abcam

Product datasheet

Anti-p53 antibody [DO-1] - BSA and Azide free ab237976

敲除验证

12 图像

概述

产品名称	Anti-p53抗体[DO-1] - BSA and Azide free
描述	小鼠单克隆抗体[DO-1] to p53 - BSA and Azide free
宿主	Mouse
特异性	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.
经测试应用	**适用于:** ICC/IF, IHC-P, ChIP, Flow Cyt (Intra), WB
种属反应性	**与反应:** Human **不与反应:** Mouse, Rat
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
表位	The epitope maps to within aa 20-25.
阳性对照	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.
常规说明	ab237976 is the carrier-free version of **ab1101**. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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.

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.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	Constituent: PBS
无载体	**是**
纯度	Protein G purified
克隆	单克隆
克隆编号	DO-1
骨髓瘤	unknown
同种型	IgG2a
轻链类型	kappa

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab237976于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

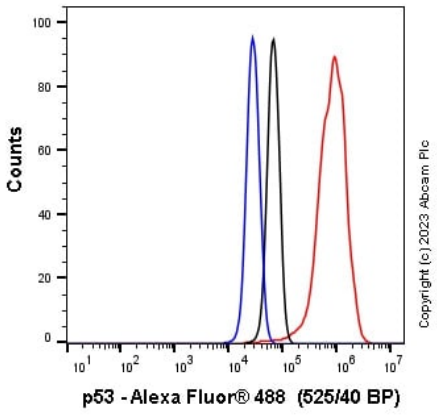
应用	Ab评论	说明
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use a concentration of 1 - 5 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000.

靶标

功能	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.
组织特异性	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.
疾病相关	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.

序列相似性	Belongs to the p53 family.
结构域	The nuclear export signal acts as a transcriptional repression domain. The TADI and TADII motifs (residues 17 to 25 and 48 to 56) correspond both to 9aaTAD motifs which are transactivation domains present in a large number of yeast and animal transcription factors.
翻译后修饰	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.
细胞定位	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.

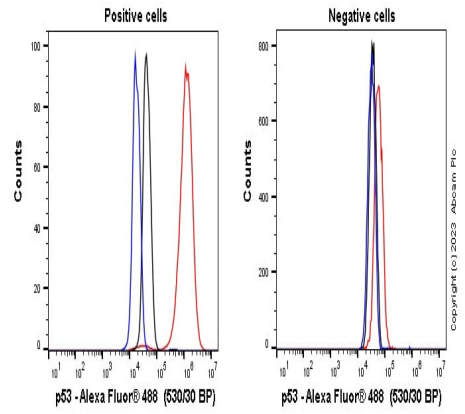
图片



Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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.

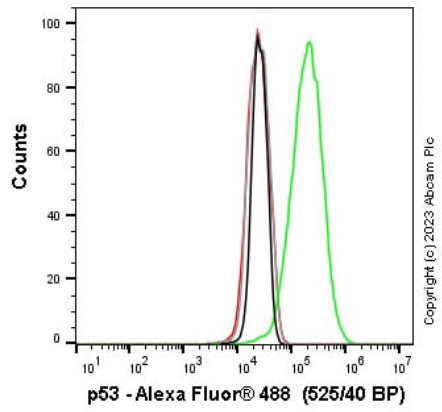


Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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) 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

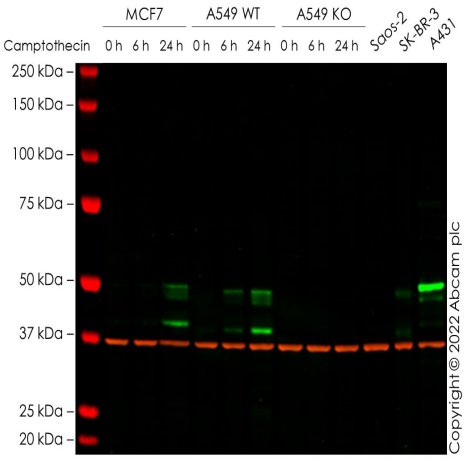
525/40 bandpass filter. This antibody gave a positive signal in Hek-293 fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Flow Cytometry (Intracellular) - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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.



