# abcam

## Product datasheet

## Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free ab241391

Recombinant RabMAb

### 12 Images

#### Overview

**Product name** Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free

**Description** Rabbit monoclonal [EPR18253] to CTCF - ChIP Grade - BSA and Azide free

**Host species** Rabbit

**Tested applications** Suitable for: ChIC/CUT&RUN-seq, ChIP-sequencing, WB, IHC-P, ICC/IF, ChIP

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, LLC, RAW 264.7, HEK-293, HepG2, MCF7, C6, PC-12 and NIH/3T3 whole cell

> lysates; human fetal brain, fetal heart, fetal kidney and fetal spleen lysates; mouse brain, heart, kidney and spleen lysates; rat brain, heart, kidney and spleen lysates. IHC-P: Human endometrium, mouse liver and rat stomach tissues. ICC/IF: HeLa and NIH/3T3 cells. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from Hela and Mouse

Embryonic Fibroblasts cells. ChlC/CUT&RUN-Seq: HeLa and F9 cells.

**General notes** ab241391 is the carrier-free version of ab188408.

> 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 cellbased 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

#### **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

ClonalityMonoclonalClone numberEPR18253

**Isotype** IgG

## **Applications**

#### The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab241391 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
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ChIP-sequencing		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 140, 130, 97, 80, 73, 70, 55 kDa (predicted molecular weight: 83, 46 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.
ICC/IF		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.

## **Target**

#### **Function**

Chromatin binding factor that binds to DNA sequence specific sites. Involved in transcriptional regulation by binding to chromatin insulators and preventing interaction between promoter and nearby enhancers and silencers. Acts as transcriptional repressor binding to promoters of vertebrate MYC gene and BAG1 gene. Also binds to the PLK and PIM1 promoters. Acts as a transcriptional activator of APP. Regulates APOA1/C3/A4/A5 gene cluster and controls MHC

class II gene expression. Plays an essential role in oocyte and preimplantation embryo development by activating or repressing transcription. Seems to act as tumor suppressor. Plays a critical role in the epigenetic regulation. Participates to the allele-specific gene expression at the imprinted IGF2/H19 gene locus. On the maternal allele, binding within the H19 imprinting control region (ICR) mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to IGF2. Plays a critical role in gene silencing over considerable distances in the genome. Preferentially interacts with unmethylated DNA, preventing spreading of CpG methylation and maintaining methylation-free zones. Inversely, binding to target sites is prevented by CpG methylation. Plays a important role in chromatin remodeling. Can dimerize when it is bound to different DNA sequences, mediating long-range chromatin looping. Mediates interchromosomal association between IGF2/H19 and WSB1/NF1 and may direct distant DNA segments to a common transcription factory. Causes local loss of histone acetylation and gain of histone methylation in the beta-globin locus, without affecting transcription. When bound to chromatin, it provides an anchor point for nucleosomes positioning. Seems to be essential for homologous X-chromosome pairing. May participate with Tsix in establishing a regulatable epigenetic switch for X chromosome inactivation. May play a role in preventing the propagation of stable methylation at the escape genes from X- inactivation. Involved in sister chromatid cohesion. Associates with both centromeres and chromosomal arms during metaphase and required for cohesin localization to CTCF sites. Regulates asynchronous replication of IGF2/H19.

Tissue specificity

Sequence similarities

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**Domain** 

Post-translational modifications

**Cellular localization** 

Ubiquitous. Absent in primary spermatocytes.

Belongs to the CTCF zinc-finger protein family.

Contains 11 C2H2-type zinc fingers.

The 11 zinc fingers are highly conserved among vertebrates, exhibiting almost identical amino acid sequences. Different subsets or combination of individual zinc fingers gives the ability to CTCF to recognize multiple DNA target sites.

CTCF to recognize multiple DNA target sites

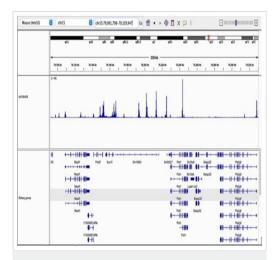
 $Sum oy lated \ on \ Lys-74 \ and \ Lys-689; sum oy lation \ of \ CTCF \ contributes \ to \ the \ repressive \ function \ of \ CTCF \ contributes \ to \ the \ repressive \ function \ of \ contributes \ of \ contribu$ 

CTCF on the MYC P2 promoter.

Nucleus > nucleoplasm. Chromosome. Chromosome > centromere. May translocate to the nucleolus upon cell differentiation. Associates with both centromeres and chromosomal arms during metaphase. Associates with the H19 ICR in mitotic chromosomes. May be preferentially

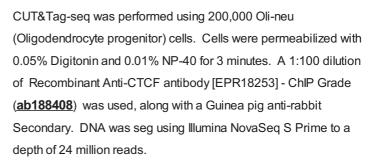
excluded from heterochromatin during interphase.

## Images



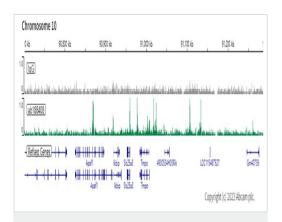
CUT&Tag - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet



This image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

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



ChIC/CUT&RUN sequencing - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391) ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL,  $2.5 \times 10^5$  F9 (Mouse embryonic testicular cancer cell line) cells and  $5\mu g$  of <u>ab188408</u> [EPR18253]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> is also shown.

