abcam

Product datasheet

Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free ab240035

Recombinant RabMAb

14 Images

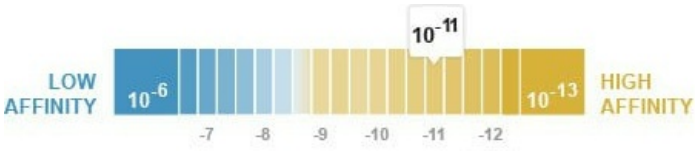
Overview

Product name	Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free
Description	Rabbit monoclonal [EPR7314(B)] to CTCF - ChIP Grade – BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ChIP-sequencing, ChIP, Flow Cyt (Intra), IHC-P, ChIC/CUT&RUN-seq, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab209492**)
Positive control	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.
General notes	ab240035 is the carrier-free version of **ab128873**. 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 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. 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. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 1.74 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR7314(B)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240035 in the following tested applications.

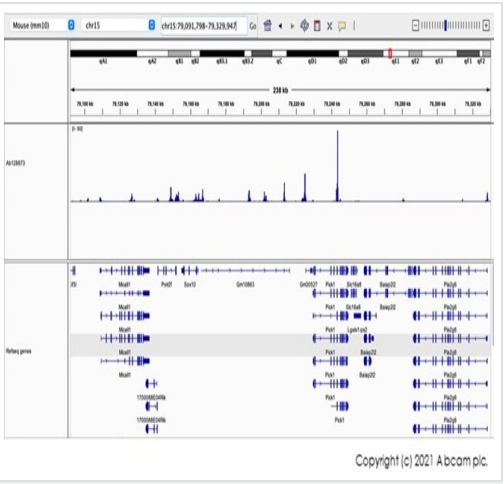
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
ChIP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 140 kDa (predicted molecular weight: 83 kDa).Can be blocked with **CTCF peptide (ab209492)**.

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	Ubiquitous. Absent in primary spermatocytes.
Sequence similarities	Belongs to the CTCF zinc-finger protein family. Contains 11 C2H2-type zinc fingers.
Domain	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.
Post-translational modifications	Sumoylated on Lys-74 and Lys-689; sumoylation of CTCF contributes to the repressive function of CTCF on the MYC P2 promoter.
Cellular localization	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 [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

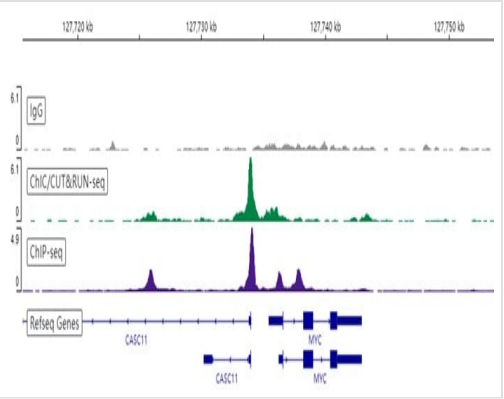
This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

This data was developed using the same antibody clone in a different buffer formulation (**ab128873**).

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.



ChIC/CUT&RUN sequencing - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

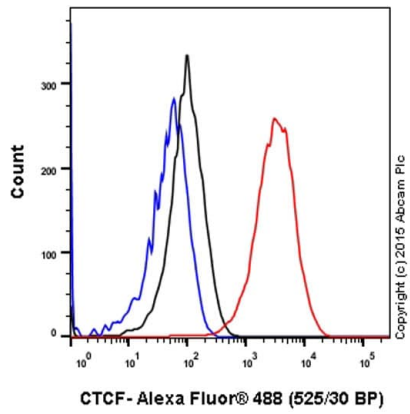
ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 µg of **ab128873** [EPR7314(B)]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative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

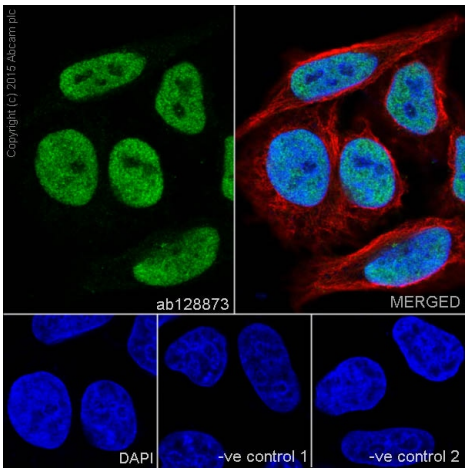
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Flow Cytometry (Intracellular) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

Intracellular 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.

