abcam

ab1101

Anti-p53 antibody [DO-1]

Anti-p53 antibody [DO-1] (ab1101) is a mouse monoclonal antibody detecting p53 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Over 160 publications
- Trusted since 2002

Lab Essentials | KO Validated

Key facts

Isotype	IgG2a
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	DO-1
Purification technique	Affinity purification Protein G
Specificity	This antibody clone recognises both wild-type and mutant forms of p53 in human samples. It is not designed to recognise any specific p53 mutation. We have confirmed this experimentally and have been able to detect p53 in different cell lines using various applications and treatments. Important note: p53 expression levels vary greatly between cell lines. It has been reported that p53 mutations render the protein more stable, hence mutated cell lines often express higher levels of the p53 protein compared to wild-type cell lines. For low expressing wild type cell lines, p53 expression can be increased with cell treatments such as camptothecin or irinotecan.
Epitope	The epitope maps to within aa 20-25.
Light chain type	kappa

Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	2 µg
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ChIP

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-

Notes	-

IHC-P

Tested

Species	Human
Dilution info	1-5 µg/mL
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Not recommended

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Mouse
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
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-p53 antibody [DO-1] (ab1101) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of p53?
Anti-p53 [DO-1] (ab1101) specifically detects a band for p53 (UniProt: P04637) at a molecular weight of 43kDa.

Trusted by the scientific community
Anti-p53 [DO-1] (ab1101) was first used in a scientific publication in 2002 and has been cited over 160 times in peer-

reviewed journals.

Reviewed by scientists
Anti-p53 [DO-1] (ab1101) has over 10 independent reviews from customers.

Specificity confirmed
The specificity of Anti-p53 antibody [DO-1] (ab1101) has been confirmed by Western blot testing in TP53 Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [DO-1] also available for your convenience: ab1101, HRP - ab204452, Carrier free - ab237976

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

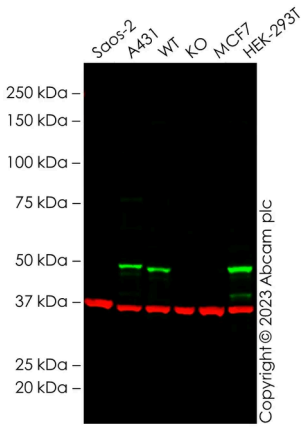
We do not recommend this combination. It is not covered by our product promise.

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.

21 product images



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

False colour image of Western blot: Anti-p53 antibody [DO-1] - ChIP Grade staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab1101 was shown to bind specifically to p53. A band was observed at 50 kDa in wild-type HAP1 cell lysate with no signal observed at this size in tp53 knockout cell line. To generate this image, wild-type and tp53 knockout HAP1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
All lanes:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 1/1000 dilution

Lane 1:
Saos-2 cell lysate at 20 µg

Lane 2:
A431 cell lysate

Lane 3:
Wild-type HAP1 cell lysate at 20 µg

Lane 4:
TP53 knockout HAP1 cell lysate at 20 µg

Lane 5:
MCF7 cell lysate at 20 µg

Lane 6:
HEK-293T cell lysate at 20 µg

Secondary

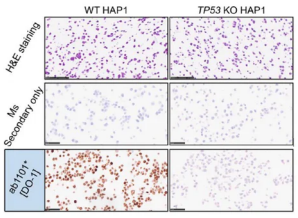
Lanes 1 - 6:
Western blot - Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/20000 dilution

Lanes 1 - 6:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 43 kDa

Observed band size: 50 kDa

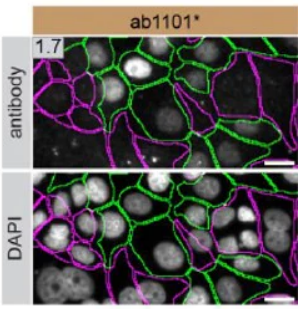


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [DO-1] (ab1101)

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

ab1101 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 ab1101 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.



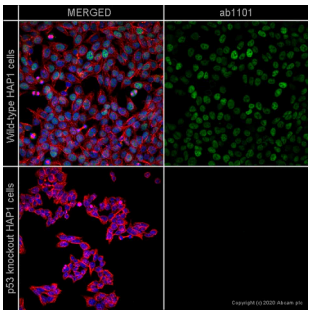
Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

ab1101 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 ab1101 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).

