abcam

Product datasheet

Sp1 Transcription Factor Assay Kit (Colorimetric) ab207226

1 References 画像数 1

製品の概要

製品名	Sp1 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:** Mouse, Rat, Human
製品の概要	Sp1 Transcription Factor Assay Kit (Colorimetric) (ab207226) is a high throughput assay to quantify Sp1 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 Sp1 consensus binding site (5' – GGGGCGGGG – 3') has been immobilized onto a 96-well plate. Active Sp1 present in the nuclear extract specifically binds to the oligonucleotide. Sp1 is detected by a primary antibody that recognizes an epitope of Sp1 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 human, mouse and rat Sp1. 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 Sp1 transcription factor is a 105 kDa protein that can activate a wide subset of mammalian genes containing upstream promoter elements called a GC box (GGGGCGGGG) and the related GT/CACCC box (GGTGTGGGG). The C-terminal domain of Sp1 harbors three contiguous Cys-X_4-Cys-X_{12}-His-X_3-His repeats, which are typical of the Cys_2His_2-type zinc-finger DNA-binding domain that was first found in the TFIIIA transcription factor. Specific variations in the

ubiquitous expression of Sp1 suggest its involvement in gene regulation of cell cycle, hormonal activation and development patterning. Sp1 knock-out embryos show a broad range of abnormalities and usually die around day 11 of gestation. Recent studies suggest that Sp1 is an important regulator of expression of the methyl-CpG-binding protein MeCP2.

The Sp zinc finger transcription factor family is composed of four members (Sp1, Sp2, Sp3 and Sp4) that share similarity within their DNA-binding domains, transactivation domains and tissue expression patterns. Sp1, Sp3 and Sp4 are more closely related to each other than to Sp2, which does not bind to a GC-box but to a GT-rich element. Sp1 contains two glutamine-rich transcriptional activation domains that mediate direct interactions with the TATA box-binding protein (TBP) involved in the TFIID-RNA polymerase II complex. These activation domains can also interact directly with TAF110 transcription factor. A cooperative interaction between Sp1 and NFκB p65 is required for the efficient stimulation of HIV-1 transcription. Sp1 has been shown to interact with YY1, Oct-1, E2F-1, E2F-3 and p74. The role of Sp3 alone is not well defined as an activator or repressor. However, gene transcription has been shown to be regulated by the ratio of Sp1 and Sp3 in different cell models. These two Sp-family members compete with each other to bind to Sp1 DNA binding sites. Sp4 expression appears to be restricted to a few tissues. Sp4 is highly expressed during the development of the mouse embryo central nervous system (CNS) and seems to be required for normal male reproductive behavior. Recently, the role of Sp1 in breast cancer and aging has been reassessed due to its effect on estrogen and progesterone receptor transcription levels. Sp1 phosphorylation has also been the focus of investigations on proapoptotic and angiogenic gene transcription regulation in vascular smooth muscle and vascular endothelial cells.

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	1 x 11ml
10X Wash Buffer	1 x 22ml	1 x 110ml
96-well Sp1 assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	1 x 55µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
Lysis Buffer	1 x 10ml	1 x 50ml
MCF-7 nuclear extract (2.5 µg/µL)	1 x 40µl	1 x 200µl
Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl
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	1 x 500µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Sp1 antibody	1 x 11µl	1 x 55µl
Stop Solution	1 x 11ml	1 x 55ml
Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl

機能

Transcription factor that can activate or repress transcription in response to physiological and pathological stimuli. Binds with high affinity to GC-rich motifs and regulates the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation and immune responses. Highly regulated by post-translational modifications (phosphorylations, sumoylation, proteolytic cleavage, glycosylation and acetylation). Binds also the PDGFR-alpha G-box promoter. May have a role in modulating the cellular response to DNA damage. Implicated in chromatin remodeling. Plays a role in the recruitment of SMARCA4/BRG1 on the c-FOS promoter. Plays an essential role in the regulation of FE65 gene expression. In complex with ATF7IP, maintains telomerase activity in cancer cells by inducing TERT and TERC gene expression.

