

Human TP53 (p53) knockout A549 cell line ab276092

4 Images

Overview

Product name	Human TP53 (p53) knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 49 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	Suitable for: WB
Biosafety level	1
General notes	<p>Recommended control: Human wild-type A549 cell line (ab275463). 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>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: F-12K + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^3-1×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 6×10^4 cells/cm² 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.

Do not exceed 7×10^4 cells/cm².

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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
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	Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4 suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis.
Tissue specificity	Ubiquitous. Isoforms are expressed in a wide range of normal tissues but in a tissue-dependent manner. Isoform 2 is expressed in most normal tissues but is not detected in brain, lung, prostate, muscle, fetal brain, spinal cord and fetal liver. Isoform 3 is expressed in most normal tissues but is not detected in lung, spleen, testis, fetal brain, spinal cord and fetal liver. Isoform 7 is expressed in most normal tissues but is not detected in prostate, uterus, skeletal muscle and breast. Isoform 8 is detected only in colon, bone marrow, testis, fetal brain and intestine. Isoform 9 is expressed in most normal tissues but is not detected in brain, heart, lung, fetal liver, salivary gland, breast or intestine.
Involvement in disease	Note=TP53 is found in increased amounts in a wide variety of transformed cells. TP53 is frequently mutated or inactivated in about 60% of cancers. TP53 defects are found in Barrett metaplasia a condition in which the normally stratified squamous epithelium of the lower esophagus is replaced by a metaplastic columnar epithelium. The condition develops as a complication in approximately 10% of patients with chronic gastroesophageal reflux disease and predisposes to the development of esophageal adenocarcinoma. Defects in TP53 are a cause of esophageal cancer (ESCR) [MIM:133239].

Defects in TP53 are a cause of Li-Fraumeni syndrome (LFS) [MIM:151623]. LFS is an autosomal dominant familial cancer syndrome that in its classic form is defined by the existence of a proband affected by a sarcoma before 45 years with a first degree relative affected by any tumor before 45 years and another first degree relative with any tumor before 45 years or a sarcoma at any age. Other clinical definitions for LFS have been proposed (PubMed:8118819 and PubMed:8718514) and called Li-Fraumeni like syndrome (LFL). In these families affected relatives develop a diverse set of malignancies at unusually early ages. Four types of cancers account for 80% of tumors occurring in TP53 germline mutation carriers: breast cancers, soft tissue and bone sarcomas, brain tumors (astrocytomas) and adrenocortical carcinomas. Less frequent tumors include choroid plexus carcinoma or papilloma before the age of 15, rhabdomyosarcoma before the age of 5, leukemia, Wilms tumor, malignant phyllodes tumor, colorectal and gastric cancers.

Defects in TP53 are involved in head and neck squamous cell carcinomas (HNSCC) [MIM:275355]; also known as squamous cell carcinoma of the head and neck.

Defects in TP53 are a cause of lung cancer (LNCr) [MIM:211980].

Defects in TP53 are a cause of choroid plexus papilloma (CPLPA) [MIM:260500]. Choroid plexus papilloma is a slow-growing benign tumor of the choroid plexus that often invades the leptomeninges. In children it is usually in a lateral ventricle but in adults it is more often in the fourth ventricle. Hydrocephalus is common, either from obstruction or from tumor secretion of cerebrospinal fluid. If it undergoes malignant transformation it is called a choroid plexus carcinoma. Primary choroid plexus tumors are rare and usually occur in early childhood.

Defects in TP53 are a cause of adrenocortical carcinoma (ADCC) [MIM:202300]. ADCC is a rare childhood tumor of the adrenal cortex. It occurs with increased frequency in patients with the Beckwith-Wiedemann syndrome and is a component tumor in Li-Fraumeni syndrome.

Sequence similarities

Belongs to the p53 family.

Domain

The nuclear export signal acts as a transcriptional repression domain. The TAD1 and TAD2 motifs (residues 17 to 25 and 48 to 56) correspond both to 9aaTAD motifs which are transactivation domains present in a large number of yeast and animal transcription factors.

Post-translational modifications

Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.

Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity). Phosphorylation at Ser-9 by HIPK4 increases repression activity on BIRC5 promoter. Phosphorylated on Thr-18 by VRK1. Phosphorylated on Ser-20 by CHEK2 in response to DNA damage, which prevents ubiquitination by MDM2. Phosphorylated on Thr-55 by TAF1, which promotes MDM2-mediated degradation. Phosphorylated on Ser-46 by HIPK2 upon UV irradiation. Phosphorylation on Ser-46 is required for acetylation by CREBBP. Phosphorylated on Ser-392 following UV but not gamma irradiation. Phosphorylated upon DNA damage, probably by ATM or ATR. Phosphorylated on Ser-15 upon ultraviolet irradiation; which is enhanced by interaction with BANP.

