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Product datasheet

Anti-p53 antibody [9D3DE3] ab154036

KO VALIDATED

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Overview

Product name	Anti-p53 antibody [9D3DE3]
Description	Mouse monoclonal [9D3DE3] to p53
Host species	Mouse
Tested applications	**Suitable for:** WB, IP, Flow Cyt, In-Cell ELISA, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Rabbit, Guinea pig, Chimpanzee, Cynomolgus monkey, Rhesus monkey, Chinese hamster **Does not react with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293 whole cell lysate, MCF7 cells treated with camptothecin. IHC-P - Human colon adenocarcinoma FFPE tissue sections ICC/IF: HEK293 cells.
General notes	This monoclonal antibody to p53 has been knockout validated in Western blot and ICC/IF. The expected signal for p53 was observed in wild type cells and the signal was not seen in knockout 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 Product was previously marketed under the MitoSciences sub-brand.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

Storage buffer	pH: 7.5 Preservative: 0.02% Sodium azide Constituents: 0.88% Sodium chloride, 0.36% HEPES
Purity	Ammonium Sulphate Precipitation
Purification notes	Near homogeneity as judged by SDS-PAGE. ab154036 was produced in vitro using hybridomas grown in serum-free medium, and then concentrated by ammonium sulfate precipitation.
Clonality	Monoclonal
Clone number	9D3DE3
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab154036 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	★★★★★ (1)	Use a concentration of 2 µg/ml. Detects a band of approximately 53 kDa.
IP	★☆☆☆☆ (1)	Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
In-Cell ELISA		Use at an assay dependent concentration.
ICC/IF		Use a concentration of 1 µg/ml.
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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

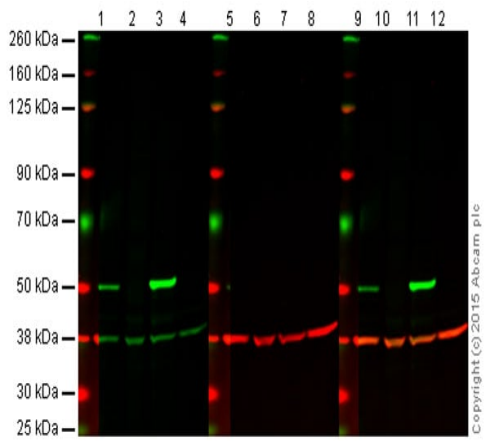
Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.
Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity).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

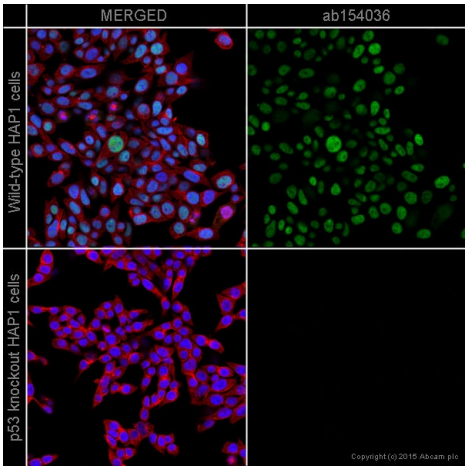


Western blot - Anti-p53 antibody [9D3DE3] (ab154036)

Lanes 1, 5 and 9: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 6 and 10: p53 knockout HAP1 cell lysate (20 µg)
Lanes 3, 7 and 11: A431 cell lysate (20 µg)
Lanes 4, 8 and 12: Saos-2 cell lysate (20 µg)
Lanes 1, 2, 3 and 4: Green signal from target – ab154036 observed at 53 kDa
Lanes 5, 6, 7 and 8: Red signal from loading control – **ab181602** observed at 37 kDa
Lanes 9, 10, 11 and 12: Merged (red and green) signal

