BRG1/SMARCA4 CUTANA™ CUT&RUN Antibody

Catalog No	13-2002	**Type**	Polyclonal
Lot No	23034002-81	**Host**	Rabbit
Pack Size	100 µL	**Concentration**	200 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. BRG1 antibody produces CUT&RUN peaks primarily flanking transcription start sites (TSSs, **Figure 1**). BRG1 peaks show a large degree of overlap with BRD4 peaks (**Figure 2**), as has been reported in the literature [1].

TECHNICAL INFORMATION

Immunogen	Between amino acids 75 and 125
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified antibody in Tris-buffered saline, 0.1% BSA, 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Immunoprecipitation**	2 - 10 µg/mg lysate
Immunohistochemistry	1:250 - 1:2,000	**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	P51532
Gene Name	SMARCA4
Protein Name	Transcription activator BRG1
Target Size	185 kDa
Alternate Names	BAF190A, BRG1, SNF2B, SNF2L4, ATP-dependent helicase SMARCA4, BRG1-associated factor 190A (BAF190A), Mitotic growth and transcription activator, Protein BRG-1, Protein brahma homolog 1

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 BRG1/SMARCA4, 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 14.4 million reads (BRG1), 14.0 million reads (H3K4me3), and 11.5 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.

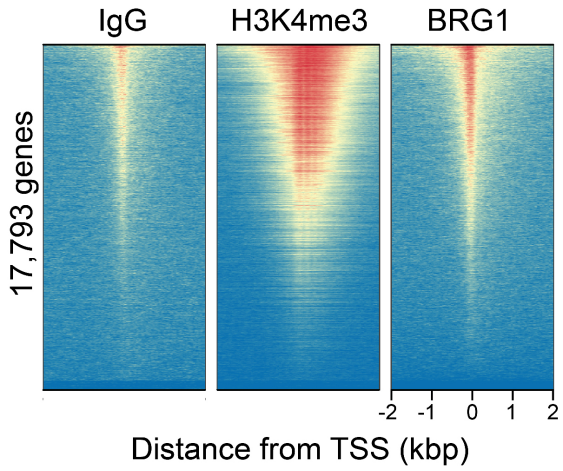

FIGURE 1 BRG1 peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmap showing BRG1 peaks relative to IgG negative control antibody and H3K4me3 positive control antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal.

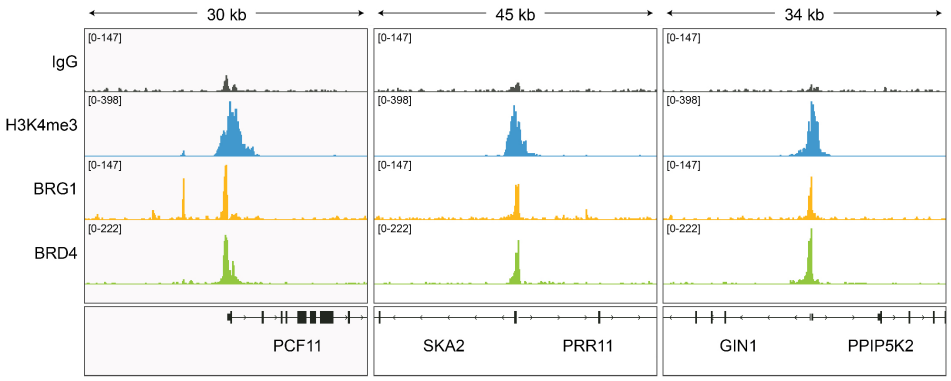

FIGURE 2 BRG1 CUT&RUN peak enrichment and functional overlap. CUT&RUN was performed as described above. BRG1 peaks overlapped with BRD4 antibody CUT&RUN peaks (EpiCypher 13-2003, top), as has been demonstrated in the literature [1]. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Three representative loci show overlap of BRG1 peaks with H3K4me3 and BRD4 peaks (bottom).

