

CTCF CUTANA™ CUT&RUN Antibody

Catalog No	13-2014	Type	Monoclonal
Lot No	25162003-81	Host	Rabbit
Pack Size	100 µL	Concentration	100 µg/mL
Applications	CUT&RUN, IHC, ICC, IP, WB	Reactivity	Human, Mouse

DESCRIPTION

This antibody meets EpiCypher's "CUTANA Compatible" criteria for performance in Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and/or Cleavage Under Targets and Tagmentation (CUT&Tag) approaches to genomic mapping. Every lot of a CUTANA Compatible antibody is tested in the indicated approach using EpiCypher optimized protocols (epicypher.com/protocols) and determined to yield peaks that show a genomic distribution pattern consistent with reported function(s) of the target protein. CTCF is a multifunctional DNA-binding protein that shapes the 3D genome by establishing chromatin boundaries, insulating enhancers from unintended promoter interactions, and regulating long-range gene expression programs; functions that are critical for lineage specification and cellular identity [1].

TECHNICAL INFORMATION

Immunogen	Between amino acids 650 and 700
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Purified recombinant monoclonal antibody in borate buffered saline pH 8.2, 0.1% BSA, 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.125 µg per reaction*	Western Blot	1:1,000
Immunohistochemistry	1:100 - 1:500 [†]	Flow Cytometry	1 µL per 1 × 10 ⁶ cells
Immunoprecipitation	20 µL/mg lysate		

*Using more than the recommended 0.125 µg per reaction has been shown to negatively impact signal in CUT&RUN.

[†]Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections.

GENE & PROTEIN INFORMATION

UniProt ID	P49711
Gene Name	CTCF
Protein Name	Transcriptional repressor CTCF
Target Size	83 kDa
Alternate Names	11-zinc finger protein, CCCTC-binding factor, CTCFL paralog

REFERENCES

[1] Dehingia et al. *EMBO Rep.* (2022). PMID: 35993175

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with either CTCF (0.125 μ g) or IgG negative control (0.5 μ g, EpiCypher 13-0042) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v5 (EpiCypher 14-1048). Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001/14-1002). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 15.2 million reads (IgG) and 16.1 million reads (CTCF). Data were aligned to the hg38 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

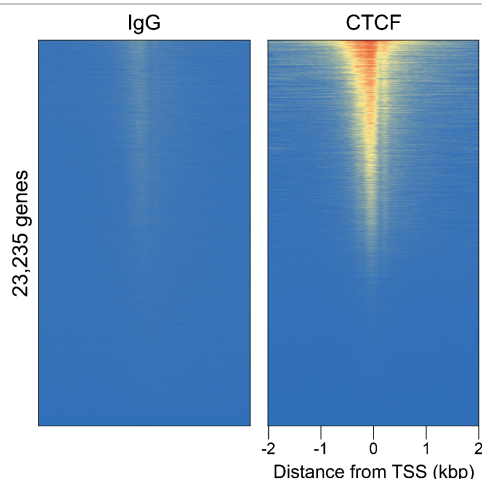


FIGURE 1 CTCF peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmaps show CTCF peaks relative to IgG negative control antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. CTCF showed expected enrichment around the TSS.

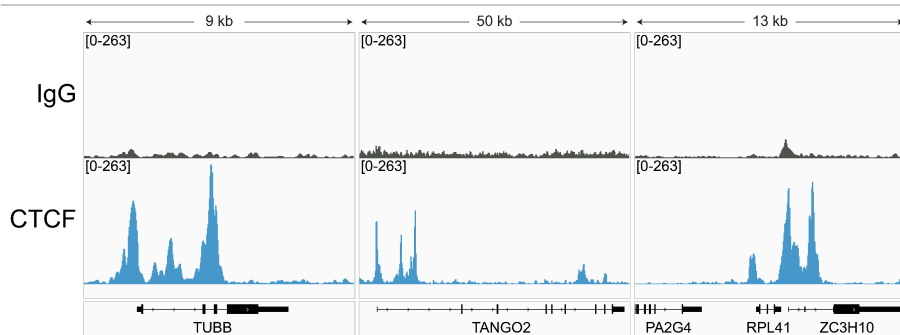


FIGURE 2 CTCF CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Three representative loci are shown.

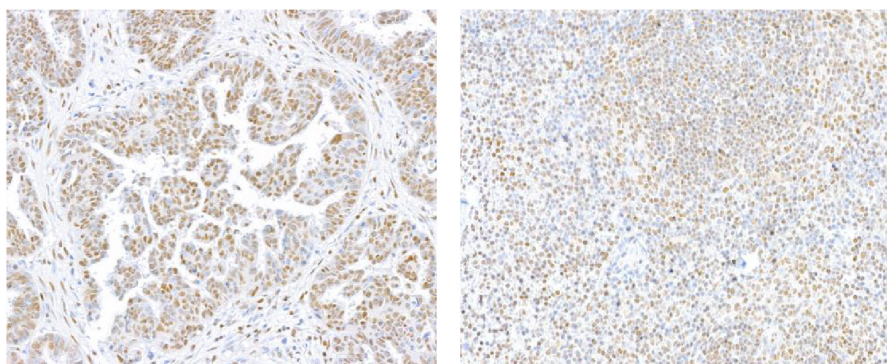


FIGURE 3 Immunohistochemistry data. FFPE sections of human ovarian carcinoma (left) and mouse spleen (right) examined using CTCF antibody at a dilution of 1:250.