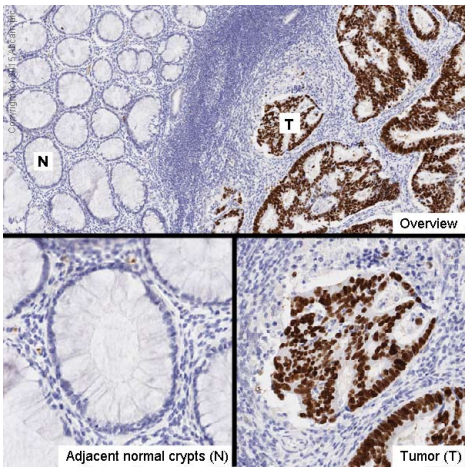
ab154036 was shown to specifically react with p53 in wild type HAP1 cells. No band was observed when p53 knockout samples were used. Wild-type and p53 knockout samples were subjected to SDS-PAGE. ab154036 and **ab181602** (loading control to GAPDH) were diluted 2 µg/mL and 1/10000 respectively and incubated overnight at 4ºC. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed **ab216772** and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed **ab216777**

secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



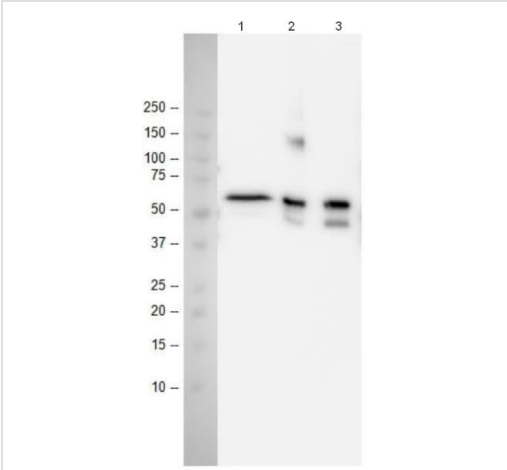
Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [9D3DE3] (ab154036)

ab154036 staining p53 in wild-type HAP1 cells (top panel) and p53 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab154036 at 1µg/ml concentration and **ab202272** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [9D3DE3] (ab154036)

IHC image of ab154036 staining beta Catenin in human colon adenocarcinoma formalin-fixed paraffin-embedded tissue sections*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab154036, 1/500 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. High magnification of the tumor region - T (lower right panel) and adjacent normal crypts - N (lower left panel) are shown.
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



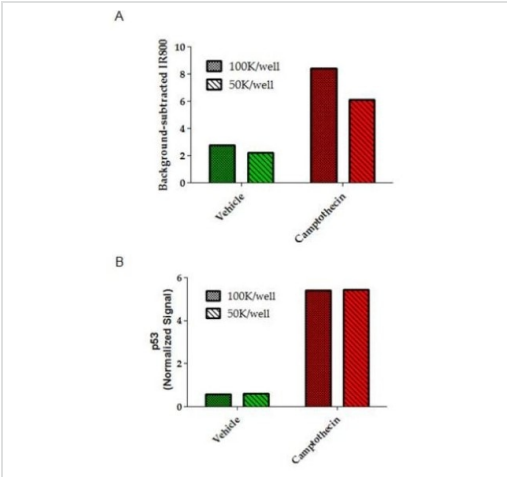
Immunoprecipitation - Anti-p53 antibody [9D3DE3] (ab154036)

Figure 2: p53 (ab154036) antibody specificity demonstrated by immunoprecipitation, followed by Western blot

Lane 1: Hek293 whole cell extract
Lane 2: anti-p53 (ab154036) IP using Hek293 cells extracted with RIPA buffer
Lane 3: anti-p53 (ab154036) IP using Hek293 cells extracted with Lauryl Maltoside (**ab109857**)

Western blot with anti-p53 (**ab32389**) 1/1000.

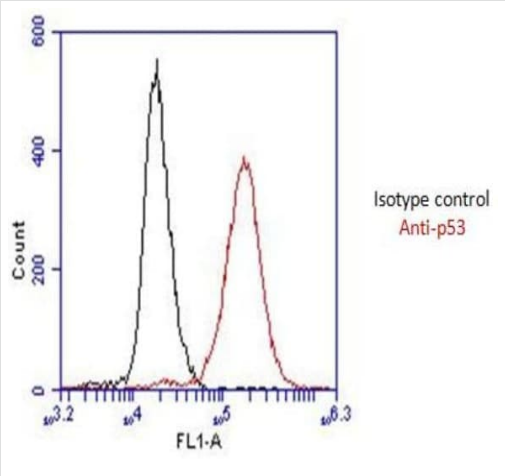
Secondary goat anti-rabbit IgG 1/5000.



