TECHNICAL DATASHEET

LOT: A2355P

HOLOGIC® Diagenode

CTCF antibody

Cat. No. C15410210

Lot:	A2355P
Size:	10 µg /50 µg
Type:	Polyclonal, **ChIP-grade ChIP-seq grade CUT&Tag-grade**
Isotype:	NA
Source:	Rabbit
Concentration:	0.6 µg/µl

Specificity:	Human, mouse, pig: positive Other species: not tested
Purity:	Affinity purified polyclonal antibody
Storage buffer:	PBS containing 0.05% azide

Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against human CTCF (CCCTC-Binding Factor), using 4 KLH coupled peptides.

Applications

Applications	Suggested dilution	References
ChIP/ChIP-seq*	1–2 µg per ChIP	Fig 1, 2
CUT&Tag	1 µg	Fig 3
ELISA	1:1,000	Fig 4
Western blotting	1:1,000	Fig 5

*Please note that of the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-10 µg per IP.

Target description

CTCF (UniProt/Swiss-Prot entry P49711) is a transcriptional regulator protein with 11 highly conserved zinc finger domains. By using different combinations of the zinc finger domains, CTCF can bind to different DNA sequences and proteins. As such it can act as both a transcriptional repressor and a transcriptional activator. By binding to transcriptional insulator elements, CTCF can also block communication between enhancers and upstream promoters, thereby regulating imprinted gene expression. CTCF also binds to the H19 imprinting control region and mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to IGF2. Mutations in the CTCF gene have been associated with invasive breast cancers, prostate cancers, and Wilms' tumor.

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Results

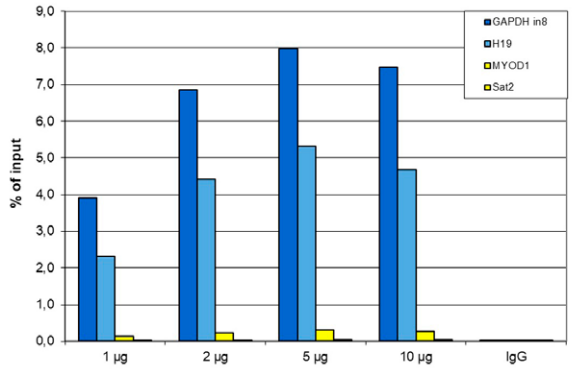

Figure 1: ChIP results obtained with the antibody directed against CTCF

ChIP was performed with the antibody against CTCF (cat. No. C15410210) on sheared chromatin from 4,000,000 HeLa cells. A titration consisting of 1, 2, 5 and 10 µg of antibody per ChIP experiment was analyzed. IgG (2 µg/IP) was used as a negative IP control. Quantitative PCR was performed with optimized primers for the H19 imprinting control region, and a specific region in the GAPDH gene, used as positive controls, and for the Sat2 satellite repeat region and the MYOD1 gene, used as a negative controls. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

