abcam

Product datasheet

Anti-SP1 antibody [EPR22648-50] - ChIP Grade ab231778

敲除验证 重组 RabMAb

13 References 13 图像

概述

产品名称	Anti-SP1抗体[EPR22648-50] - ChIP Grade
描述	兔单克隆抗体[EPR22648-50] to SP1 - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** ChIP-sequencing, Flow Cyt (Intra), ChIC/CUT&RUN-seq, WB, IHC-P, ICC/IF, IP, ChIP
种属反应性	**与反应:** Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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.
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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR22648-50

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab231778**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
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
Flow Cyt (Intra)		1/60.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
WB		1/1000. Predicted molecular weight: 81 kDa.
IHC-P		1/500. 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		1/100.
IP		1/30.
ChIP		Use 5 µg for 25 µg of chromatin.

靶标

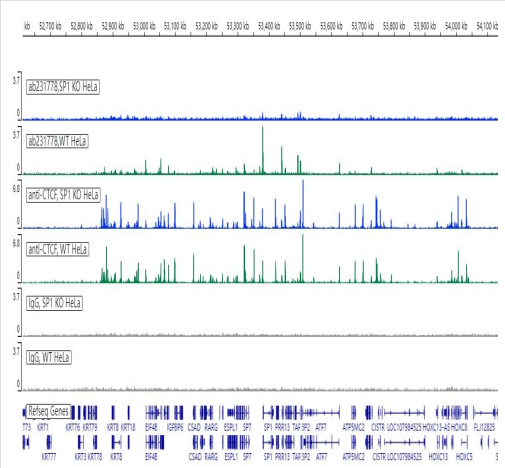
功能	Transcription factor that can activate or repress transcription in response to physiological and pathological stimuli. Binds with high affinity to GC-rich motifs and regulates the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation 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

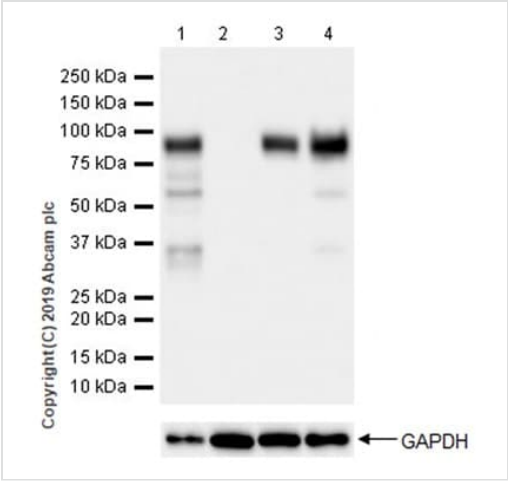


ChIC/CUT&RUN sequencing - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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 downloaded **here**.

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



Western blot - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SP1 knockout HAP1 whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 4 : 293T (human embryonic kidney epithelial cell) whole cell lysate

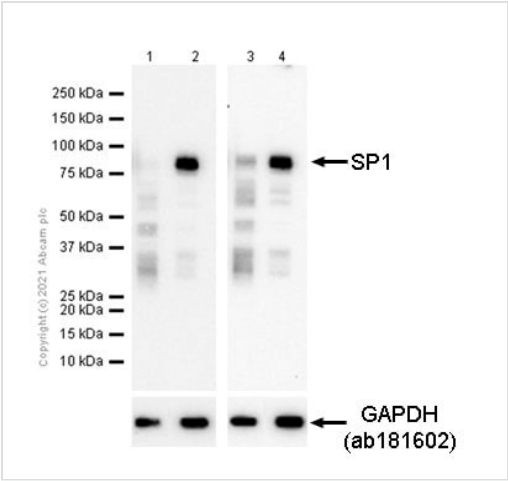
Lysates/proteins at 20 µg per lane.

Predicted band size: 81 kDa
Observed band size: 98 kDa

Exposure time: 8 seconds

ab231778 was shown to specifically react with SP1 in wild-type HAP1 cells as signal was lost in SP1 knockout cells. Wild-type and SP1 knockout samples were subjected to SDS-PAGE. ab231778 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument using the ECL technique.

Degraded fragments (29-97kDa) of SP1 have been descried in the literature (PMID: 10329728).