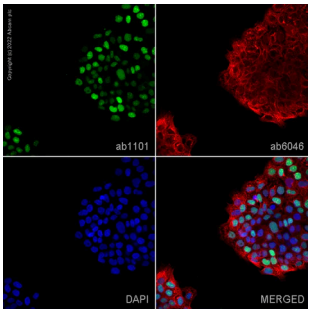
This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate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



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

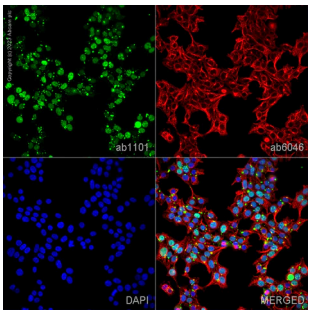
ab1101 staining p53 in wild-type Hap1 cells (top panel) and p53 knockout Hap1 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab1101 at 1µg/ml concentration and ab6046 (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution 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) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (ab150080) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining of A431 cells using mouse Anti-p53 antibody

ab1101 staining mutant p53 in A431 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab1101 at 0.2µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 100% methanol (5 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

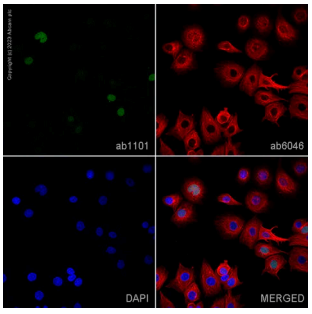


Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining of Hek293 cells (a high expressing cell line) using mouse Anti-p53 antibody

ab1101 staining wild-type p53 in Hek293 cells (a high expressing cell line). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then

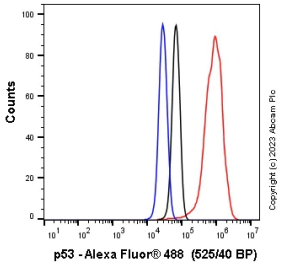
blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab1101 at 0.2µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining of MCF7 cells (a low expressing cell line) using mouse Anti-p53 antibody

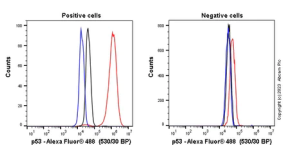
ab1101 staining wild-type p53 in MCF7 cells (a low expressing cell line). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab1101 at 0.2µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] (ab1101)

P53 Flow Cytometry (Intracellular) staining of A431 cells using mouse Anti-p53 antibody

Flow cytometry overlay histogram showing mutant p53 in A-431 cells stained with ab1101 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab1101) (1x 106 cells in 100µl at 0.04µg/ml (1/25000)) for 30min at 22°C. The secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody (black line) Mouse IgG2a, Kappa Monoclonal [MOPC-173] - Isotype Control - ChIP Grade (ab18413) was used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control. Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. This antibody gave a positive signal in A-431 fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

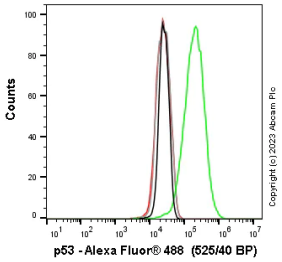


Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] (ab1101)

P53 Flow Cytometry (Intracellular) staining using mouse Anti-p53 antibody

Flow cytometry overlay histogram showing wild-type p53 in Hek-293 positive cells (left) and MCF7 negative cells (right) stained with ab1101 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab1101) (1x 106 cells in 100µl at 0.2µg/ml (1/5000)) for 30min at 22°C. The secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 488)

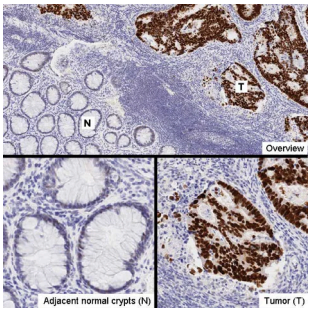
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Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] (ab1101)

