# abcam

## Product datasheet

## Anti-p53 antibody ab131442



★★★★★ 7 Abreviews 238 References 13 Images

#### Overview

Product name Anti-p53 antibody

**Description** Rabbit polyclonal to p53

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide corresponding to Human p53 aa 1-100 conjugated to keyhole limpet

haemocyanin.

Database link: P04637-1

Run BLAST with
Run BLAST with

Positive control WB: Wild type HAP1 whole cell lysate, Saos2 and A431 cell lysates; IHC-P: Human lung, liver,

lung cancer, and stomach cancer tissues. Rat spleen tissue. Mouse liver, kidney, and testis

tissues.

**General notes**The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

## **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at -20°C.

Storage buffer pH: 7.4

Preservative: 0.02% Sodium azide

Constituents: 49% PBS, 50% Glycerol, 0.5% BSA

**Purity** Affinity purified

**Purification notes** ab131442 was purified by affinity-chromatography using epitope-specific peptide.

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**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise quarantee covers the use of ab131442 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	<b>★★★★☆</b> (2)	1/500 - 1/1000. Predicted molecular weight: 53 kDa. Blocking for 1 hour at room temperature in 5% non-fat milk, 1xTBS, 0.1% Tween-20. Primary incubation overnight at 4°C.
IHC-P	<b>★★★★★</b> (3)	1/50 - 1/100.

#### **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 transactivator 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

Domain

Post-translational modifications

Belongs to the p53 family.

The nuclear export signal acts as a transcriptional repression domain. The TADI and TADII 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.

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.

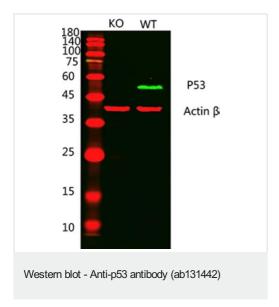
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#### **Cellular localization**

Sumoylated by SUMO1.

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.

### **Images**



All lanes: Anti-p53 antibody (ab131442) at 1/1000 dilution

**All lanes :** Lysate from p53 knockout A431 (human epidermoid carcinoma cell line) cells

#### **Secondary**

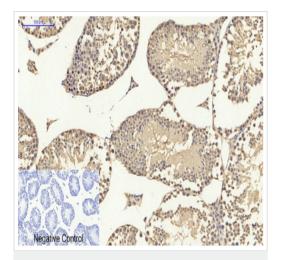
**All lanes :** Dylight 800 secondary antibody (green) at 1/10000 dilution

Predicted band size: 53 kDa

**Loading control:** Beta actin monoclonal antibody (5B7) at 1/5000 dilution followed by Dylight 680 secondary antibody at 1/10000 dilution (red).

Primary antibodies incubated overnight at 4°C.

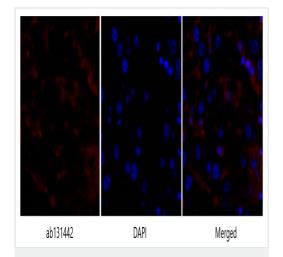
Secondary antibodies incubated for 1 hour at 37°C.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

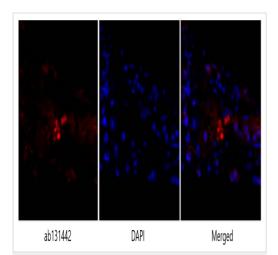
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse testis tissue labeling p53 with ab131442 at 1/200 dilution (overnight at 4°C). Heat mediated antigen retrieval was performed using Sodium citrate buffer pH 6 (>98°C, 20 minutes). Secondary antibody was used at 1/200 dilution for 30 minutes at room temperature.

Negative control: Secondary antibody only.



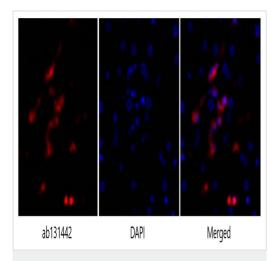
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling p53 with ab131442 at 1/200 (4°C, overnight). Cy3 labeled secondary antibody was diluted at 1/300 and incubated for 50 minutes at room temperature (red). Nuclei stained with DAPI (blue).



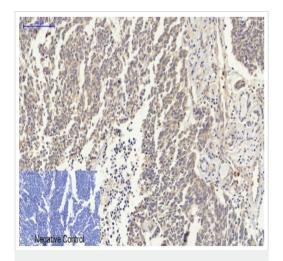
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemical analysis of paraffin-embedded human lung tissue labeling p53 with ab131442 at 1/200 (4°C, overnight). Cy3 labeled secondary antibody was diluted at 1/300 and incubated for 50 minutes at room temperature (red). Nuclei stained with DAPI (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

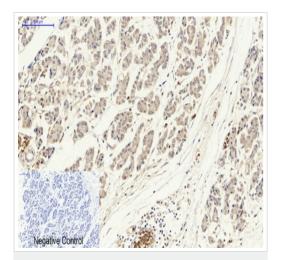
Immunohistochemical analysis of paraffin-embedded human liver tissue labeling p53 with ab131442 at 1/200 (4°C, overnight). Cy3 labeled secondary antibody was diluted at 1/300 and incubated for 50 minutes at room temperature (red). Nuclei stained with DAPI (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung cancer tissue labeling p53 with ab131442 at 1/200 dilution (overnight at 4°C). Heat mediated antigen retrieval was performed using Sodium citrate buffer pH 6 (>98°C, 20 minutes). Secondary antibody was used at 1/200 dilution for 30 minutes at room temperature.

