abcam

Product datasheet

Anti-CTCF antibody [EPR7314(B)] - ChIP Grade ab128873

重组 RabMAb

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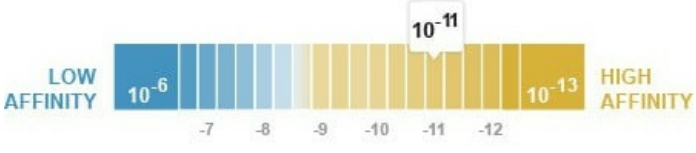
概述

产品名称	Anti-CTCF抗体[EPR7314(B)] - ChIP Grade
描述	兔单克隆抗体[EPR7314(B)] to CTCF - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** ChIP-sequencing, ChIP, WB, IHC-P, ICC/IF, Flow Cyt (Intra), ChIC/CUT&RUN-seq
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human CTCF aa 700-800. The exact sequence is proprietary. (Peptide available as **ab209492**)
阳性对照	WB: HeLa and 293T cell lysates, Human colon tissue, Mouse brain tissue and rat heart tissue. IHC: Human breast carcinoma, mouse and rat kideny ICC/IF: HeLa cell lysates Flow Cyt (intra): 293T cell lysates ChIP-seq: HeLa cells. ChIC/CUT&RUN-Seq: 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. Stable for 12 months at -20°C.
解离常数(K_D)	$K_D = 1.74 \times 10^{-11}$ M



Learn more about K_D

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.2% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR7314(B)
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab128873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	★★★★★ (1)	Use 4 µg for 30 µg of chromatin.
ChIP		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 140 kDa (predicted molecular weight: 83 kDa).Can be blocked with **CTCF peptide (ab209492)**.
IHC-P		1/250 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		1/250 - 1/500.
Flow Cyt (Intra)		1/40 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 2 µg

靶标

功能 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.

组织特异性 Ubiquitous. Absent in primary spermatocytes.

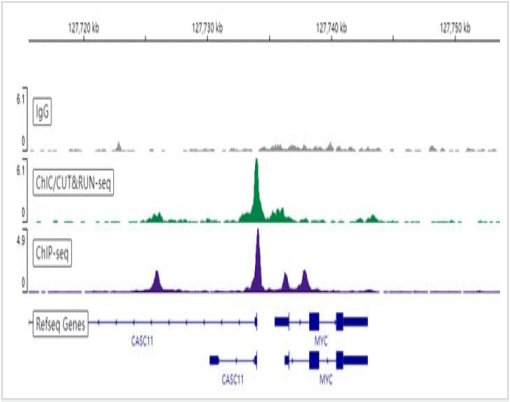
序列相似性 Belongs to the CTCF zinc-finger protein family.
Contains 11 C2H2-type zinc fingers.

结构域 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.

翻译后修饰 Sumoylated on Lys-74 and Lys-689; sumoylation of CTCF contributes to the repressive function of CTCF on the MYC P2 promoter.

细胞定位 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.

图片

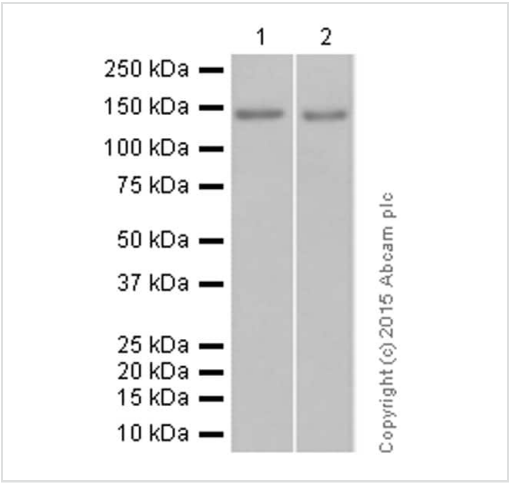


ChIC/CUT&RUN sequencing - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 µg of ab128873 [EPR7314(B)]. 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.
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 4 µg of ab128873. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

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-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

All lanes : Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873) at 1/10000 dilution

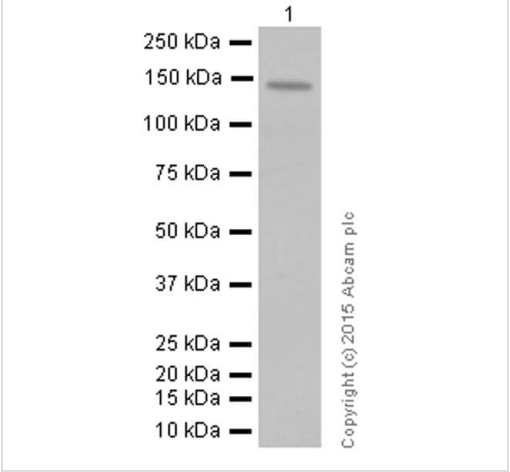
Lane 1 : HeLa (human cervix adenocarcinoma) whole cell lysate
Lane 2 : 293T (human embryonic kidney) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 83 kDa
Observed band size: 140 kDa