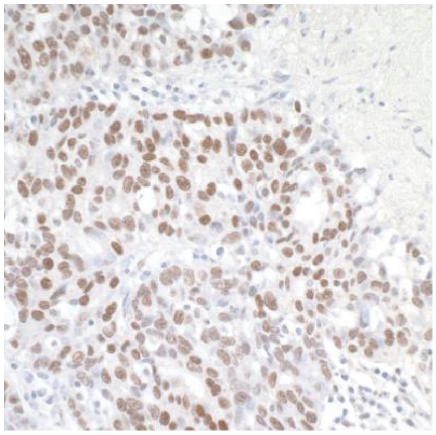

FIGURE 3 Immunohistochemistry data. FFPE section of human ovarian carcinoma using BRG1 antibody at a dilution of 1:1,000.

VALIDATION DATA

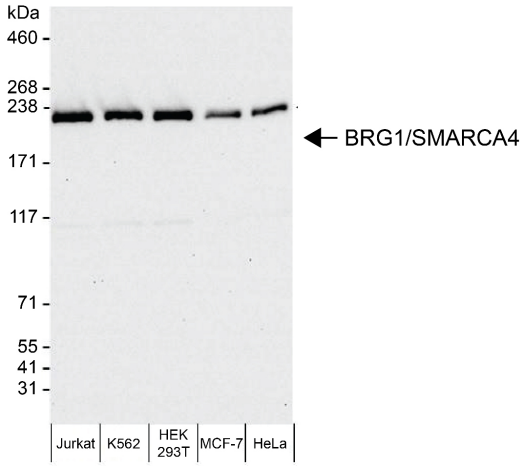

FIGURE 4 Western blot data. Western analysis of BRG1 in whole cell lysates isolated from Jurkat, K562, HEK293T, MCF-7, and HeLa cells using NETN lysis buffer. Lysates (10 µg) were loaded onto a 4-8% SDS-PAGE gel and analyzed under standard western blot conditions using BRG1 antibody (0.04 µg/mL).

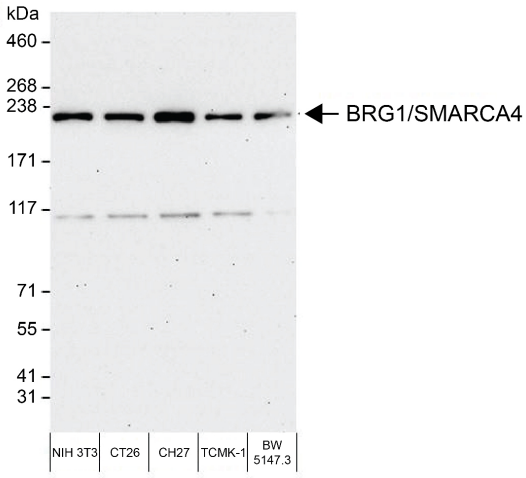

FIGURE 5 Western blot data. Western analysis of BRG1 in whole cell lysates isolated from NIH 3T3, CT26, CH27, TCMK-1, and BW5147.3 cells using NETN lysis buffer. Lysates (10 µg) were loaded onto a 4-8% SDS-PAGE gel and analyzed under standard western blot conditions using BRG1 antibody (0.04 µg/mL).

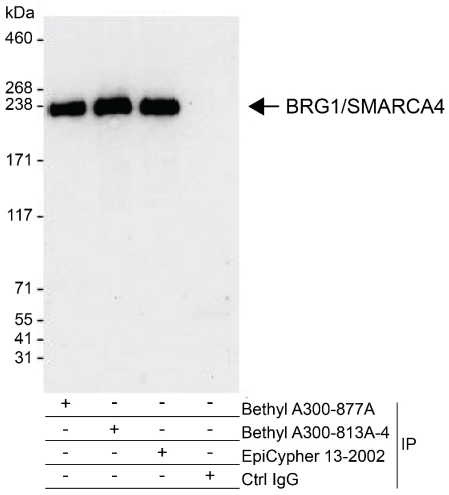

FIGURE 6 Immunoprecipitation data. EpiCypher BRG1 antibody (6 µg) was used to immunoprecipitate whole cell lysates isolated from HeLa cells using NETN lysis buffer (1 mg per IP). A negative control IgG antibody and positive control antibodies to the same BRG1 epitope (Bethyl Laboratories A300-813A-4) and to a different BRG1 epitope (Bethyl Laboratories A300-877A) were also used to demonstrate specificity of the IP. Immunoprecipitates were loaded onto a 4-20% SDS-PAGE gel (5% of IP loaded) and probed via western blot with EpiCypher BRG1 antibody (0.04 µg/mL).