abcam

Product datasheet

Human NR3C1 (Glucocorticoid Receptor) knockout HeLa cell line ab261766

2 Images

Overview

Product name	Human NR3C1 (Glucocorticoid Receptor) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 2. The cells can still express the functionally active isoforms GRα -D1, D2, D3 from alternative start codons.
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.
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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
Antibiotic resistance	Puromycin 1.00µg/ml
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	Receptor for glucocorticoids (GC). Has a dual mode of action: as a transcription factor that binds to glucocorticoid response elements (GRE) and as a modulator of other transcription factors. Affects inflammatory responses, cellular proliferation and differentiation in target tissues. Could act as a coactivator for STAT5-dependent transcription upon growth hormone (GH) stimulation and could reveal an essential role of hepatic GR in the control of body growth. Involved in chromatin remodeling. Plays a significant role in transactivation. Involved in nuclear translocation.
Tissue specificity	Widely expressed. In the heart, detected in left and right atria, left and right ventricles, aorta, apex, intraventricular septum, and atrioventricular node as well as whole adult and fetal heart.
Involvement in disease	Defects in NR3C1 are a cause of glucocorticoid resistance (GCRES) [MIM:138040]; also known as cortisol resistance. It is a hypertensive, hyperandrogenic disorder characterized by increased serum cortisol concentrations. Inheritance is autosomal dominant.
Sequence similarities	Belongs to the nuclear hormone receptor family. NR3 subfamily. Contains 1 nuclear receptor DNA-binding domain.
Domain	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.
Post-translational modifications	Increased proteasome-mediated degradation in response to glucocorticoids. Phosphorylated in the absence of hormone; becomes hyperphosphorylated in the presence of glucocorticoid. The Ser-203-phosphorylated form is mainly cytoplasmic, and the Ser-211-

	phosphorylated form is nuclear. Transcriptional activity correlates with the amount of phosphorylation at Ser-211. Sumoylated; this reduces transcription transactivation. Ubiquitinated; restricts glucocorticoid-mediated transcriptional signaling.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand, nuclear after ligand-binding and Nucleus. Localized largely in the nucleus.

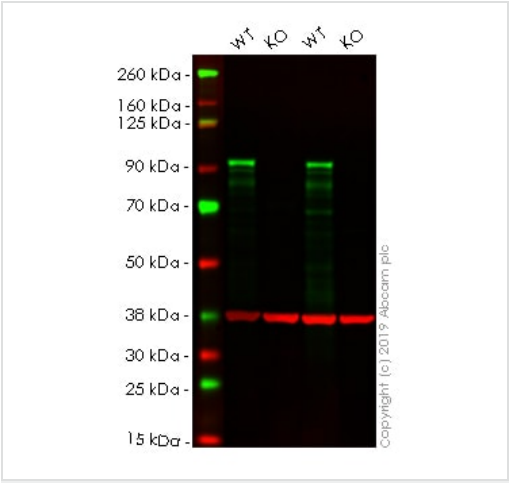
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261766 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: 85 kDa.

Images



Western blot - Human NR3C1 (Glucocorticoid Receptor) knockout HeLa cell line (ab261766)

All lanes : Anti-Glucocorticoid Receptor antibody [EPR19621] (**ab183127**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NR3C1 knockout HeLa cell lysate
Lane 3 : Wild-type A549 cell lysate
Lane 4 : NR3C1 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 90-100 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab183127** observed at 90-100 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab183127 Anti-Glucocorticoid Receptor antibody [EPR19621] was

shown to specifically react with Glucocorticoid Receptor in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261766 (knockout cell lysate **ab257009**) was used. Wild-type and Glucocorticoid Receptor knockout samples were subjected to SDS-PAGE. **ab183127** and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) 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 NR3C1 knockout HeLa cell line (ab261766)

Homozygous: 1 bp insertion in exon 2.

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