FOXA1/HNF3A CUTANA™ CUT&RUN Antibody

Catalog No	13-2001	**Type**	Polyclonal
Lot No	20240001-17	**Host**	Rabbit
Pack Size	100 µL	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, IP, WB	**Reactivity**	Human, Mouse, Rat (predicted)

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. FOXA1 antibody produces CUT&RUN peaks above background primarily in intronic, intergenic, and promoter regions (**Figure 1**) that overlap with known FOXA1 DNA-binding motifs (**Figure 2**).

TECHNICAL INFORMATION

Immunogen	Between amino acids 422 and 472
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	**Western Blot**	1:2,000 - 1:10,000
Immunoprecipitation	2 - 10 µg/mg lysate		

GENE & PROTEIN INFORMATION

UniProt ID	P55317
Gene Name	FOXA1
Protein Name	Hepatocyte nuclear factor 3-alpha
Target Size	49 kDa
Alternate Names	HNF-3-alpha, HNF-3A, Forkhead box protein A1, Transcription factor 3A (TCF-3A), HNF3A, TCF3A

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k MCF7 cells with 0.5 µg of either FOXA1/HNF3A 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 2.5 million reads (FOXA1) and 2.4 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.

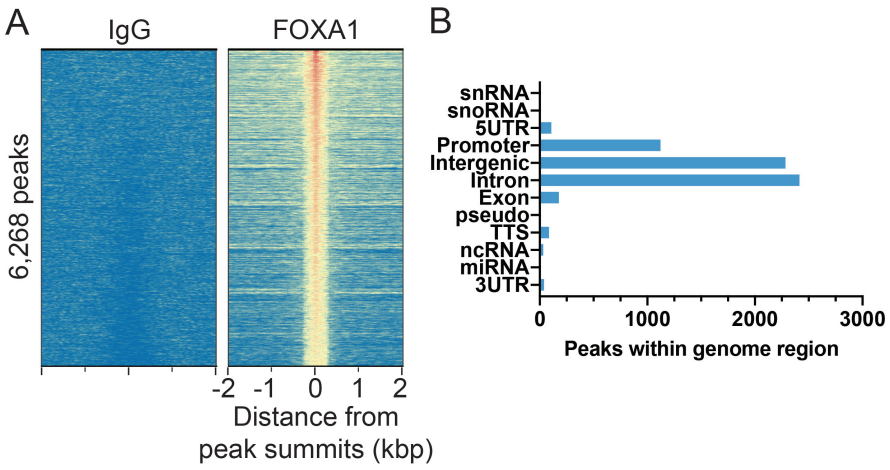

FIGURE 1 FOXA1 peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. (**A**) Heatmap showing FOXA1 peaks relative to IgG negative control antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. (**B**) The number of peaks that fall into distinct classes of functionally annotated genomic regions are shown.

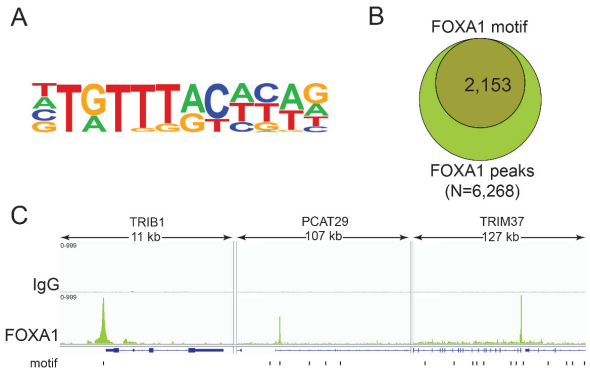

FIGURE 2 FOXA1 transcription factor binding motif analysis in CUT&RUN. (**A**) Homer analysis determined that the FOXA1 consensus motif, represented as a sequence logo position weight matrix, was enriched under FOXA1 CUT&RUN peaks. (**B**) The number of FOXA1 peaks containing consensus motifs from panel A is represented by a Venn Diagram. (**C**) Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Three representative loci show overlap of FOXA1 peaks with consensus motifs.

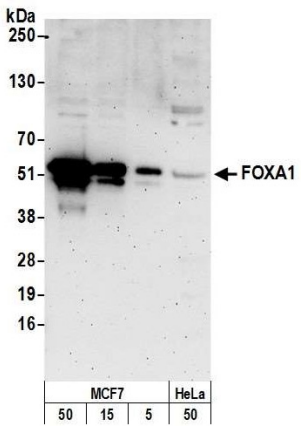

FIGURE 3 Western blot data. Western analysis of FOXA1 in whole cell lysates from MCF-7 and HeLa cells using NETN lysis buffer. The indicated amounts (µg) of lysate were loaded onto 4-20% SDS-PAGE gel and analyzed under standard western blot conditions using FOXA1 antibody (0.1 µg/mL).

VALIDATION DATA

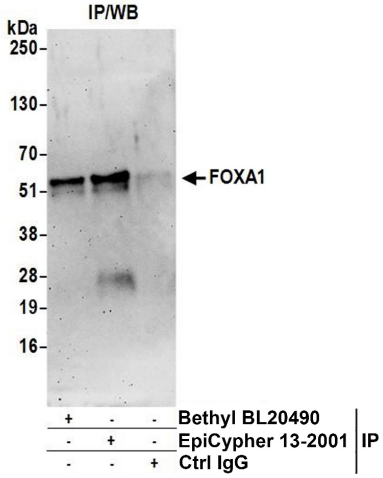

FIGURE 4 Immunoprecipitation data. EpiCypher FOXA1 antibody (6 µg) was used to immunoprecipitate whole cell lysates isolated from HeLa cells using NETN lysis buffer (1 mg per IP). A negative control IgG antibody and positive control antibody to a different FOXA1 epitope (Bethyl Laboratories) were also used to demonstrate specificity of the IP. Immunoprecipitates were loaded onto 4-20% SDS-PAGE gel (20% of IP loaded) and probed via western blot with EpiCypher FOXA1 antibody (1 µg/mL).