

CTCF polyclonal antibody - Classic

Cat. No. C15410210-50

Type: Polyclonal	Specificity: Human, mouse
Size: 50 µg	Isotype: NA
Concentration: 1.5 µg/µl	Source: Rabbit
Lot No.: A2359-00234P	Purity: Affinity purified
Storage buffer: PBS containing 0.05% azide and 0.05% ProClin 300.	Storage conditions: 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

Alternative name: **MRD21**

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

Applications

Applications	Suggested dilution	References
ChIP *	1 µg per ChIP	Fig 1, 2
ELISA	1:10,000	Fig 3
Western Blotting	1:1,000	Fig 4, 5
Immunofluorescence	1:200	Fig 6

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 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.

Validation Data

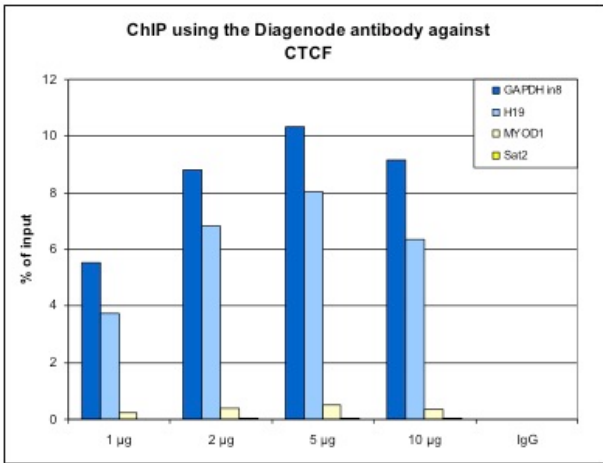


Figure 1. ChIP results obtained with the Diagenode antibody directed against CTCF

ChIP was performed with the Diagenode 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 MYOD1 gene and the Sat2 satellite repeat region, used as a negative control. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

