

Anti-p53 antibody [E47] ab32509

KO 評価済 リコンビナント RabMAb

★★★★☆ 2 Abreviews 12 References 画像数 13

製品の概要

製品名	Anti-p53 antibody [E47]
製品の詳細	Rabbit monoclonal [E47] to p53
由来種	Rabbit
特異性	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.

アプリケーション

適用あり: Flow Cyt (Intra), IHC-P, ICC/IF, WB, IP

種交差性

交差種: Human

免疫原

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール

Wild type p53: A549, HEK293, HepG2, MCF7, U-87 MG. Mutant p53: A431 (R273H), DU 145 (P223L and V274F), HAP1 (S215G), Jurkat (R196*), MDA-MB-435 (G266E), Raji (R213Q and Y234H), Ramos (I254N), SK-BR-3 (R175H), T-47D (L194F). Cell lines expressing the highest levels of p53 without induction are HEK293 (WT p53), A431 and HAP1 (mutant p53). Negative cell line: Saos-2. IHC-P controls: Bladder, Skin Cancer, Glioma, Gastric adenocarcinoma, Human breast and lung carcinoma tissue, Human colon adenocarcinoma.

特記事項

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 production

For more information [see here](#).

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

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

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	E47
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab32509の使用に適用されます**
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご確認ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	1/100 - 1/250.
WB		1/1000 - 1/10000. Detects a band of approximately 53 kDa (predicted molecular weight: 44 kDa).
IP		1/50.

ターゲット情報

機能	Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4 suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis.
組織特異性	Ubiquitous. Isoforms are expressed in a wide range of normal tissues but in a tissue-dependent manner. Isoform 2 is expressed in most normal tissues but is not detected in brain, lung, prostate, muscle, fetal brain, spinal cord and fetal liver. Isoform 3 is expressed in most normal tissues but is

not detected in lung, spleen, testis, fetal brain, spinal cord and fetal liver. Isoform 7 is expressed in most normal tissues but is not detected in prostate, uterus, skeletal muscle and breast. Isoform 8 is detected only in colon, bone marrow, testis, fetal brain and intestine. Isoform 9 is expressed in most normal tissues but is not detected in brain, heart, lung, fetal liver, salivary gland, breast or intestine.

関連疾患

Note=TP53 is found in increased amounts in a wide variety of transformed cells. TP53 is frequently mutated or inactivated in about 60% of cancers. TP53 defects are found in Barrett metaplasia a condition in which the normally stratified squamous epithelium of the lower esophagus is replaced by a metaplastic columnar epithelium. The condition develops as a complication in approximately 10% of patients with chronic gastroesophageal reflux disease and predisposes to the development of esophageal adenocarcinoma.

Defects in TP53 are a cause of esophageal cancer (ESCR) [MIM:133239].

Defects in TP53 are a cause of Li-Fraumeni syndrome (LFS) [MIM:151623]. LFS is an autosomal dominant familial cancer syndrome that in its classic form is defined by the existence of a proband affected by a sarcoma before 45 years with a first degree relative affected by any tumor before 45 years and another first degree relative with any tumor before 45 years or a sarcoma at any age.

Other clinical definitions for LFS have been proposed (PubMed:8118819 and PubMed:8718514) and called Li-Fraumeni like syndrome (LFL). In these families affected relatives develop a diverse set of malignancies at unusually early ages. Four types of cancers account for 80% of tumors occurring in TP53 germline mutation carriers: breast cancers, soft tissue and bone sarcomas, brain tumors (astrocytomas) and adrenocortical carcinomas. Less frequent tumors include choroid plexus carcinoma or papilloma before the age of 15, rhabdomyosarcoma before the age of 5, leukemia, Wilms tumor, malignant phyllodes tumor, colorectal and gastric cancers.

Defects in TP53 are involved in head and neck squamous cell carcinomas (HNSCC) [MIM:275355]; also known as squamous cell carcinoma of the head and neck.

Defects in TP53 are a cause of lung cancer (LNCr) [MIM:211980].

Defects in TP53 are a cause of choroid plexus papilloma (CPLPA) [MIM:260500]. Choroid plexus papilloma is a slow-growing benign tumor of the choroid plexus that often invades the leptomeninges. In children it is usually in a lateral ventricle but in adults it is more often in the fourth ventricle. Hydrocephalus is common, either from obstruction or from tumor secretion of cerebrospinal fluid. If it undergoes malignant transformation it is called a choroid plexus carcinoma. Primary choroid plexus tumors are rare and usually occur in early childhood.

Defects in TP53 are a cause of adrenocortical carcinoma (ADCC) [MIM:202300]. ADCC is a rare childhood tumor of the adrenal cortex. It occurs with increased frequency in patients with the Beckwith-Wiedemann syndrome and is a component tumor in Li-Fraumeni syndrome.

配列類似性

Belongs to the p53 family.

ドメイン

The nuclear export signal acts as a transcriptional repression domain. The TAD1 and TAD2 motifs (residues 17 to 25 and 48 to 56) correspond both to 9aaTAD motifs which are transactivation domains present in a large number of yeast and animal transcription factors.

翻訳後修飾

Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.

Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity). Phosphorylation at Ser-9 by HIPK4 increases repression activity on BIRC5 promoter. Phosphorylated on Thr-18 by VRK1. Phosphorylated on Ser-20 by CHEK2 in response to DNA damage, which prevents ubiquitination by MDM2. Phosphorylated on Thr-55 by TAF1, which promotes MDM2-mediated degradation. Phosphorylated on Ser-46 by HIPK2 upon UV irradiation. Phosphorylation on Ser-46 is required for acetylation by CREBBP. Phosphorylated on Ser-392 following UV but not gamma irradiation. Phosphorylated upon DNA damage, probably by ATM or ATR. Phosphorylated on Ser-15 upon ultraviolet irradiation; which is enhanced by

interaction with BANP.

Dephosphorylated by PP2A-PPP2R5C holoenzyme at Thr-55. SV40 small T antigen inhibits the dephosphorylation by the AC form of PP2A.

May be O-glycosylated in the C-terminal basic region. Studied in EB-1 cell line.

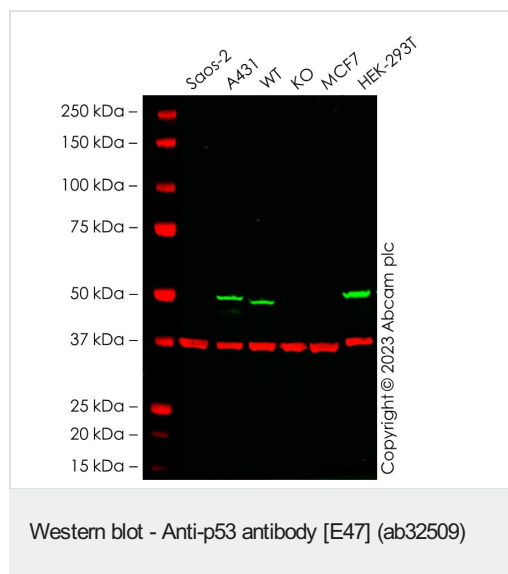
Ubiquitinated by MDM2 and SYVN1, which leads to proteasomal degradation. Ubiquitinated by RFWD3, which works in cooperation with MDM2 and may catalyze the formation of short polyubiquitin chains on p53/TP53 that are not targeted to the proteasome. Ubiquitinated by MKRN1 at Lys-291 and Lys-292, which leads to proteasomal degradation. Deubiquitinated by USP10, leading to its stabilization. Ubiquitinated by TRIM24, which leads to proteasomal degradation. Ubiquitination by TOPORS induces degradation. Deubiquitination by USP7, leading to stabilization. Isoform 4 is monoubiquitinated in an MDM2-independent manner.

Monomethylated at Lys-372 by SETD7, leading to stabilization and increased transcriptional activation. Monomethylated at Lys-370 by SMYD2, leading to decreased DNA-binding activity and subsequent transcriptional regulation activity. Lys-372 monomethylation prevents interaction with SMYD2 and subsequent monomethylation at Lys-370. Dimethylated at Lys-373 by EHMT1 and EHMT2. Monomethylated at Lys-382 by SETD8, promoting interaction with L3MBTL1 and leading to repress transcriptional activity. Demethylation of dimethylated Lys-370 by KDM1A prevents interaction with TP53BP1 and represses TP53-mediated transcriptional activation. Sumoylated by SUMO1.

