abcam

Product datasheet

Human TNFRSF1A (TNF Receptor I) knockout HeLa cell line ab265972

5 Images

Overview

Product name	Human TNFRSF1A (TNF Receptor I) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 2 and 2 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 (**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.

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
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 TNFSF2/TNF-alpha and homotrimeric TNFSF1/lymphotoxin-alpha. The adapter molecule FADD recruits caspase-8 to the activated receptor. The resulting death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. Contributes to the induction of non-cytocidal TNF effects including anti-viral state and activation of the acid sphingomyelinase.
Involvement in disease	Familial hibernian fever Multiple sclerosis 5
Sequence similarities	Contains 1 death domain. Contains 4 TNFR-Cys repeats.
Domain	The domain that induces A-SMASE is probably identical to the death domain. The N-SMASE activation domain (NSD) is both necessary and sufficient for activation of N-SMASE. Both the cytoplasmic membrane-proximal region and the C-terminal region containing the death domain are involved in the interaction with TRPC4AP.
Post-translational modifications	The soluble form is produced from the membrane form by proteolytic processing.
Cellular localization	Cell membrane. Golgi apparatus membrane. Secreted. A secreted form is produced through proteolytic processing and Secreted. Lacks a Golgi-retention motif, is not membrane bound and therefore is secreted.

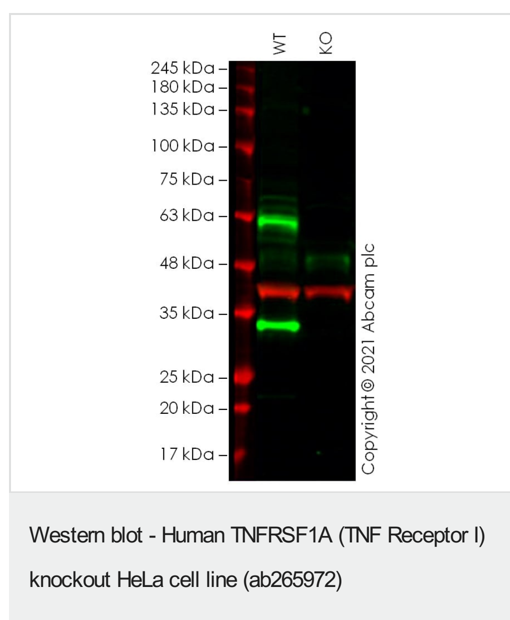
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265972 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: 50 kDa.

Images



Western blot - Human TNFRSF1A (TNF Receptor I) knockout HeLa cell line (ab265972)

All lanes : Anti-TNFRSF1A antibody at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

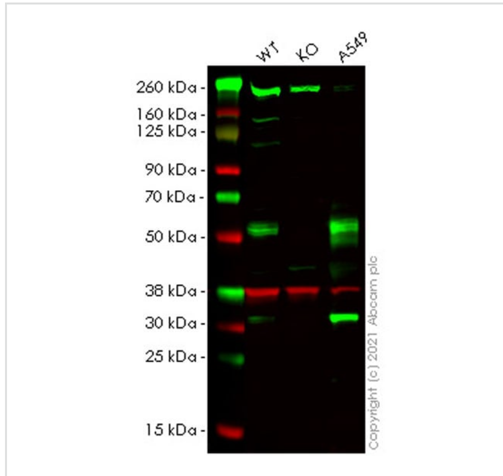
Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 33,63 kDa

False colour image of Western blot: Anti-TNFRSF1A antibody 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, the antibody was shown to bind specifically to TNFRSF1A. A band was observed at 33/63 kDa in wild-type HeLa cell lysates with no signal observed at this size in TNFRSF1A knockout cell line ab265972 (knockout cell lysate **ab257751**). To generate this image, wild-type and TNFRSF1A 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.



Western blot - Human TNFRSF1A (TNF Receptor I) knockout HeLa cell line (ab265972)

All lanes : Anti-TNF Receptor I antibody [EPR23742-65] (**ab259817**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : TNFRSF1A (TNF Receptor I) knockout HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : A549 (human lung carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

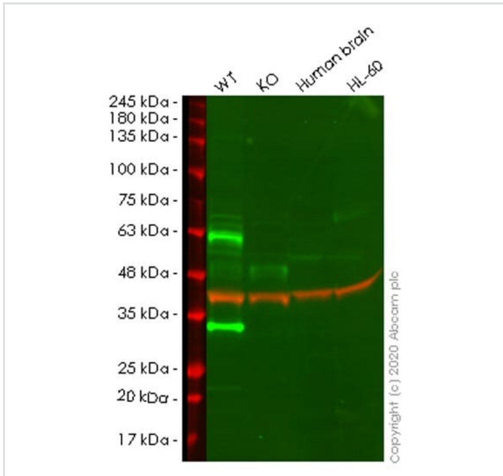
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

Predicted band size: 50 kDa
Observed band size: 50-70 kDa

Blocking and diluting buffer and concentration: 3% NFDM/TBST

Lanes 1-3: Merged signal (red and green). Green - **ab259817** observed at 51 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab259817 Anti-TNF Receptor I antibody [EPR23742-65] was shown to specifically react with TNF Receptor I in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265972 (knockout cell lysate **ab257751**) was used. Wild-type and TNF Receptor I knockout samples were subjected to SDS-PAGE. **ab259817** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4? 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.



Western blot - Human TNFRSF1A (TNF Receptor I) knockout HeLa cell line (ab265972)

All lanes : Anti-TNF-R1 Rabbit monoclonal antibody at 1/1000 dilution

Lane 1 : Human wild-type HeLa cell lysate
Lane 2 : TNFRSF1A knockout HeLa cell lysate
Lane 3 : Human brain cell lysate
Lane 4 : HL-60 cell lysate

Predicted band size: 50 kDa
Observed band size: 51 kDa

Lanes 1-4: Merged signal (red and green). Green - anti-TNF-R1 Rabbit monoclonal antibody observed at 51 kDa. Red - loading control, **ab8245** observed at 36 kDa.

Anti-TNF-R1 Rabbit monoclonal antibody and anti-GPADH antibody [6C5] - Loading control (**ab8245**) were incubated overnight at 4°C at a 1/1000 and 1/20000 dilution respectively before imaging. Blots were developed with Goat anti-Rabbig IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10,000 dilution for 1 hour at room temperature before imaging.

Mut ACACTATCTCTCTTCTCCCTGTCCCCTA--TGAGGGACCAGTCCAATAACCCCTGAGGGG
WT ACACTATCTCTCTTCTCCCTGTCCCCTAGGTGAGGGACCAGTCCAATAACCCCTGAGGGG

Sanger Sequencing - Human TNFRSF1A knockout HeLa cell line (ab265972)

Allele-1: 2 bp deletion in exon 2.

Mut ACACTATCTCTCTTCTCCCTGTCCCCTA-GTGAGGGACCAGTCCAATAACCCCTGAGGGG
WT ACACTATCTCTCTTCTCCCTGTCCCCTAGGTGAGGGACCAGTCCAATAACCCCTGAGGGG

Sanger Sequencing - Human TNFRSF1A knockout HeLa cell line (ab265972)

Allele-2: 1 bp deletion in exon 2.

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