

Anti-p53 antibody [E26] ab32389

KO 評価済 リコンビナント RabMAb

83 References 画像数 8

製品の概要

製品名	Anti-p53 antibody [E26]
製品の詳細	Rabbit monoclonal [E26] to p53
由来種	Rabbit
特異性	The antibody should recognize human wild-type and mutant p53.
アプリケーション	適用あり: WB, IHC-P, Flow Cyt, ICC/IF
種交差性	交差種: Human 非交差種: Mouse, Rat
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	WB: Wild type HAP1, HEK293, A431, wild-type A549 and wild-type Jurkat whole cell lysate. ICC/IF: Wild type HAP1 and A431 cells
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 .

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% 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	E26

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ab32389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検討ください。

アプリケーション	Abreviews	特記事項
WB		1/10000. Detects a band of approximately 53 kDa (predicted molecular weight: 44 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		1/500.
ICC/IF		1/50 - 1/100.

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 phylloides 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.

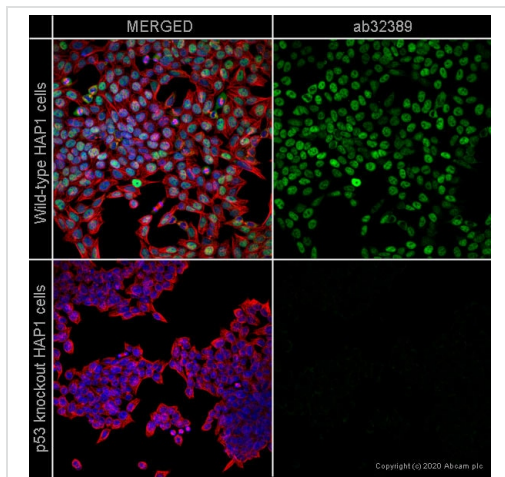
Monomethylated at Lys-372 by SETD7, leading to stabilization and increased transcriptional activation. Monomethylated at Lys-370 by SMYD2, leading to decreased DNA-binding activity and subsequent transcriptional regulation activity. Lys-372 monomethylation prevents interaction with SMYD2 and subsequent 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.

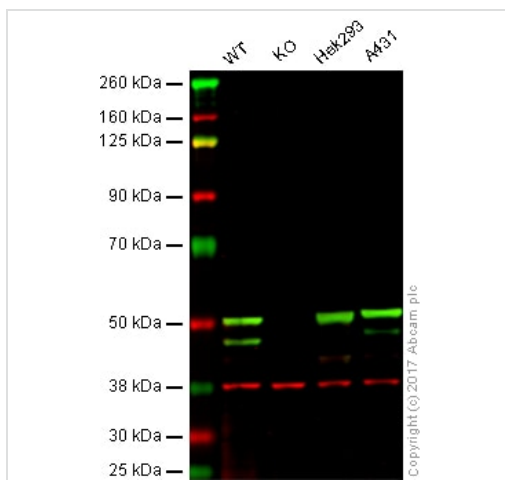
画像



Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [E26] (ab32389)

ab32389 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 ab32389 at 1 µg/ml concentration and **ab7291** (Mouse monoclonal to alpha 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 rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) 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™).



Western blot - Anti-p53 antibody [E26] (ab32389)

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

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

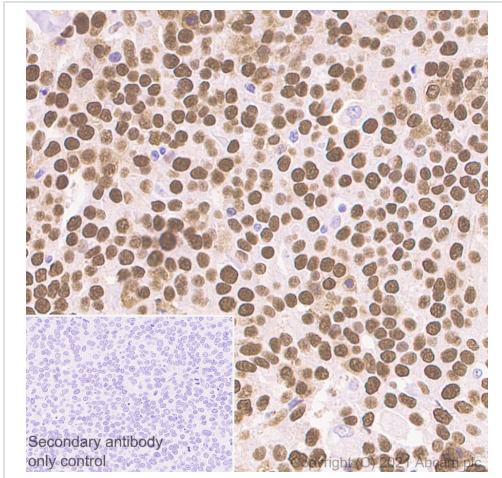
Lane 3: HEK293 whole cell lysate (20 µg)

Lane 4: A431 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab32389 observed at 50 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

