abcam

Product datasheet

Anti-SP1 antibody [EPR6662(B)] ab124804

敲除验证 重组 RabMAb

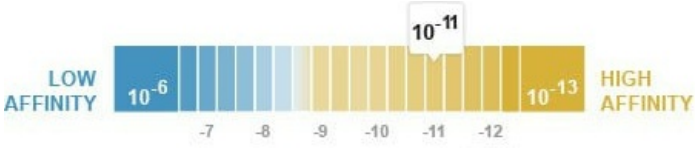
★★★★★ 1 Abreviews 27 References 12 图像

概述

产品名称	Anti-SP1抗体[EPR6662(B)]
描述	兔单克隆抗体[EPR6662(B)] to SP1
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, ChIC/CUT&RUN-seq **不适用于:** IP
种属反应性	**与反应:** Human **不与反应:** Mouse, Rat
免疫原	Synthetic peptide within Human SP1 aa 550-650. The exact sequence is proprietary.
阳性对照	WB: Ramos, HAP1, HeLa K562 and Raji cell lysates. IHC-P: human gastric carcinoma, Human colon tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. ChIC/CUT&RUN-Seq: HeLa cells.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
解离常数 (K_D)	K_D = 3.30 x 10^{-11} M



Learn more about K_D

存储溶液	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR6662(B)
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab124804于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/100.
WB	★★★★★ (1)	1/1000 - 1/10000. Detects a band of approximately 90 kDa (predicted molecular weight: 81 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. (Heat to 98&degC, allow to cool for 10-20 minutes) See **IHC antigen retrieval protocols**
ICC/IF		1/100 - 1/250.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg

应用说明 Is unsuitable for IP.

靶标

功能 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.

组织特异性 Up-regulated in adenocarcinomas of the stomach (at protein level).

序列相似性 Belongs to the Sp1 C2H2-type zinc-finger protein family.
Contains 3 C2H2-type zinc fingers.

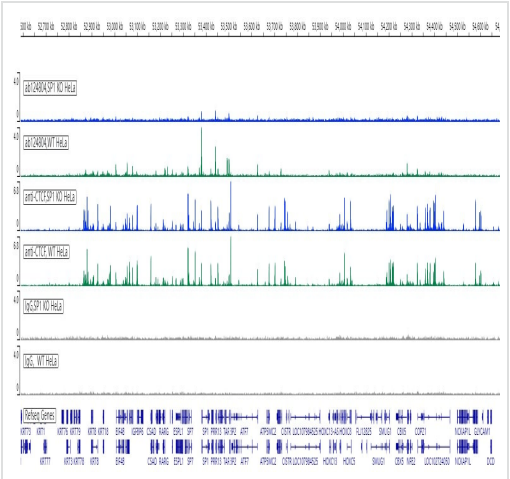
翻译后修饰 Phosphorylated on multiple serine and threonine residues. Phosphorylation is coupled to ubiquitination, sumoylation and proteolytic processing. Phosphorylation on Ser-59 enhances proteolytic cleavage. Phosphorylation on Ser-7 enhances ubiquitination and protein degradation.

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).

细胞定位

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

图片

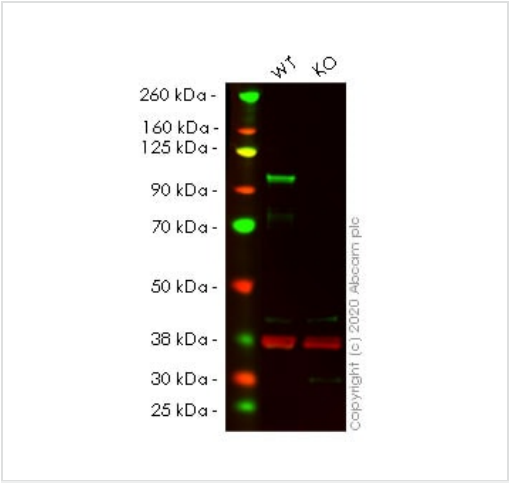


ChIC/CUT&RUN sequencing - Anti-SP1 antibody [EPR6662(B)] (ab124804)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL. 2.5X10^5 of Human wild-type HeLa cell line (**ab255928**) or Human SP1 knockout HeLa cell line (**ab265519**) were used along with 5µg of ab124804 [EPR6662(B)]. Assay Quality Control was conducted using 5µg Anti-CTCF (**ab188408**) on the same cell lines. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-SP1 antibody [EPR6662(B)] (ab124804)

