abcam

Product datasheet

Human STAT3 knockout HeLa cell line ab255436

4 Images

Overview

Product name	Human STAT3 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. 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.
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**.

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

Function	Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transctivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
Involvement in disease	Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant Autoimmune disease, multisystem, infantile-onset
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF, KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation is important for the formation of stable DNA-binding STAT3 homodimers and maximal

transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

Cellular localization

Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

Applications

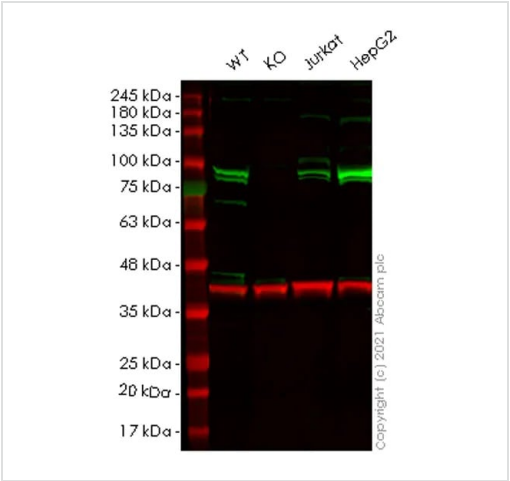
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab255436 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: 88 kDa.

Images



Western blot - Human STAT3 knockout HeLa cell line (ab255436)

All lanes : Anti-STAT3 (phospho Y705) antibody [EPR23968-52] (**ab267373**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 2 : STAT3 knockout HeLa serum starved overnight, then treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 3 : Jurkat (human t cell leukemia cell line from peripheral blood) treated with 50 ng/ml IFN alpha for 30 minutes, whole cell lysate

Lane 4 : HepG2 (human hepatocellar carcinoma epithelial cell) serum starved overnight, then treated with 100 ng/ml IL-6 for 30 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD)

(**ab216776**) at 1/10000 dilution

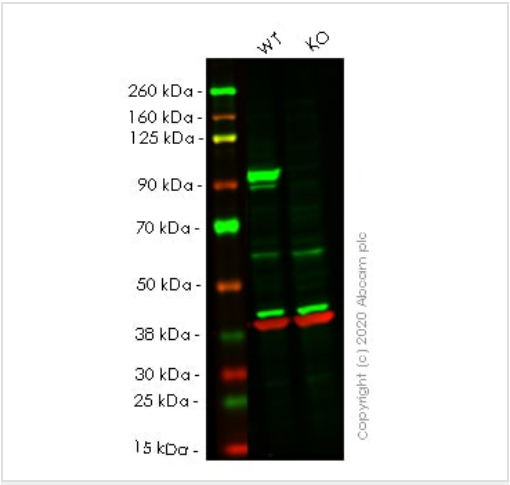
Predicted band size: 88 kDa
Observed band size: 88 kDa

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.

Lanes 1-4: Merged signal (red and green). Green - **ab267373** observed at 88 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

Lanes 1-2: **ab267373** Anti-STAT3 (phospho Y705) antibody [EPR23968-52] was shown to specifically react with STAT3 in wild-type serum starved and then IFN alpha treated HeLa cells. Loss of signal was observed when serum starved and then IFN alpha treated knockout cell line ab255436 (knockout cell lysate **ab263797**) was used. Wild-type and STAT3 knockout samples were subjected to SDS-PAGE. **ab267373** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight 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 10000 dilution for 1 hour at room temperature before imaging.

Lysates loaded onto lanes 3-4 were made freshly and used in WB immediately to minimize protein degradation.



Western blot - Human STAT3 knockout HeLa cell line (ab255436)

All lanes : Anti-STAT3 antibody [EPR787Y] (**ab68153**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT3 knockout HeLa cell lysate

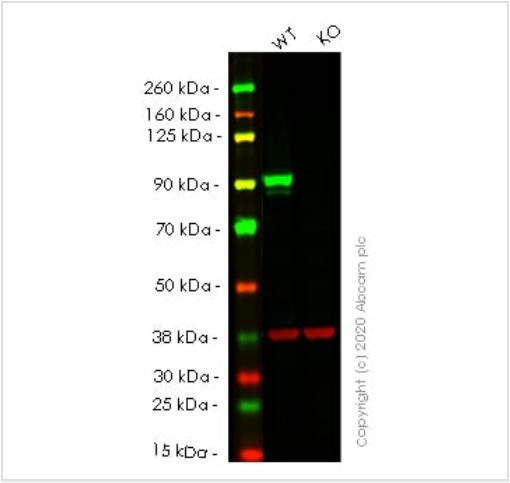
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 92 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab68153** observed at 92 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab68153 was shown to react with STAT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255436 (knockout cell lysate **ab263797**) was used. Wild-type HeLa and STAT3 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. **ab68153** 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.



Western blot - Human STAT3 knockout HeLa cell line (ab255436)

All lanes : Anti-STAT3 antibody [EPR361] (**ab109085**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : STAT3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 92 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab109085** observed at 92 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109085 was shown to react with STAT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255436 (knockout cell lysate **ab263797**) was used. Wild-type HeLa and STAT3 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. **ab109085** 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.

Sanger Sequencing - Human STAT3 knockout HeLa cell line (ab255436)

Homozygous: 1 bp deletion in exon 2.

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