Blocking and diluting buffer 5% NFDM/TBST



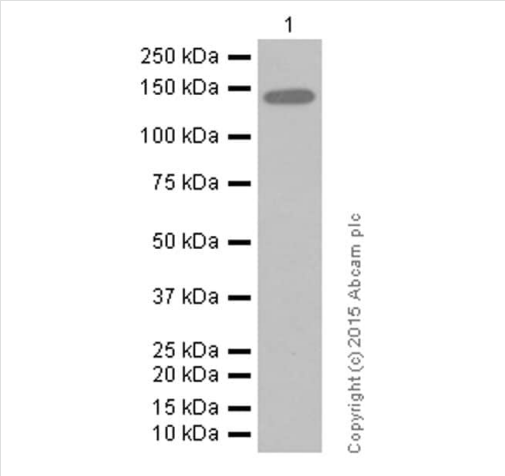
Western blot - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873) at 1/10000 dilution + Mouse brain at 20 µg

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

Predicted band size: 83 kDa
Observed band size: 140 kDa

Blocking and diluting buffer 5% NFDM/TBST



Western blot - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873) at 1/10000 dilution + Rat heart at 20 µg

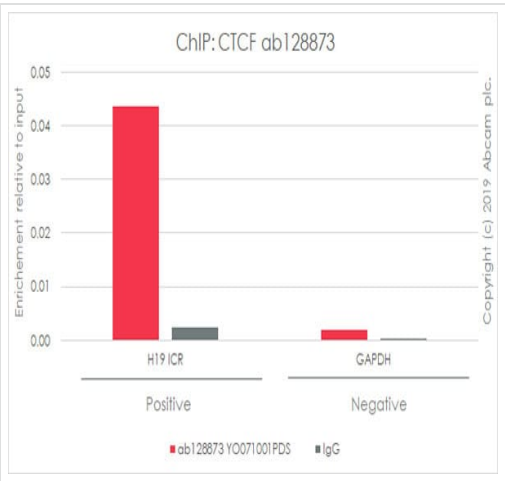
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 83 kDa

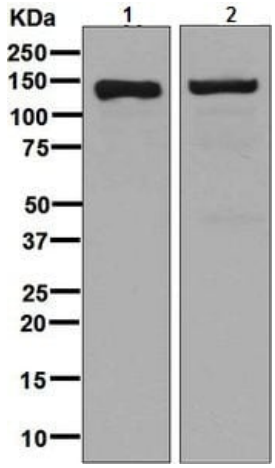
Observed band size: 140 kDa

Blocking and diluting buffer 5% NFDM/TBST



ChIP - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab128873 (red), and 20µl of protein A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Western blot - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

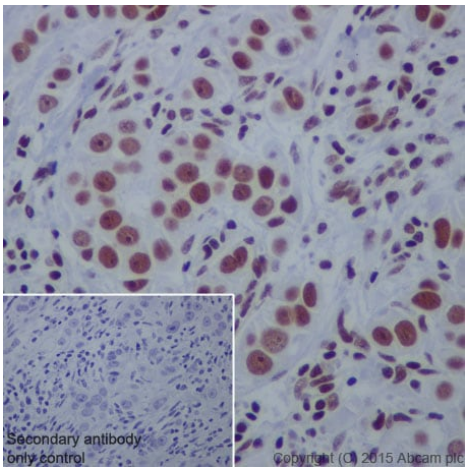
All lanes : Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873) at 1/1000 dilution (un-purified)

Lane 1 : HeLa cell lysate
Lane 2 : 293T cell lysate

Lysates/proteins at 10 µg per lane.

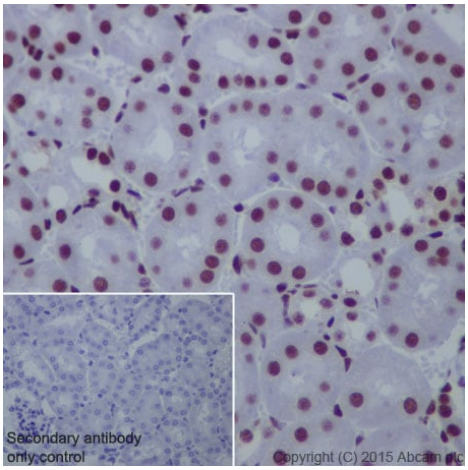
Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Predicted band size: 83 kDa