Additional screenshots of mapped reads can be downloaded <u>here</u>.

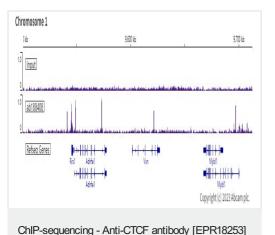
The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab188408</u>).

Chromatin was prepared from Mouse Embryonic Fibroblast cells.

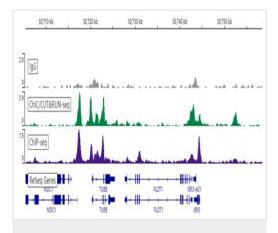
Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was

performed with 10<sup>7</sup> cells and 8 μg of [Anti-CTCF antibody](
ab188408). ChIP DNA was sequenced on the Illumina NovaSeq



- ChIP Grade - BSA and Azide free (ab241391)

6000 to a depth of 30 million reads. The Input control is also shown. Additional screenshots of mapped reads can be downloaded **here**. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab188408**).



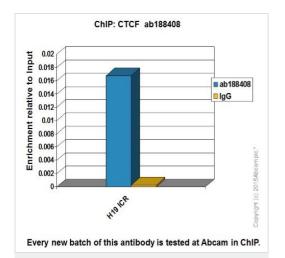
ChIC/CUT&RUN sequencing - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391) ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5  $\mu$ g of **ab188408** [EPR18253]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative lgG control **ab172730** is also shown.

The ChIP data was conducted on chromatin prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 8  $\mu$ g of <u>ab188408</u>. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded <u>here</u>.

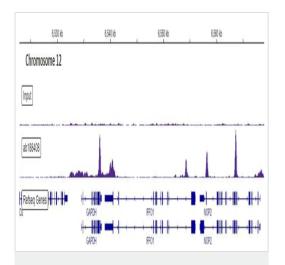
The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab188408</u>).



ChIP - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391) Chromatin was prepared from HeLa (Human epithelial cells from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab188408 (blue), and 20µl of Anti Rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).



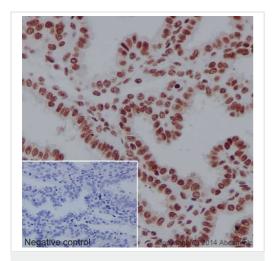
ChIP-sequencing - Anti-CTCF antibody [EPR18253]

- ChIP Grade - BSA and Azide free (ab241391)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab188408</u>).

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30  $\mu$ g of chromatin and 4  $\mu$ g of Anti-CTCF antibody [EPR18253] - ChIP Grade (ab188408). 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTCF antibody

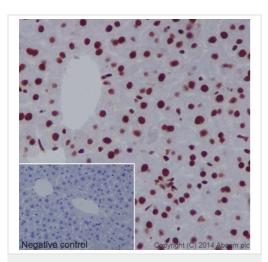
[EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

Immunohistochemical analysis of paraffin-embedded human endometrium tissue labeling CTCF with <u>ab188408</u> at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on human endometrium is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTCF antibody

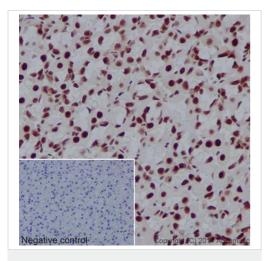
[EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling CTCF with <u>ab188408</u> at 1/4000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on hepatocytes of mouse liver is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).

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



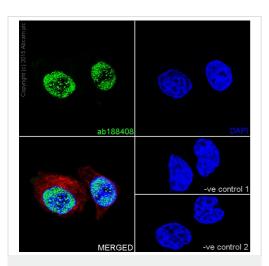
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

Immunohistochemical analysis of paraffin-embedded rat stomach tissue labeling CTCF with <u>ab188408</u> at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on the epithelium cells of rat stomach is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).

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



Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling CTCF with <u>ab188408</u> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb ( $\underline{ab7291}$ ) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor  $^{\circledR}$  594) ( $\underline{ab150120}$ ) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1:  $\underline{ab188408}$  at 1/2000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) ( $\underline{ab150120}$ ) secondary antibody at 1/1000 dilution.

-ve control 2: <u>ab7291</u> Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).

ab188408 DAPI

-ve control 1

Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR18253] - ChIP Grade - BSA and Azide free (ab241391)

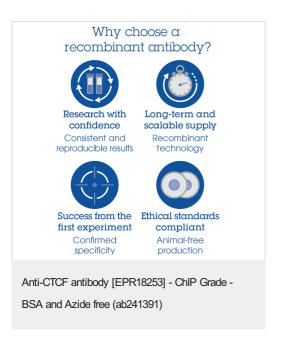
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyronic fibroblast cells) cells labeling CTCF with <a href="mailto:ab188408">ab188408</a> at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb ( $\underline{ab7291}$ ) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor  $^{\circledR}$  594) ( $\underline{ab150120}$ ) seconday antibody at 1/1000 dilution (red). The negative controls are as follows:

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This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab188408).



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