

Product datasheet

Anti-SP1 antibody [EPR22648-50] - BSA and Azide free ab255289

KO VALIDATED Recombinant RabMAb

[1 References](#) [10 Images](#)

Overview

Product name	Anti-SP1 antibody [EPR22648-50] - BSA and Azide free
Description	Rabbit monoclonal [EPR22648-50] to SP1 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ChIP-sequencing, ChIC/CUT&RUN-seq, ChIP, WB, IHC-P, ICC/IF, IP
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, K-562 and HEK-293T whole cell lysate. IHC-P: Human breast carcinoma and gastric carcinoma tissue. ICC/IF: HeLa cells. Flow Cyt: HeLa cells. IP: HeLa cell lysate. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells. ChIC/CUT&RUN-Seq: Wild-type HeLa cells.
General notes	<p>ab255289 is the carrier-free version of ab231778.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>

Properties

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

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22648-50
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255289 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

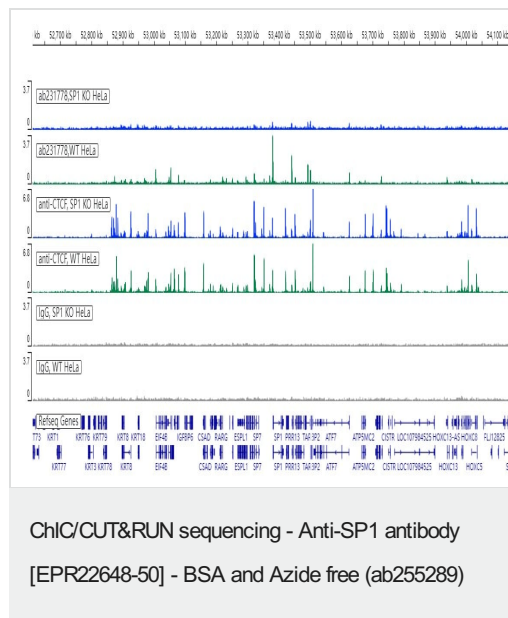
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIP-sequencing		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 81 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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.
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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	<p>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 artery 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.</p> <p>Acetylated. Acetylation/deacetylation events affect transcriptional activity. Deacetylation leads to an increase in the expression the 12(s)-lipooxygenase gene through recruitment of p300 to the promoter.</p> <p>Ubiquitinated. Ubiquitination occurs on the C-terminal proteolytically-cleaved peptide and is triggered by phosphorylation.</p> <p>Sumoylated by SUMO1. Sumoylation modulates proteolytic cleavage of the N-terminal repressor domain. Sumoylation levels are attenuated during tumorigenesis. Phosphorylation mediates SP1 desumoylation.</p> <p>Proteolytic cleavage in the N-terminal repressor domain is prevented by sumoylation. The C-terminal cleaved product is susceptible to degradation.</p> <p>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 peroxisome proliferator receptor gamma (PPARgamma).</p>
Cellular localization	Nucleus. Cytoplasm. Nuclear location is governed by glycosylated/phosphorylated states. Insulin promotes nuclear location, while glucagon favors cytoplasmic location.

Images

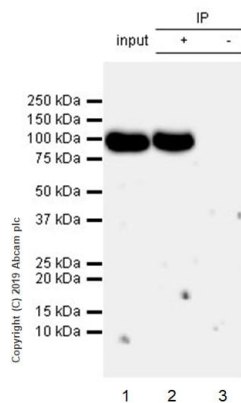


ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL. 2.5×10^5 of Human wild-type HeLa cell line (**ab255928**) or Human SP1 knockout HeLa cell line (**ab265519**) were used along with 5 μ g of **ab231778** [EPR22648-50]. Assay Quality Control was conducted using 5 μ g Anti-CTCF (**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.

All lanes : Anti-SP1 antibody [EPR22648-50] - ChIP Grade
(**ab231778**) at 1/1000 dilution

This data was developed using the same antibody clone in a different buffer formulation (**ab231778**).

was observed when knockout cell line **ab265519** (knockout cell lysate **ab257698**) was used. Wild-type and SP1 knockout samples were subjected to SDS-PAGE. **ab231778** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 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.



Immunoprecipitation - Anti-SP1 antibody
[EPR22648-50] - BSA and Azide free (**ab255289**)

SP1 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate using **ab231778** at 1/30 dilution. Western blot was performed on the immunoprecipitate using **ab231778** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used as the secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (input).

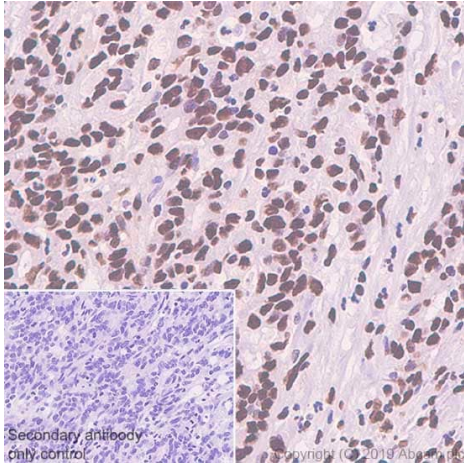
Lane 2: **ab231778** IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab231778** in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab231778**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

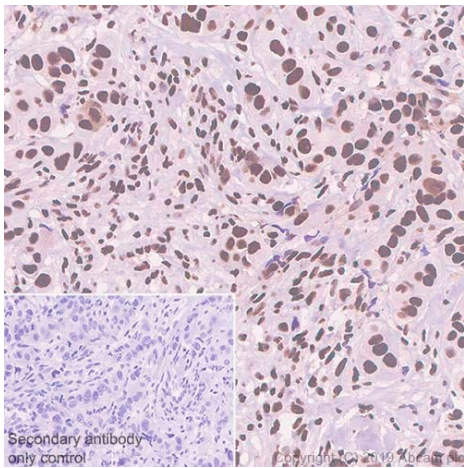
Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue labeling SP1 using [ab231778](#) at 1/500 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). Counterstained with hematoxylin.