P53 Flow Cytometry (Intracellular) staining using mouse Anti-p53 antibody

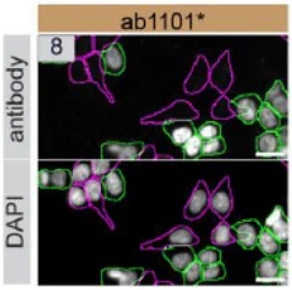
Flow cytometry overlay histogram showing mutant p53 in wild-type HAP1 (green line) and TP53 knockout HAP1 cells (red line) stained with ab1101. The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab1101) (1x 106 cells in 100µl at 0.04 µg/ml (1/25000)) for 30min at 22°C.The secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (ab150117) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody Mouse IgG2a, Kappa Monoclonal [MOPC-173] - Isotype Control - ChIP Grade (ab18413) was used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line, TP53 knockout HAP1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. This antibody gave a positive signal in HAP1 fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-p53 antibody

IHC image of ab1101 staining p53 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 (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab1101, 1/500 dilution, for 15 minutes 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

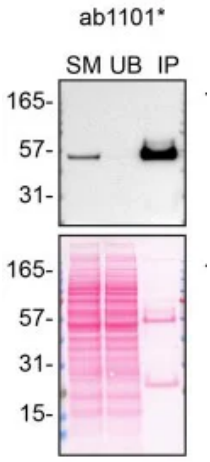


Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] (ab1101)

P53 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-p53 antibody

ab1101 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 ab1101 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.

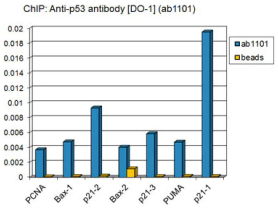


Immunoprecipitation - Anti-p53 antibody [DO-1] (ab1101)

Immunoprecipitation of TP53 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2 µg of ab1101 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

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All lanes:
Immunoprecipitation - Anti-p53 antibody [DO-1] (ab1101) at 2 µg

All lanes:
HAP1 cells



ChIP - Anti-p53 antibody [DO-1] (ab1101)

Chromatin was prepared from HEK-293 (human epithelial cell line from embryonic kidney) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 2 µg of ab1101, and 10 ml of protein A sepharose beads,10 ml of protein G sepharose beads. No IgG was added to the beads control. The immunoprecipitated DNA was quantified by real time PCR. Primers were as follows:
Bax-1, forward: GGGTTATCTCTTGGGCTCACAA.

Bax-1, reverse: GAGCTCTCCCCAGCGCA.

Bax-2, forward: TGG AGC TGC AGA GGA TGA TTG

Bax-2, reverse: CCA GTT GAA GTT GCC GTC AGA

PUMA, forward: ATG CCT GCC TCA CCT TCA TC

PUMA, reverse: TCA CAC GTC GCT CTC TCT AAA CC

p21-1, forward: GCT GTG GCT CTG ATT GGC TTT

p21-1, reverse: ACA GGC AGC CCA AGG ACA AA

p21-2, forward: CAT CCC CAC AGC AGA GGA GAA

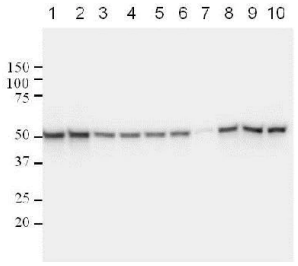
p21-2, reverse: ACC CAG GCT TGG AGC AGC TA

p21-3, forward: GAG TCC TGT TTG CTT CTG GGC A

p21-3, reverse: CTG CAT TGG GGC TGC CTA TGT A

PCNA, forward: CCA CCA TAA AGC TGG GGC TT

PCNA, reverse: TCT CCC CGC CTC TTT GAC TC



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

Developed using the ECL technique with 30 second exposure.
Blocking: 5% milk in PBS for 3 hours at RT.
Primary antibody in 5% BSA + PBS was incubated overnight at 4°C.
Secondary antibody in 5% milk + PBS was incubated for 2 hours at RT.
All lanes:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 0.4 µg/mL

Lane 1:
Extract of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells incubated with vehicle at 20 µg

Lane 2:
Extract of HEK-293T cells incubated with etoposide at 20 µg

Lane 3:
Extract of HEK-293T cells incubated with etoposide at 7.5 µg

Lane 4:
Lambda phosphatase (diluted 1/400)-treated extract of HEK-293T cells incubated with etoposide at 7.5 µg

