abcam

ab154036

Anti-p53 antibody [9D3DE3]

Lab Essentials | KO Validated

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.5 Preservative: 0.02% Sodium azide Constituents: 0.88% Sodium chloride, 0.36% HEPES
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	9D3DE3
Purification technique	Precipitation Ammonium Sulphate
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
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.

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/1000

Notes	-

Not recommended

Species	Mouse, Rat, Chimpanzee, Cynomolgus monkey, Rhesus monkey
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Not recommended

Species	Mouse, Rat, Chimpanzee, Cynomolgus monkey, Rhesus monkey
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	2 µg
Notes	-

Not recommended

Species	Mouse, Rat, Chimpanzee, Cynomolgus monkey, Rhesus monkey
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Mouse
Dilution info	-
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Species	Rat
Dilution info	-
Notes	ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Species	Chimpanzee
Dilution info	-
Notes	-

Species	Cynomolgus monkey
Dilution info	-
Notes	-

Species	Rhesus monkey
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	2 µg/mL

Notes	-

Not recommended

Species	Mouse, Rat, Chimpanzee, Cynomolgus monkey, Rhesus monkey
Dilution info	-
Notes	-

In-Cell ELISA

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Chimpanzee, Cynomolgus monkey, Rhesus monkey
Dilution info	-
Notes	-

Target data

See full target information TP53

Function

Multifunctional transcription factor that induces cell cycle arrest, DNA repair or apoptosis upon binding to its target DNA sequence (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492, PubMed:24652652, PubMed:35618207, PubMed:36634798, PubMed:38653238, PubMed:9840937). Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17189187, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492, PubMed:24652652, PubMed:38653238, PubMed:9840937). Negatively regulates cell division by controlling expression of a set of genes required for this process (PubMed:11025664, PubMed:12524540, PubMed:12810724, PubMed:15186775, PubMed:15340061, PubMed:17317671, PubMed:17349958, PubMed:19556538, PubMed:20673990, PubMed:20959462, PubMed:22726440, PubMed:24051492,

PubMed:24652652, PubMed:9840937). 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 (PubMed:12524540, PubMed:17189187). Its pro-apoptotic activity is activated via its interaction with PPP1R13B/ASPP1 or TP53BP2/ASPP2 (PubMed:12524540). However, this activity is inhibited when the interaction with PPP1R13B/ASPP1 or TP53BP2/ASPP2 is displaced by PPP1R13L/iASPP (PubMed:12524540). In cooperation with mitochondrial PPIF is involved in activating oxidative stress-induced necrosis; the function is largely independent of transcription. Induces the transcription of long intergenic non-coding RNA p21 (lincRNA-p21) and lincRNA-Mkln1. LincRNA-p21 participates in TP53-dependent transcriptional repression leading to apoptosis and seems to have an effect on cell-cycle regulation. Implicated in Notch signaling cross-over. Prevents CDK7 kinase activity when associated to CAK complex in response to DNA damage, thus stopping cell cycle progression. 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. Regulates the circadian clock by repressing CLOCK-BMAL1-mediated transcriptional activation of PER2 (PubMed:24051492).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

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.
Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The protein p53 also known as TP53 or tumor protein p53 has a molecular weight of approximately 53 kDa. It acts as a transcription factor and plays a major role in

	cell cycle regulation apoptosis and maintaining genomic stability. This protein mainly expresses in the nucleus of cells and acts as a critical regulator of cellular responses to stress signals including DNA damage. Scientists commonly use p53 antibodies in various assays like western blot and p53 immunofluorescence to detect and study its expression and functional status in cells.
Biological function summary	P53 functions to control cell division and apoptosis serving as a guardian of the genome by preventing mutation accumulation. It does not form part of a larger complex under normal conditions but interacts with various other molecules to execute its functions. p53 can activate or suppress the transcription of numerous genes involved in cell cycle arrest DNA repair and programmed cell death allowing it to halt the progression of damaged cells and trigger repair mechanisms or eliminate those that cannot be repaired.
Pathways	P53 acts within several key biological pathways such as the p53 signaling pathway and the intrinsic apoptotic pathway. Its activity involves interaction with proteins like MDM2 which regulates p53 through ubiquitin-mediated degradation and ATM kinase which phosphorylates p53 in response to DNA damage. These interactions ensure appropriate cellular responses during stress and are vital for maintaining homeostasis.
Associated diseases and disorders	P53 mutation or inactivation is often associated with the development of cancer given its role in controlling cell division and preventing tumor formation. Specifically its dysfunction has been linked to cancers such as breast cancer and lung cancer. Additionally p53 can interact with other mutant proteins like Ras compounding mutations that contribute to tumor progression and aggressive cancer phenotypes. Understanding these interactions and the status of p53 can be important in developing targeted cancer therapies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

