

Product datasheet

Anti-p53 antibody [PAb 1801] ab28

KO VALIDATED

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Overview

Product name	Anti-p53 antibody [PAb 1801]
Description	Mouse monoclonal [PAb 1801] to p53
Host species	Mouse
Specificity	This monoclonal p53 antibody recognizes both wild type and mutant forms of human p53 protein. The epitope has been mapped to amino acids 46-55 of human p53. Due to low sequence homology with the epitope, we do not expect ab28 to react with mouse and rat samples so cannot guarantee reactivity in these species. Please note that the following publications suggest that the PAb 1801 clone also cross reacts with an unknown component of processing bodies (P-bodies) (PMID: 11313875; PMID: PMC3349707). Please ensure that appropriate controls (e.g. MW ladder, treatment to induce p53 expression) are included to ensure the positive signal is from p53.
Tested applications	Suitable for: ChIP, WB, IP, ELISA, RIA
Species reactivity	Reacts with: Human
Immunogen	Fusion protein corresponding to Human p53 (N terminal). Database link: P04637
Epitope	aa 46-55 of human p53
Positive control	WB: A431 cell lysate, MDA-MB-231 cell lysate.
General notes	This monoclonal p53 antibody has been knockout validated in Western blot. The expected band for p53 was observed in HCT116 cells treated with irinotecan and the band was not seen in <i>TP53</i> knockout HCT116 cell lysate. For Western blot, we recommend using 3% milk as the blocking agent. We recommend using ab1101 or ab154036 , alternative mouse monoclonal antibodies, to detect human p53 by IHC, ICC/IF or flow cytometry since they perform better in these applications. This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here .

Properties

Form Liquid

Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
	Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.
Purity	Protein G purified
Clonality	Monoclonal
Clone number	PAb 1801
Myeloma	NS1
Isotype	IgG1
Light chain type	kappa

Applications

Our [Abpromise guarantee](#) covers the use of **ab28** 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
ChIP		Use at an assay dependent concentration. Use at an assay dependent concentration.
WB	★★★★☆	Use a concentration of 5 µg/ml. Detects a band of approximately 53 kDa (predicted molecular weight: 53 kDa). We recommend using 3% milk as the blocking agent for Western blot.
IP	★★★★★	Use at 10 µg/mg of lysate.
ELISA		Use at an assay dependent concentration.
RIA		Use at an assay dependent concentration.

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.

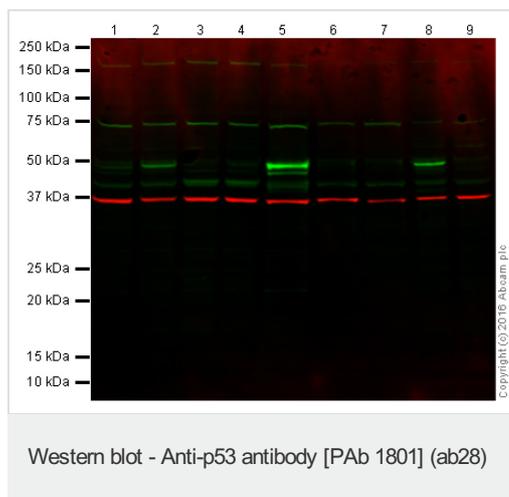
Ubiquitinated by MDM2 and SYVN1, which leads to proteasomal degradation. Ubiquitinated by RFWD3, which works in cooperation with MDM2 and may catalyze the formation of short polyubiquitin chains on p53/TP53 that are not targeted to the proteasome. Ubiquitinated by MKRN1 at Lys-291 and Lys-292, which 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.

Images



All lanes : Anti-p53 antibody [PAb 1801] (ab28) 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

Lane 7 : MEF cell lysate at 20 µg

Lane 8 : Wild-type HAP1 cell lysate at 20 µg

Lane 9 : p53 knockout HAP1 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 53 kDa

Lanes 1-9: Merged (red and green) signal.

Ab28 was shown to specifically react with p53 in wild-type HAP1 and HCT116 cells treated with irinotecan. No band was observed in p53 knockout HAP1 or HCT116 cells. Wild-type and p53 knockout samples, positive and negative controls were subjected to SDS-PAGE. Ab28 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.