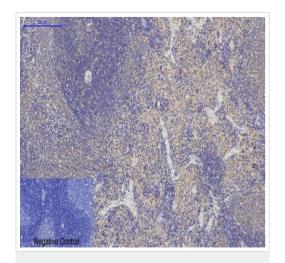
Negative control: Secondary antibody only.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach cancer tissue labeling p53 with ab131442 at 1/200 dilution (overnight at 4°C). Heat mediated antigen retrieval was performed using Sodium citrate buffer pH 6 (>98°C, 20 minutes). Secondary antibody was used at 1/200 dilution for 30 minutes at room temperature.

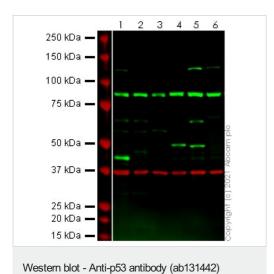
Negative control: Secondary antibody only.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat spleen tissue labeling p53 with ab131442 at 1/200 dilution (overnight at 4°C). Heat mediated antigen retrieval was performed using Sodium citrate buffer pH 6 (>98°C, 20 minutes). Secondary antibody was used at 1/200 dilution for 30 minutes at room temperature.

Negative control: Secondary antibody only.



All lanes: Anti-p53 antibody (ab131442) at 1/1000 dilution

Lane 1 : Saos-2 (p53 null) cell lysate
Lane 2 : HL-60 (p53 null) cell lysate

Lane 3 : MCF7 (wildtype p53) cell lysate
Lane 4 : A431 (mutant p53) cell lysate
Lane 5 : Wild-type HAP1 cell lysate

Lane 6: TP53 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

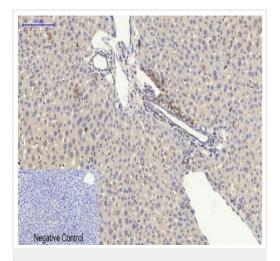
Performed under reducing conditions.

**Predicted band size:** 53 kDa **Observed band size:** 53 kDa

**Lanes 1 - 6:** Merged signal (red and green). Green - ab131442 observed at 53 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

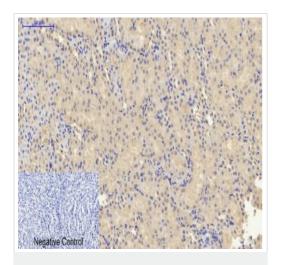
ab131442 was shown to react with p53 in wild-type HAP1 cells in Western blot with loss of signal observed in TP53 knockout sample. Wild-type HAP1 and TP53 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab131442 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW)

preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



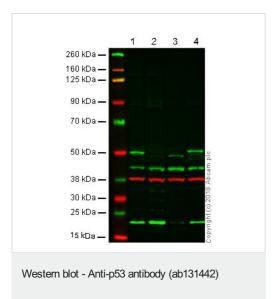
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling p53with ab131442 at 1/200 (4°C, overnight). Sodium citrate pH 6.0 was used for antibody retrieval (>98°C, 20 min). Secondary antibody was diluted at 1/200 (RT, 30min). Negative control was used by secondary antibody only.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody (ab131442)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling p53with ab131442 at 1/200 (4°C, overnight). Sodium citrate pH 6.0 was used for antibody retrieval (>98°C, 20 min). Secondary antibody was diluted at 1/200 (RT, 30min). Negative control was used by secondary antibody only.



All lanes: Anti-p53 antibody (ab131442) at 1/500 dilution

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

Lane 2: p53 knockout HAP1 whole cell lysate (20 μg)

**Lane 3**: Saos2 (20 μg) **Lane 4**: A431 (20 μg)

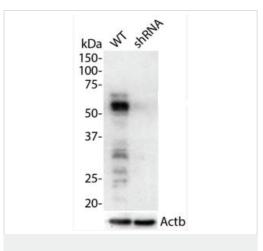
Performed under reducing conditions.

Predicted band size: 53 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab131442 observed at 53 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Wild-type and p53 knockout samples were subjected to SDS-PAGE. ab131442 and <u>ab8245</u> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

Other recommended antibodies to this target are <u>ab32389</u> and <u>ab26</u>.



Western blot - Anti-p53 antibody (ab131442)

**All lanes:** Anti-p53 antibody (ab131442)

Lane 1 : Wild-type PC-12 (Rat adrenal gland pheochromocytoma

cell line) cells

Lane 2: p53 shRNA knockout PC-12 (Rat adrenal gland pheochromocytoma cell line) cells

Predicted band size: 53 kDa

Western blot analysis showing p53 expression in wild-type and p53 shRNA knockout in rat PC-12 cells. Beta-actin served as the loading control.

This analysis shows the antibodies specificity due to detection in the endogenous p53 protein in wild-type but not in shRNA knockdown cell lysate.

The lower band may represent the physiologically degraded p53 protein

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