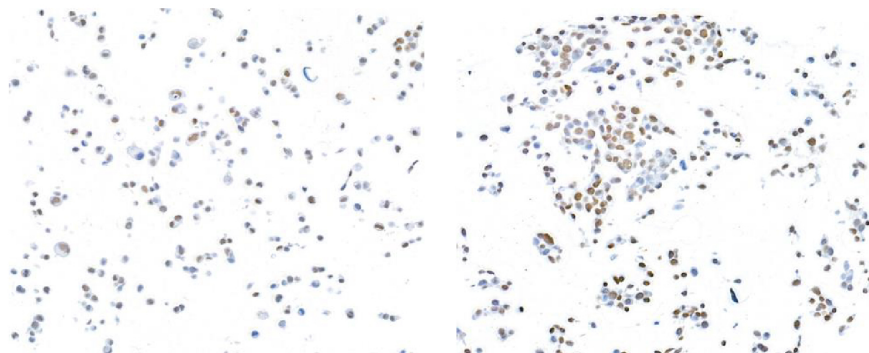


FIGURE 4 Immunocytochemistry data. FFPE sections of human MCF-7 cells (left) and mouse CT26 cells (right) examined using CTCF antibody at a dilution of 1:250.

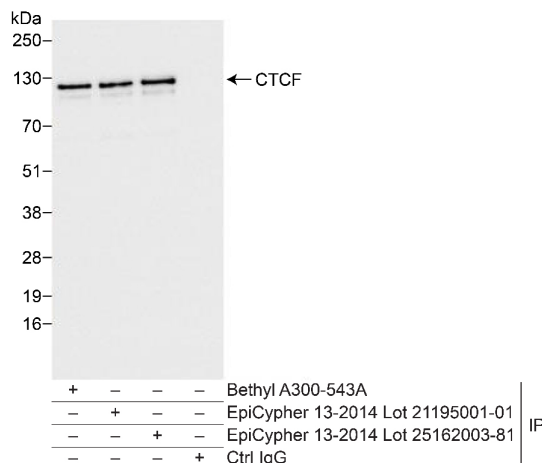


FIGURE 5 Immunoprecipitation data. EpiCypher CTCF antibody (20 μ L/mg lysate) was used to immunoprecipitate whole cell lysates (1 mg, 20% of IP loaded) isolated from HEK293T cells. A negative control IgG antibody, previous lot of this antibody (EpiCypher 13-2014 Lot 21195001-01), and positive control antibody targeting CTCF (Bethyl Laboratories) were also used to demonstrate specificity of the IP. All CTCF antibodies target the same CTCF epitope; however, A300-543A is polyclonal. For blotting immunoprecipitates, EpiCypher CTCF antibody was used at a 1:1,000 dilution.

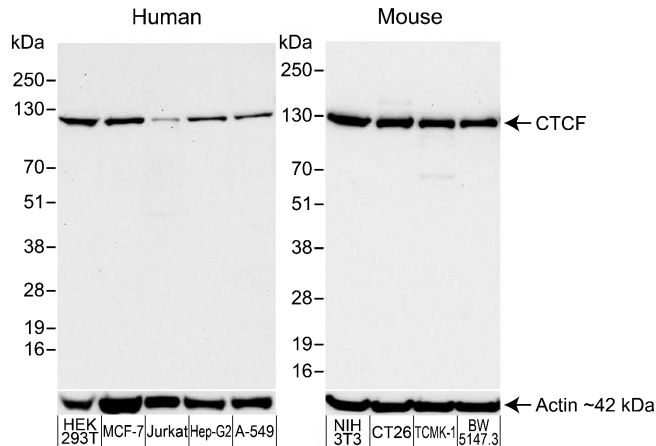


FIGURE 6 Western blot data. Western analysis of CTCF in whole cell extracts from human cells (HEK293T, MCF-7, Jurkat, Hep-G2, and A-549; **left**) and mouse cells (NIH3T3, CT26, TCMK-1, and BW5147.3; **right**). Fifty micrograms of lysate (10 μ g for Jurkat cells) was resolved via SDS-PAGE and detected with a 1:1,000 dilution of CTCF antibody.

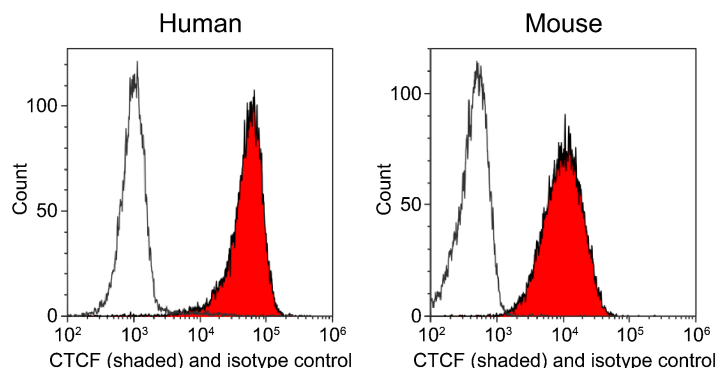


FIGURE 7 Flow cytometry data. Flow cytometry detection of human CTCF in K562 cells (**left**) and mouse CTCF in A20 cells (**right**). Cells were stained with either EpiCypher CTCF antibody, shown in shaded histogram, or an isotype control (unshaded). CTCF antibody was used at a concentration of 1 μ L per 1×10^6 cells. A DyLight® 650-conjugated goat anti-rabbit IgG secondary antibody (Bethyl Laboratories A120-101D5) was used for visualization.

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