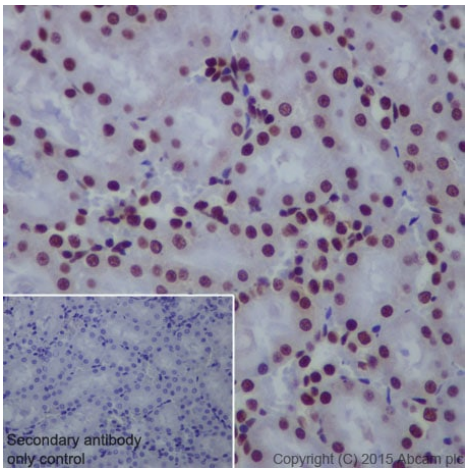
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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.

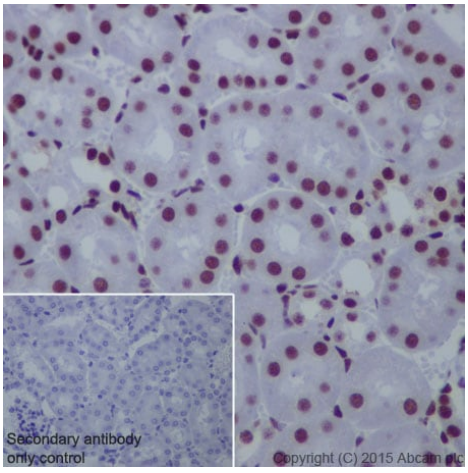
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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.

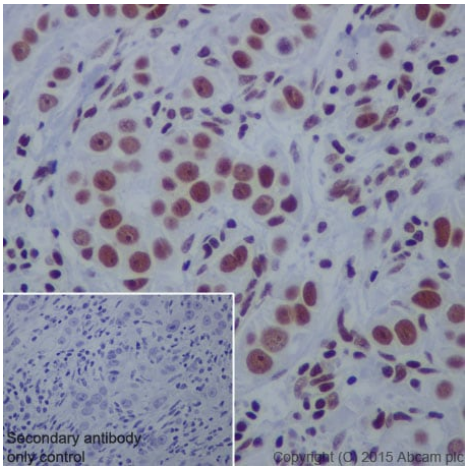
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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.

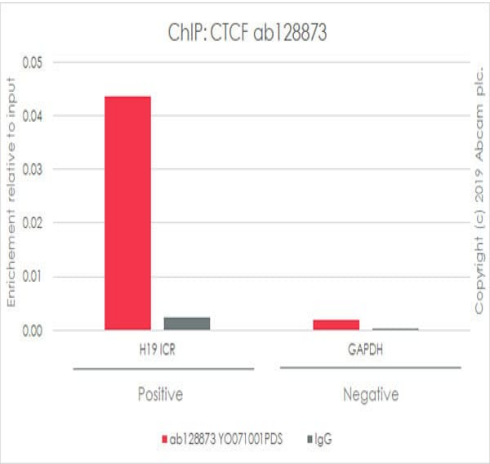
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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.

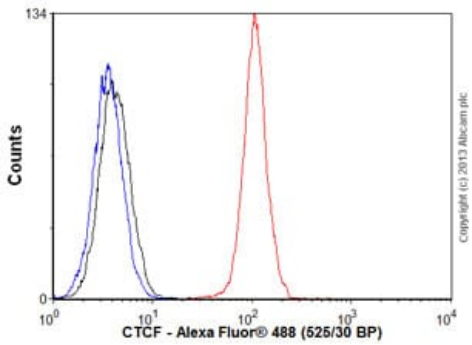
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



ChIP - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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).