細胞内局在

Cytoplasm; Cytoplasm. Nucleus. Nucleus > PML body. Endoplasmic reticulum. Interaction with BANP promotes nuclear localization. Recruited into PML bodies together with CHEK2; Nucleus. Cytoplasm. Localized in both nucleus and cytoplasm in most cells. In some cells, forms foci in the nucleus that are different from nucleoli; Nucleus. Cytoplasm. Localized in the nucleus in most cells but found in the cytoplasm in some cells; Nucleus. Cytoplasm. Localized mainly in the nucleus with minor staining in the cytoplasm; Nucleus. Cytoplasm. Predominantly nuclear but localizes to the cytoplasm when expressed with isoform 4 and Nucleus. Cytoplasm. Predominantly nuclear but translocates to the cytoplasm following cell stress.

画像



All lanes : Anti-p53 antibody [E47] (ab32509) at 1/1000 dilution

Lane 1 : Saos-2 cell lysate

Lane 2 : A431 cell lysate

Lane 3 : Wild-type HAP1 cell lysate

Lane 4 : TP53 knockout HAP1 cell lysate

Lane 5 : MCF7 cell lysate

Lane 6 : HEK-293T cell lysate

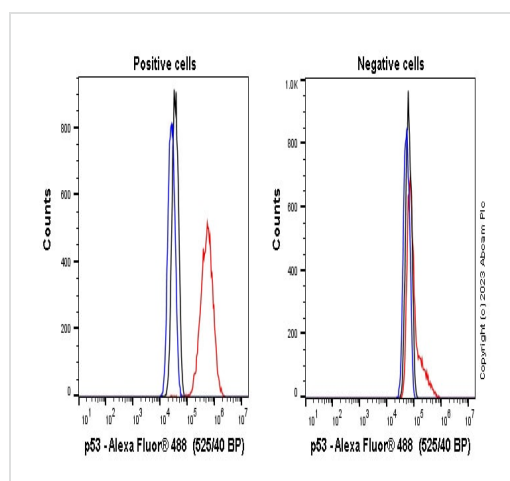
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 44 kDa

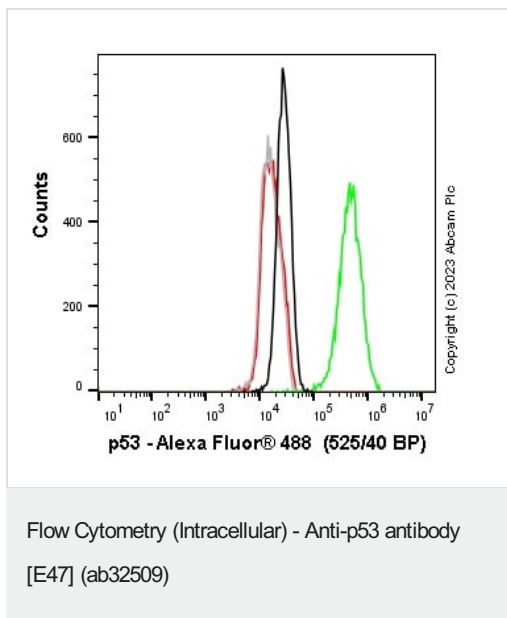
Observed band size: 50 kDa

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.

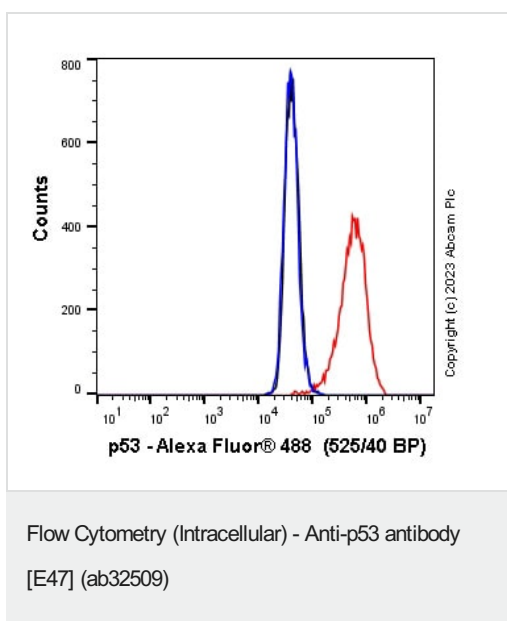


Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] (ab32509)

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.