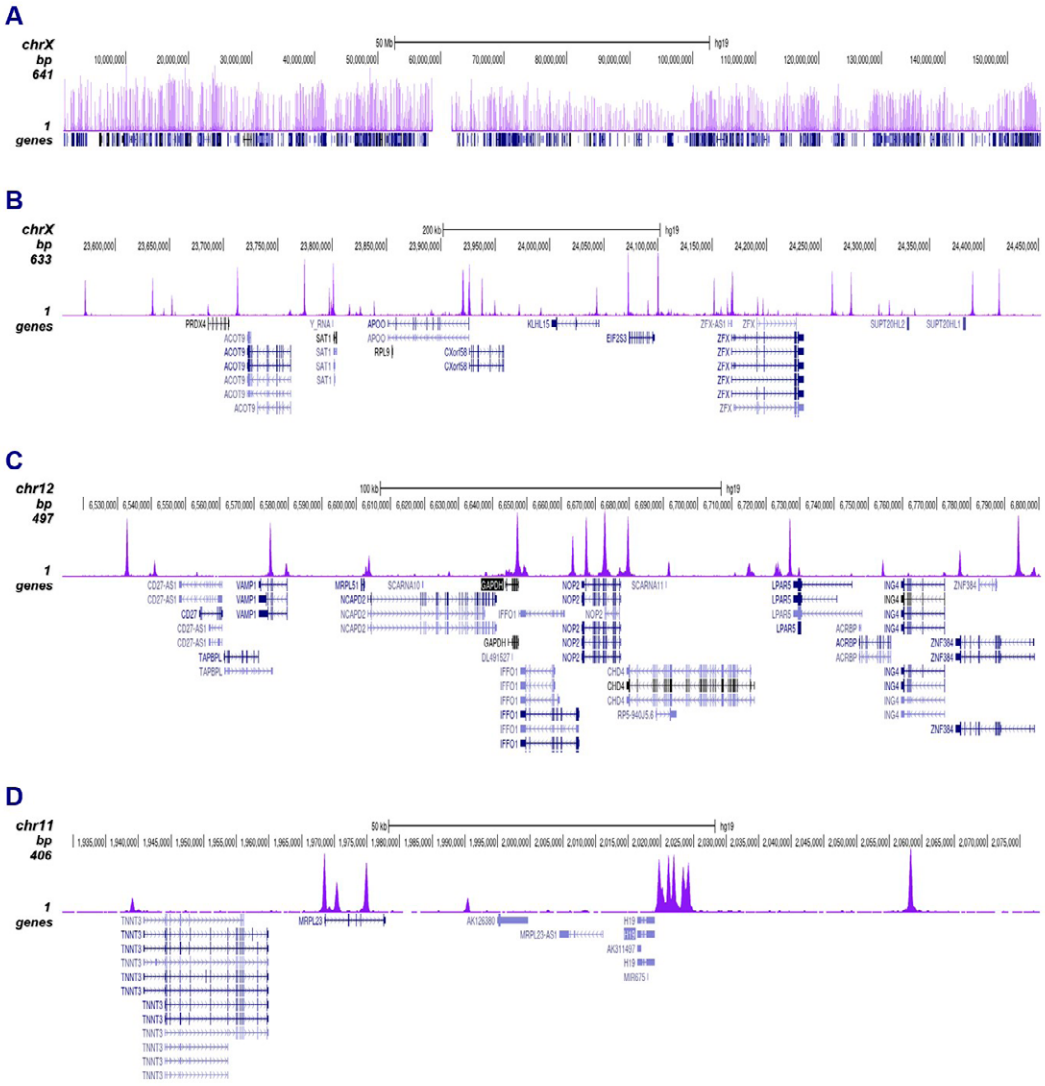

Figure 2: ChIP-seq results obtained with the antibody directed against CTCF

ChIP was performed on sheared chromatin from 4,000,000 HeLa cells using 1 µg of the antibody against CTCF (cat. No. C15410210) as described above. The IP'd DNA was subsequently analysed on an Illumina NovaSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 50 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the peak distribution along the complete sequence and a 60 kb region of the human X-chromosome (figure 2A and B) and in two regions surrounding the GAPDH and H19 positive control genes, respectively (figure 2C and D).

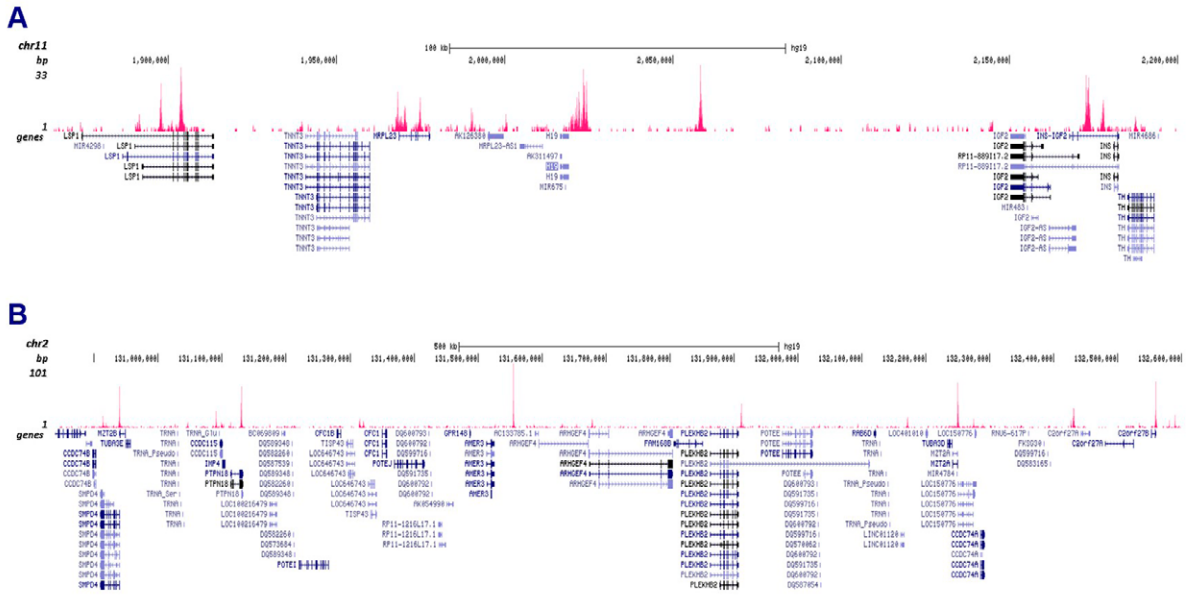

Figure 3: Cut&Tag results obtained with the antibody directed against CTCF

CUT&Tag was performed on 50,000 K562 cells using 1 µg of the antibody against CTCF (cat. No. C15410210) and the iDeal CUT&Tag kit (C01070020). The libraries were subsequently analysed on an Illumina NextSeq 500 sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. Figure 3 shows the peak distribution in 2 genomic regions surrounding the h19 imprinting control gene on chromosome 11 and the AMER3 gene on chromosome 2 (figure 3A and B, respectively).

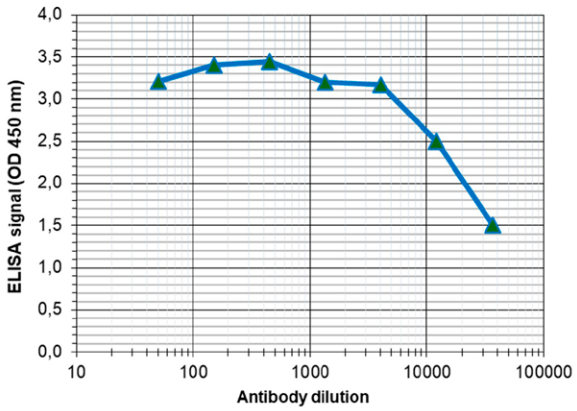

Figure 4: Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody against CTCF (cat. No. C15410210). The plates were coated with the peptides used for immunization of the rabbit. By plotting the absorbance against the antibody dilution (Figure 4), the titer of the antibody was estimated to be 1:29,500.

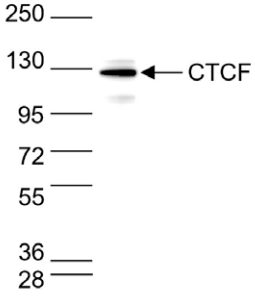

Figure 5: Western blot analysis using the antibody directed against CTCF

Whole cell extracts (25 µg) from HeLa cells were analysed by Western blot using the antibody against CTCF (cat. No. C15410210) diluted 1:1,000 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.