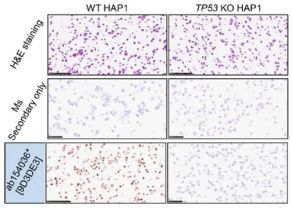
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

12 product images

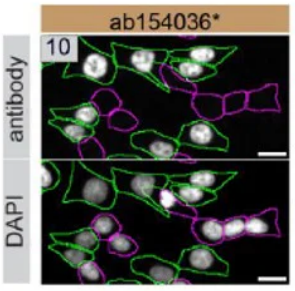


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [9D3DE3] (ab154036)

P53 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of wild-type HAP1 cells using mouse Anti-p53 antibody

ab154036 was shown to react with TP53 in wild-type HAP1 cells in immunohistochemistry with loss of signal observed in a TP53 knockout cell line. HAP1 WT and TP53 KO cells were fixed and paraffin embedded. Sections were mounted onto slides and stained with ab154036 and the corresponding multimer HRP secondary. Chromogen image acquisition was performed, and representative images of each section are shown. Scale bar = 50 µm.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

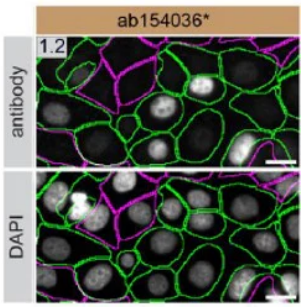


Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [9D3DE3] (ab154036)

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

ab154036 was shown to react with TP53 in wild-type HAP1 cells in immunocytochemistry with loss of signal observed in a TP53 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab154036 at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

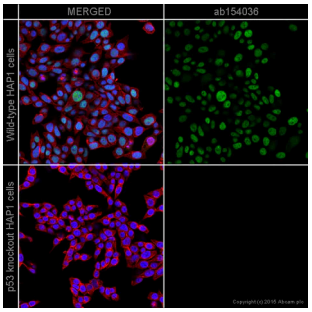


Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [9D3DE3] (ab154036)

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

ab154036 was shown to react with TP53 in wild-type MCF7 cells in immunocytochemistry with loss of signal observed in TP53 knockout cell line ab286440. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab154036 at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

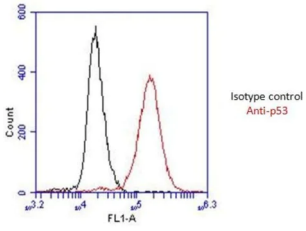
This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



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

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

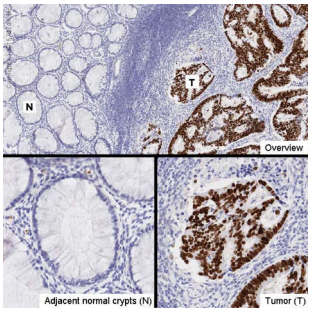
ab154036 staining p53 in wild-type HAP1 cells (top panel) and p53 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells 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.



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

P53 Flow Cytometry staining using mouse Anti-p53 antibody

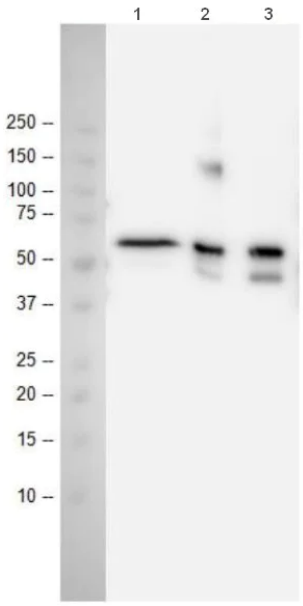
Figure 5: Flow Cytometry with anti-p53 antibody (ab154036) using Hek293 cells
Standard flow cytometry procedure was performed on Hek293 cells that were fixed and permeabilized with methanol and stained with 1 µg/mL of anti-p53 antibody (red) or a negative isotype control antibody (black). 1% BSA in PBS was used as the blocking reagent for all the blocking steps.