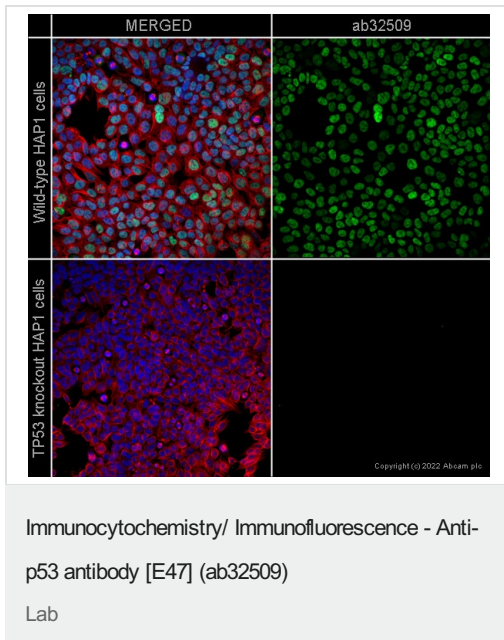


Flow cytometry overlay histogram showing mutant p53 in wild-type HAP1 cells (green line) and TP53 knockout HAP1 cells (red line) stained with ab32509. The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab32509) (1×10^6 cells in 100 μ l at 0.2 μ g/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 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) (1×10^6 cells in 100 μ l at 0.2 μ g/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

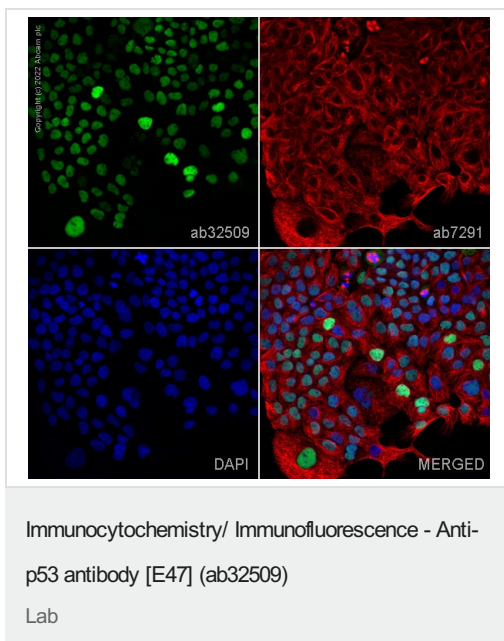
same conditions.



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).

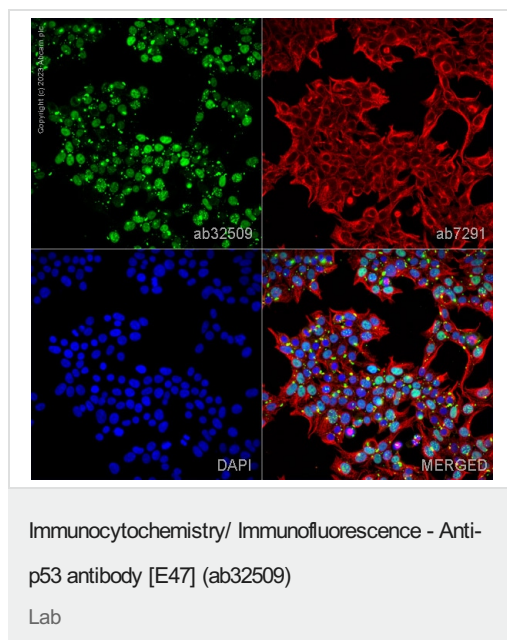
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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).