Lane 5:
Lambda phosphatase (diluted 1/100)-treated extract of HEK-293T cells incubated with etoposide at 7.5 µg

Lane 6:
Lambda phosphatase (diluted 1/25)-treated extract of HEK-293T cells incubated with etoposide at 7.5 µg

Lane 7:
Extract of MCF7 (human breast adenocarcinoma cell line) cells incubated with vehicle at 20 µg

Lane 8:
Extract of MCF7 cells incubated 6 hours with camptothecin at 20 µg

Lane 9:
Extract of MCF7 cells incubated 16 hours with camptothecin at 20 µg

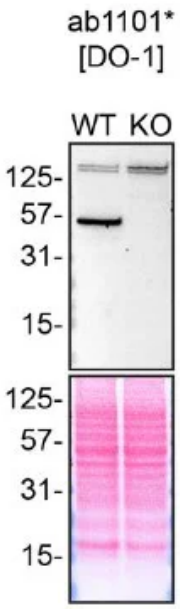
Lane 10:
Extract of MCF7 cells incubated 24 hours with camptothecin at 20 µg

Secondary

All lanes:
Goat anti mouse IgG(H&L)-HRP at 1/10000 dilution

Predicted band size: 43 kDa

Observed band size: 53 kDa



Western blot - Anti-p53 antibody [DO-1] (ab1101)

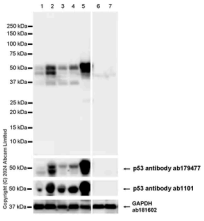
P53 Western blot staining using mouse Anti-p53 antibody

ab1101 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 ab1101 overnight at 4 °C at a 1/1000 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 [DO-1] (ab1101) at 1/1000 dilution

Lane 1:
Wild-type HAP1 lysate at 20 µg

Lane 2:
TP53 knock-out HAP1 lysate at 20 µg



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: HL-60 (p53 null), NCI-H1299 (p53 null).

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 21941372, PMID: 21779513, PMID: 25996291).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-p53 antibody [DO-1] - ChIP Grade (ab1101) staining at 1/1000 dilution.

In Western blot, Anti-p53 antibody [EPR17343] (ab179477) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-TP53 (phospho S376+S377+S392) antibody [RM1161] (ab322465) at 1/1000 dilution

Lane 1:
Untreated HCT 116 (human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HCT 116 treated with 20uM etoposide for 15 hours whole cell lysate at 20 µg

Lane 3:
Untreated A549 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
A549 treated with 500nM doxorubicin for 24 hours whole cell lysate at 20 µg

Lane 5:
A431 (human epidermoid carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
HL-60 (human acute promyelocytic leukemia promyeloblast) whole cell lysate at 20 µg

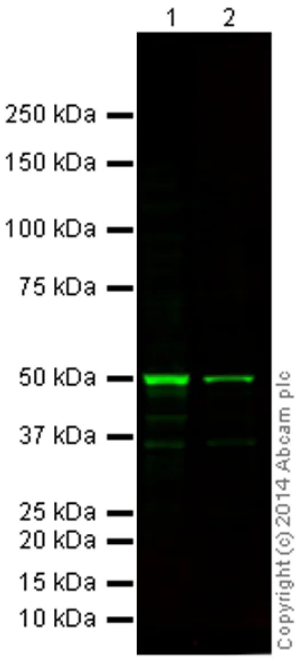
Lane 7:
NCI-H1299 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 53 kDa, 26-48 kDa, 36 kDa

Exposure time: 10s



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

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 5% Milk before being incubated with ab1101 overnight at 4°C. Antibody binding was detected using ab175783 at a 1/10,000 dilution for 1 hour at room temperature and then imaged using the Licor Odyssey CLx.
All lanes:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney cell line) whole cell lysate at 20 µg

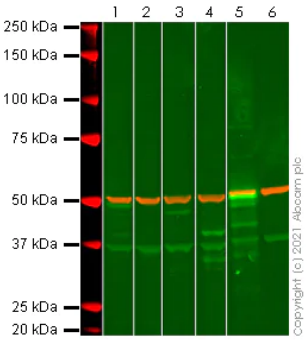
Lane 2:
DU 145 (human prostate carcinoma cell line) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Predicted band size: 43 kDa