Dephosphorylated by PP2A-PPP2R5C holoenzyme at Thr-55. SV40 small T antigen inhibits the dephosphorylation by the AC form of PP2A.

May be O-glycosylated in the C-terminal basic region. Studied in EB-1 cell line.

Ubiquitinated by MDM2 and SYVN1, which leads to proteasomal degradation. Ubiquitinated by RFWD3, which works in cooperation with MDM2 and may catalyze the formation of short polyubiquitin chains on p53/TP53 that are not targeted to the proteasome. Ubiquitinated by MKRN1 at Lys-291 and Lys-292, which leads to proteasomal degradation. Deubiquitinated by USP10, leading to its stabilization. Ubiquitinated by TRIM24, which leads to proteasomal degradation. Ubiquitination by TOPORS induces degradation. Deubiquitination by USP7, leading to stabilization. Isoform 4 is monoubiquitinated in an MDM2-independent manner.

Monomethylated at Lys-372 by SETD7, leading to stabilization and increased transcriptional activation. Monomethylated at Lys-370 by SMYD2, leading to decreased DNA-binding activity

and subsequent transcriptional regulation activity. Lys-372 monomethylation prevents interaction with SMYD2 and subsequent monomethylation at Lys-370. Dimethylated at Lys-373 by EHMT1 and EHMT2. Monomethylated at Lys-382 by SETD8, promoting interaction with L3MBTL1 and leading to repress transcriptional activity. Demethylation of dimethylated Lys-370 by KDM1A prevents interaction with TP53BP1 and represses TP53-mediated transcriptional activation. Sumoylated by SUMO1.

Cellular localization

Cytoplasm; Cytoplasm. Nucleus. Nucleus > PML body. Endoplasmic reticulum. Interaction with BANP promotes nuclear localization. Recruited into PML bodies together with CHEK2; Nucleus. Cytoplasm. Localized in both nucleus and cytoplasm in most cells. In some cells, forms foci in the nucleus that are different from nucleoli; Nucleus. Cytoplasm. Localized in the nucleus in most cells but found in the cytoplasm in some cells; Nucleus. Cytoplasm. Localized mainly in the nucleus with minor staining in the cytoplasm; Nucleus. Cytoplasm. Predominantly nuclear but localizes to the cytoplasm when expressed with isoform 4 and Nucleus. Cytoplasm. Predominantly nuclear but translocates to the cytoplasm following cell stress.

Applications

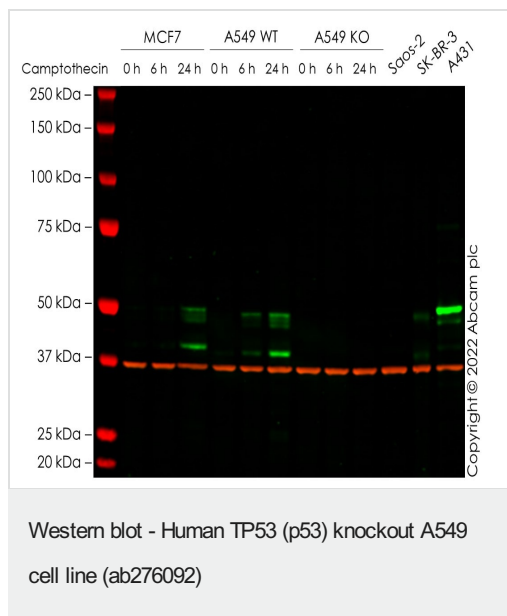
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab276092 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.

Images



All lanes : Anti-p53 antibody [DO-1] - ChIP Grade (**ab1101**) at 1/1000 dilution