組織特異性

Up-regulated in adenocarcinomas of the stomach (at protein level).

配列類似性

Belongs to the Sp1 C2H2-type zinc-finger protein family.
Contains 3 C2H2-type zinc fingers.

翻訳後修飾

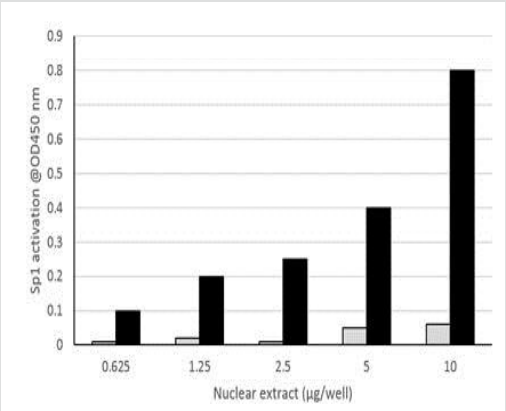
Phosphorylated on multiple serine and threonine residues. Phosphorylation is coupled to ubiquitination, sumoylation and proteolytic processing. Phosphorylation on Ser-59 enhances proteolytic cleavage. Phosphorylation on Ser-7 enhances ubiquitination and protein degradation. Hyperphosphorylation on Ser-101 in response to DNA damage has no effect on transcriptional activity. MAPK1/MAPK3-mediated phosphorylation on Thr-453 and Thr-739 enhances VEGF transcription but, represses FGF2-triggered PDGFR-alpha transcription. Also implicated in the repression of RECK by ERBB2. Hyperphosphorylated on Thr-278 and Thr-739 during mitosis by MAPK8 shielding SP1 from degradation by the ubiquitin-dependent pathway. Phosphorylated in the zinc-finger domain by calmodulin-activated PKCzeta. Phosphorylation on Ser-641 by PKCzeta is critical for TSA-activated LHR gene expression through release of its repressor, p107. Phosphorylation on Thr-668, Ser-670 and Thr-681 is stimulated by angiotensin II via the AT1 receptor inducing increased binding to the PDGF-D promoter. This phosphorylation is increased in injured artey wall. Ser-59 and Thr-681 can both be dephosphorylated by PP2A during cell-cycle interphase. Dephosphorylation on Ser-59 leads to increased chromatin association during interphase and increases the transcriptional activity. On insulin stimulation, sequentially glycosylated and phosphorylated on several C-terminal serine and threonine residues.
Acetylated. Acetylation/deacetylation events affect transcriptional activity. Deacetylation leads to an increase in the expression the 12(s)-lipooxygenase gene though recruitment of p300 to the promoter.
Ubiquitinated. Ubiquitination occurs on the C-terminal proteolytically-cleaved peptide and is triggered by phosphorylation.
Sumoylated by SUMO1. Sumoylation modulates proteolytic cleavage of the N-terminal repressor domain. Sumoylation levels are attenuated during tumorigenesis. Phosphorylation mediates SP1 desumoylation.
Proteolytic cleavage in the N-terminal repressor domain is prevented by sumoylation. The C-

terminal cleaved product is susceptible to degradation.
O-glycosylated; contains at least 8 N-acetylglucosamine side chains. Levels are controlled by insulin and the SP1 phosphorylation states. Insulin-mediated O-glycosylation locates SP1 to the nucleus, where it is sequentially deglycosylated and phosphorylated. O-glycosylation affects transcriptional activity through disrupting the interaction with a number of transcription factors including ELF1 and NFYA. Also inhibits interaction with the HIV1 promoter. Inhibited by peroxisomome proliferator receptor gamma (PPARgamma).

細胞内局在

Nucleus. Cytoplasm. Nuclear location is governed by glycosylated/phosphorylated states. Insulin promotes nuclear location, while glucagon favors cytoplasmic location.

画像



Different amounts of untreated (Black) and H2O2-post treated (Gray) nuclear extracts from MCF-7 cells were tested for Sp1 activation.

Different amounts of H_2O_2-post treated (grey) and untreated nuclear extracts (black) from MCF-7 cells were tested for Sp1 activation. These curves are provided for demonstration only.

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