Observed band size: 50 kDa



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

False colour image of Western blot: Anti-p53 antibody [DO-1] - ChIP Grade staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (ab52866) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab1101 was shown to bind specifically to p53. A band was observed at 49 kDa in wild-type A549 and Jurkat cell lysates with no signal observed at this size in tp53 knockout cell lines ab276092, ab276112 (knockout cell lysates ab282999, ab283832). To generate this image, wild-type and tp53 knockout A549 and Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
All lanes:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
Western blot - Human TP53 (p53) knockout A549 cell line (ab276092) at 20 µg

Lane 3:
Wild-type Jurkat cell lysate at 20 µg

Lane 4:
Western blot - Human TP53 (p53) knockout Jurkat cell line (ab276112) at 20 µg

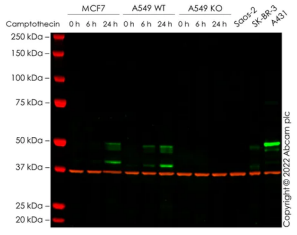
Lane 5:
A431 cell lysate at 20 µg

Lane 6:
Saos-2 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 43 kDa

Observed band size: 49 kDa



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

False colour image of Western blot: Anti-p53 antibody [DO-1] - ChIP Grade staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab1101 was shown to bind specifically to p53. A band was observed at 40/50 kDa in treated wild-type A549 cell lysates with no signal observed at this size in tp53 knockout cell line ab276092. To generate this image, wild-type and tp53 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye®

800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.
Lanes 1 - 12:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 1/1000 dilution

Lanes 1 - 12:
Western blot - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

Lane 1:
MCF7 Treated Camptothecin (20 ug/ml, 0 h) cell lysate at 15 µg

Lane 2:
MCF7 Treated Camptothecin (20 ug/ml, 6 h) cell lysate at 15 µg

Lane 3:
MCF7 Treated Camptothecin (20 ug/ml, 24 h) cell lysate at 15 µg

Lane 4:
Wild-type A549 Treated Camptothecin (20 ug/ml, 0 h) cell lysate at 15 µg

Lane 5:
Wild-type A549 Treated Camptothecin (20 ug/ml, 6 h) cell lysate at 15 µg

Lane 6:
Wild-type A549 Treated Camptothecin (20 ug/ml, 24 h) cell lysate at 15 µg

Lane 7:
TP53 knockout A549 Treated Camptothecin (20 ug/ml, 0 h) cell lysate at 15 µg

Lane 8:
TP53 knockout A549 Treated Camptothecin (20 ug/ml, 6 h) cell lysate at 15 µg

Lane 9:
TP53 knockout A549 Treated Camptothecin (20 ug/ml, 24 h) cell lysate at 15 µg

Lane 10:
Saos-2 cell lysate at 15 µg

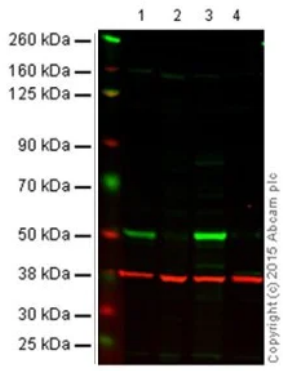
Lane 11:
SK-BR-3 cell lysate at 15 µg

Lane 12:
A431 cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) at 1/20000 dilution

Performed under reducing conditions.



Western blot - Anti-p53 antibody [DO-1] (ab1101)

P53 Western blot staining using mouse Anti-p53 antibody

This blot was produced using a 4-12% Bis-tris gel under the MES 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 before being incubated with ab1101 overnight at 4°C. Antibody binding was detected using the Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 at a 1/10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.
Image shows merged signal (red and green). Green - ab1101 observed at 53 kDa. Red - GAPDH loading control, ab181602, observed at 37 kDa using as secondary Goat Anti-Rabbit IgG H&L (IRDye® 680RD)- ab216777

All lanes:
Western blot - Anti-p53 antibody [DO-1] (ab1101) at 1/1000 dilution

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

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

Lane 3:
A431 cell lysate (20 µg)

Lane 4:
Saos-2 cell lysate (20 µg)

Secondary

All lanes:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) at 1/10000 dilution

Predicted band size: 36 kDa

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