BRD4 Antibody: CUTANA™ Compatible (CUT&RUN)

Catalog No. 13-2003
Lot No. 20240001-49
Pack Size 50 µL

EpiCypher®
www.epicypher.com

Type Polyclonal
Host Rabbit
Target Size 152 kDa
Format Aff. Pur. IgG
Reactivity H, M
Applications CUT&RUN, WB, IP, IHC

Product 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 CUTANA approach using EpiCypher optimized protocols (EpiCypher.com/resources/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 (Conrad *et al.*, 2017).

Immunogen:

A synthetic peptide corresponding to human BRD4 amino acids 1312 to 1362.

Formulation:

Antigen affinity-purified antibody (1 mg/mL) in Tris-citrate/phosphate buffer pH 7 to 8, 0.09% sodium azide.

Storage and Stability:

Stable for 1 year at 4°C from date of receipt.

Application Notes:

Recommended Dilutions:

CUT&RUN: 0.5 µg
IP: 2 - 5 µg/mg lysate
WB: 1:2,000 - 1:10,000
IHC: 1:1,000 - 1:5,000*

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

References:

Conrad et al (2017) Mol Cell 12:42.

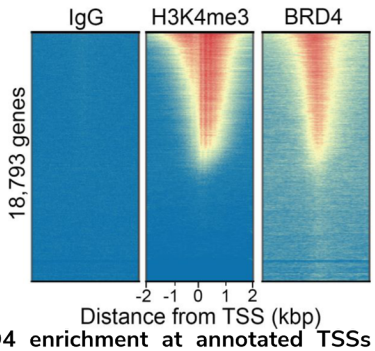

Figure 1: BRD4 enrichment at annotated TSSs in CUT&RUN. CUT&RUN was performed using 500,000 K562 cells with BRD4 antibody as well as control antibodies (IgG negative control, EpiCypher 13-0042; H3K4me3 positive control, EpiCypher 13-0041). Sequencing reads were aligned to annotated TSSs (+/- 2 kbp) of 18,793 genes. High, medium, and low signal is ranked by intensity (top to bottom) and reflected by red, yellow, and blue colors, respectively. All rows aligned relative to H3K4me3 antibody.

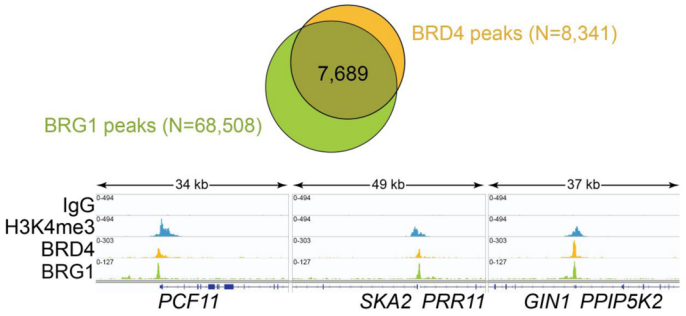

Figure 2: BRD4 CUT&RUN peak enrichment and functional overlap. The CUT&RUN data from Figure 1 was subjected to peak calling using MACS2. BRD4 peaks overlapped with BRG1/SMARCA4 antibody CUT&RUN peaks (EpiCypher 13-2002, top), as has been demonstrated in the literature (Conrad *et al.*, 2017). Three representative loci show BRD4 peaks in relation to control and BRG1 antibodies (bottom, Integrative Genomics Viewer).

Applications Key: ChIP: Chromatin immunoprecipitation; CUT&RUN: Cleavage Under Targets and Release Using Nuclease; CUT&Tag: Cleavage Under Targets and Tagmentation; E: ELISA; FACS: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; L: Luminex; WB: Western Blot. **Reactivity Key**: B: Bovine; Ce: *C. elegans*; Ch: Chicken; Dm: *Drosophila*; Eu: Eukaryote; H: Human; M: Mouse; Ma: Mammal; R: Rat; Sc: *S.cerevesiae*; Sp: *S. pombe*; WR: Wide Range (predicted); X: Xenopus; Z: Zebrafish