Western blot - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

All lanes : Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778) at 1/10000 dilution

Lanes 1-2 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lanes 3-4 : 293T (human embryonic kidney epithelial cell) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 81 kDa
Observed band size: 98 kDa

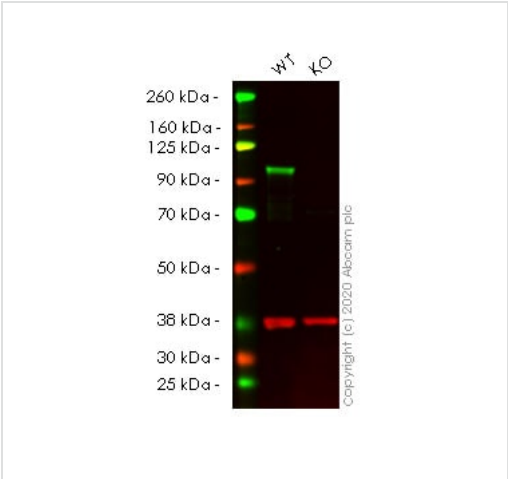
Exposure time: 40 seconds

Lanes 1 and 3: Samples were made from freeze-thaw cycled lysate.

Lanes 2 and 4: Samples were freshly made and used immediately to minimize protein degradation.

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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

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

Lysates/proteins at 20 µg per lane.

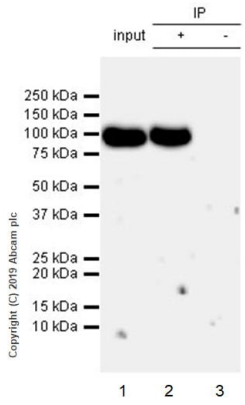
Performed under reducing conditions.

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

Lanes 1-2: Merged signal (red and green). Green - 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.



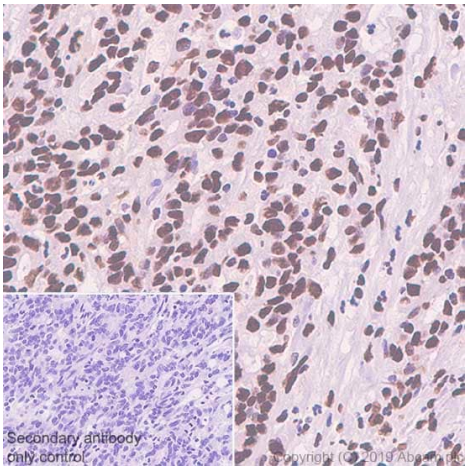
Immunoprecipitation - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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.



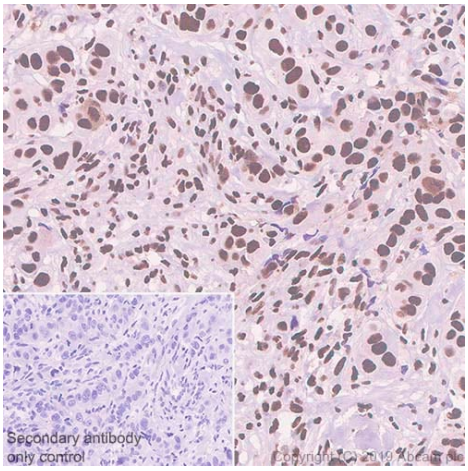
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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**).

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



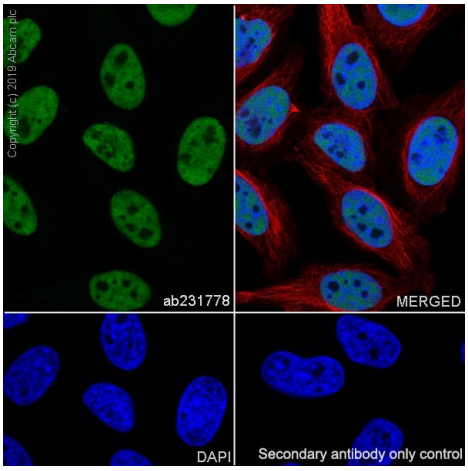
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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**).