Nuclear staining on tumor cells of human gastric carcinoma (PMID: 14695137). The section was incubated with [ab231778](#) for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab231778](#)).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

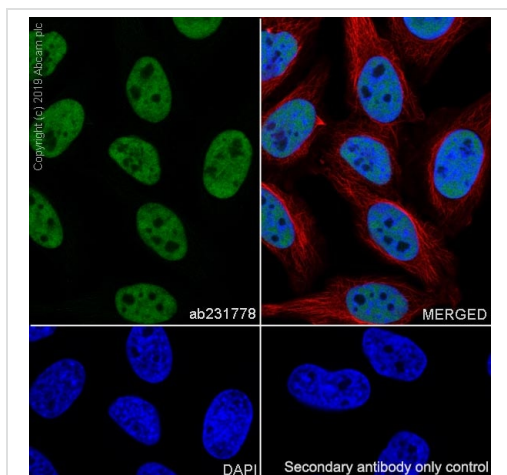
Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling SP1 using [ab231778](#) at 1/500 dilution, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). Counterstained with hematoxylin.

Nuclear staining on tumor cells of human breast carcinoma (PMID: 14695137). The section was incubated with [ab231778](#) for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab231778](#)).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

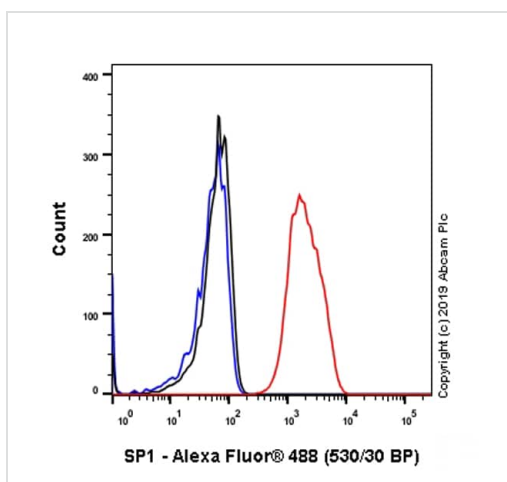


Immunocytochemistry/ Immunofluorescence - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling SP1 (green) with **ab231778** at 1/100 dilution, followed by an AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution. Confocal image showing nuclear staining in HeLa cell line. The nuclear counterstain is DAPI (Blue). Tubulin was stained using an Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (Red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**).

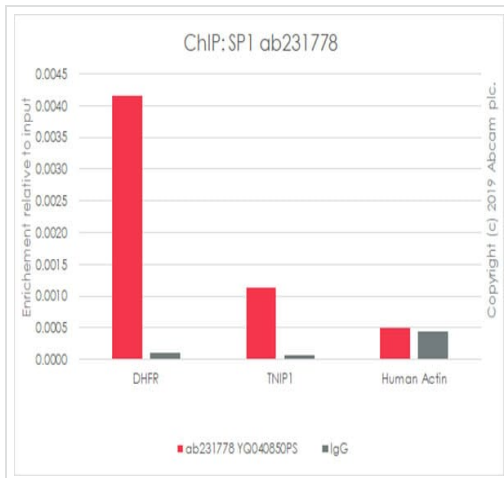
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab231778**).



Flow Cytometry (Intracellular) - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling SP1 using **ab231778** at 1/60 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**, Black) isotype control and a unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). The secondary antibody was a Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at a 1/2000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab231778**).



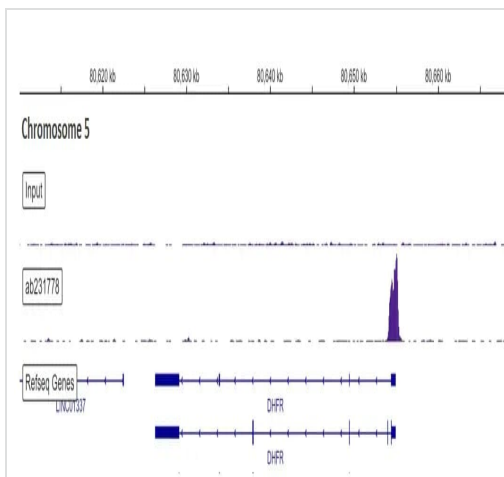
ChIP - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of **ab231778** (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab231778**).



ChIP-sequencing - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

This data was developed using the same antibody clone in a different buffer formulation (ab255289).

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-SP1 antibody [EPR22648-50] - ChIP Grade (**ab231778**). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

Additional screenshots of mapped reads can be downloaded [here](#).

Why choose a recombinant antibody?



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Consistent and reproducible results



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Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

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