CTCF CUTANA™ CUT&RUN Antibody

Catalog No	13-2014	**Type**	Monoclonal
Lot No	22222002-81	**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 regulates transcription by binding DNA and preventing interaction between promoters and nearby enhancers or silencers.

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	**Immunoprecipitation**	20 µL/mg lysate
Immunohistochemistry	1:100 - 1:1,000	**Western Blot**	1:1,000

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

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with either CTCF (0.125 µg) or IgG negative control (0.5 µg, EpiCypher 13-0042) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v3.0 (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). Both kit protocols were adapted for high throughput Tecan liquid handling. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 7.6 million reads (IgG) and 10.4 million reads (CTCF). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

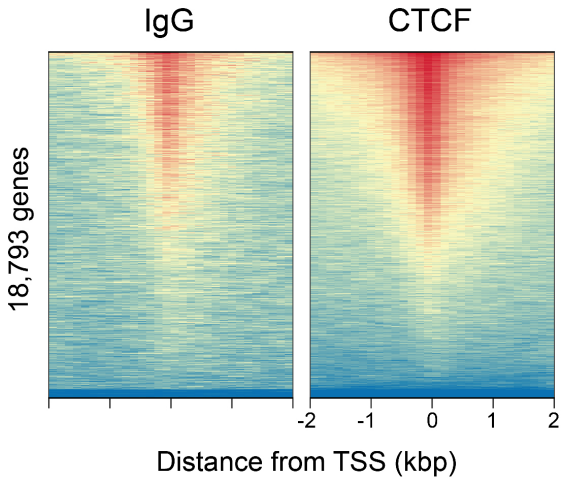

FIGURE 1 CTCF peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmaps show CTCF peaks relative to IgG negative control antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes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

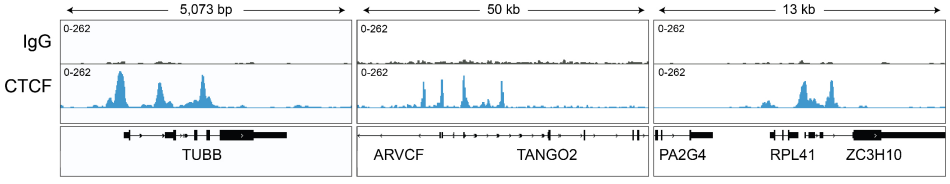

FIGURE 2 CTCF CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Three representative loci are shown.

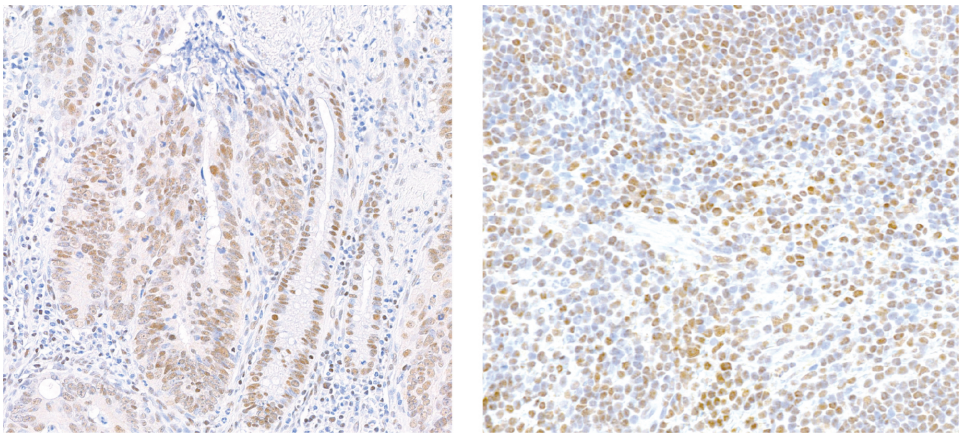

FIGURE 3 Immunohistochemistry data. FFPE sections of human colon carcinoma (left) and mouse spleen (right) using CTCF antibody at a dilution of 1:1,000.

VALIDATION DATA

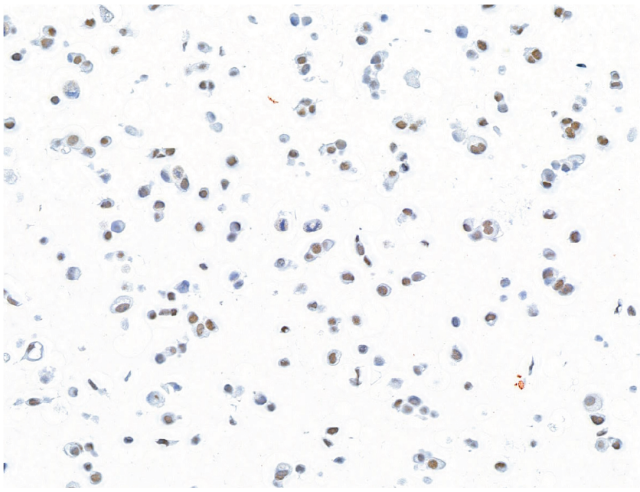

FIGURE 4 Immunocytochemistry data. FFPE section of human MCF-7 cells examined using CTCF antibody at a dilution of 1:1,000.

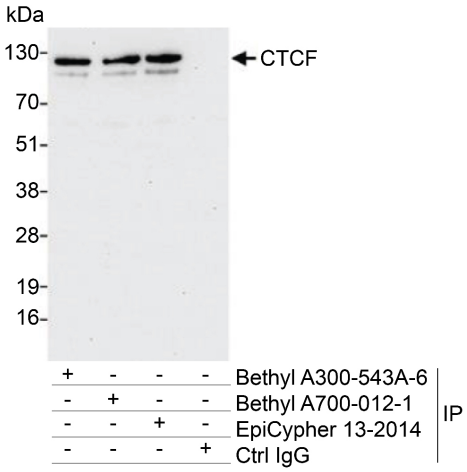

FIGURE 5 Immunoprecipitation data. EpiCypher CTCF antibody (6 µg/mg lysate) was used to immunoprecipitate whole cell lysates (1 mg, 20% of IP loaded) isolated from HEK293T cells. A negative control IgG antibody and positive control antibodies (Bethyl Laboratories) were also used to demonstrate specificity of the IP. All CTCF antibodies target the same CTCF epitope, however, A300-534A-6 is hosted in rabbit. For blotting immunoprecipitates, EpiCypher CTCF antibody was used at a 1:1,000 dilution.

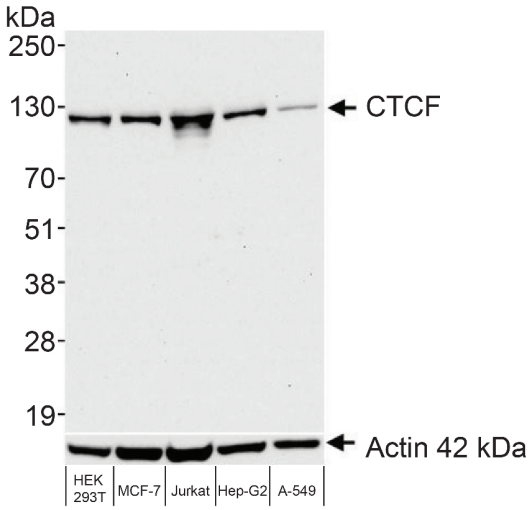

FIGURE 6 Western blot data. Western analysis of CTCF in whole cell extracts from HEK293T, MCF-7, Jurkat, Hep-G2, and A-549 cells. Fifty micrograms of lysate was resolved via SDS-PAGE and detected with a 1:1,000 dilution of CTCF antibody.