abcam

Product datasheet

Anti-SP1 (phospho T453) antibody ab59257

6 Abreviews 19 References 5 Images

Overview

Product name	Anti-SP1 (phospho T453) antibody
Description	Rabbit polyclonal to SP1 (phospho T453)
Host species	Rabbit
Specificity	ab59257 detects endogenous levels of SP1 only when phosphorylated at threonine 453.
Tested applications	**Suitable for:** ELISA, IHC-P, WB, ChIP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic phosphopeptide derived from human SP1 around the phosphorylation site of threonine 453 (I-R-T^{P}-P-T).
Positive control	IHC-P: Human brain tissue. WB: A549 cell extracts.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 49% PBS, 50% Glycerol, 0.87% Sodium chloride Without Mg+2 and Ca+2
Purity	Immunogen affinity purified
Purification notes	ab59257 was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab59257** 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
ELISA		1/5000.
IHC-P		Use at an assay dependent concentration.
WB	★★★★☆	1/500 - 1/1000. Detects a band of approximately 90 kDa (predicted molecular weight: 81 kDa).
ChIP		Use at an assay dependent concentration. PubMed: 20375015
ICC/IF		Use at an assay dependent concentration. PubMed: 21115498

Target

Function

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.

Tissue specificity

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

Sequence similarities

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

Post-translational modifications

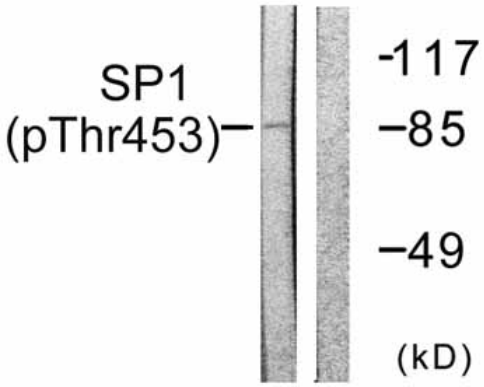
Phosphorylated on multiple serine and threonine residues. Phosphorylation is coupled to ubiquitination, sumoylation and proteolytic processing. Phosphorylation on Ser-59 enhances proteolytic cleavage. Phosphorylation on Ser-7 enhances ubiquitination and protein degradation. Hyperphosphorylation on Ser-101 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).

Cellular localization

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

Images

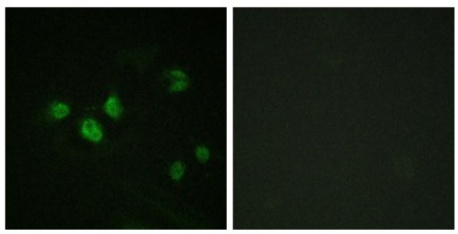


Western blot - Anti-SP1 (phospho T453) antibody (ab59257)

All lanes : Anti-SP1 (phospho T453) antibody (ab59257) at 1/500 dilution

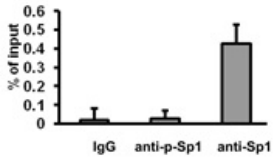
Lane 1 : A549 cell extracts
Lane 2 : A549 cell extracts with immunising phospho peptide

Predicted band size: 81 kDa
Observed band size: 90 kDa
why is the actual band size different from the predicted?



Immunocytochemistry/ Immunofluorescence - Anti-SP1 (phospho T453) antibody (ab59257)

Immunofluorescence analysis of HeLa cells, using ab59257 Antibody. The picture on the right is treated with the synthesized peptide.

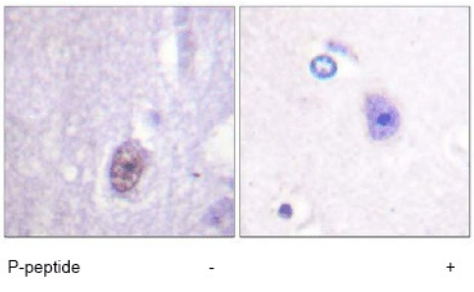


ChIP - Anti-SP1 (phospho T453) antibody (ab59257)

Image from Yi et al., J Biol Chem. 2010 Jun 4;285(23):17811-20. Fig. 6. doi: 10.1074/jbc.M110.130740.

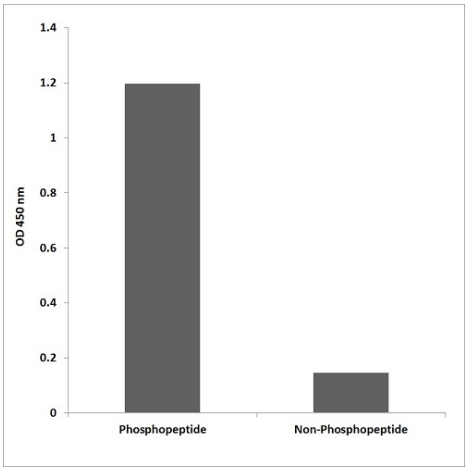
Wild type MEF cells (2×10^7 cells) were cross-linked with formaldehyde, quenched with glycine, resuspended in SDS lysis buffer (1% SDS, 10 mm EDTA, 50 mm Tris-HCl, pH 8.0, with protease inhibitors and phosphatase inhibitors), sonicated on ice, and centrifuged at 4 °C. Supernatant (400 µl) were diluted to a final volume of 4 ml in a mixture of 9 parts dilution buffer (1% Triton X-100, 150 mm NaCl, 2 mm EDTA, 20 mm Tris-HCl, with protease inhibitors, pH 8.0) and 1 part lysis buffer.

Mixtures were incubated with 4 µg of anti-p-Sp1 (ab 59257) or anti-Sp1 antibodies with rotating at 4 °C overnight followed with incubation with 100 µl of protein A beads with rotating at 4 °C for 4 h. After gentle centrifugation (2000 rpm), beads were resuspended in 1 ml of wash buffer (1% Triton X-100, 0.1% SDS, 150 mm NaCl, 2 mm EDTA, 20 mm Tris-HCl, with protease inhibitors, pH 8.0) and washed with wash buffer 3 times followed by one wash with a final wash buffer (1% Triton X-100, 0.1% SDS, 500 mm NaCl, 2 mm EDTA, 20 mm Tris-HCl, pH 8.0, with protease inhibitors). The immune complexes were eluted with elution buffer (1% SDS, 100 mm $NaHCO_3$) followed by incubation with proteinase K and RNase A (500 µg/ml each) at 37 °C for 30 min. Reverse cross-links were performed by placing the tubes at 65 °C overnight. Immunoprecipitated DNA was extracted and dissolved in sterile water and Q-PCR was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 (phospho T453) antibody (ab59257)

ab59257, at 1/50 dilution, staining SP1 in paraffin embedded human brain tissue by Immunohistochemistry in the absence or presence of the immunising peptide.



ELISA - Anti-SP1 (phospho T453) antibody (ab59257)

ab59257 (1:1000) Antibody detects endogenous levels of SP1 only when phosphorylated at Thr453.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors