Datasheet for ABIN6731034

Recombinant anti-CTCF antibody

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Overview

Quantity:	100 µg
Target:	CTCF
Reactivity:	Human
Host:	Rabbit
Antibody Type:	Recombinant Antibody
Clonality:	Monoclonal
Conjugate:	This CTCF antibody is un-conjugated
Application:	Western Blotting (WB), ChIP DNA-Sequencing (ChIP-seq), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Brand:	AbFlex®
Immunogen:	This antibody was raised against a peptide within human CTCF.
Isotype:	IgG
Purification:	Protein A Chromatography

Target Details

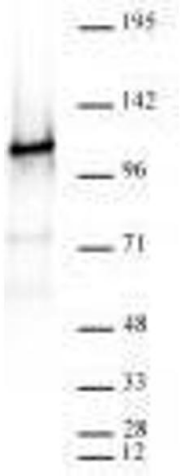
Target:	CTCF
Alternative Name:	CTCF (CTCF Products)
Molecular Weight:	120 kDa

Application Details

Application Notes:	**Validated Applications:** **ChIP-seq:** 4 µg per ChIP **WB:** 0.1-1 µg/mL **Note**: Many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt & Sonication Protocol when preparing nuclear extracts.
Comment:	AbFlex® antibodies are recombinant antibodies (rAbs) that have been generated using defined DNA sequences to produce highly specific, reproducible antibodies. Each AbFlex antibody contains a 6XHis tag, an avidin tag sequence for enzymatic biotin conjugation using the biotin ligase, BirA, and a sortase recognition motif (LPXTG) to attach a variety of labels directly to the antibody including fluorophores, enzymatic substrates (HRP, AP), peptides, drugs as well as solid supports. AbFlex CTCF antibody was expressed in CHO cells and contains rabbit immunoglobulin heavy and light chains.
Restrictions:	For Research Use only

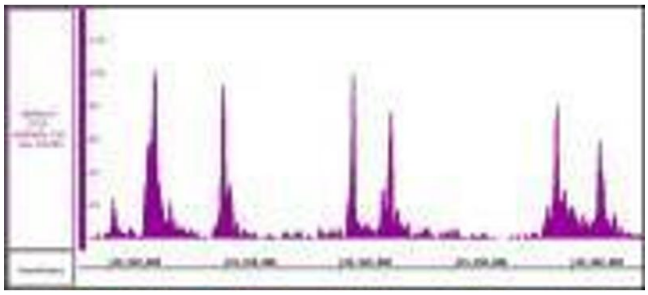
Handling

Format:	Liquid
Buffer:	Purified IgG in 140 mM Hepes, pH 7.5, 70 mM NaCl, 32 mM NaOAc, 0.035 % sodium azide, 30 % glycerol.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C



Western Blotting

Image 1. CTCF antibody (rAb) tested by Western blot. 20 µg of HeLa cell nuclear extract was run on SDS-PAGE and probed with CTCF antibody at 0.2 µg/ml.



ChIP DNA-Sequencing

Image 2. CTCF recombinant antibody (rAb) tested by ChIP-Seq Chromatin immunoprecipitation (ChIP) was performed using the High Sensitivity Kit with 30 µg of MCF7 chromatin and 4 µg of CTCF antibody. ChIP DNA was sequenced on the Illumina NextSeq and 8.9 million sequence tags were mapped to identify CTCF binding sites on chromosome 9.

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104605

Date: Feb 22 2024

Target:	CTCF
Lot Number:	09120003-11
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Anti H3K4me (antibodies-online, ABIN3023251)
Negative Control:	Guinea Pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed.
Primary Antibody:	ABIN6731034

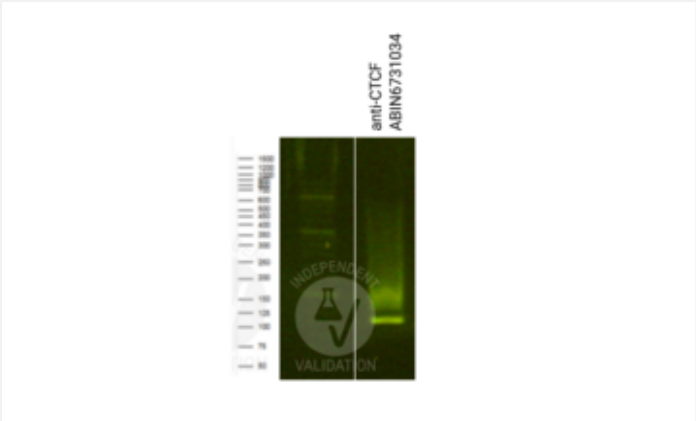
Protocol:

- Cell harvest and nuclear extraction
  - Harvest 250,000 HCT116 cells per antibody
  - Centrifuge cell solution 5 min at 600 x g at RT.
  - Remove the liquid carefully.
  - Gently resuspend cells in 2 mL of Nuclear Extraction Buffer (20 mM HEPES-KOH pH 8.2, 20% Glycerol, 0.05% IGEPAL, 0.5 mM Spermidine, 10 mM KCl, Roche Complete Protease Inhibitor EDTA-free).
  - Move the solution to a 2 mL centrifuge tube.
  - Pellet the nuclei 800 x g for 5 min.
  - Repeat the NE wash twice for a total of three washes.
  - Resuspend the nuclei in 40 µL NE Buffer per sample.
- Concanavalin A beads preparation
  - Prepare one 2 mL microcentrifuge tube.
  - Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6923139).
  - Pipette 10 µL Con A Beads slurry for each sample into the 2 mL microcentrifuge tube.
  - Place the tube on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Pipette 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2) into the tube and resuspend ConA beads by gentle pipetting.
  - Spin down the liquid from the lid with a quick pulse in a table-top centrifuge.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Repeat the wash twice for a total of three washes.
  - Gently resuspend the ConA Beads in 44 µL binding buffer.

- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 44 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly, incubate 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 1 mL of EDTA wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free, 2 mM EDTA).
  - Incubate 5 min at RT.
  - Place the tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 200 µL of wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) for each sample.
- Primary antibody binding
  - Divide nuclei suspension into separate 200 µL PCR tubes, one for each antibody.
  - Add 2 µL antibody (CTCF ABIN6731034, anti-H3K4me positive control ABIN3023251, and guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Incubate at 4 °C ON.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash five times for a total of six washes.
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 200 µL of pAG mix per sample (200 µL of wash buffer + 120 ng pAG-MNase per sample).
  - Place the PCR tubes with the sample on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove tubes from the magnetic stand.
  - Resuspend the beads in 200 µL of pAG-MNase premix.
  - Incubate 30 min at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash five times for a total of six washes.
  - Resuspend in 100 µL of Wash Buffer.
- MNase digestion and release of pAG-MNase-antibody-chromatin complexes
  - Place PCR tubes on ice and allow to chill.
  - Prepare a 1.5 mL microcentrifuge tube with 102 µL of 2 mM CaCl2 mix per sample (100 µL Wash Buffer + 2 µL 100 mM CaCl2) and let it chill on ice.
  - Always in ice, place the samples on the magnetic rack and when the liquid is clear remove the supernatant.
  - Resuspend the samples in 100 µL of the 2 mM CaCl2 mix and incubate in ice for exactly 30 min.

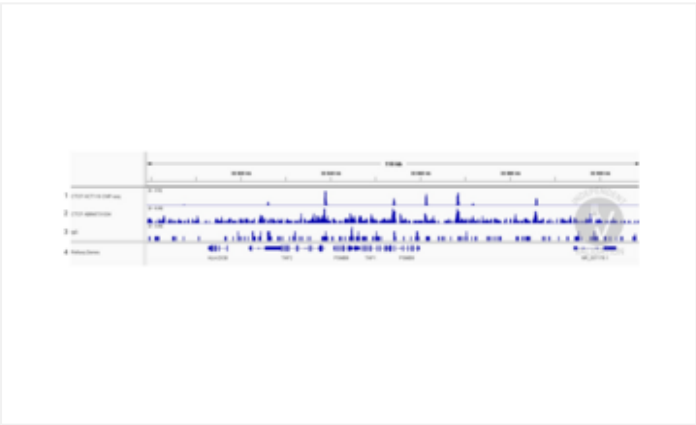
- Place the sample on the magnet stand and when the liquid is clear remove the supernatant.
- Resuspend the sample in 50 µL of 1x Urea STOP Buffer (8.5 M Urea, 100 mM NaCl, 2 mM EGTA, 2 mM EDTA, 0.5% IGEPAL).
- Incubate the samples 1h at 4°C.
- Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to fresh 200 µL PCR tubes.

- DNA Clean up (Mag-Bind® TotalPure NGS - M1378-01)
  - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are RT.
  - Add 2x volume of beads to each sample (e.g., 100 µL of beads for 50 µL of sample).
  - Incubate the beads and the sample for 15 min at RT.
  - During incubation prepare fresh EtOH 80%.
  - Place the PCR tubes on a magnet stand and when the liquid is clear remove the supernatant.
  - Add 200 µL of fresh 80% EtOH to the sample without disturbing the beads (Important! Do NOT resuspend the beads or remove the tubes from the magnet stand or the sample will be lost).
  - Incubate 30 sec at RT.
  - Remove the EtOH from the sample.
  - Repeat the wash with 80% EtOH.
  - Resuspend the beads in 25 µL of 10 mM Tris.
  - Incubate the sample for 2 min at RT.
  - Repeat the 2x beads clean up as described before (this time with 50 µL of beads for each sample).
  - Resuspend the beads + DNA in 20 µL of 10 mM Tris.
- Library preparation and sequencing
  - Prepare Libraries using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
  - Sequence samples on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36bp PE.
- Peak calling
  - Trim reads using bbTools bbduk (BBMap - Bushnell B. - sourceforge.net/projects/bbmap/) to remove adapters, artifacts and repeat sequences.
  - Aligned reads were mapped to the hg38 human genome using bowtie with options -m 1 -v 0 -l 0 -X 500.
  - Use SAMtools to convert SAM files to BAM files and remove duplicates.
  - Use BEDtools genomecov to produce Bedgraph files.
  - Call peaks using SEACR with a 0.001 threshold and the option norm stringent.



Validation image no. 1 for anti-CCCTC-Binding Factor (Zinc Finger Protein) (CTCF) antibody (ABIN6731034)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using CTCF ABIN6731034 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



Validation image no. 2 for anti-CCCTC-Binding Factor (Zinc Finger Protein) (CTCF) antibody (ABIN6731034)

1. Alignment tracks from ENCODE ChIP-seq data for CTCF in HCT116 cells. 2. Alignment tracks from CUT&RUN targeting CTCF in HCT116 cells using ABIN6731034 antibody showing the TAP1 locus. 3. Alignment tracks for CUT&RUN with the IgG negative control ABIN101961. 4. RefSeq Genes.