BRD4 CUTANA™ CUT&RUN Antibody

Catalog No	13-2003	**Type**	Polyclonal
Lot No	22179002-81	**Host**	Rabbit
Pack Size	50 µL	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, IHC, 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. BRD4 antibody produces CUT&RUN peaks primarily flanking transcription start sites (TSSs, **Figure 1**). BRD4 peaks show a large degree of overlap with BRG1/SMARCA4 peaks (**Figure 2**), as has been reported in the literature [1].

TECHNICAL INFORMATION

Immunogen	Between amino acids 1312 and 1362
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified antibody in Tris-citrate/phosphate buffer pH 7-8, 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Immunoprecipitation**	2 - 5 µg/mg lysate
Immunohistochemistry	1:1,000 - 1:5,000*	**Western Blot**	1:2,000 - 1:10,000

*Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections

GENE & PROTEIN INFORMATION

UniProt ID	O60885
Gene Name	BRD4
Protein Name	Bromodomain-containing protein 4
Target Size	152 kDa
Alternate Names	HUNK1, Protein HUNK1, bromodomain-containing protein 4

REFERENCES

[1] Conrad et al. *Mol Cell* (2017). PMID: 28844864

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with 0.5 µg of either BRD4, BRG1 (EpiCypher 13-2002), H3K4me3 positive control (EpiCypher 13-0041), or IgG negative control (EpiCypher 13-0042) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v2.0 (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 9.4 million reads (BRD4), 7.5 million reads (BRG1), 11.7 million reads (H3K4me3), and 8.3 million reads (IgG). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

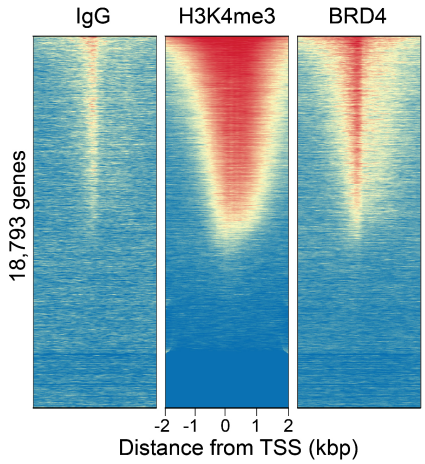

FIGURE 1 BRD4 peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmaps show BRD4 peaks relative to IgG negative control antibody and H3K4me3 positive control antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. All rows were aligned relative to H3K4me3 antibody.

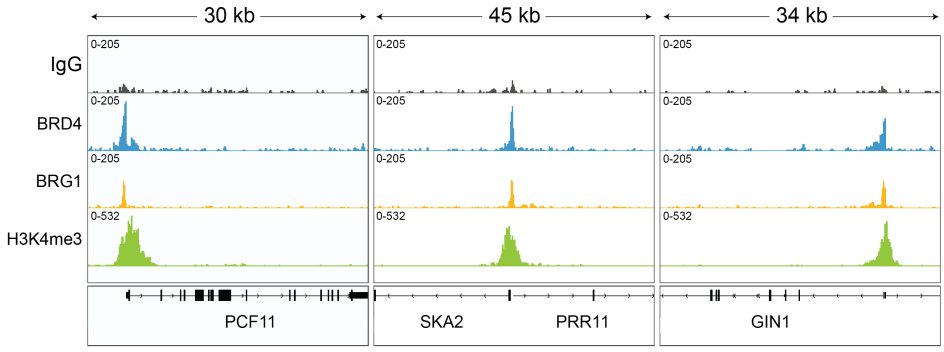

FIGURE 2 BRD4 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Three representative loci show overlap of BRD4 and BRG1 peaks.

