abcam

Product datasheet

Human TRIM21 (SS-A) knockout A549 cell line ab267025

6 Images

Overview

Product name	Human TRIM21 (SS-A) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 13 bp deletion in exon 4 and 1 bp insertion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type A549 cell line (**ab255450**). 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:** F-12K + 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 $2x10^3$-$1x10^4$ 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 $6x10^4$ 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.
Do not exceed $7x10^4$ cells/cm^2.
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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	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 vWA: 14 TH01: 8,9.3 TPOX: 8,11 CSF1PO: 10, 12
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	E3 ubiquitin-protein ligase whose activity is dependent on E2 enzymes, UBE2D1, UBE2D2, UBE2E1 and UBE2E2. Forms a ubiquitin ligase complex in cooperation with the E2 UBE2D2 that is used not only for the ubiquitination of USP4 and IKBKB but also for its self-ubiquitination. Component of cullin-RING-based SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complexes such as SCF(SKP2)-like complexes. A TRIM21-containing SCF(SKP2)-like complex is shown to mediate ubiquitination of CDKN1B ('Thr-187' phosphorylated-form), thereby promoting its degradation by the proteasome. Monoubiquitinates IKBKB that will negatively regulates Tax-induced NF-kappa-B signaling. Negatively regulates IFN-beta production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. Mediates the ubiquitin-mediated proteasomal degradation of IgG1 heavy chain, which is linked to the VCP-mediated ER-associated degradation (ERAD) pathway. Promotes IRF8 ubiquitination, which enhanced the ability of IRF8 to stimulate cytokine genes transcription in macrophages. Plays a role in the regulation of the cell cycle progression. Enhances the decapping activity of DCP2. Exists as a ribonucleoprotein particle present in all mammalian cells studied and composed of a single polypeptide and one of four small RNA molecules. At least two isoforms are present in nucleated and red blood cells, and tissue specific differences in RO/SSA proteins have been identified. The common feature of these proteins is their ability to bind HY RNAs.2.
Tissue specificity	Isoforms 1 and 2 are expressed in fetal and adult heart and fetal lung.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Belongs to the TRIM/RBCC family.

	Contains 1 B box-type zinc finger. Contains 1 B30.2/SPRY domain. Contains 1 RING-type zinc finger.
Domain	The coiled-coil is necessary for the cytoplasmic localization. The B30.2/SPRY domain is necessary for the cytoplasmic localization, the interaction with IRF3 and for the IRF3-driven interferon beta promoter activity. The RING-type zinc finger is necessary for ubiquitination and for the IRF3-driven interferon beta promoter activity. Interacts with SKP2 and CUL1 in a RING finger-independent manner.
Post-translational modifications	Autoubiquitinated; does not lead to its proteasomal degradation. Deubiquitinated by USP4; leading to its stabilization.
Cellular localization	Cytoplasm. Nucleus. Cytoplasm > P-body. Enters the nucleus upon exposure to nitric oxide. Localizes to small dot- or rod-like structures in the cytoplasm, called cytoplasmic bodies (P-body) that are located underneath the plasma membrane and also diffusely in the cytoplasm and are highly motil in cells. Cytoplasmic bodies are located along the microtubules and do not share the same cytoplasmic bodies with TRIM5. Colocalizes with DCP2 in P-body.

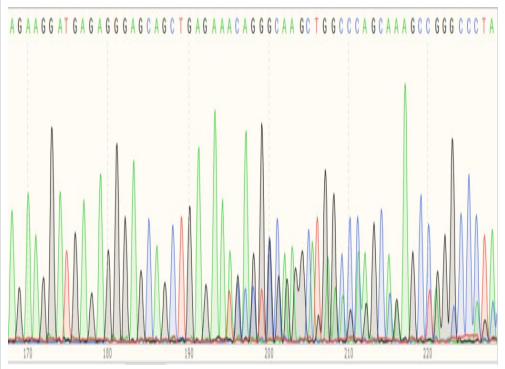
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab267025 in the following tested applications.