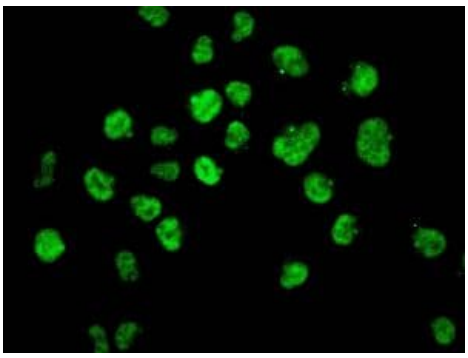
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Flow Cytometry (Intracellular) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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.

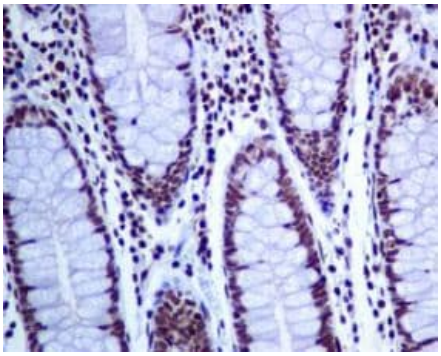
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Immunocytochemistry/ Immunofluorescence - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).

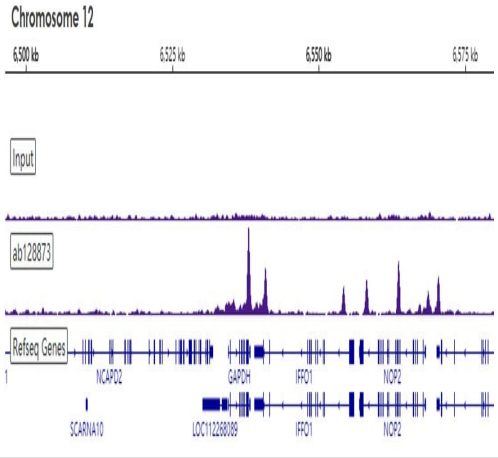


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).

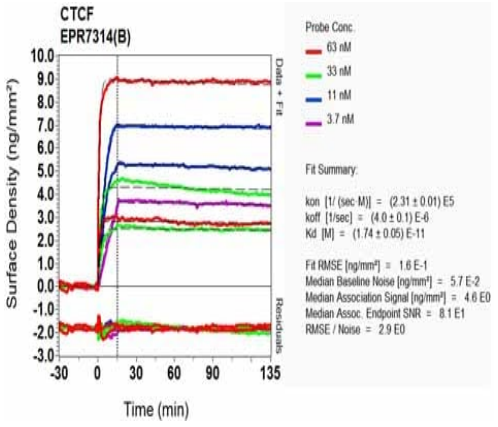
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



ChIP-sequencing - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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 **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**.



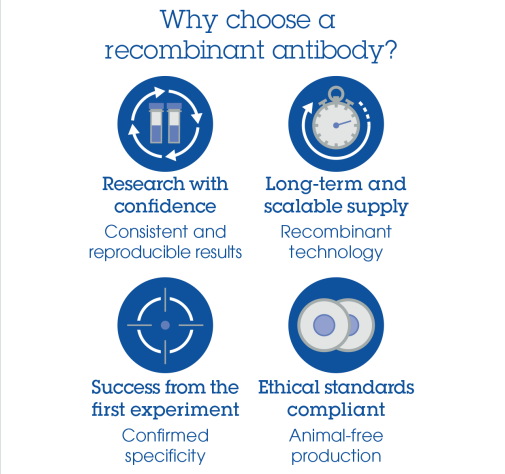
OI-RD Scanning - Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab128873**).



Anti-CTCF antibody [EPR7314(B)] - ChIP Grade - BSA and Azide free (ab240035)

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