abcam

Product datasheet

Anti-p53 antibody [PAb 240] ab26

KO VALIDATED

54 Abreviews 296 References 8 Images

Overview

Product name	Anti-p53 antibody [PAb 240]
Description	Mouse monoclonal [PAb 240] to p53
Host species	Mouse
Tested applications	**Suitable for:** IP, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Cow, Dog, Monkey, Chinese hamster, Syrian hamster
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Epitope	The epitope has been mapped to between amino acids 212 and 217 on human p53 (PMID: 1376364).
Positive control	WB: Wild-type HCT116 treated with irinotecan, A431, HeLa treated with bleomycin, NIH/3T3 treated with doxorubin, MCF7, MDA231. ICC/IF: A431. IP: HCT116 cell lysates
General notes	ab26 has been knockout validated in Western blot. The expected band was seen in wild type HCT116 cells treated with the DNA damaging agent irinotecan and no band was seen in *TP53* knockout HCT116 cells. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	PAb 240
Myeloma	Sp2
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab26 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
IP	★★★★★ (2)	Use at an assay dependent concentration.
ICC/IF	★★★★★ (25)	Use a concentration of 0.5 - 1 µg/ml.
WB	★★★★☆ (18)	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 53 kDa (predicted molecular weight: 53 kDa). Please note that expression of target protein may be very low without stimulation/treatment (e.g. DNA damaging agent). We recommend using 3% milk as the blocking agent for Western blot.

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 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.

Post-translational modifications

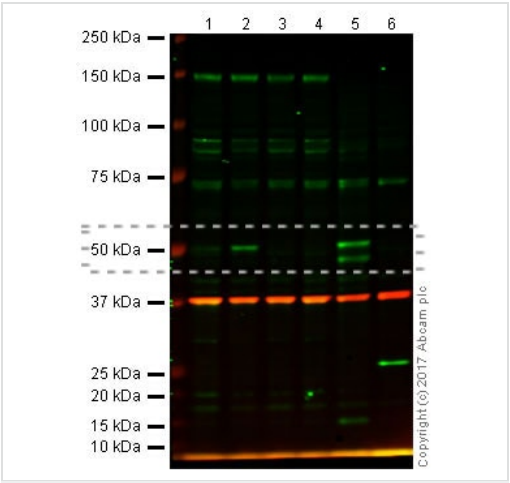
Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.
Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity). 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.

Images



Western blot - Anti-p53 antibody [PAb 240] (ab26)

All lanes : Anti-p53 antibody [PAb 240] (ab26) at 5 µg/ml

Lane 1 : Wild-type HCT116 cell lysate at 30 µg
Lane 2 : Wild-type HCT116 + irinotecan (10 µM, 24 hours) cell lysate at 30 µg
Lane 3 : p53 knockout HCT116 cell lysate at 30 µg
Lane 4 : p53 knockout HCT116 + irinotecan (10 µM, 24 hours) cell lysate at 30 µg
Lane 5 : A431 cell lysate (positive control) at 20 µg
Lane 6 : Saos-2 cell lysate (negative control) at 20 µg

Performed under reducing conditions.

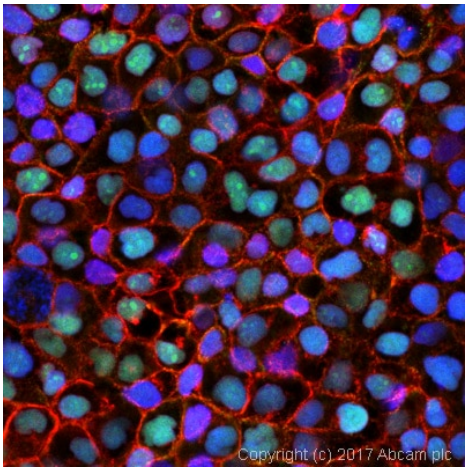
Predicted band size: 53 kDa
Observed band size: 53 kDa

Lanes 1-6: Merged (red and green) signal.

Ab26 was shown to specifically react with p53 in wild type HCT116 cells treated with irinotecan. No band was observed in p53 knockout HCT116 cells. Wild-type and p53 knockout samples,

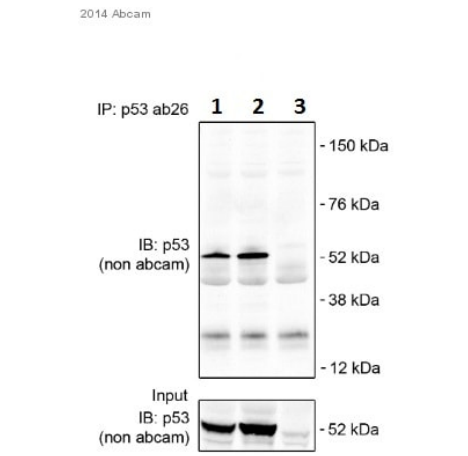
positive and negative controls were subjected to SDS-PAGE. Ab26 and **ab181602**(loading control to GAPDH) were diluted 5 µg/mL and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.

Wild-type and p53 knockout HCT116 cell lysates were kindly provided by a collaborator.



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [PAb 240] (ab26)

ab26 stained in A431 cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with ab26 at 1µg/ml and **ab6046** (Rabbit polyclonal to beta tubulin) at 1ug/ml overnight at +4°C. The secondary antibodies were **ab150177** (colored green) used at 1 ug/ml and **ab150087** (pseudo-colored red) used at 2ug/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Immunoprecipitation - Anti-p53 antibody [PAb 240] (ab26)

p53 was immunoprecipitated from 7x10^6 HCT116 (human colon carcinoma cell line) cells with ab26 at 1/150 dilution. Western blot was performed from the immunoprecipitate using anti-p53 antibody. Donkey Anti-Mouse IgG H&L (Alexa Fluor® 750) preadsorbed (**ab175739**) was used as secondary antibody at 1/5000 dilution.