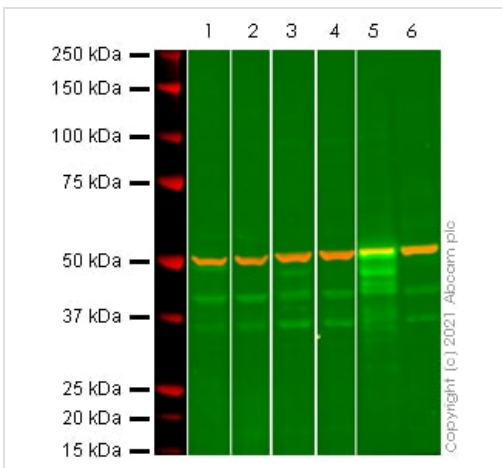
ab32389 was shown to specifically react with p53 in wild type HAP1 cells. No band was observed when p53 knockout samples were used. Wild-type and p53 knockout samples were subjected to SDS-PAGE. ab32389 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and

Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53 antibody [E26] (ab32389)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium cancer tissue sections labelling p53 with purified ab32389 at 1/5000 dilution (0.09 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1/0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-p53 antibody [E26] (ab32389)

All lanes : Anti-p53 antibody [E26] (ab32389) 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.

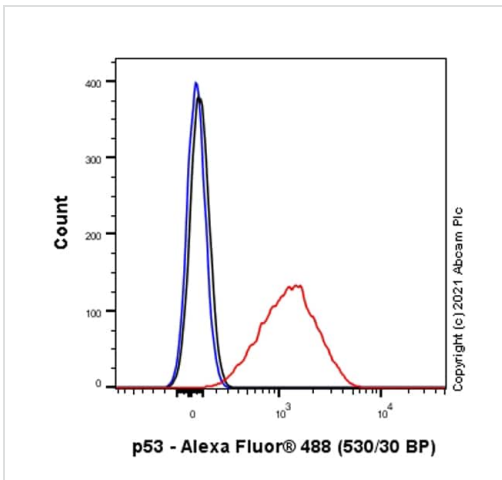
Performed under reducing conditions.

Predicted band size: 44 kDa

Observed band size: 49 kDa

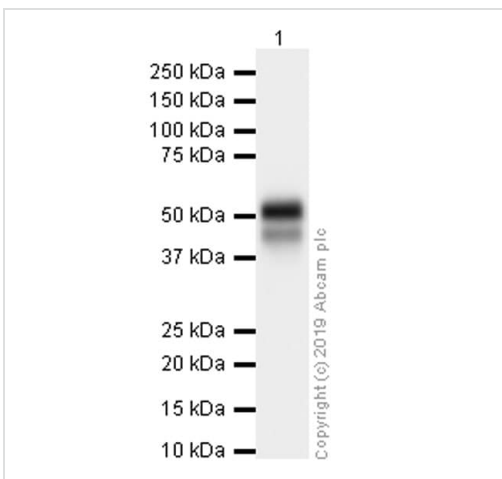
False colour image of Western blot: Anti-p53 antibody [E26] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32389 was shown to bind specifically to p53. A band was observed at 49 kDa 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-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 - Anti-p53 antibody [E26] (ab32389)

Flow cytometric analysis of 4% Paraformaldehyde fixed 90% Methanol permeabilized HEK-293 (Human embryonic kidney epithelial cell) cells labelling p53 with ab32389 at 1/500 dilution (1 µg/ml) compared with a Rabbit monoclonal IgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor[®] 488, [ab150077](#)) at 1/2000 was used as the secondary antibody.



Western blot - Anti-p53 antibody [E26] (ab32389)

Anti-p53 antibody [E26] (ab32389) at 1/10000 dilution (Purified) + HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate at 15 µg

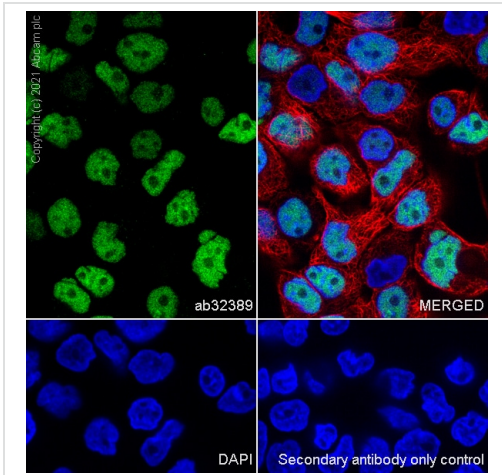
Secondary

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

Predicted band size: 44 kDa

Observed band size: 46, 53 kDa





The isoforms of p53 produced by alternative splicing of the intron 9 have been described by the literatures (PMID: 16131611, 29235495 and 22647703).



Immunocytochemistry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling p53 with purified ab32389 at 1/50 dilution (8.7 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunocytochemistry/ Immunofluorescence - Anti-p53 antibody [E26] (ab32389)

Why choose a recombinant antibody?

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Anti-p53 antibody [E26] (ab32389)

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