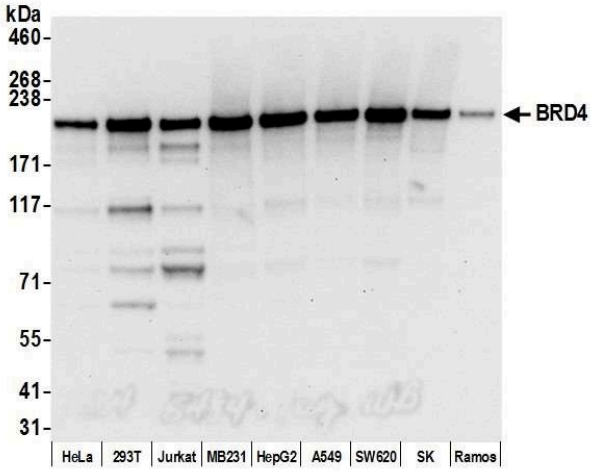

Figure 3: Western blot detection of human BRD4. Whole cell lysates were isolated from HeLa, HEK293T ("293T"), Jurkat, MB231, Hep-G2, A-549, SW620, SK-MEL -28 ("SK"), and Ramos cells using NETN lysis buffer. Lysates (15 µg) were loaded onto 4-8% SDS-PAGE gel and analyzed under standard western blot conditions using BRD4 antibody (1:1,000 dilution).

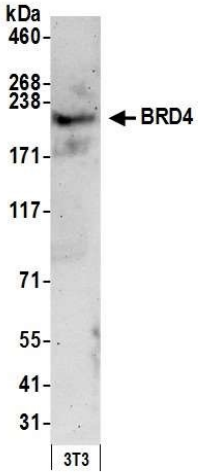

Figure 4: Western blot detection of mouse BRD4. Whole cell lysates were isolated from NIH 3T3 ("3T3") cells using NETN lysis buffer. Lysates (15 µg) were loaded onto 4-8% SDS-PAGE gel and analyzed under standard western blot conditions using BRD4 antibody (0.1 µg/mL).

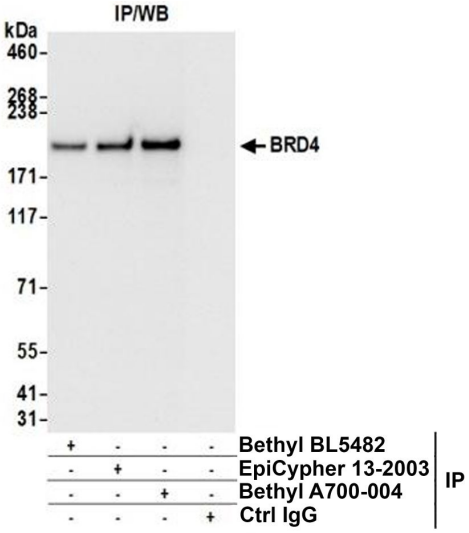

Figure 5: Immunoprecipitation of human BRD4. EpiCypher BRD4 antibody (3 µg) was used to immunoprecipitate whole cell lysates isolated from HeLa cells using NETN lysis buffer (1 mg per IP). A negative control IgG antibody and positive control antibodies to different BRD4 epitopes (Bethyl Laboratories) were also used to demonstrate specificity of the IP. Immunoprecipitates were loaded onto 4-8% SDS-PAGE gel (20% of IP loaded) and probed via western blot with a different BRD4 antibody (Bethyl Laboratories, Catalog No. A700-004, 1:1,000 dilution).

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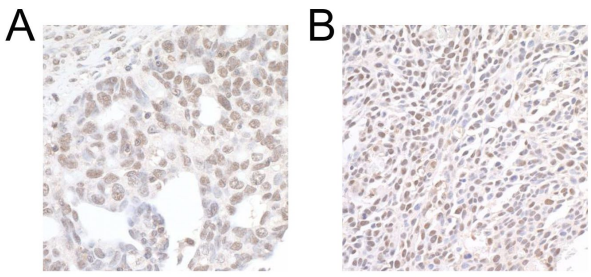

Figure 6: Immunohistochemistry detection of human and mouse BRD4. A) FFPE section of human ovarian carcinoma examined using BRD4 antibody (1:5,000 dilution, 0.2 µg/mL). **B)** FFPE section of mouse CT26 colon carcinoma examined using BRD4 antibody (1:1,000 dilution, 1 µg/mL).