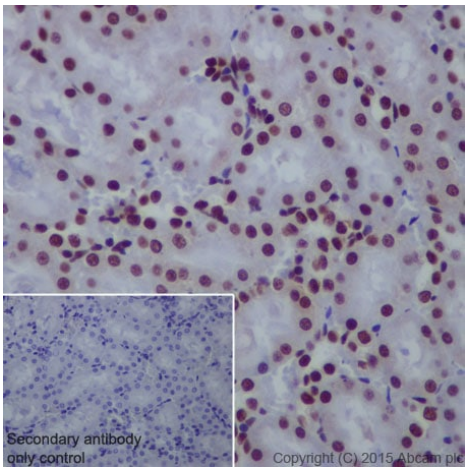
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Immunohistochemical staining of paraffin-embedded human breast carcinoma sections labelling CTCF with purified ab128873 at dilution of 1:1000. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



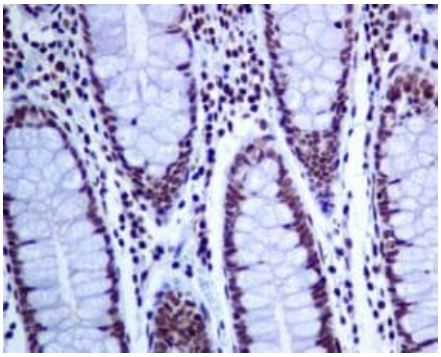
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Immunohistochemical staining of paraffin-embedded mouse kidney sections labelling CTCF with purified ab128873 at dilution of 1:1000. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

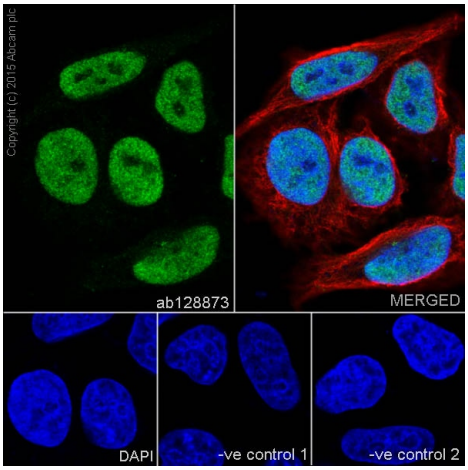
Immunohistochemical staining of paraffin-embedded rat kidney sections labelling CTCF with purified ab128873 at dilution of 1:1000. The secondary antibody used was **ab97051**; a goat anti-rabbit IgG H&L (HRP) at dilution of 1/500. The sample was counter-stained with hematoxylin. Antigen retrieval was performed using EDTA Buffer; pH 9.0. PBS was used instead of the primary antibody as the negative control and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

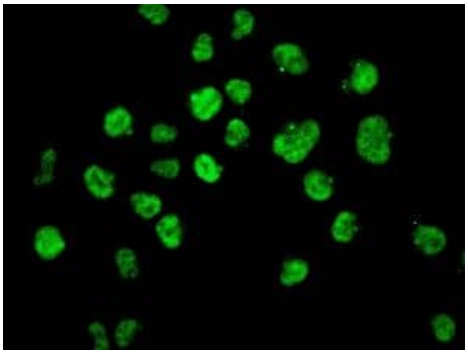
Immunohistochemical analysis of paraffin embedded Human colon tissue labelling CTCF with un-purified ab128873 at 1/250 dilution.

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



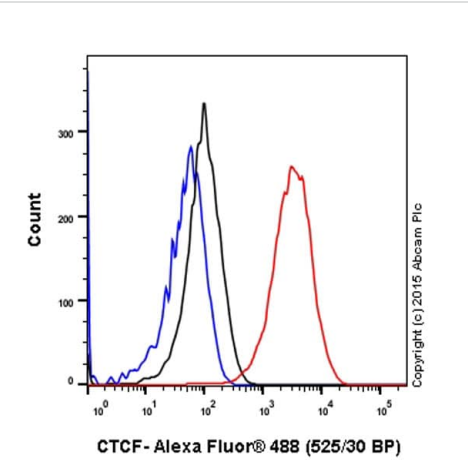
Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Immunocytochemistry/immunofluorescence staining of 4% paraformaldehyde fixed; 0.1% triton X 100 permeabilized HeLa (human cervix adenocarcinoma) cells with purified ab128873 at dilution of 1/500. The secondary antibody used was Alexa Fluor® 488; goat anti-rabbit IgG (**ab150077**) at a dilution of 1/1000. Nucleus was counter-stained with DAPI (blue). **ab7291**, a mouse anti-tubulin antibody (1/1000) was used to stain tubulin along with **ab150120** (AlexaFluor®594 goat anti-mouse secondary, 1/1000) shown in the top right hand panel. The negative controls are shown in the bottom middle and right hand panels- for negative control 1 rabbit primary antibody and anti-mouse secondary antibody (**ab150120**) was used. For negative control 2 mouse primary antibody (**ab7291**) and anti-rabbit secondary antibody (**ab150077**) was used.



