abcam

Product datasheet

Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free ab240001

KO VALIDATED Recombinant RabMAb

8 Images

Overview

Product name	Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free
Description	Rabbit monoclonal [EPR6662(B)] to SP1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, ChIC/CUT&RUN-seq, ICC/IF, WB, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human **Does not react with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab240001 is the carrier-free version of **ab124804**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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

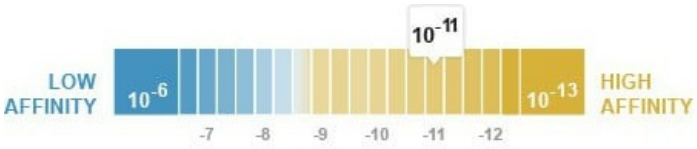
monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 3.30 \times 10^{-11}$ M



Learn more about K_D

Storage buffer pH: 7.2
Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number EPR6662(B)

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240001 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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. (heat to 98 degrees C, allow to cool for 10-20 minutes)
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 90 kDa (predicted molecular weight: 81 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration.

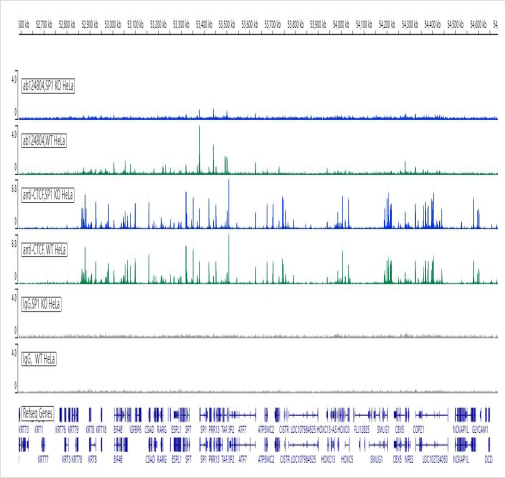
Application notes Is unsuitable for IP.

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



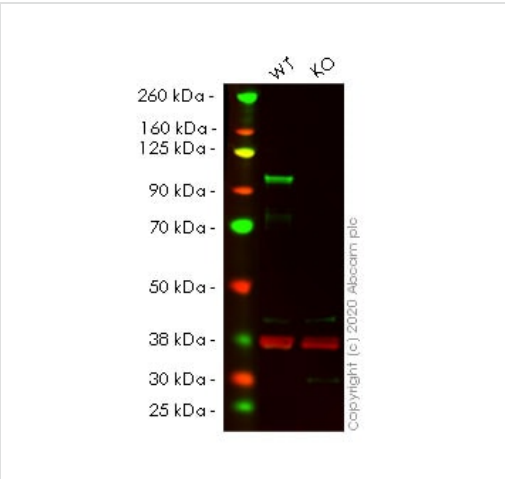
ChIC/CUT&RUN sequencing - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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.

This data was developed using the same antibody clone in a different buffer formulation (**ab124804**).



Western blot - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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.

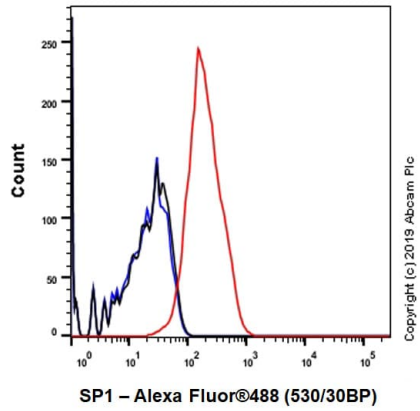
Predicted band size: 81 kDa
Observed band size: 100 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab124804**).

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

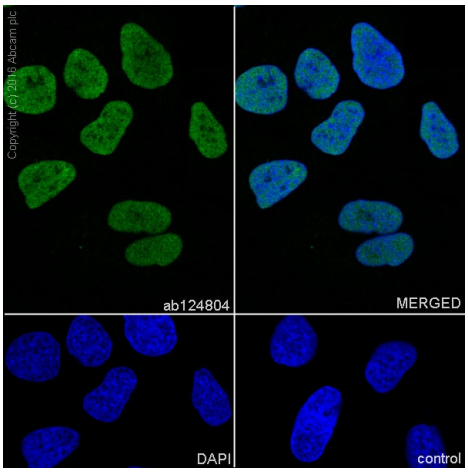
ab124804 Anti-SP1 antibody [EPR6662(B)] was shown to specifically react with SP1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265519** (knockout cell lysate **ab257698**) was used. Wild-type and SP1 knockout samples were subjected to SDS-PAGE. **ab124804** and Anti-GAPDH 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.



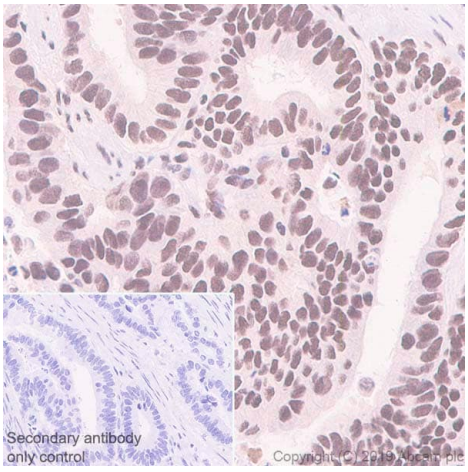
Flow Cytometry (Intracellular) - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124804**)



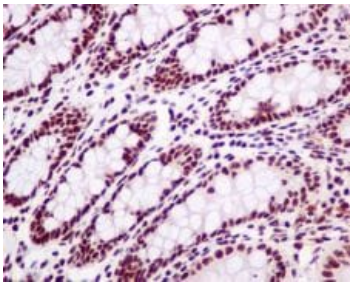
Immunocytochemistry/ Immunofluorescence - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124804**)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124804**)

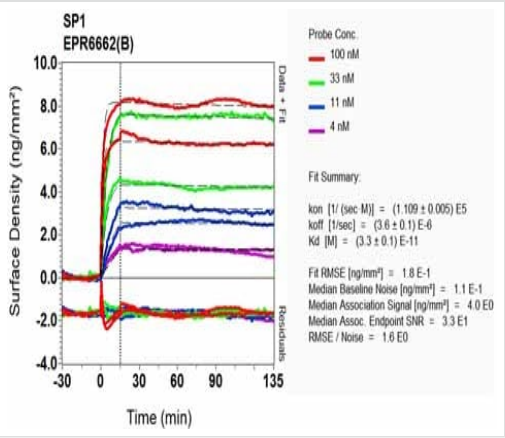


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124804**).

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



OI-RD Scanning - Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124804**).

Anti-SP1 antibody [EPR6662(B)] - BSA and Azide free (ab240001)

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