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

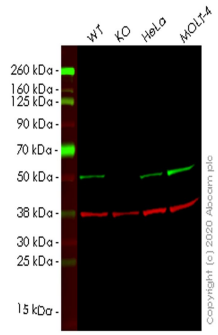
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 54 kDa.

Images



Sanger Sequencing - Human TRIM21 (SS-A) knockout A549 cell line (ab267025)

Sequencing chromatogram displaying sequence edit in exon 4



Western blot - Human TRIM21 (SS-A) knockout A549 cell line (ab267025)

All lanes : Anti-TRIM21/SS-A antibody [EPR20290] (**ab207728**) at 1/500 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : TRIM21 knockout A549 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

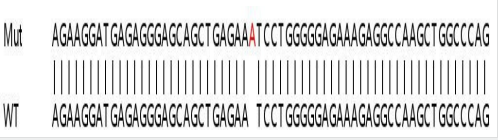
Predicted band size: 54 kDa
Observed band size: 50 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab207728** observed at 50 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab207728 Anti-TRIM21/SS-A antibody [EPR20290] was shown to specifically react with TRIM21/SS-A in wild-type A549 cells. Loss of signal was observed when knockout cell line ab267025 (knockout cell lysate **ab257767**) was used. Wild-type and TRIM21/SS-A knockout samples were subjected to SDS-PAGE. **ab207728** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 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.

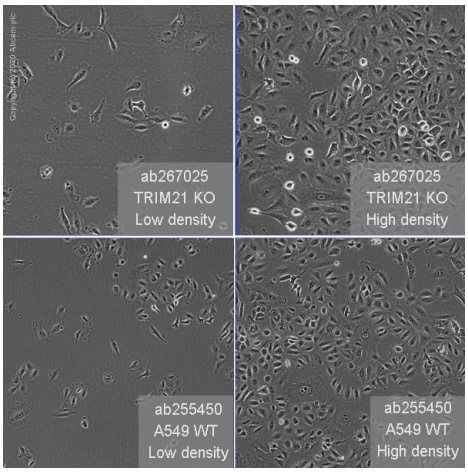
Sanger Sequencing - Human TRIM21 knockout A549 cell line (ab267025)

Allele-1: 13 bp deletion in exon4



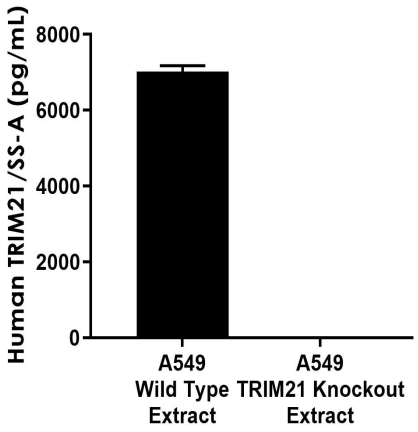
Sanger Sequencing - Human TRIM21 knockout A549 cell line (ab267025)

Allele-2: 1 bp insertion in exon 4.



Cell Culture - Human TRIM21 (SS-A) knockout A549 cell line (ab267025)

Representative images of TRIM21 knockout A549 cells, low and high confluency examples (top left and right respectively) and wild-type A549 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.



Sandwich ELISA - Human TRIM21 (SS-A) knockout A549 cell line (ab267025)

Interpolated concentrations of native TRIM21/SS-A in human control wild type A549 (human lung carcinoma cell) and TRIM21 (TRIM21/SS-A) knockout A549 cell based on 200 µg/mL extract loads. The concentrations of TRIM21/SS-A were measured in duplicate and interpolated from the TRIM21/SS-A standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean TRIM21/SS-A concentration was determined to be 7026.2 pg/mL in wild type A549 extract (Human wild-type A549 cell line **ab255450**) and undetectable in TRIM21 (TRIM21/SS-A) knockout A549 extract (Human TRIM21 (TRIM21/SS-A) knockout A549 cell line ab267025).

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