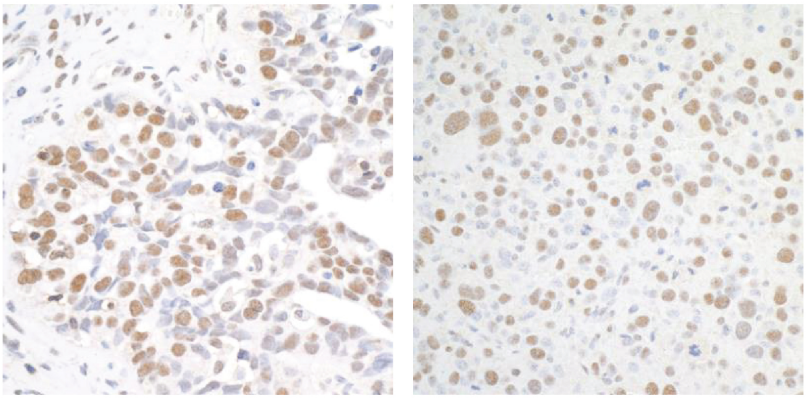

FIGURE 3 Immunohistochemistry data. FFPE sections of human ovarian carcinoma (**left**) and mouse renal cell carcinoma (**right**) using BRD4 antibody at a dilution of 1:5,000.

VALIDATION DATA

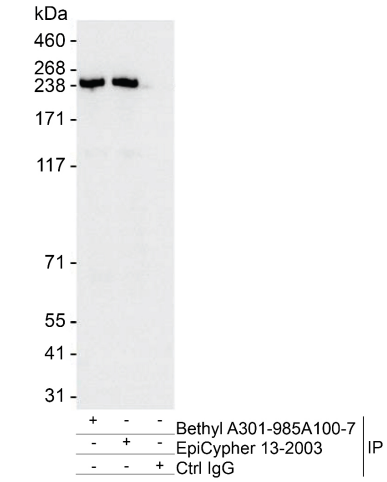

FIGURE 4 Immunoprecipitation data. EpiCypher BRD4 antibody (6 µg) was used to immunoprecipitate whole cell lysates (1 mg, 20% of IP loaded) isolated from HeLa cells. A negative control IgG antibody and positive control antibody targeting BRD4 (Bethyl Laboratories) were also used to demonstrate the specificity of the IP. For blotting immunoprecipitates, Bethyl Laboratories BRD4 antibody (A700-004) was used at a dilution of 1:1,000.

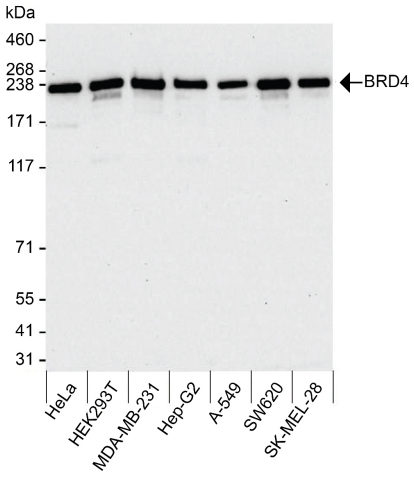

FIGURE 5 Western blot data. Western analysis of BRD4 in whole cell extracts from HeLa, HEK293T, MDA-MB-231, Hep-G2, A-549, SW620, and SK-MEL-28 cells. Ten micrograms of lysate was resolved via SDS-PAGE and detected with a 1:25,000 dilution of EpiCypher BRD4 antibody.

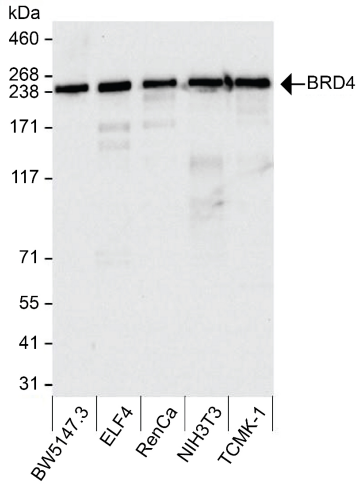

FIGURE 6 Western blot data. Western analysis of BRD4 in whole cell extracts from BW5147.3, ELF4, RenCa, NIH3T3, and TCMK-1 cells. Fifteen micrograms of lysate was resolved via SDS-PAGE and detected with a 1:25,000 dilution of EpiCypher BRD4 antibody.