vehicle control: hIFN-a1 (0 ng/ml, 16 h) cell lysate
**Lane 5 :** THP-1 cell lysate
**Lane 6 :** ACHN cell lysate

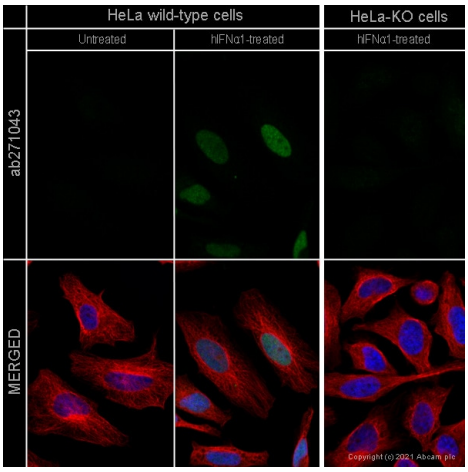
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 44 kDa
**Observed band size:** 48 kDa

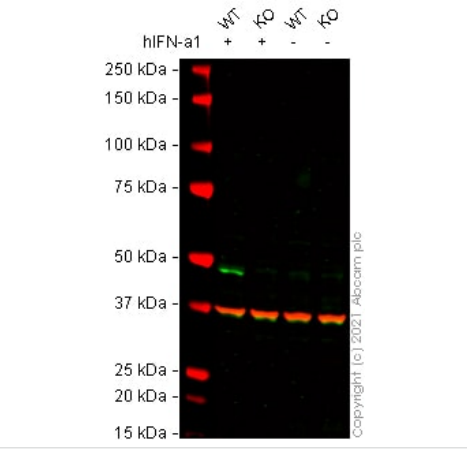
False colour image of Western blot: Anti-Interferon regulatory factor 9/IRF-9 antibody [EPR24260-55] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab271043** was shown to bind specifically 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.




Immunocytochemistry - Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell line (ab266051)

**ab271043** staining Interferon regulatory factor 9 in wild-type HeLa cells and IRF9 knockout HeLa cells treated with interferon-a1 (**ab48750**) at 10 ng/ml for 16 hours. The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab271043** at 0.4 µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).




Western blot - Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell line (ab266051)

**All lanes :** Anti-Interferon regulatory factor 9/IRF-9 antibody [14HCLC] (**ab277803**) at 1/1000 dilution

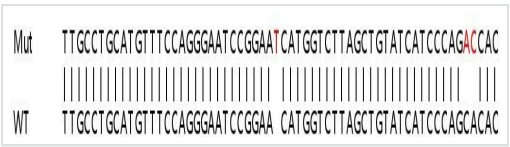
**Lane 1 :** Wild-type HeLa treated hIFN-a1 (10 ng/ml, 16 h) cell lysate
**Lane 2 :** IRF9 knockout HeLa treated hIFN-a1 (10 ng/ml, 16 h) cell lysate
**Lane 3 :** Wild-type HeLa vehicle control hIFN-a1 (0 ng/ml, 16 h) cell lysate
**Lane 4 :** IRF9 knockout HeLa vehicle control hIFN-a1 (0 ng/ml, 16 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

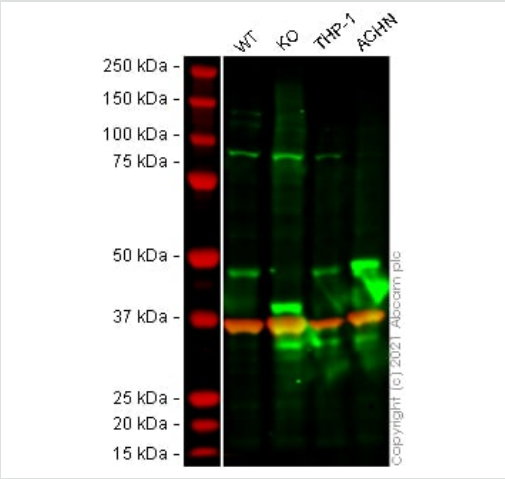
**Predicted band size:** 44 kDa
**Observed band size:** 48 kDa

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.




Sanger Sequencing - Human IRF9 knockout HeLa cell line (ab266051)

Allele-1: 1 bp insertion in exon2




Western blot - Human IRF9 (Interferon regulatory factor 9) knockout HeLa cell line (ab266051)

**All lanes :** Anti-IRF9 antibody at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** IRF9 knockout HeLa cell lysate
**Lane 3 :** THP-1 cell lysate
**Lane 4 :** ACHN cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 44 kDa
**Observed band size:** 48 kDa

**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 and the functional properties of the gene product have not been determined. 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 line (ab266051)

Allele-2: 2 bp deletion in exon 2.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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