All lanes : Anti-SP1 antibody [EPR6662(B)] (ab124804) at 1/5000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SP1 knockout HeLa cell lysate

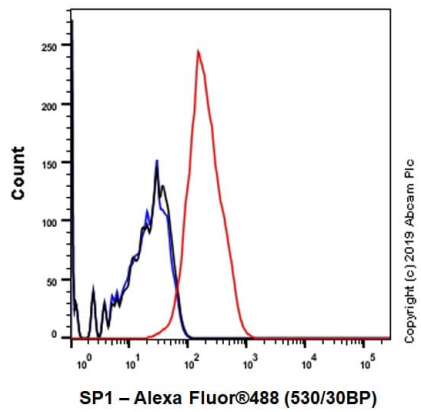
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 100 kDa

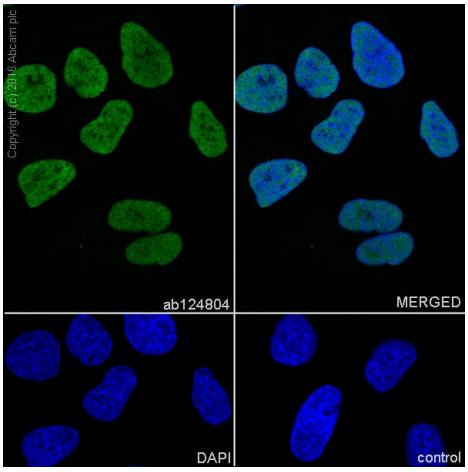
Lanes 1-2: Merged signal (red and green). Green - ab124804 observed at 100 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab124804 Anti-SP1 antibody [EPR6662(B)] was shown to specifically react with SP1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265519** (knockout cell lysate **ab257698**) was used. Wild-type and SP1 knockout samples were subjected to SDS-PAGE. ab124804 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 5000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



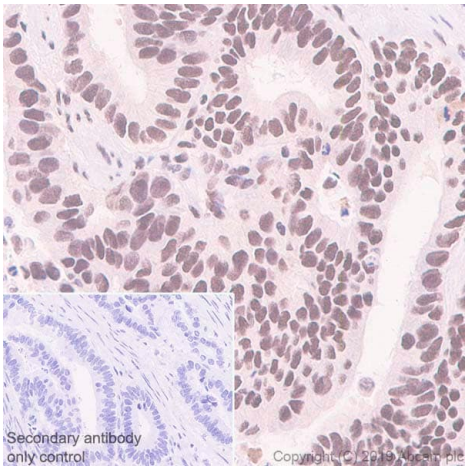
Flow Cytometry (Intracellular) - Anti-SP1 antibody [EPR6662(B)] (ab124804)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SP1 with Purified ab124804 at 1/100 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



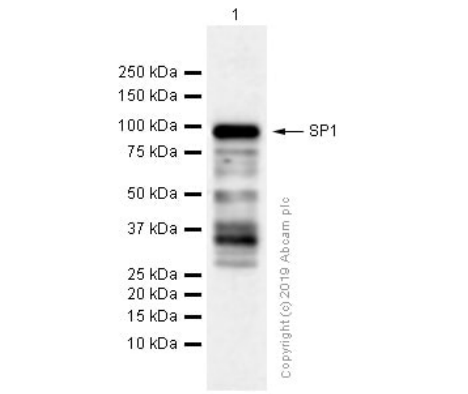
Immunocytochemistry/ Immunofluorescence - Anti-SP1 antibody [EPR6662(B)] (ab124804)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SP1 with Purified ab124804 at 1:200 dilution (4 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with None. Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR6662(B)] (ab124804)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric carcinoma tissue sections labeling SP1 with Purified ab124804 at 1:2000 dilution (0.41 µg/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Purified)ImmunoHistoProbe one step HRP Polymer (ready to use) was used for detection. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-SP1 antibody [EPR6662(B)] (ab124804)

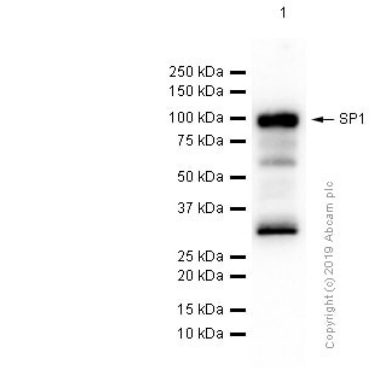
All lanes : Anti-SP1 antibody [EPR6662(B)] (ab124804) at 1/5000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 81 kDa
Observed band size: 100 kDa

We are unsure how to define the extra bands.



