abcam

Product datasheet

Anti-p53 (mono methyl K372) antibody ab16033

11 References 3 Images

Overview

Product name	Anti-p53 (mono methyl K372) antibody
Description	Rabbit polyclonal to p53 (mono methyl K372)
Host species	Rabbit
Specificity	In vivo (HEK293 cells) this antibody recognizes p53 (monomethyl K372) only through IP-Western. It can detect the target protein in straight WB but only in HEK293 cells with Set7/9 wt. In vitro the antibody works both for IP and in WB. Blocking with non-methylated peptide (ab16204) should reduce non-specific binding.
Tested applications	**Suitable for:** WB, IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Dog, Chimpanzee, Non human primates, Cynomolgus monkey, Macaque monkey, African green monkey
Immunogen	Synthetic peptide corresponding to Human p53 aa 350 to the C-terminus (internal sequence) (mono methyl K372) conjugated to keyhole limpet haemocyanin. Database link: P04637 (Peptide available as ab16203)
Positive control	This antibody gave a positive signal in the following whole cell lysates: HEK293 Overexpressing p53.
General notes	Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee. Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

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 or -80°C. Avoid freeze / thaw cycle. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab16033** 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
WB		Use a concentration of 0.05 µg/ml. Detects a band of approximately 44 kDa (predicted molecular weight: 53 kDa).
IP		Use a concentration of 1 µg/ml.

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 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.
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

Belongs to the p53 family.

Domain

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.

Post-translational modifications

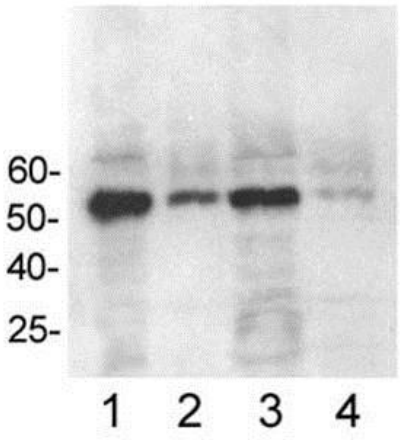
Acetylated. Acetylation of Lys-382 by CREBBP enhances transcriptional activity. Deacetylation of Lys-382 by SIRT1 impairs its ability to induce proapoptotic program and modulate cell senescence.
Phosphorylation on Ser residues mediates transcriptional activation. Phosphorylated by HIPK1 (By similarity). 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.

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



Immunoprecipitation - Anti-p53 (mono methyl K372) antibody (ab16033)
This image is courtesy of T. Ivanova, Tufts University

Whole cell extracts derived from HEK293 cells stably expressing either Set7/9 wt or Set7/9 DN mutant were subjected to immunoprecipitation with anti-p53 monomethyl K372 antibody, ab16033.

The input and immunoprecipitated materials were resolved by SDS-PAGE and analyzed by immunoblotting using p53-specific antibody (Ab6-Oncogene).

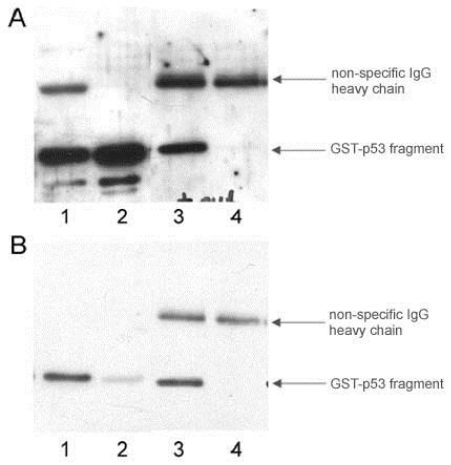
Lanes 1 and 3 represent 2% of input materials for Set7/9wt and Set7/9DN mutant cell lines, respectively.
Lanes 2 and 4 represent immunoprecipitated materials from Set7/9wt and Set7/9DN extracts, respectively.

The data shows that p53 monomethyl K372 was succssfully IPed from the Set7/9 wt but not the Set 7/9 DN mutant cells.

NB:

The antibody was exhausted twice with 5 ug of control p53 peptide each time to eliminate non-specific binding to unmethylated p53. For westerns 5 ug of the blocking peptide was used per 10 ml of p53-K372me1 antibody in 1:5000 dilution.

Western blots were blocked in PBS with 2% non-fa



Immunoprecipitation - Anti-p53 (mono methyl K372) antibody (ab16033)

This image is courtesy of T. Ivanova, Tufts University

In vitro methylated or mock-methylated GST-p53 fragments (fragment = AAs300-393) were subjected to immunoprecipitation with anti-p53 monomethyl K372 antibody, ab16033.

(A) Western blotting with GST-specific antibody.
Lanes 1 and 2 – input materials representing 20% of methylated and mock-methylated GST-p53, respectively.
Lanes 3 and 4 – immunoprecipitated methylated and mock-methylated p53 proteins.

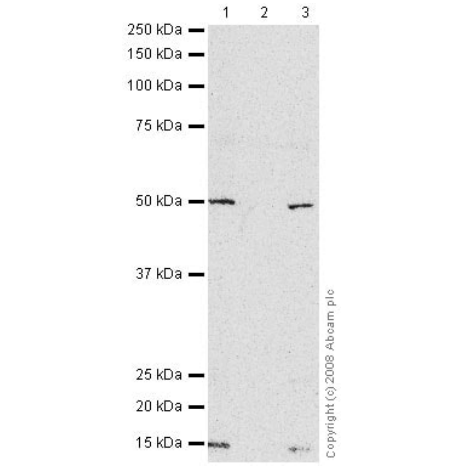
ab16033 was able to IP methylated but not unmethylated (mock methylated) GST-p53 fragments.

(B) Western blotting with anti-p53 monomethyl K372 antibody, ab16033.
Lanes 1 and 2 – input materials representing 20% of methylated and mock-methylated GST-p53, respectively.
Lanes 3 and 4 – immunoprecipitated methylated and mock-methylated p53 proteins.

ab16033 was able to IP and recognise by Western Blot methylated but not unmethylated (mock methylated) GST-p53 fragments.

NB:

The antibody was exhaus



Western blot - Anti-p53 (mono methyl K372) antibody (ab16033)

All lanes : Anti-p53 (mono methyl K372) antibody (ab16033) at 1 µg/ml

Lane 1 : HEK293 Whole Cell Lysate Overexpressing p53
Lane 2 : HEK293 Whole Cell Lysate Overexpressing p53 with Human p53 (mono methyl K372) peptide (ab16203) at 1 µg/ml
Lane 3 : HEK293 Whole Cell Lysate Overexpressing p53 with Human p53 (unmodified) peptide (ab16204) at 1 µg/ml

Lysates/proteins at 5 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 53 kDa

Observed band size: 50 kDa

why is the actual band size different from the predicted?

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