Lane 1: HCT116 whole cell lysate 10 µg (Input).

Lane 2: **ab207799** IP in etoposide treated HCT116 whole cell lysate.

Lane 3: **ab207799** IP in etoposide treated HCT116 p53-/- whole cell lysate (negative control).

All lanes : Anti p53 antibody

All lanes :

Secondary

All lanes : Donkey Anti-Mouse IgG H&L (Alexa Fluor® 750)

preadsorbed (**ab175739**) at 1/5000 dilution

Western blot - Anti-p53 antibody [PAb 240] (ab26)

Anti-p53 antibody [PAb 240] (ab26) at 1 µg/ml + HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Secondary
Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

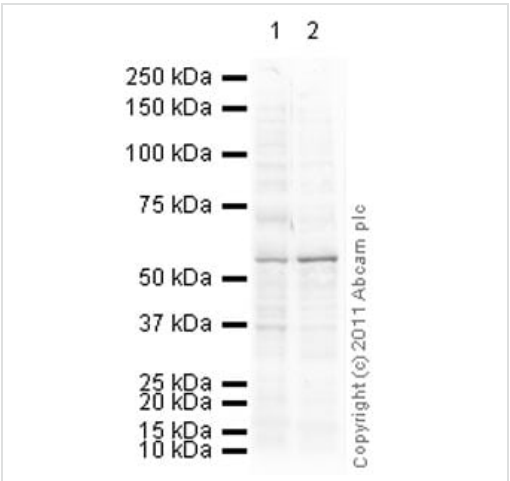
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 8 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab26 overnight at 4°C. Antibody binding was detected using an **anti-mouse HRP** secondary antibody, and visualised using ECL development solution **ab133406**



Western blot - Anti-p53 antibody [PAb 240] (ab26)

All lanes : Anti-p53 antibody [PAb 240] (ab26) at 5 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Hela Whole Cell Lysate - Bleomycin Treated (40U/ml)

Lysates/proteins at 20 µg per lane.

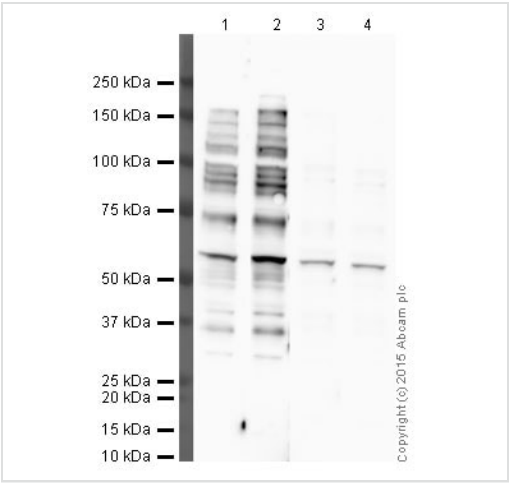
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 53 kDa

Exposure time: 4 minutes



Western blot - Anti-p53 antibody [PAb 240] (ab26)

Lanes 1-2 : Anti-p53 antibody [PAb 240] (ab26) at 1 µg/ml
Lanes 3-4 : Anti-p53 antibody [PAb 240] (ab26) at 5 µg/ml

All lanes : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

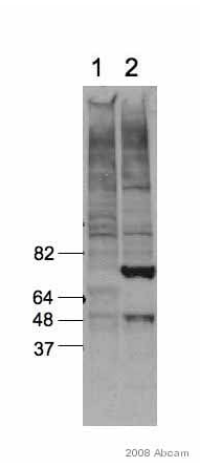
Predicted band size: 53 kDa

Exposure time: 4 minutes

Lanes 1-2: 1% BSA blocking buffer

Lanes 3-4: 3% Milk blocking buffer

We recommend using 3% milk as the blocking agent for Western blot.



Western blot - Anti-p53 antibody [PAb 240] (ab26)

This image is courtesy of an Abreview submitted by Dr Cherie Blenkiron

All lanes : Anti-p53 antibody [PAb 240] (ab26) at 1/2000 dilution

Lane 1 : Human breast cancer cell-line, MCF7 cells (p53 WT), whole cell lysate
Lane 2 : Human breast cancer cell-line, MDA231 cells (p53 Mutant), whole cell lysate

Lysates/proteins at 20 µg per lane.

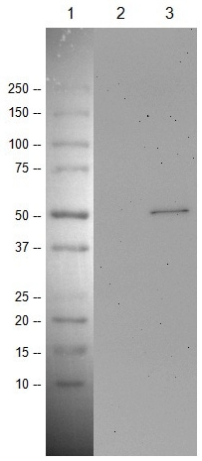
Secondary
All lanes : HRP conjugated donkey anti-mouse antibody at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 53 kDa
Observed band size: 53 kDa
Additional bands at: 72 kDa (possible non-specific binding)

Exposure time: 10 seconds



Western blot - Anti-p53 antibody [PAb 240] (ab26)

Primary: All Lanes: Anti-p53 antibody (ab26) at 5 µg/mL. Lane 1: MW marker. Lane 2: NIH/3T3 cells treated with vehicle for 24 hours. Lane 3: NIH/3T3 cells treated with 1 µM doxorubicin for 24 hours Secondary: All Lanes: HRP-conjugated VeriBlot anti-Mouse IgG (**ab131368**) 1:1000. Lysates at 20 µg/lane. Performed under denaturing conditions. Developed using ECL technique. Blocking buffer: 5% milk in PBS.

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