abcam

ab255289

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

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR22648-50
Purification technique	Affinity purification Protein A
Concentration	1.018 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-

Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins.

Target data

See full target information SP1

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). Also binds the PDGFR-alpha G-box promoter. May have a role in modulating the cellular response to DNA damage. Implicated in chromatin remodeling. 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. Isoform 3 is a stronger activator of transcription than isoform 1. Positively regulates the transcription of the core clock component BMAL1 (PubMed:10391891, PubMed:11371615, PubMed:11904305, PubMed:14593115, PubMed:16377629, PubMed:16478997, PubMed:16943418, PubMed:17049555, PubMed:18171990, PubMed:18199680, PubMed:18239466, PubMed:18513490, PubMed:18619531, PubMed:19193796, PubMed:20091743, PubMed:21046154, PubMed:21798247). Plays a role in the recruitment of SMARCA4/BRG1 on the c-FOS promoter. Plays a

role in protecting cells against oxidative stress following brain injury by regulating the expression of RNF112 (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab255289 is the carrier-free version of ab231778.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility

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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

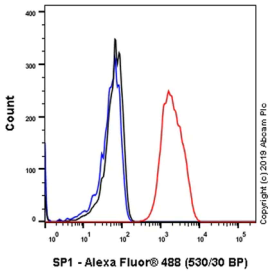
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

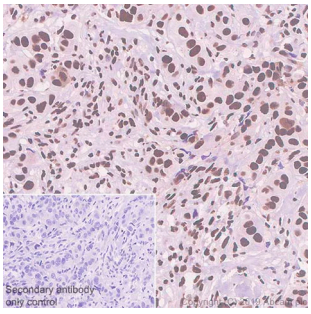
10 product images



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

SP1 Flow Cytometry (Intracellular) staining using rabbit Anti-SP1 antibody

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).



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

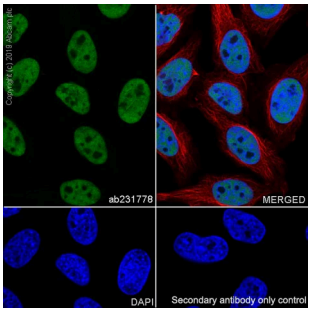
SP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SP1 antibody

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.

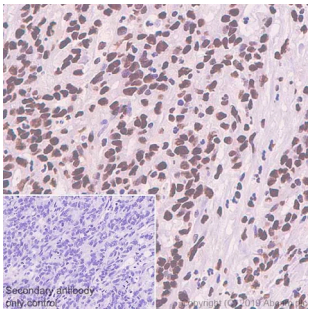


Immunocytochemistry/ Immunofluorescence - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

SP1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-SP1 antibody

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).



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

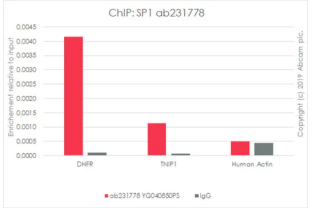
SP1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-SP1 antibody

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).

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

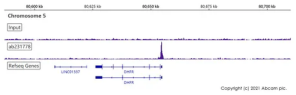


ChIP - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

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).

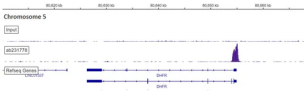
Primers and probes are located in the first kb of the transcribed region.

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



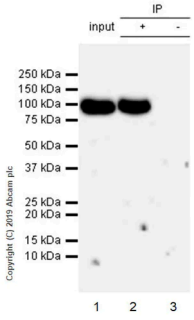
ChIP-sequencing - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

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 ab231778. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. ChIP-Seq validation performed with ChIP-Kit Transcription Factors ChIP-Seq (ab270813).
Additional screenshots of mapped reads can be downloaded here.



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 (ab231778). 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.



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.

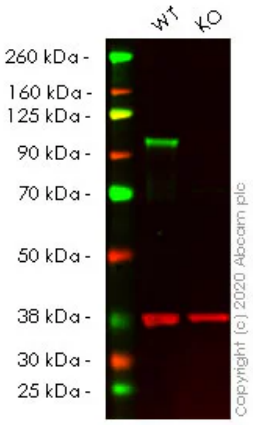
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

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).

All lanes:
Immunoprecipitation - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

Predicted band size: 81 kDa



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

SP1 Western blot staining using rabbit Anti-SP1 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab231778).
Lanes 1-2: Merged signal (red and green). Green - ab231778 observed at 100 kDa. Red - loading control ab8245 observed at 37 kDa.

ab231778 Anti-SP1 antibody [EPR22648-50] 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. 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.

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

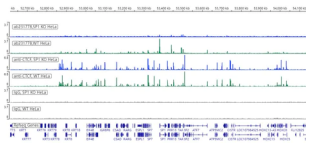
Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human SP1 knockout HeLa cell lysate (ab257698) at 20 µg

Performed under reducing conditions.

Predicted band size: 81 kDa

Observed band size: 100 kDa



ChIC/CUT&RUN sequencing - Anti-SP1 antibody [EPR22648-50] - BSA and Azide free (ab255289)

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 ab231778 [EPR22648-50]. 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 found in the Protocol booklet in the Product Protocol section. 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 (ab231778).

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.