Western blot - Anti-p53 antibody [PAb 1801] (ab28)

Anti-p53 antibody [PAb 1801] (ab28) at 5 µg/ml + MDA-MB-231 (Human breast adenocarcinoma cell line) Whole Cell Lysate at 20 µg

Secondary

Goat Anti-Mouse IgG H&L (HRP) preadsorbed ([ab97040](#)) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

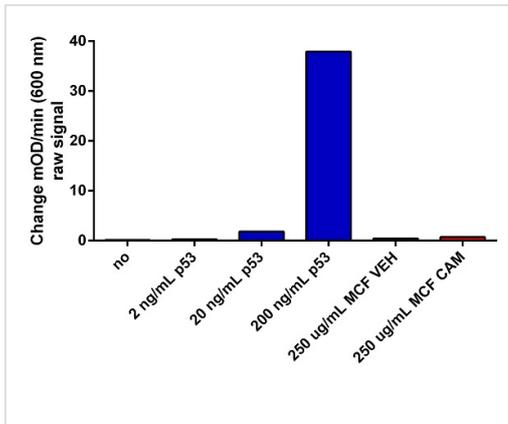
Predicted band size: 53 kDa

Additional bands at: 53 kDa, 60 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 20 minutes

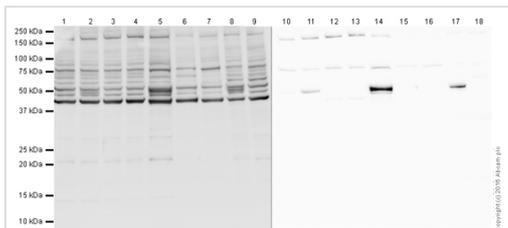
This blot was produced using a 10% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab28 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution.

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



ELISA - Anti-p53 antibody [PAb 1801] (ab28)

Analysis of recombinant p53 protein ([ab43615](#)) in MCF7 cells treated with vehicle (VEH) as well as MCF7 cells treated with camptothecin (CAM) and no material (NO) by sandwich ELISA with the use of anti-53 antibody (ab28) as capture and anti-p53 antibody ([ab32389](#)) as detector.



Western blot - Anti-p53 antibody [PAb 1801] (ab28)

All Lanes: Anti-p53 antibody [PAb 1801] (ab28) at 5 µg/ml

Lane 1 and 10: Wild-type HCT116 cell lysate

Lane 2 and 11: Wild-type HCT116 + irinotecan (10 µM, 24 hours) cell lysate

Lane 3 and 12: p53 knockout HCT116 cell lysate

Lane 4 and 13: p53 knockout HCT116 + irinotecan (10 µM, 24 hours) cell lysate

Lane 5 and 14: A431 cell lysate (positive control)

Lane 6 and 15: Saos-2 cell lysate (negative control)

Lane 7 and 16: MEF cell lysate

Lane 8 and 17: Wild-type HAP1 cell lysate

Lane 9 and 18: p53 knockout HAP1 cell lysate

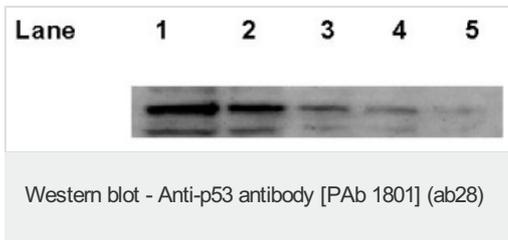
Lanes 1-9: 1% BSA blocking buffer

Lanes 10-18: 3% Milk blocking buffer

Secondary

Goat Anti-Mouse IgG H&L (HRP) at 1/5000 dilution

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



The image shows a Western blot for ab28, testing the threshold detection of the p53 antibody. The lanes are as follows:

Lane 1 - 20ug of A549 lysate, lane 2 - 10ug of A549 lysate, lane 3 - 5ug of A549 lysate, lane 4 - 2.5ug of A549 lysate, lane 5 - 1.5ug of A549 lysate.

This picture was submitted as part of the review by Craig Carson.

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