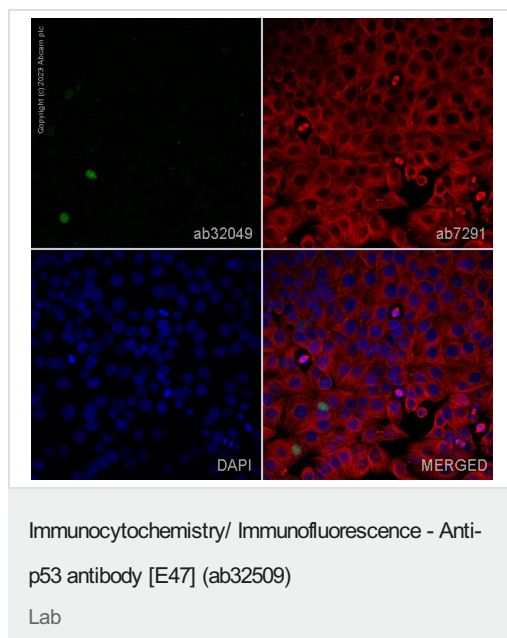
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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).

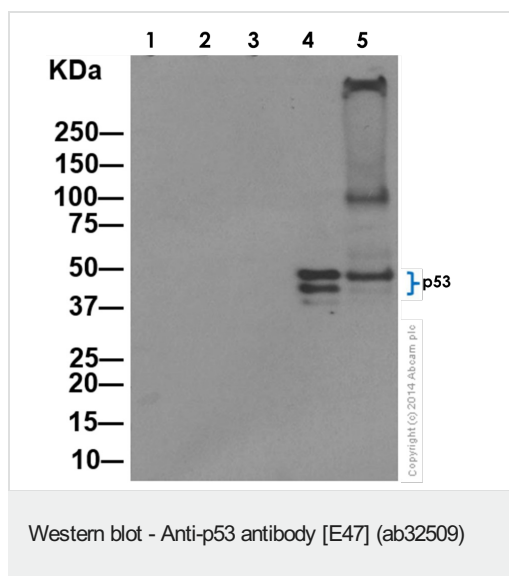
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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.



All lanes : Anti-p53 antibody [E47] (ab32509) at 1/200 dilution

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

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

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

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

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

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 44 kDa

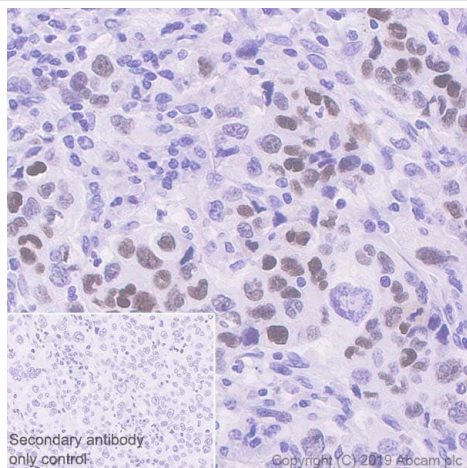
Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 180 seconds

Lane 1-3: Wildtype p53 cell lines

Lane 4-5: Mutant p53 cell lines

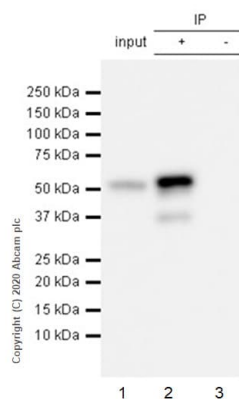
Observed MW: 50kDa, 39kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [E47] (ab32509)

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).

Nuclear staining on human lung carcinoma.



Immunoprecipitation - Anti-p53 antibody [E47] (ab32509)

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.

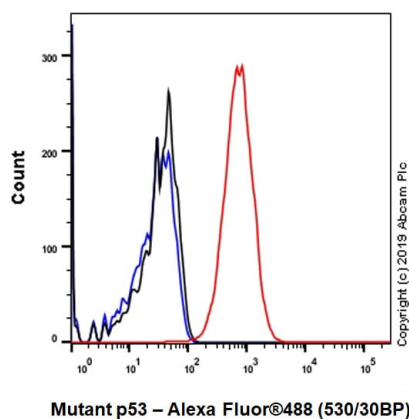
Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab32509 in HEK-293 whole cell lysate.

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



Flow Cytometry (Intracellular) - Anti-p53 antibody [E47] (ab32509)

Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling p53 with purified ab32509 at 1/100 dilution (10 µg/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).

Why choose a recombinant antibody?



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Anti-p53 antibody [E47] (ab32509)

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