abcam

ab247264

Anti-p53 antibody [E47] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	E47
Purification technique	Affinity purification Protein A
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.
Concentration	1.033 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
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 conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab247264 is the carrier-free version of ab32509.

Species reactivity

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

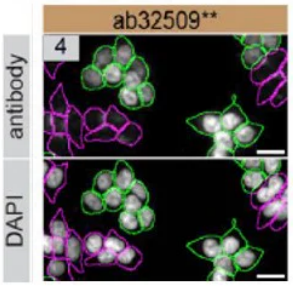
We do not recommend this combination. It is not covered by our product promise.

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.

14 product images



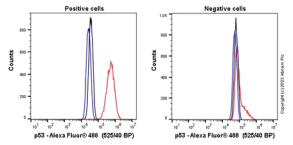
Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

P53 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p53 antibody

This data was developed using ab32509, the same antibody clone in a different buffer formulation.

ab32509 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 ab32509 at 1/100 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.

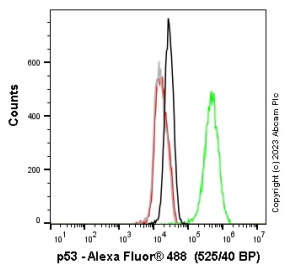


Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509).

Flow cytometry overlay histogram showing wild-type p53 in Hek-293 positive cells (left) and MCF7 negative cells (right) stained with ab32509 (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 (ab32509) (1x 106 cells in 100µl at 0.2µg/ml (1/11865)) for 30min at 22°C. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody (black line) Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) 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 Hek-293 fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

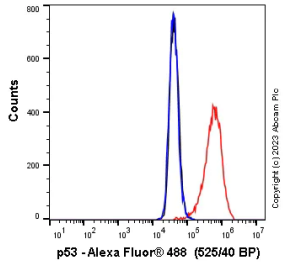


Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509).

Flow cytometry overlay histogram showing mutant p53 in wild-type HAP1 cells (green line) and TP53 knockout HAP1 cells (red line) stained with ab32509. 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 (ab32509) (1x 106 cells in 100µl at 0.2 µg/ml (1/550)) for 30min at 22°C. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) 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.

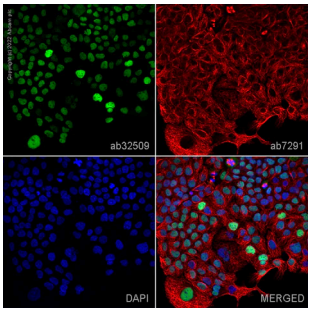


Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509).

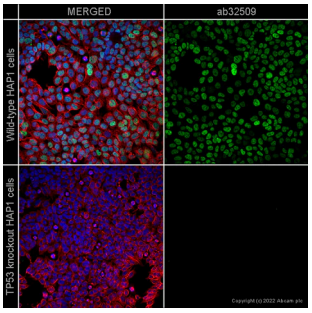
Flow cytometry overlay histogram showing mutant p53 in A-431 cells stained with ab32509 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32509) (1x 106 cells in 100µl at 0.2µg/ml (1/550)) for 30min at 22°C. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) was incubated at 1/4000 for 30min at 22°C. Isotype control antibody (black line) Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) 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.



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509). ab32509 staining mutant p53 in A431 cells. 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 ab32509 at 0.2µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at

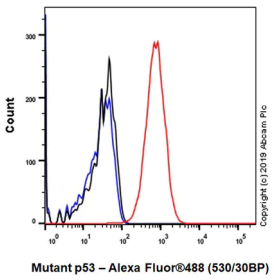
1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image 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 [E47] - BSA and Azide free (ab247264)

P53 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-p53 antibody

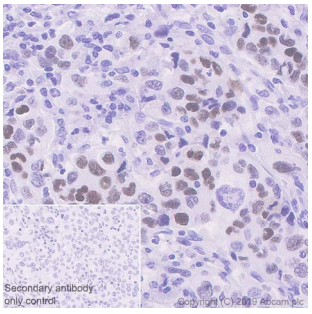
This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509). ab32509 staining mutant p53 in wild-type Hap1 cells (top panel) and p53 knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min), permeabilized 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 ab32509 at 0.2µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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.



Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

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

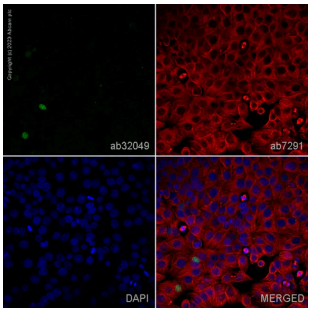
This data was developed using ab32509, the same antibody clone in a different buffer formulation.Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling p53 with purified ab32509 at 1/100 dilution (10µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cells without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

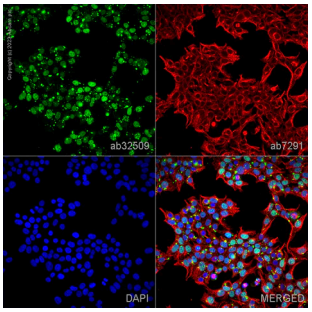
P53 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-p53 antibody

This data was developed using ab32509, the same antibody clone in a different buffer formulation.Immunohistochemical analysis of paraffin-embedded human lung carcinoma sections labeling mutant p53 with ab32509 at 1/2000 dilution (0.49 µg/mL). Sections were counterstained with Hematoxylin. Goat Anti-Rabbit IgG H&L (HRP Polymer) was used as the secondary antibody. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).



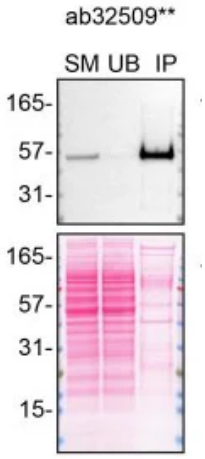
Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509). ab32509 staining wild-type p53 inMCF7 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 ab32509 at 0.2µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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 [E47] - BSA and Azide free (ab247264)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509). ab32509 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 ab32509 at 0.2µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed 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.

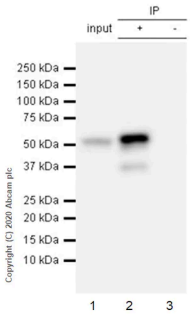


Immunoprecipitation - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

This data was developed using ab32509, the same antibody clone in a different buffer formulation.

Immunoprecipitation of TP53 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2 µg of ab32509 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

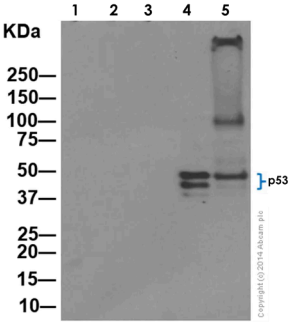
This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Immunoprecipitation - Anti-p53 antibody [E47] (ab32509) at 2 µg



Immunoprecipitation - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

This data was developed using ab32509, the same antibody clone in a different buffer formulation.
Purified ab32509 at 1/50 dilution (2µg) immunoprecipitating p53 in HEK-293 whole cell lysate.
Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32509 + HEK-293 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32509 in HEK-293 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 53 kDa
All lanes:
Immunoprecipitation - Anti-p53 antibody [E47] (ab32509)

Predicted band size: 43 kDa



Western blot - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

P53 Western blot staining using rabbit Anti-p53 antibody

This data was developed using ab32509, the same antibody clone in a different buffer formulation.
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 180 seconds

Lane 1-3: Wildtype p53 cell lines

Lane 4-5: Mutant p53 cell lines

Observed MW: 50kDa, 39kDa

All lanes:
Western blot - Anti-p53 antibody [E47] - BSA and Azide free (ab247264) at 1/200 dilution

Lane 1:
A549 (Human lung carcinoma epithelial cell), Whole cell lysate at 20 µg

Lane 2:
HepG2 (Human hepatocellular carcinoma epithelial cell), Whole cell lysate at 20 µg

Lane 3:
MCF-7 (Human breast adenocarcinoma epithelial cell), Whole cell lysate at 20 µg

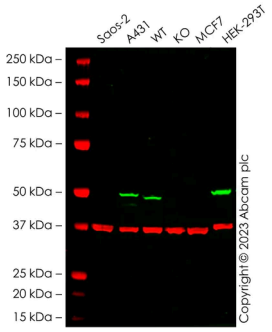
Lane 4:
T-47D (Human ductal breast epithelial tumor epithelial cell), Whole cell lysate at 20 µg

Lane 5:
A431 (Human epidermoid carcinoma epithelial cell), Whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 43 kDa



Western blot - Anti-p53 antibody [E47] - BSA and Azide free (ab247264)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (ab32509). False colour image of Western blot: Anti-p53 antibody [E47] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32509 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-p53 antibody [E47] (ab32509) at 1/1000 dilution

Lane 1:
Saos-2 cell lysate at 20 µg

Lane 2:
A431 cell lysate at 20 µg

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-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/20000 dilution

Lanes 1 - 6:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 43 kDa

Observed band size: 50 kDa

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