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

All lanes : Anti-p53 antibody [DO-1] - ChIP Grade (**ab1101**) at 1/1000 dilution

Lane 1 : MCF7 Treated Camptothecin (20 ug, 0 h) cell lysate
Lane 2 : MCF7 Treated Camptothecin (20 ug, 6 h) cell lysate
Lane 3 : MCF7 Treated Camptothecin (20 ug, 24 h) cell lysate
Lane 4 : Wild-type A549 Treated Camptothecin (20 ug, 0 h) cell lysate
Lane 5 : Wild-type A549 Treated Camptothecin (20 ug, 6 h) cell lysate
Lane 6 : Wild-type A549 Treated Camptothecin (20 ug, 24 h) cell lysate
Lane 7 : TP53 knockout A549 Treated Camptothecin (20 ug, 0 h) cell lysate
Lane 8 : TP53 knockout A549 Treated Camptothecin (20 ug, 6 h) cell lysate

Lane 9 : TP53 knockout A549 Treated Camptothecin (20 ug, 24 h) cell lysate
Lane 10 : Saos-2 cell lysate
Lane 11 : SK-BR-3 cell lysate
Lane 12 : A431 cell lysate

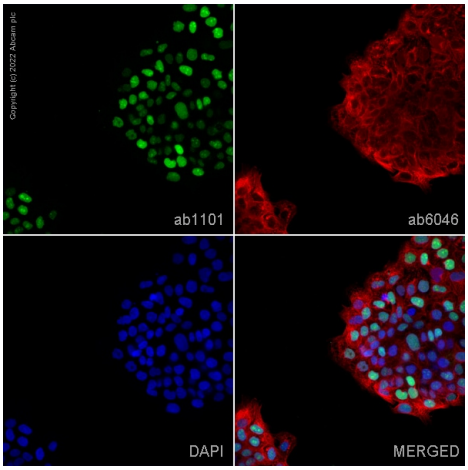
Lysates/proteins at 15 µg per lane.

Performed under reducing conditions.

Observed band size: 50 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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.



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

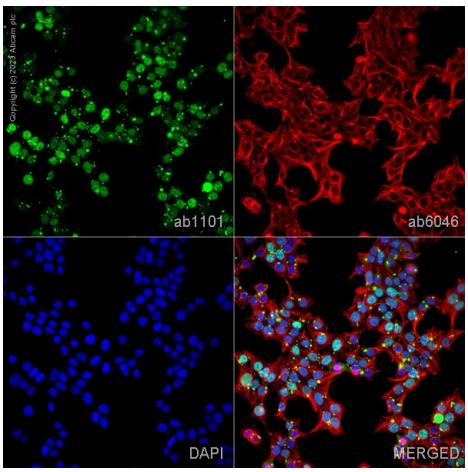
Lab

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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] - BSA and Azide free (ab237976)

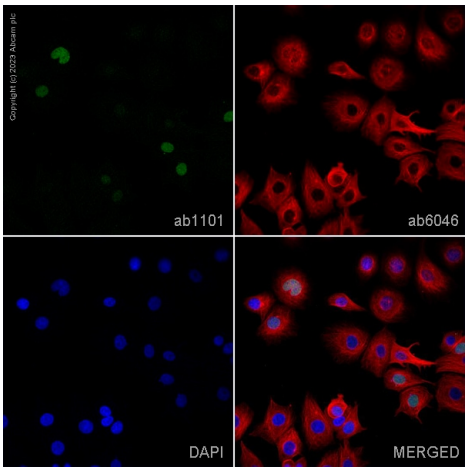
Lab

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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] - BSA and Azide free (ab237976)

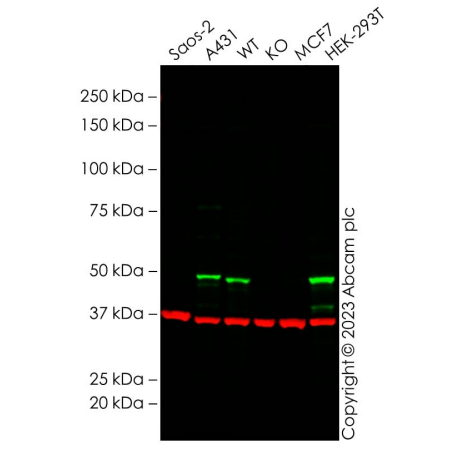
Lab

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).