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

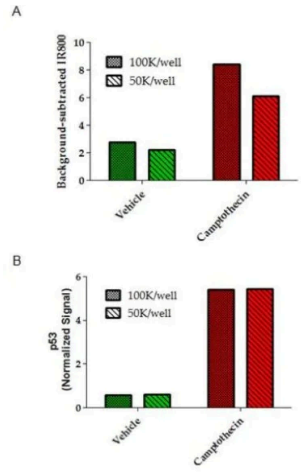
Lane 1: Hek293 whole cell extract
Lane 2: anti-p53 (ab154036) IP using Hek293 cells extracted with RIPA buffer
Lane 3: anti-p53 (ab154036) IP using Hek293 cells extracted with Lauryl Maltoside (ab109857)

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

Secondary goat anti-rabbit IgG 1/5000.
All lanes:
Immunoprecipitation - Anti-p53 antibody [9D3DE3] (ab154036)

Predicted band size: 43 kDa

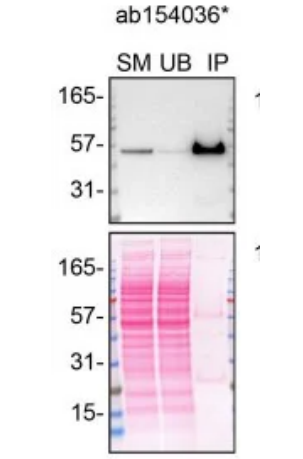
Observed band size: 53 kDa



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).

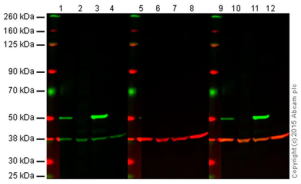


Immunoprecipitation - Anti-p53 antibody [9D3DE3] (ab154036)

Immunoprecipitation of TP53 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2 µg of ab154036 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Immunoprecipitation - Anti-p53 antibody [9D3DE3] (ab154036) at 2 µg

All lanes:
HAP1 cells



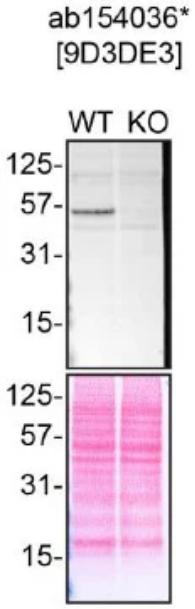
Western blot - Anti-p53 antibody [9D3DE3] (ab154036)

P53 Western blot staining using mouse Anti-p53 antibody

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 4°C. 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.

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

Predicted band size: 43 kDa



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

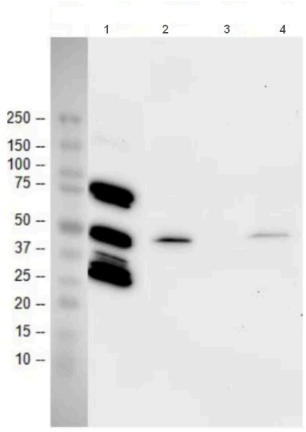
P53 Western blot staining using mouse Anti-p53 antibody

ab154036 was shown to react with TP53 in wild-type HAP1 cells in Western blot with loss of signal observed in a TP53 knockout cell line. Wild-type HAP1 and TP53 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab154036 overnight at 4 °C at a 1/500 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-p53 antibody [9D3DE3] (ab154036) at 1/500 dilution

Lanes 1 - 2:
Wild-type HAP1 lysate at 20 µg

Lanes 1 - 2:
TP53 knock-out HAP1 lysate at 20 µg



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

P53 Western blot staining using mouse Anti-p53 antibody

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

Lane 1:
Western blot - Recombinant Human p53 protein (GST tag N-Terminus) (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

Predicted band size: 43 kDa

Observed band size: 53 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.