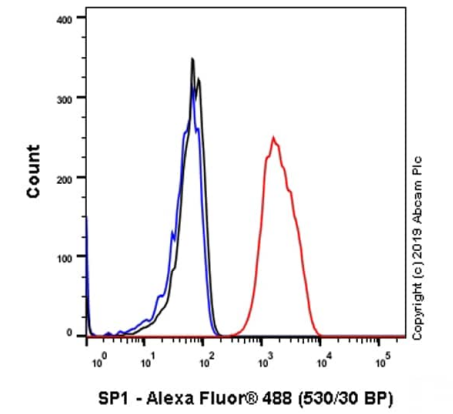
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

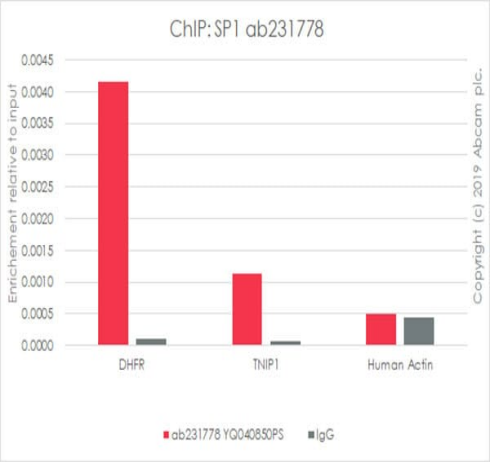
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling SP1 (green) with ab231778 at 1/100 dilution, followed by an AlexaFluor® 488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution. Confocal 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**).



Flow Cytometry (Intracellular) - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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.

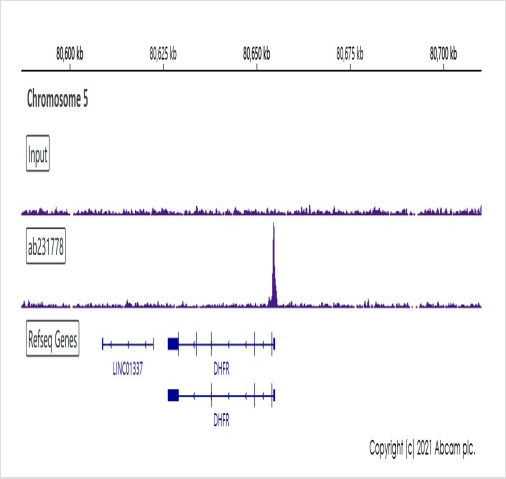


ChIP - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (ab231778)

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).

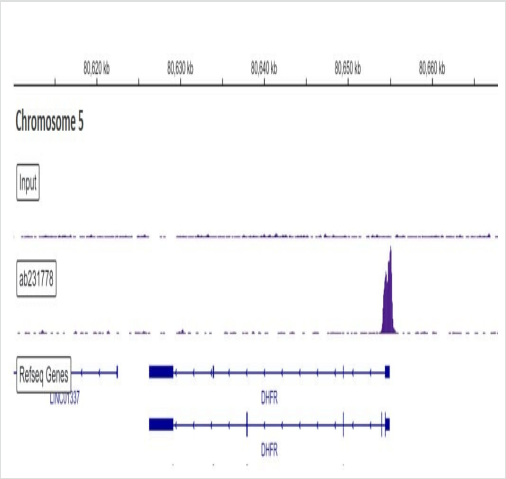
Primers and probes are located in the first kb of the transcribed region.



ChIP-sequencing - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (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 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**).

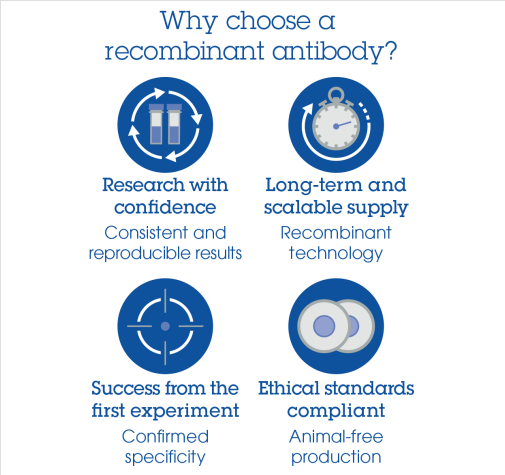
Additional screenshots of mapped reads can be downloaded **here**.



ChIP-sequencing - Anti-SP1 antibody [EPR22648-50] - ChIP Grade (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**.



Anti-SP1 antibody [EPR22648-50] - ChIP Grade

(ab231778)

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