Lane 1 : MCF7 Treated Camptothecin (20 ug, 0 h) cell lysate

Lane 2 : MCF7 Treated Camptothecin (20 ug, 6 h) cell lysate

Lane 3 : MCF7 Treated Camptothecin (20 ug, 24 h) cell lysate

Lane 4 : Wild-type A549 Treated Camptothecin (20 ug, 0 h) cell lysate

Lane 5 : Wild-type A549 Treated Camptothecin (20 ug, 6 h) cell lysate

Lane 6 : Wild-type A549 Treated Camptothecin (20 ug, 24 h) cell lysate

Lane 7 : TP53 knockout A549 Treated Camptothecin (20 ug, 0 h) cell lysate

Lane 8 : TP53 knockout A549 Treated Camptothecin (20 ug, 6 h) cell lysate

Lane 9 : TP53 knockout A549 Treated Camptothecin (20 ug, 24 h) cell lysate

Lane 10 : Saos-2 cell lysate

Lane 11 : SK-BR-3 cell lysate

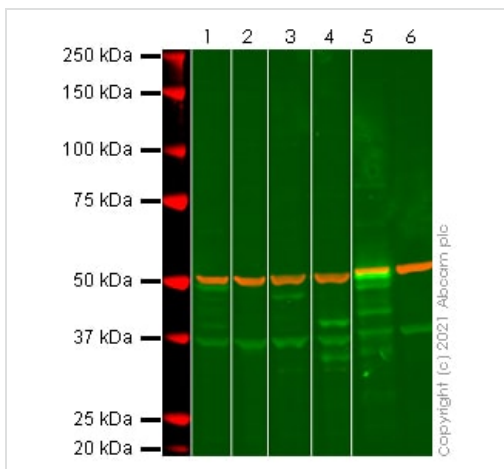
Lane 12 : A431 cell lysate

Lysates/proteins at 15 µg per lane.

Performed under reducing conditions.

Observed band size: 40,50 kDa

False colour image of Western blot: Anti-p53 antibody [DO-1] - ChIP Grade staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] ([ab181602](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab1101](#) was shown to bind specifically to p53. A band was observed at 40/50 kDa in treated wild-type A549 cell lysates with no signal observed at this size in tp53 knockout cell line [ab276092](#). To generate this image, wild-type and tp53 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed ([ab216772](#)) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ([ab216777](#)) at 1/20000 dilution.



Western blot - Human TP53 (p53) knockout A549 cell line ([ab276092](#))

All lanes : Anti-p53 antibody [DO-1] - ChIP Grade ([ab1101](#)) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : TP53 knockout A549 cell lysate

Lane 3 : Wild-type Jurkat cell lysate

Lane 4 : TP53 knockout Jurkat cell lysate

Lane 5 : A431 cell lysate

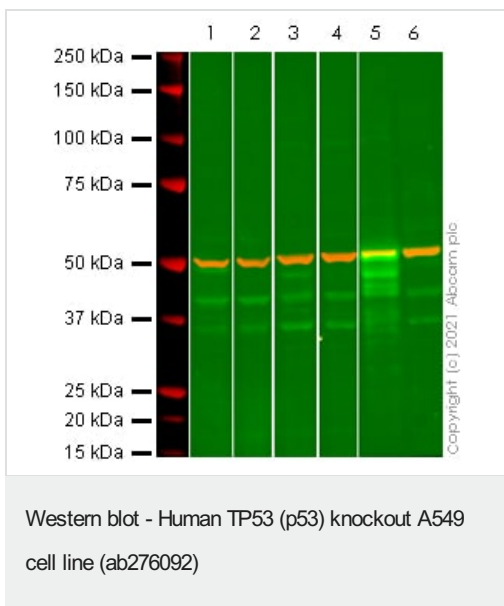
Lane 6 : Saos-2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 49 kDa

False colour image of Western blot: Anti-p53 antibody [DO-1] - ChIP Grade staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] ([ab52866](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab1101](#) was shown to bind specifically to p53. A band was observed at 49 kDa in wild-type A549 and Jurkat cell lysates with no signal observed at this size in tp53 knockout cell lines [ab276092](#), [ab276112](#) (knockout cell lysates [ab282999](#), [ab283832](#)). To generate this image, wild-type and tp53 knockout A549 and Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween® 20 (TBS-T) 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed ([ab216772](#)) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ([ab216777](#)) at 1/20000 dilution.



All lanes : Anti-p53 antibody [E26] ([ab32389](#)) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : TP53 knockout A549 cell lysate

Lane 3 : Wild-type Jurkat cell lysate

Lane 4 : TP53 knockout Jurkat cell lysate

Lane 5 : A431 cell lysate

Lane 6 : Saos-2 cell lysate

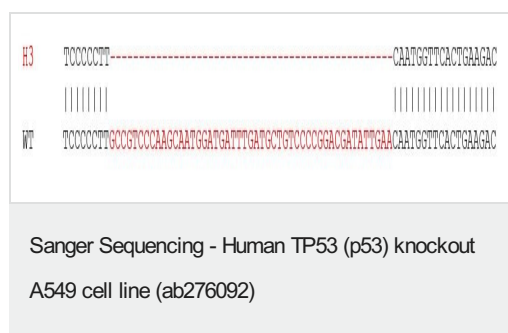
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 49 kDa

False colour image of Western blot: Anti-p53 antibody [E26] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] ([ab7291](#)) loading control staining at 1/20000

dilution, shown in red. In Western blot, **ab32389** was shown to bind specifically to p53. A band was observed at 49 kDa in wild-type A549 and Jurkat cell lysates with no signal observed at this size in tp53 knockout cell lines ab276092, **ab276112** (knockout cell lysates **ab282999**, **ab283832**). To generate this image, wild-type and tp53 knockout A549 and Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween[®] 20 (TBS-T) 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.



49 bp deletion in exon 4

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