Western blot - Anti-SP1 antibody [EPR6662(B)] (ab124804)

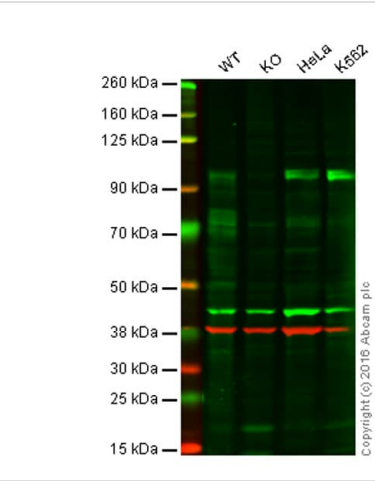
Anti-SP1 antibody [EPR6662(B)] (ab124804) at 1/5000 dilution (Purified) + Ramos (Human Burkitt's lymphoma B lymphocyte) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 81 kDa
Observed band size: 100 kDa

We are unsure how to define the extra bands.



Western blot - Anti-SP1 antibody [EPR6662(B)] (ab124804)

All lanes : Anti-SP1 antibody [EPR6662(B)] (ab124804) at 1/1000 dilution

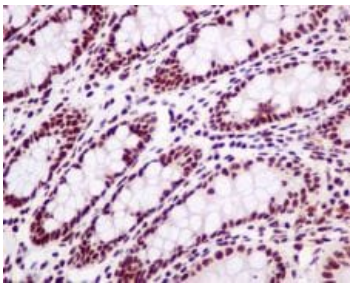
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : SP1 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : K562 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 81 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab124804 observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

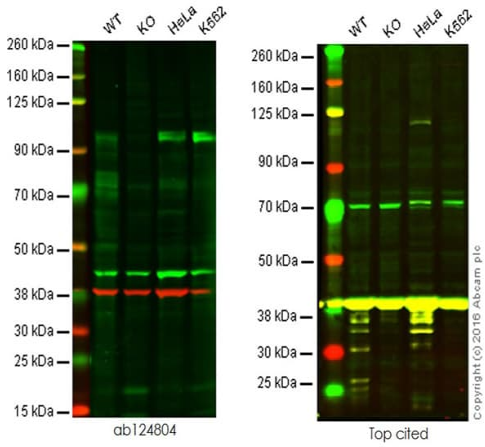
Ab16032 detected the expected band for SP1 in wild-type HAP1 cells along with additional cross-reactive bands. The band was not seen in SP1 knockout HAP1 cells. Wild-type and SP1 knockout samples were subjected to SDS-PAGE. ab124804 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR6662(B)] (ab124804)

ab124804, at 1/100 dilution staining SP1 in paraffin-embedded Human colon tissue, by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-SP1 antibody [EPR6662(B)] (ab124804)

All lanes : Anti-SP1 antibody [EPR6662(B)] (ab124804)

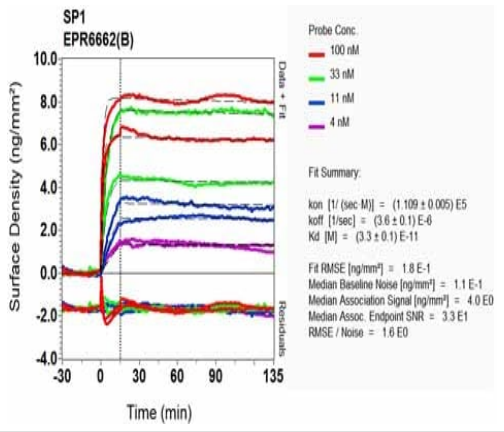
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : SP1 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : K562 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 81 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab124804 observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This western blot image is a comparison between ab124804 and a competitor's top cited rabbit polyclonal antibody.



OI-RD Scanning - Anti-SP1 antibody [EPR6662(B)] (ab124804)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-SP1 antibody [EPR6662(B)] (ab124804)

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