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Product datasheet

Human p53 ELISA Kit (pSer15) ab156027

1 References 4 Images

Overview

Product name Human p53 ELISA Kit (pSer15)

Detection method Colorimetric

Precision

Sample	n	Mean	SD	CV%
overall	3			8.5%

Inter-assay

Intra-assav

Sample	n	Mean	SD	CV%
Overall	3			14.2%

Sample type Cell culture extracts, Adherent cells, Suspension cells, Cell Lysate, Tissue Homogenate, Tissue

Lysate

Assay type Sandwich (quantitative)

 $8 \ \mu g/ml$ Assay time $4h \ 15m$

Assay duration Multiple steps standard assay

Species reactivity Reacts with: Human

Product overview Abcam's p53 pSer15 Human ELISA kit is an in vitro enzyme-linked immunosorbent assay for the

accurate quantitative measurement of phosphorylated Ser15 of p53 protein in human cell and tissue lysates. The assay employs an antibody specific to p53 protein coated onto well plate strips. Standards and samples are pipetted into the wells and p53 present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti-p53 phospho Ser15 detector antibody is added. After washing away unbound detector antibody, HRP-conjugated label specific for the detector antibody is pipetted into the wells. The wells are again washed, a TMB substrate solution is added to the wells and blue color develops in proportion to the amount of phosphorylated Ser15 of bound p53. The reaction is stopped by adding hydrochloric acid which changes the color from blue to yellow and the color intensity is measured at 450 nm.

Get higher sensitivity in only 90 minutes with Human p53 ELISA Kit ($\underline{ab171571}$) from our SimpleStep ELISA[®] range.

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Notes

p53 (TP53 gene) acts as a tumor suppressor in many tumor types and induces growth arrest or apoptosis depending on the physiological circumstances and cell type. p53 is 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. p53 mediated apoptosis induction seems to be by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. p53 is also implicated in Notch signaling crossover.

The p53 protein is found in increased amounts in a wide variety of transformed cells. p53 is mutated or inactivated in about 60% of cancers. 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.

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Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Blocking Solution	1 x 8ml
10X Buffer	1 x 6ml
10X HRP Label	1 x 1ml
10X p53 pSer15 Detector Antibody	1 x 700µl
10X Wash Buffer	1 x 40ml
Extraction Buffer	1 x 15ml
HRP Development Solution	1 x 12ml
p53 Microplate	1 unit
Stop Solution	1 x 12ml

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

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.

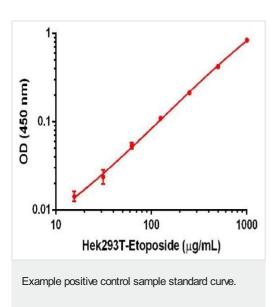
Ubiquitinated by MDM2 and SYVN1, which leads to proteasomal degradation. Ubiquitinated by RFWD3, which works in cooperation with MDM2 and may catalyze the formation of short polyubiquitin chains on p53/TP53 that are not targeted to the proteasome. Ubiquitinated by MKRN1 at Lys-291 and Lys-292, which 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.

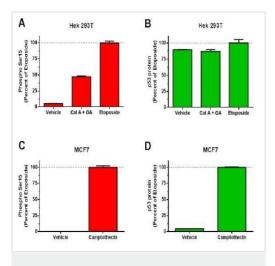
Cellular localization

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.

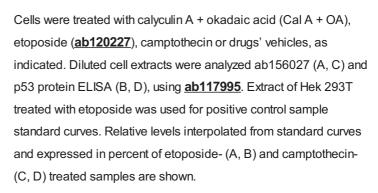
Images

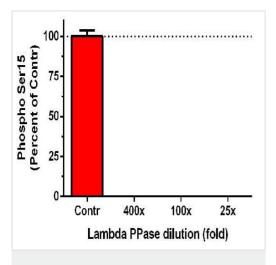


A dilution series of extract in Incubation Buffer in the working range of the assay. The extract was prepared from pellets of Hek293T cells treated with etoposide (<u>ab120227</u>).



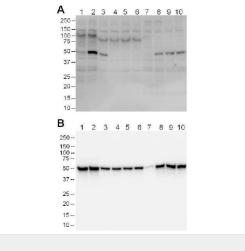
Example experimental analysis of drug treatment of Hek 293T and MCF7 cells.





The p53 pSer15 ELISA specifically measures the phosphorylated Serine.

Extracts of Hek 293T cells (induced with etoposide) were treated with increasing concentrations of λ protein phosphatase (400x= 400-times diluted, 100x=100-times diluted, 25x=25-times diluted), or left untreated (Contr), and relative phospho Ser15 levels were determined using this kit. Dilutions of extracts of Hek 293T cells treated with etoposide were used to construct the standard curve.



The detector antibody used in this kit specifically detects the phosphorylated p53 as determined by Western blotting.

Hek293T cells were treated with vehicle (lane 1) or etoposide (lanes 2-6). MCF7 cells were treated with vehicle (lane 7) or camptothecin for 6 (lane 8), 16 (lane 9) and 24 (lane 10) hours. Extracts of Hek293T cells (induced with etoposide) were treated with increasing concentrations of λ protein phosphatase (lane 4, 400x diluted; lane 5, 100x diluted; lane 6, 25x diluted) or left untreated (lane 3). Samples (lanes 1-2 and 7-10, 20 μ g/lane; lanes 3-6, 8 μ g/lane) were analyzed by Western blotting using the p53 pSer15 Detector Antibody of ab156027 kit (A), or a p53 antibody (ab1101) was used to detect total p53 protein (B).

The detector antibody used in this kit specifically detects the phosphorylated p53 as determined by Western blotting.

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