CUTANA™ ChIC/CUT&RUN Kit

Catalog No	14-1048	**Pack Size**	48 Reactions
Lot No	22326005-81	**Kit Version**	v3

DESCRIPTION

The CUTANA™ ChIC/CUT&RUN Kit enables streamlined chromatin profiling of histone post-translational modifications (PTMs) and chromatin associated proteins while providing increased throughput and reproducibility with multi-channel pipetting. Positive (H3K4me3) and negative (IgG) control antibodies are included to pair with SNAP-CUTANA™ spike-in controls for assay optimization and continuous monitoring (**Figure 2**). *E. coli* DNA is included for data normalization. The kit is compatible with a variety of inputs including cells or nuclei derived from native, cryopreserved, or cross-linked samples. While it is recommended to start with 500,000 cells, comparable data can be generated using as few as 5,000 cells. The inclusion of controls, as well as compatibility with diverse target types, sample inputs, and low cell numbers, make this kit the go-to solution for chromatin mapping experiments.

KIT CONTENTS

Item	CAT	Item	CAT
ConA Beads	21-1401	SNAP-CUTANA K-MetStat Panel	19-1002k
E. coli Spike-in DNA	18-1401	8-strip Tubes	10-0009
Bead Activation Buffer	21-1001	DNA Cleanup Columns	10-0010
Pre-Wash Buffer	21-1002	DNA Collection Tubes	10-0011
Stop Buffer	21-1003	0.5 M EDTA	21-1006
5% Digitonin	21-1004	100 mM Calcium Chloride	21-1007
1 M Spermidine	21-1005	DNA Binding Buffer	21-1008
pAG-MNase	15-1016	DNA Wash Buffer	21-1009
H3K4me3 Positive Control Antibody	13-0041k	DNA Elution Buffer	21-1010
Rabbit IgG Negative Control Antibody	13-0042k		

TECHNICAL INFORMATION

Storage OPEN KIT IMMEDIATELY and store components at room temperature, 4°C, and -20°C as indicated (see **User Manual corresponding to Kit Version 3**). Stable for 6 months upon date of receipt.

Instructions for Use See User Manual corresponding to Kit Version 3. This kit is not compatible with previous user manuals.

VALIDATION DATA

CUT&RUN Methods CUT&RUN was performed using the CUTANA™ ChIC/CUT&RUN Kit starting with 500k K562 cells with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0041), H3K27me3 (ThermoFisher MA5-11198), or 0.125 µg of CTCF (EpiCypher 13-2014) antibodies. Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ Library Prep Kit (EpiCypher 14-1001/14-1002). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 3.4 million reads (IgG Rep 1), 3.5 million reads (IgG Rep 2), 4.1 million reads (H3K4me3 Rep 1), 2.9 million reads (H3K4me3 Rep 2), 3.1 million reads (H3K27me3), and 7.5 million reads (CTCF). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

VALIDATION DATA

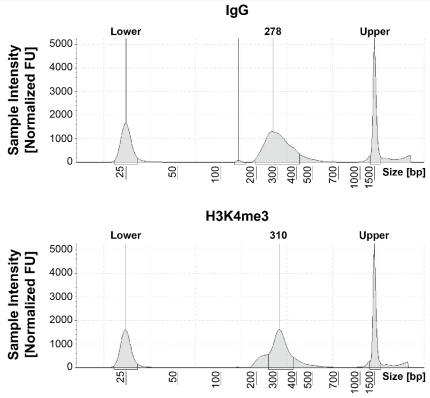

FIGURE 1 CUT&RUN DNA fragment size distribution analysis. CUT&RUN was performed as described above. Library DNA was analyzed by Agilent Tapestation®. This analysis confirmed that mononucleosomes were predominantly enriched in CUT&RUN (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters).