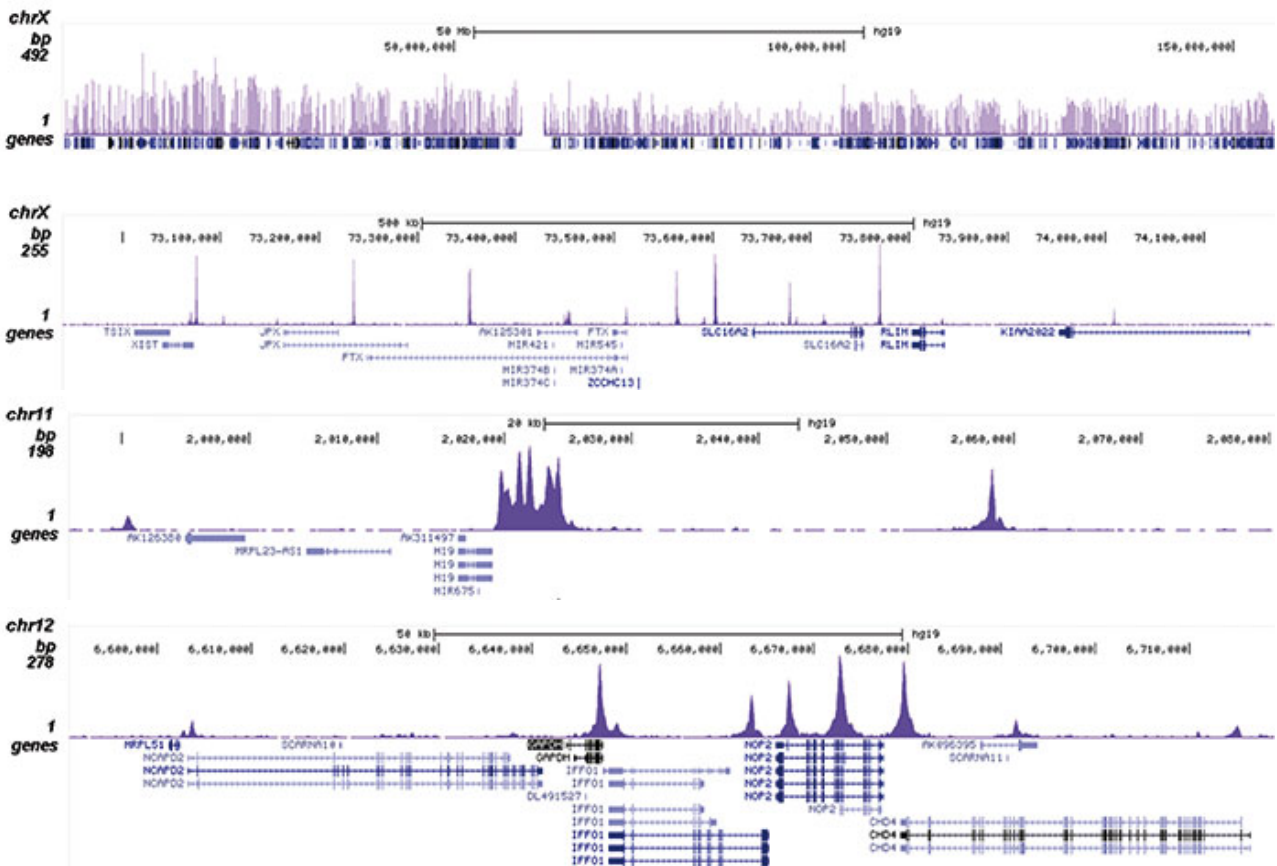


Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against CTCF

ChIP was performed on sheared chromatin from 4,000,000 HeLa cells using 1 µg of the Diagenode antibody against CTCF (Cat. No. C15410210) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq 2000. 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 1 mb region of the X-chromosome (figure 2A and B) and in two regions surrounding the H19 and GAPDH positive control genes, respectively (figure 2C and D).

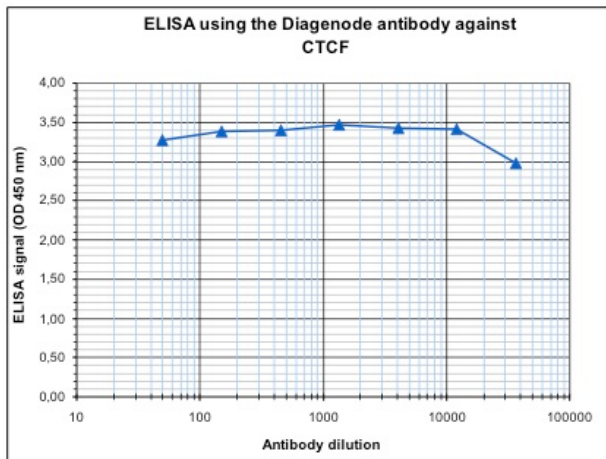


Figure 3. Determination of the antibody titer

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 3), the titer of the antibody was estimated to be >1:500,000.

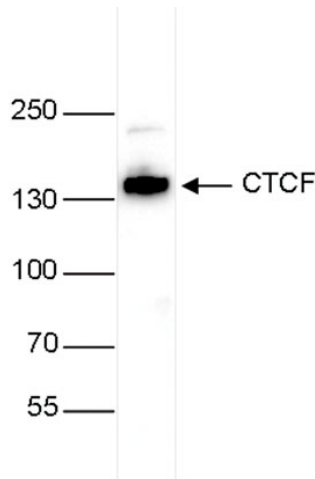


Figure 4. Western blot analysis using the Diagenode antibody directed against CTCF

Whole cell extracts (25 µg) from HeLa cells were analysed by Western blot using the Diagenode 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.

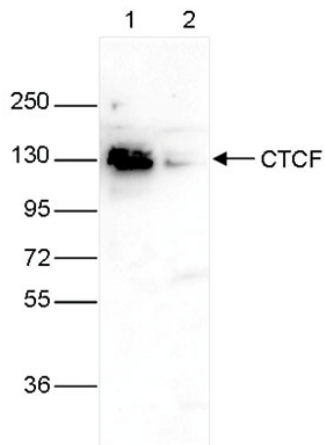


Figure 5. Western blot analysis using the Diagenode antibody directed against CTCF

Whole cell extracts (40 μ g) from HeLa cells transfected with CTCF siRNA (lane 2) and from an untransfected control (lane 1) were analysed by Western blot using the Diagenode 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.



Figure 6. Immunofluorescence using the Diagenode antibody directed against CTCF

HeLa cells were stained with the Diagenode antibody against CTCF (Cat. No. C15410210) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labeled with the CTCF antibody (left) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.