MLL1/KMT2A CUTANA™ CUT&RUN Antibody

Catalog No	13-2004	**Type**	Monoclonal
Lot No	22094002-82	**Host**	Rabbit
Pack Size	100 µL	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, IP, WB	**Reactivity**	Human

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. MLL1 antibody produces CUT&RUN peaks above background (**Figure 1**) that overlap with H3K4me3 (**Figures 1-2**), consistent with its known role as the catalytic subunit of the MLL1/MLL histone methyltransferase complex.

TECHNICAL INFORMATION

Immunogen	Between amino acids 720 and 780
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Purified recombinant monoclonal antibody in borate buffered saline (BBS) pH 8.2, 0.09% sodium azide, BSA free

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Western Blot**	1:1,000
Immunoprecipitation	10 µL/0.5 mg lysate		

REFERENCES

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with 0.5 µg of either MLL1, IgG negative control (EpiCypher 13-0042), or H3K4me3 positive control (EpiCypher 13-0041) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v2.0 (EpiCypher 14-1048). Library preparation was performed with 5 ng 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 10.0 million reads (IgG), 13.5 million reads (MLL1), and 8.8 million reads (H3K4me3). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

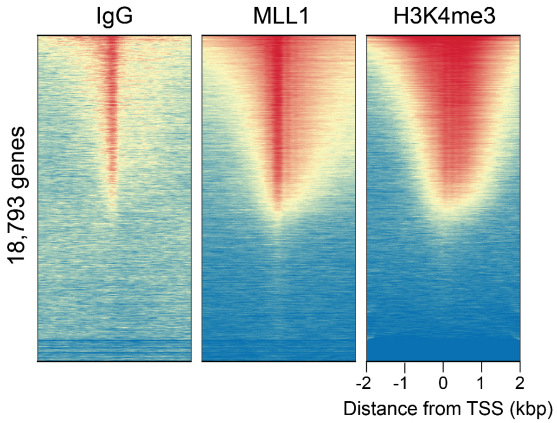

FIGURE 1 MLL1 peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmaps show MLL1 peaks relative to IgG negative control or H3K4me3 positive control antibodies in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. MLL1 and H3K4me3 antibodies showed expected enrichment around the TSS. Despite some observable background in the IgG control, differential enrichment with the target antibodies is as expected.

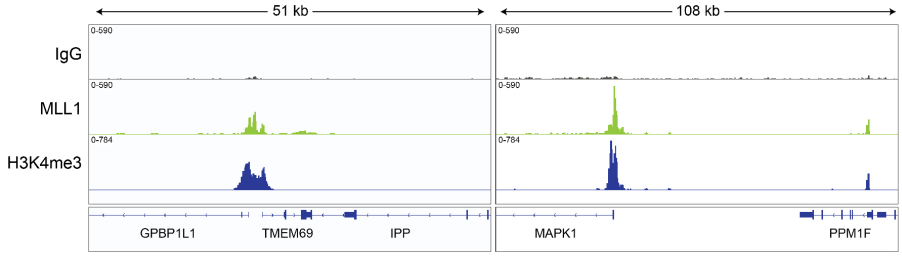

FIGURE 2 MLL1 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two representative loci show overlap of MLL1 and H3K4me3 peaks.

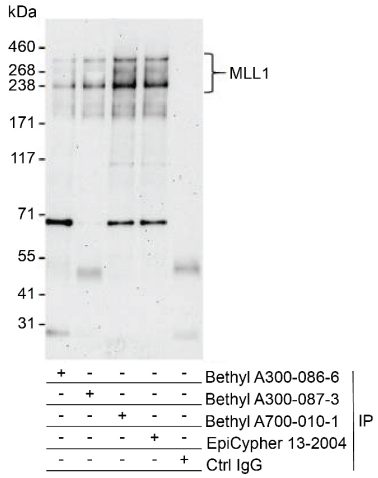

FIGURE 3 Immunoprecipitation data. EpiCypher MLL1 antibody (6 µL/mg lysate) was used to immunoprecipitate whole cell lysates (1 mg per IP reaction; 20% of IP loaded) isolated from HEK293T cells using NETN lysis buffer. A negative control IgG antibody and positive control antibodies targeting MLL1 (Bethyl Laboratories) were also used to demonstrate specificity of the IP. EpiCypher 13-2004, Bethyl A700-010-1, and Bethyl A300-086-6 target the same epitope, while Bethyl A300-087-3 targets a different epitope (between amino acids 1320 and 1380) For blotting immunoprecipitates, EpiCypher MLL1 antibody was used at a dilution of 1:1,000.

VALIDATION DATA

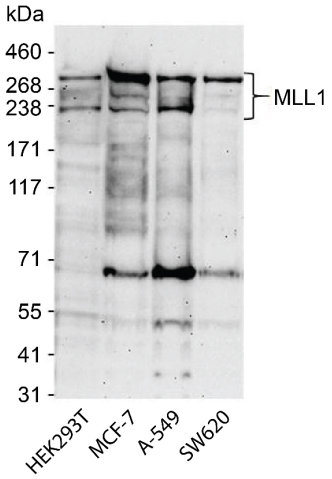

FIGURE 4 **Western blot data.** Western analysis of MLL1 in whole cell extracts from HEK293T, MCF-7, A-549, and SW620 cells. Fifty micrograms of lysate was resolved via SDS-PAGE and detected with a 1:1,000 dilution of MLL1 antibody.