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.



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

All lanes : Anti-p53 antibody [DO-1] - ChIP Grade (**ab1101**) 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

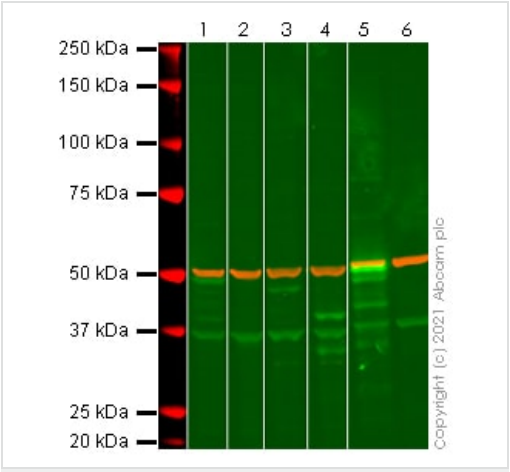
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 50 kDa

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab1101**).



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

All lanes : Anti-p53 antibody [DO-1] - ChIP Grade (**ab1101**) at 1/1000 dilution

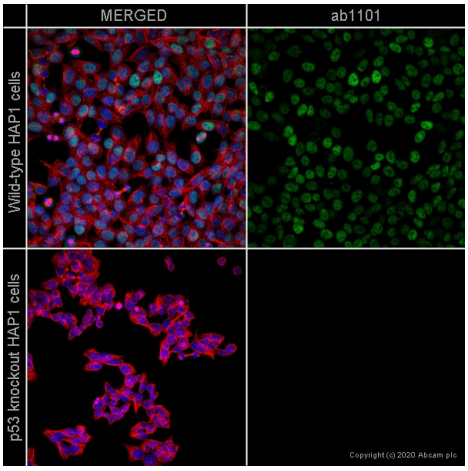
Lane 1 : Wild-type A549 cell lysate
Lane 2 : TP53 knockout A549 cell lysate
Lane 3 : Wild-type Jurkat cell lysate
Lane 4 : TP53 knockout Jurkat cell lysate
Lane 5 : A431 cell lysate
Lane 6 : Saos-2 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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 cell lysates with no signal observed at this size in tp53 knockout cell line **ab276092** (knockout cell lysate **ab282999**). 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.

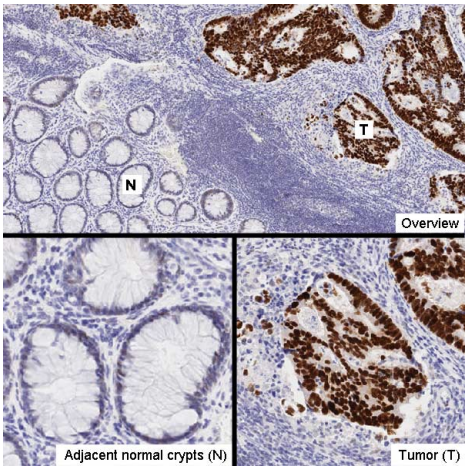


Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

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™).

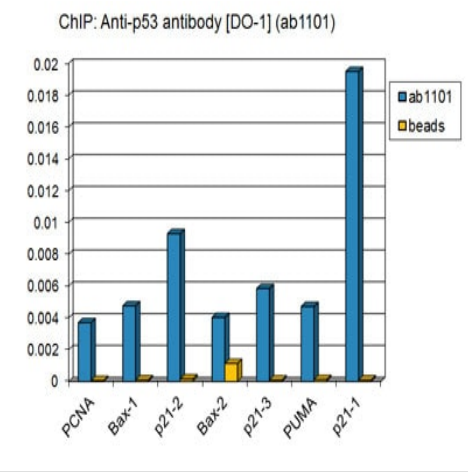
This data was developed using the same antibody clone in a different buffer formulation (**ab1101**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

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.

This image was produced using the same antibody clone but in a different formulation; PBS and sodium azide (**ab1101**).



ChIP - Anti-p53 antibody [DO-1] - BSA and Azide free (ab237976)

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

This image was produced using the same antibody clone but in a different formulation; PBS and sodium azide (**ab1101**).

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