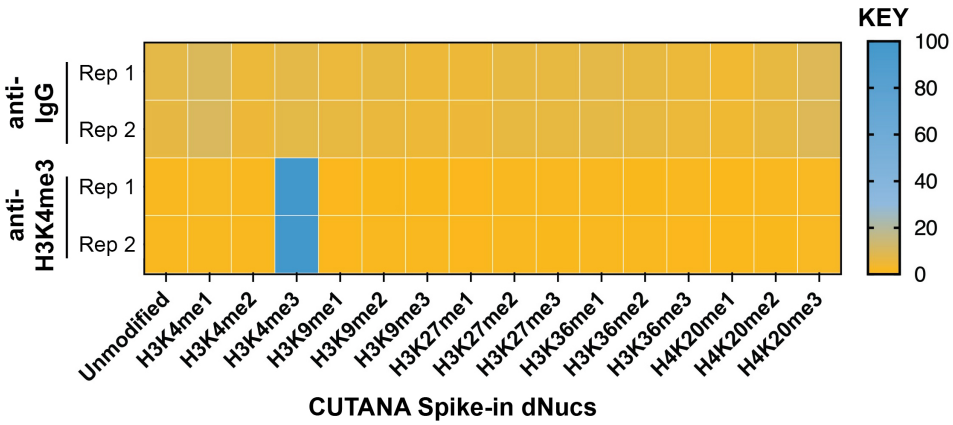

FIGURE 2 SNAP-CUTANA™ K-MetStat Spike-in Controls. DNA-barcoded designer nucleosomes (dNucs) representing 16 K-methyl PTMs: mono-, di-, and tri-methylation at H3K4, H3K9, H3K27, H3K36, and H4K20, as well as unmodified control, were spiked into CUT&RUN reactions prior to the addition of antibodies (IgG, H3K4me3). Spike-in barcodes were counted and normalized from raw fastq files using the shell script and analysis sheet available at epicypher.com/19-1002. Barcodes for IgG (top; normalized to total reads) and H3K4me3 (bottom; normalized to on-target) antibodies are shown. The spike-ins confirmed optimal experimental conditions (H3K4me3 antibody specifically recovered the target dNuc, while IgG showed no preferential enrichment).

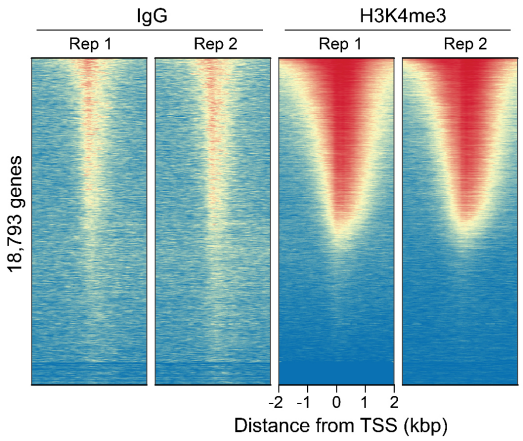

FIGURE 3 CUT&RUN genome-wide heatmaps. CUT&RUN was performed as described above. Peaks were called with MACS2. Heatmaps show two replicates ("Rep") of IgG (left) and H3K4me3 (right) kit control antibodies in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal.

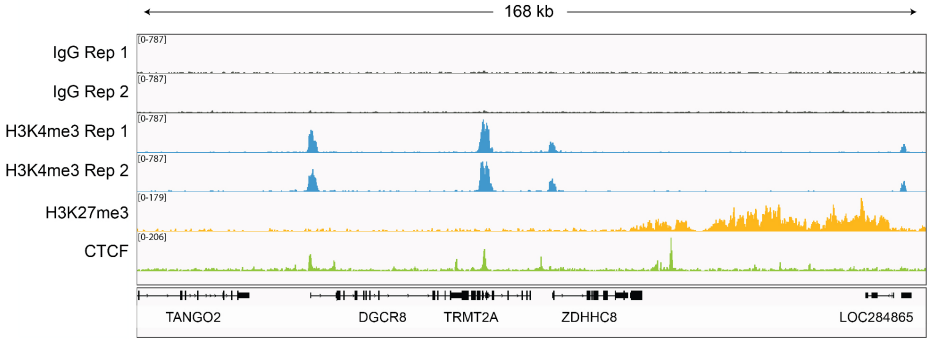

FIGURE 4 Representative gene browser tracks. CUT&RUN was performed as described above. A representative 168 kb window at the TRMT2A gene is shown for two replicates ("Rep") of IgG and H3K4me3 kit control antibodies. Representative tracks are also shown for antibodies to H3K27me3 and the transcription factor CTCF. The CUT&RUN kit produced the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).