abcam

Product datasheet

Anti-p53 antibody [SP161] ab227655





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Overview

Product name Anti-p53 antibody [SP161]

Rabbit monoclonal [SP161] to p53 **Description**

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF, IHC-P

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Rabbit, Cow, Dog, Pig

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Human colon adenocarcinoma, stomach adenocarcinoma, ovarian adenocarcinoma,

bladder transitional cell carcinoma, lung squamous cell carcinoma, B-cell lymphoma and breast

ductal carcinoma tissue. ICC/IF: MCF7 cells. Flow Cyt (Intra): HAP1 and MCF7 cells.

General notes 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.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.60

> Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Purity Protein A/G purified

Purification notes Purified from TCS by protein A/G.

Clonality Monoclonal

Clone number SP161

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab227655 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
ICC/IF		1/20.
IHC-P		1/100. Boil tissue section in Citrate buffer, pH 6.0 for 10 minutes followed by cooling at room temperature for 20 minutes. Incubate with primary antibody for 10 minutes at room temperature.

Target

Function

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 transactivator 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.

Tissue specificity

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.

Involvement in disease

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.

Sequence similarities

Domain

Post-translational modifications

Belongs to the p53 family.

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.

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

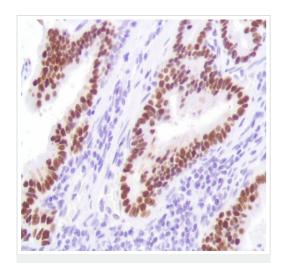
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with SMYD2 and subsequent monomethylation at Lys-370. Dimethylated at Lys-373 by EHMT1 and EHMT2. Monomethylated at Lys-382 by SETD8, promoting interaction with L3MBTL1 and leading to repress transcriptional activity. Demethylation of dimethylated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

Cellular localization

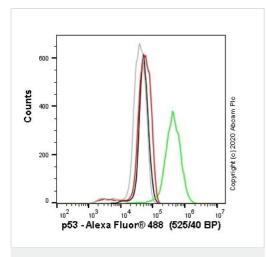
Cytoplasm; Cytoplasm. Nucleus. Nucleus > PML body. Endoplasmic reticulum. Interaction with BANP promotes nuclear localization. Recruited into PML bodies together with CHEK2; Nucleus. Cytoplasm. Localized in both nucleus and cytoplasm in most cells. In some cells, forms foci in the nucleus that are different 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.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)

Formalin-fixed, paraffin-embedded human stomach adenocarcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.



Flow Cytometry (Intracellular) - Anti-p53 antibody [SP161] (ab227655)

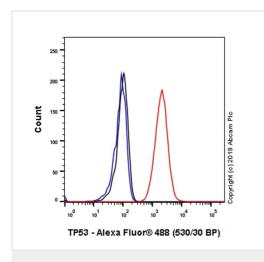
Flow cytometry overlay histogram showing wild-type HAP1 (green line) and TP53 knockout HAP1 cells stained with ab227655 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab227655) (1x10 6 in 100 μ l at 0.008 μ g/ml) for 30 min at 22°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor[®] 488, pre-adsorbed) (<u>ab150177</u>) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was Rabbit IgG (monoclonal) (<u>ab172730</u>) 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).

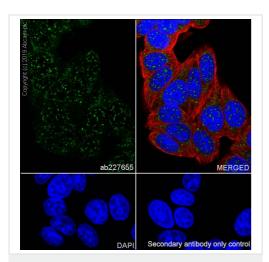
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



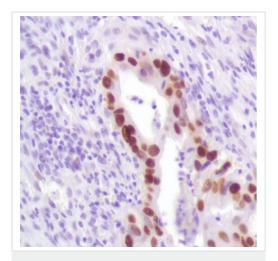
Flow Cytometry (Intracellular) - Anti-p53 antibody [SP161] (ab227655)

Flow Cytometry analysis of MCF7 (human breast adenocarcinoma epithelial cell) cells labeling p53 with purified ab227655 at 1/20 dilution (7.5 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (<u>ab172730</u>) / Black. Unlabeled control - Unlabelled cells / blue.



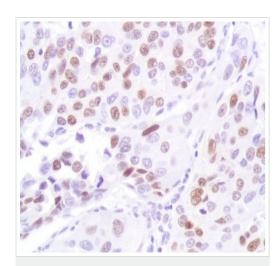
Immunocytochemistry/ Immunofluorescence - Antip53 antibody [SP161] (ab227655)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (human breast adenocarcinoma epithelial cell) cells labeling p53 with purified ab227655 at 1/20 (7.25μg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 μg/ml). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) 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.



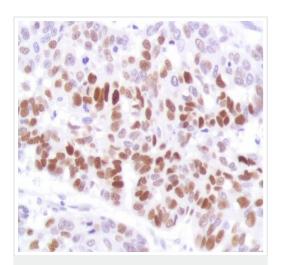
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)

Formalin-fixed, paraffin-embedded human colon adenocarcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.



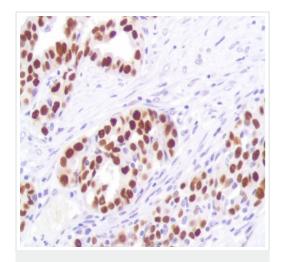
Formalin-fixed, paraffin-embedded human bladder transitional cell carcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)



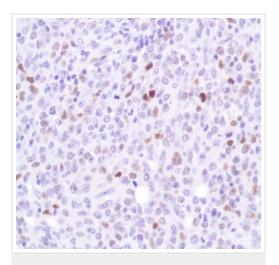
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)

Formalin-fixed, paraffin-embedded human lung squamous cell carcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.



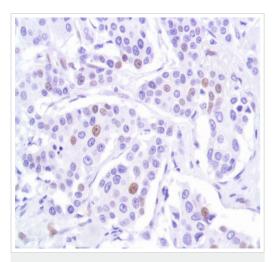
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)

Formalin-fixed, paraffin-embedded human ovarian adenocarcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)

Formalin-fixed, paraffin-embedded human B-cell lymphoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.



Formalin-fixed, paraffin-embedded human breast ductal carcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p53 antibody [SP161] (ab227655)



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