CUTANA™ pAG-MNase for ChIC/CUT&RUN Workflows

Catalog No	15-1016	**Species**	*S. aureus*
Lot No	22181005-02	**Source**	*E. coli*
Pack Size	50 Reactions	**Epitope Tag**	6xHis
Concentration	20X	**MW**	43.7 kDa

DESCRIPTION

CUTANA™ pAG-MNase is the key reagent for performing Chromatin Immunocleavage (ChIC) [1] and Cleavage Under Targets and Release Using Nuclease (CUT&RUN) [2,3]. As a fusion of Proteins A and G to Micrococcal Nuclease, CUTANA pAG-MNase is compatible with target antibodies from various host species and is highly purified to remove contaminating *E. coli* DNA, which can complicate analysis at low cell numbers. This enzyme enables efficient mapping of chromatin features in ChIC/CUT&RUN, allowing for significant improvements in signal to noise and sequencing depth compared to ChIP-seq.

RECOMMENDED ACCESSORY REAGENTS

Item	CAT	Item	CAT
CUTANA™ CUT&RUN Library Prep Kit	14-1001	CUT&RUN Antibodies	epicypher.com/antibodies
CUTANA™ DNA Purification Kit	14-0050	CUTANA™ ConA Beads	21-1401
Magnetic Separation Rack, 0.2 mL	10-0008	CUT&RUN 8-strip Tubes	10-0009
Magnetic Separation Rack, 1.5 mL	10-0012	*E. coli* Spike-in DNA	18-1401
SNAP-CUTANA™ K-MetStat Panel	19-1002		

TECHNICAL INFORMATION

Storage	Stable for one year at -20°C from date of receipt
Formulation	10 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, and 50% glycerol

APPLICATION NOTES

Add 2.5 µL of the supplied enzyme to a 50 µL CUT&RUN reaction (20X dilution). For more, see epicypher.com/protocols.

REFERENCES

[1] Schmid et al. *Mol. Cell* (2004). PMID: 15469830
[2] Skene & Henikoff *eLife* (2017). PMID: 28079019
[3] Skene et al. *Nat. Protoc.* (2018). PMID: 29651053

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k K562 cells with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0041), or H3K27me3 (Thermo MA5-11198) antibodies using CUTANA™ pAG-MNase (1:20 dilution) and 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). Both 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 3.6 million reads (IgG), 4.3 million reads (H3K4me3), and 5.2 million reads (H3K27me3). Data were aligned to the hg19 genome using Bowtie2 and filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

VALIDATION DATA

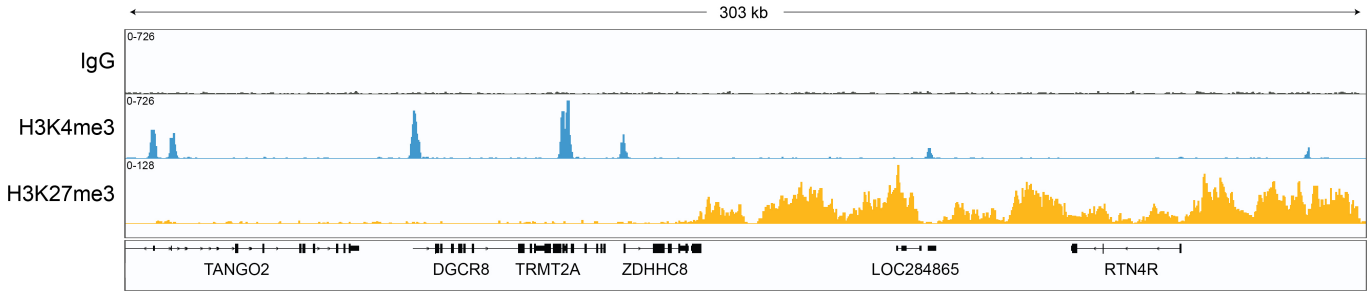

FIGURE 1 CUT&RUN gene browser tracks. CUT&RUN was performed as described above. Data verifies low non-specific MNase digestion with the absence of peaks in the IgG track, an expected H3K4me3 profile with sharp promoter peaks, and broad peaks in heterochromatin regions consistent with H3K27me3. Image was generated using the Integrative Genomics Viewer (IGV, Broad Institute).

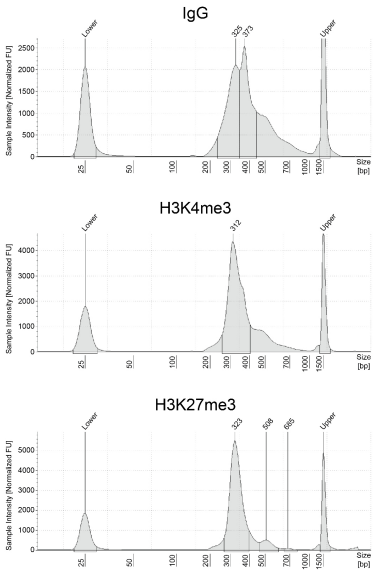

FIGURE 2 Size distribution of released chromatin. CUT&RUN was performed as described above. Excised DNA is highly enriched for mononucleosomes (peaks at ~300 bp represent 150 bp nucleosomes + sequencing adapters).

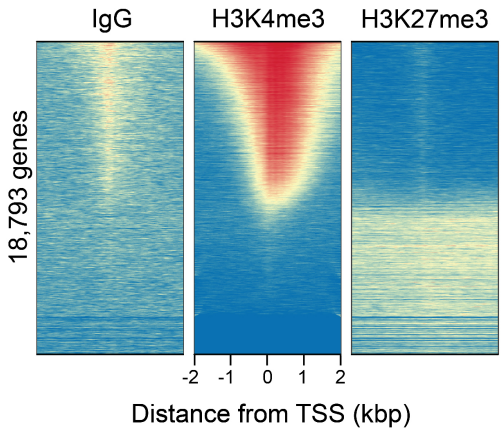

FIGURE 3 CUT&RUN genome-wide heatmaps. CUT&RUN was performed as described above. Heatmaps show CUT&RUN signal aligned to annotated transcription start sites (TSS, +/- 2kb). High and low signal are ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. Gene rows in each heatmap are aligned and sorted from high to low signal relative to H3K4me3 (middle).

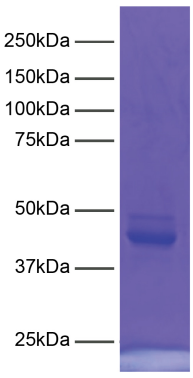

FIGURE 4 Protein gel data. CUTANA™ pAG-MNase (1 µg) was resolved via SDS-PAGE and stained with Coomassie blue. The migration and molecular weight of the protein standards are indicated.