CUTANA™ IgG Negative Control Antibody for CUT&RUN and CUT&Tag

Catalog No	13-0042	**Type**	Polyclonal
Lot No	23152006-81	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	0.5 mg/mL
Applications	CUT&RUN, CUT&Tag	**Reactivity**	Negative Control

DESCRIPTION

Cleavage Under Targets & Release Using Nuclease (CUT&RUN) and Cleavage Under Targets and Tagmentation (CUT&Tag) are next generation assays for genomic mapping of chromatin features to replace Chromatin Immunoprecipitation (ChIP-seq) [1]. Unlike in ChIP-seq, where baseline signal is established by sequencing the fragmented input chromatin, reactions performed with this rabbit IgG antibody are used to determine background signal arising from non-specific MNase digestion and Tn5 tagmentation. Pair with the H3K4me3 positive control antibody (EpiCypher 13-0041) for a well-controlled experiment.

TECHNICAL INFORMATION

Immunogen	None
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Affinity-purified antibody in PBS pH 7.6

RECOMMENDED DILUTION

CUT&RUN:	0.5 µg per reaction	**CUT&Tag:**	0.5 µg per reaction

REFERENCES

[1] Skene & Henikoff *eLife* (2017). PMID: 28079019

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k native K562 cells with 0.5 µg of either IgG or H3K4me3 (EpiCypher 13-0041) antibodies in duplicate using the CUTANA™ ChIC/CUT&RUN Kit v3 (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. Sample sequencing depth was 11.3/9.0 million reads (IgG Rep 1/Rep 2) and 11.0/13.0 million reads (H3K4me3 Rep 1/Rep 2). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

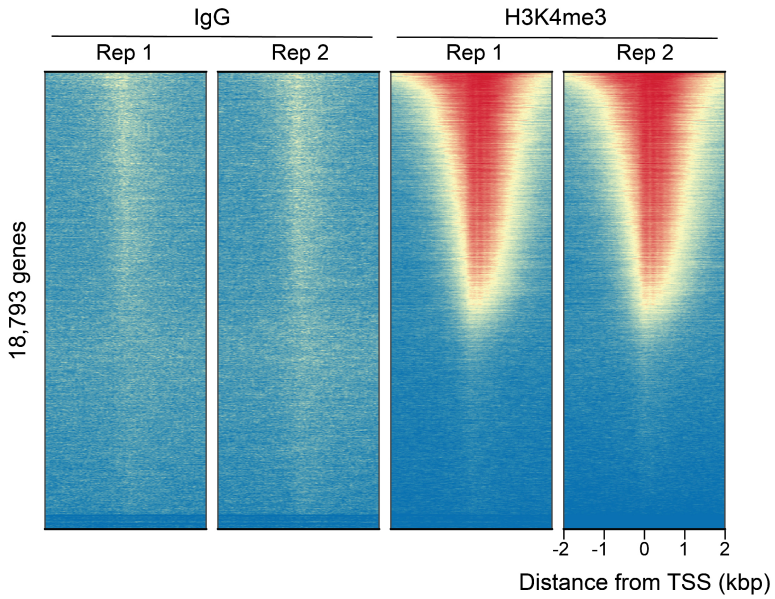

FIGURE 1 CUT&RUN genome wide enrichment. CUT&RUN was performed as described above. Heatmaps show H3K4me3 peaks relative to IgG antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. H3K4me3 antibody showed expected enrichment around the TSS, while the IgG antibody displayed minimal background, as expected.

