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Product datasheet

p53 Transcription Factor Assay Kit (Colorimetric) ab207225

3 References 画像数 1

医薬用外劇物

製品の概要

製品名	p53 Transcription Factor Assay Kit (Colorimetric)
検出方法	Colorimetric
サンプルの種類	Nuclear Extracts
アッセイタイプ	Semi-quantitative
検出感度	< 600 ng/well
全工程の試験時間	3h 30m
種交差性	**交差種:** Human
製品の概要	p53 Transcription Factor Assay Kit (Colorimetric) (ab207225) is a high throughput assay to quantify p53 activation in nuclear extracts. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor activation. A specific double stranded DNA sequence containing the p53 consensus binding site (5' – GGACATGCCCGGGCATGTCC – 3') has been immobilized onto a 96-well plate. Active p53 present in the nuclear extract specifically binds to the oligonucleotide. p53 is detected by a primary antibody that recognizes an epitope of p53 accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout that at OD 450 nm. This product detects only human p53. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.6 µg nuclear extract/well. Detection range: 0.6 – 10 µg nuclear extract/well.
特記事項	The tumor suppressor protein p53 is a transcription factor that switches on a series of protective genes when the cell is exposed to stressful events. Many solid tumors contain defective forms of p53 that are unable to stop cells from proliferating when, for example, their DNA has been damaged. Therefore, p53 functions to selectively destroy stressed or abnormal cells, thereby protecting the organism from cancer development. Stress events include radiation, low pH, heat

shock, hypoxia, genotoxins, DNA damage, RNA polymerase II block and oxidant injury. Two human p53 homologues, p73 and p63 were recently identified with roles in stem cell identity, neurogenesis, natural immunity and homeostatic control. These homologues can drive gene expression from promoters similar to that bound by p53, but neither of these have been found to be highly mutated in cancers, nor is p73 bound to viral oncoproteins that neutralize p53 protein activity, so their function in regulating p53-dependent cancer progression is unclear.

p53 possesses a modular architecture with an N-terminal transactivation domain, a strongly conserved core DNA-binding domain, a tetramerization domain, and a regulatory C terminus. The p53 DNA-binding domain comprises several hot spot regions for mutation that inactivate p53 in more than half of all human tumors. Tetrameric p53 binds specifically to a DNA consensus sequence consisting of two consecutive palindromic 10-bp half-sites 5´-RRRCWWGYYY-3´ (R = A or G, Y = C or T, W = A or T), which can be separated from 0 to 13 bp. The tetramer assembly stabilizes the p53 monomer folding and increases the DNA-binding activity of p53. p53 stays inactive in the nucleus when bound to MDM2 protein, an E3 ubiquitin ligase that targets both p53 and itself for ubiquitination. MDM2 represses p53 activity by inducing its nuclear export and degradation in proteasomes. Stress signals, such as DNA damage, activate protein kinases that lead to p53 phosphorylation of numerous sites and subsequent activation of p53 by inhibiting p53-MDM2 interaction. MDM2 gene expression is regulated by p53, creating a feedback loop in which p53 activates expression of MDM2, which keeps p53 levels low during normal growth and development.

試験プラットフォーム Microplate reader

製品の特性

保存方法 Please refer to protocols.

内容	1 x 96 tests	5 x 96 tests
10X Antibody Binding Buffer	1 x 2.2ml	5 x 2.2ml
10X Wash Buffer	1 x 22ml	5 x 22ml
96-well p53 assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	5 x 11µl
Binding Buffer	1 x 10ml	5 x 10ml
Developing Solution	1 x 11ml	5 x 11ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	5 x 100µl
Lysis Buffer	1 x 10ml	5 x 10ml
MCF-7 (H_2O_2) nuclear extract (2.5 mg/mL)	1 x 40µl	5 x 40µl
Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
p53 antibody (0.2 mg/mL)	1 x 11µl	5 x 11µl
Plate sealer	1 unit	5 units

内容	1 x 96 tests	5 x 96 tests
Poly [d(I-c)] (17 µg/µL)	1 x 100µl	5 x 100µl
Protease Inhibitor Cocktail	1 x 100µl	5 x 100µl
Stop Solution	1 x 11ml	5 x 11ml
Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl

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 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.

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 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.

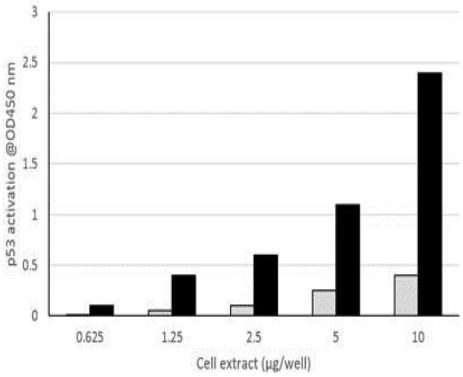
翻訳後修飾

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



Different amounts of nuclear extracts from untreated (Gray) and H2O2-treated (Black) MCF-7 cells are tested for p53 activation by using the p53 TF Assay Kit.

Different amounts of nuclear extracts from untreated (grey) and H_2O_2-treated (black) MCF-7 cells were tested for p53 activation. These curves are provided for demonstration only.

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