In-Cell ELISA - Anti-p53 antibody [9D3DE3] (ab154036)

Figure 4: In-cell ELISA with anti-p53 antibody (ab154036)

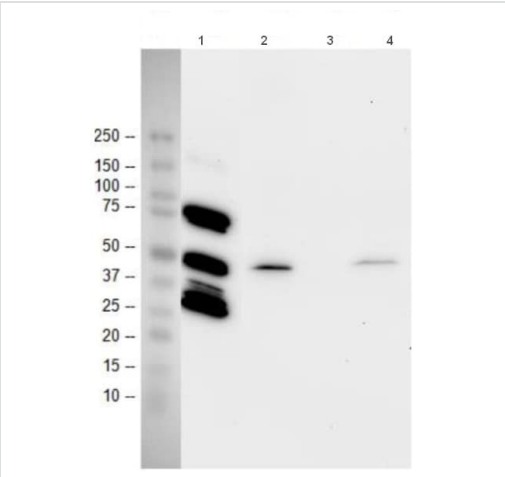
Standard In-Cell ELISA protocol was performed on vehicle- and camptothecin-treated MCF7 cells using 1 µg/mL anti-p53 primary antibody and IR800-conjugated goat anti-mouse IgG secondary antibody. Cells were imaged using a LI-COR® Odyssey near-infrared scanner. The data is presented as background-subtracted IR800 signals (A) or Janus green-normalized signals (B).



Flow Cytometry - Anti-p53 antibody [9D3DE3] (ab154036)

Figure 5: Flow Cytometry with anti-p53 antibody (ab154036) using Hek293 cells

Standard flow cytometry procedure was performed on Hek293 cells that were fixed and permeabilized with methanol and stained with 1 µg/mL of anti-p53 antibody (red) or a negative isotype control antibody (black). 1% BSA in PBS was used as the blocking reagent for all the blocking steps.



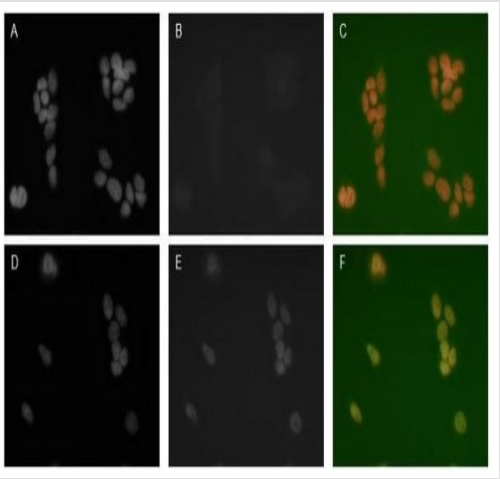
Western blot - Anti-p53 antibody [9D3DE3] (ab154036)

All lanes : Anti-p53 antibody [9D3DE3] (ab154036) at 2 µg/ml

Lane 1 : Recombinant Human p53 protein (**ab43615**) at 0.002 µg
Lane 2 : Hek293 cells at 40 µg
Lane 3 : 6 hour vehicle-treated MCF7 cells at 40 µg
Lane 4 : 6 hour camptothecin-treated MCF7 cells at 40 µg

Secondary
All lanes : Goat polyclonal to Mouse IgG - HRP at 1/5000 dilution

Observed band size: 53 kDa



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [9D3DE3] (ab154036)

Figure 3: Immunocytochemistry with anti-p53 antibody (ab154036) using camptothecin-treated MCF7 cells

MCF7 cells were treated with vehicle (A, B and C) or 1 µM camptothecin (D, E, and F) for 6 hours to induce p53 expression. The cells were fixed, permeabilized, blocked and incubated with 1 µg/mL of the p53 antibody. Samples were further processed for fluorescence immunocytochemistry and co-stained with the DNA stain DAPI. Images of DAPI signals (A and D), p53 signals (B and E) and overlays of DAPI (artificially colored red for better contrast) and anti-p53 (artificially colored green) images (C and F) are shown.

Note that the p53 antibody specifically labels nuclei of camptothecin-treated cells.

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