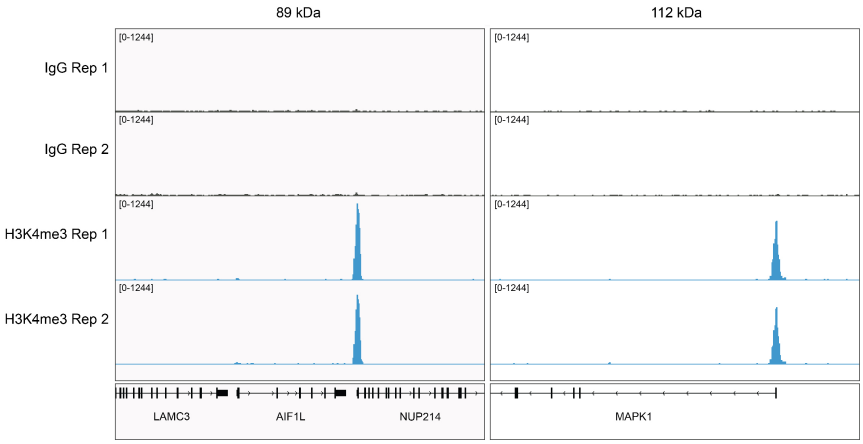

FIGURE 2 Rabbit IgG CUT&RUN tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two gene loci show H3K4me3 peaks and minimal background in the IgG track, as expected.

VALIDATION DATA

CUT&Tag Methods

CUT&Tag was performed on 100k native K562 nuclei with 0.5 µg of either IgG or H3K4me3 (EpiCypher 13-0041) antibodies in singlicate using the CUTANA™ CUT&Tag Kit v1 (EpiCypher 14-1102/14-1103). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50bp). Sample sequencing depth was 0.9 million reads (IgG) and 2.9 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.

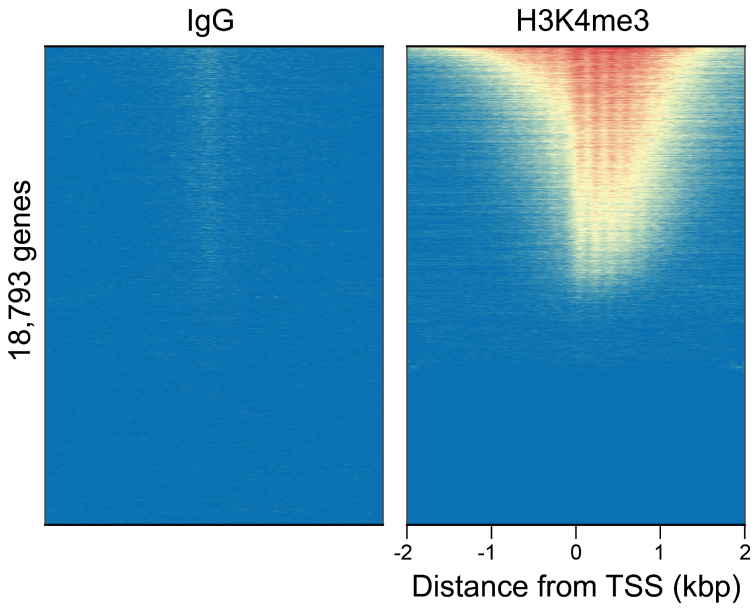

FIGURE 3 CUT&Tag genome wide enrichment. CUT&Tag was performed as described above. Heatmaps show H3K4me3 peaks relative to IgG antibody in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. H3K4me3 antibody showed expected enrichment around the TSS, while the IgG antibody displayed minimal background, as expected.

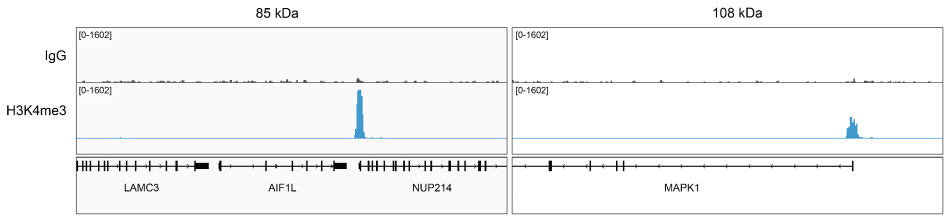

FIGURE 4 Rabbit IgG CUT&Tag tracks. CUT&Tag was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two gene loci show H3K4me3 peaks and minimal background in the IgG track, as expected.