

Product datasheet

Human ATF3 knockout HeLa cell line ab264908

4 Images

Overview

<b>Product name</b>	Human ATF3 knockout HeLa cell line
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 2 bp deletion in exon 2 and 5 bp deletion in exon 2
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255448</a>). 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.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> DMEM (High Glucose) + 10% FBS</p> <p><b>Initial handling guidelines:</b> 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.</p> <ol style="list-style-type: none"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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.</li> <li>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 <math>2 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>2 \times 10^4</math> cells/cm<sup>2</sup> is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

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## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Viability</b>	~80%
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Cervix
<b>Cell type</b>	epithelial
<b>Disease</b>	Adenocarcinoma
<b>Gender</b>	Female
<b>STR Analysis</b>	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
<b>Antibiotic resistance</b>	Puromycin 1.00µg/ml
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	This protein binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Represses transcription from promoters with ATF sites. It may repress transcription by stabilizing the binding of inhibitory cofactors at the promoter. Isoform 2 activates transcription presumably by sequestering inhibitory cofactors away from the promoters.
<b>Sequence similarities</b>	Belongs to the bZIP family. ATF subfamily. Contains 1 bZIP domain.
<b>Cellular localization</b>	Nucleus.

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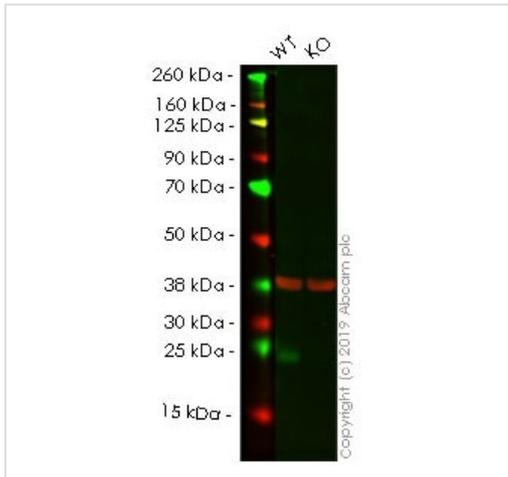
## Applications

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**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab264908 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
<b>WB</b>		Use at an assay dependent concentration. Predicted molecular weight: 21 kDa.

## Images



Western blot - Human ATF3 knockout HeLa cell line (ab264908)

**All lanes :** Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** ATF3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 21 kDa

**Observed band size:** 21 kDa

**Lanes 1- 2:** Merged signal (red and green). Green - ab207434 observed at 21 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab207434 was shown to react with ATF3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264908 (knockout cell lysate ab257073) was used. Wild-type HeLa and ATF3 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab207434 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 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.

Mut	GCTAACCTGACGCCCTTG-----GAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC
WT	GCTAACCTGACGCCCTTGTCAAGGAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC

Sanger Sequencing - Human ATF3 knockout HeLa cell line (ab264908)

Allele-1: 5 bp deletion in exon 2.

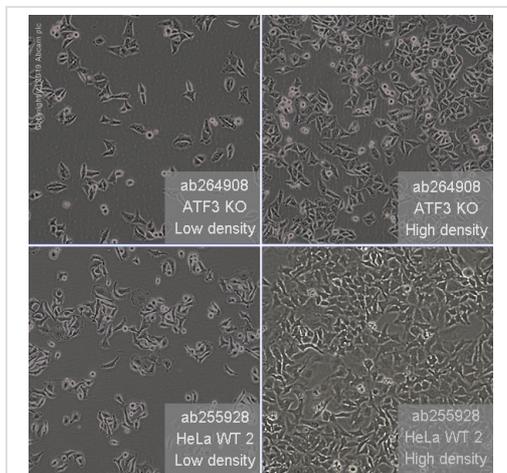
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Mut  GCTAACCTGACGCCCTTTG--AAGGAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC
      |||
WT   GCTAACCTGACGCCCTTTGTCAAGGAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC

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Allele-2: 2 bp deletion in exon 2.

Sanger Sequencing - Human ATF3 knockout HeLa cell line (ab264908)



Representative images of ATF3 knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

Cell Culture - Human ATF3 knockout HeLa cell line (ab264908)

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