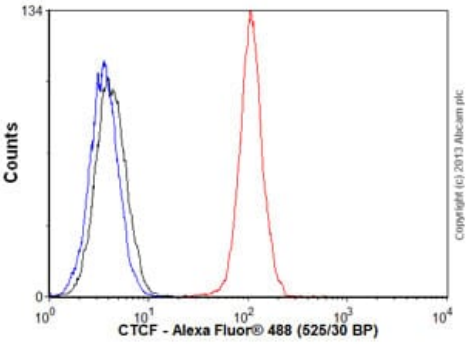
Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling CTCF with un-purified ab128873 at 1/250 dilution.



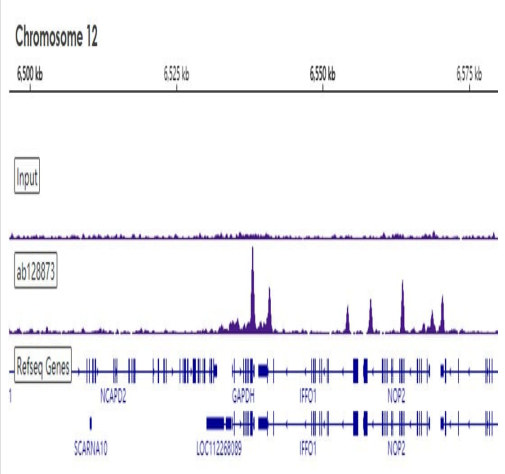
Flow Cytometry (Intracellular) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Flow cytometry analysis showing 4% paraformaldehyde fixed 293T (human embryonic kidney) cells labelling CTCF with purified ab128873 at dilution of 1/40 followed by the secondary antibody; Alexa Fluor® 488 goat-anti-rabbit IgG at dilution of 1/500 (red line). A non-specific IgG antibody (rabbit monoclonal) was used as isotype control (black line). The blue line shows cells without incubation with primary antibody and secondary antibody.



Flow Cytometry (Intracellular) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

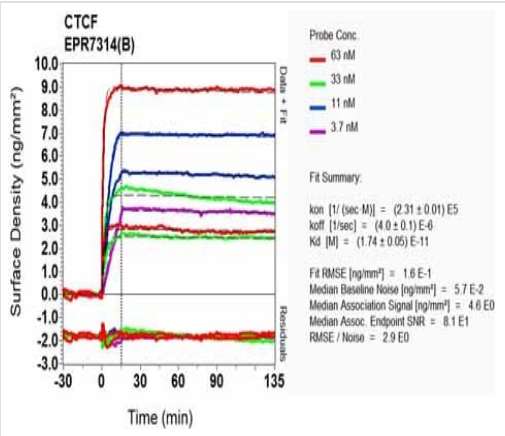
Overlay histogram showing Hela cells stained with un-purified ab128873 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab128873, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



ChIP-sequencing - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with $1x10^7$ HeLa cells and 4 µg of ab128873. 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**.

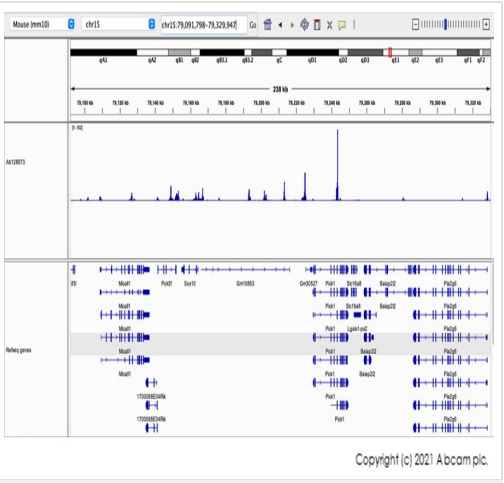


OI-RD Scanning - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



CUT&Tag sequencing - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

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

CUT&Tag-seq was performed using 200,000 Oli-neu (Oligodendrocyte progenitor) cells. Cells were permeabilized with 0.05% Digitonin and 0.01% NP-40 for 3 minutes. A 1:100 dilution of Recombinant Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873) was used, along with a Guinea pig anti-rabbit Secondary. DNA was seg using Illumina NovaSeq S Prime to a depth of 24 million reads.

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

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

Anti-CTCF antibody [EPR7314(B)] - ChIP Grade (ab128873)

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