CUTANA™ ChIC/CUT&RUN Kit

Catalog No	14-1048	**Pack Size**	48 Reactions
Lot No	23298006-81	**Kit Version**	v4

DESCRIPTION

The CUTANA™ ChIC/CUT&RUN Kit enables streamlined chromatin profiling of histone post-translational modifications (PTMs) and chromatin associated proteins. The CUT&RUN Kit Version 4 (v4) now uses SPRI magnetic beads for DNA purification instead of DNA spin columns, enabling multi-channel pipetting throughout for increased throughput and reproducibility. Positive (H3K4me3) and negative (IgG) control antibodies are included to pair with SNAP-CUTANA™ spike-in controls for assay optimization and continuous assay 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
8-strip Tubes	10-0009a	Pre-Wash Buffer	21-1002
0.5 M EDTA	21-1006	Stop Buffer	21-1003
100 mM Calcium Chloride	21-1007	5% Digitonin	21-1004k
SPRIselect Reagent from Beckman Coulter, Inc.	21-1405	1 M Spermidine	21-1005
0.1X TE Buffer	21-1025	SNAP-CUTANA K-MetStat Panel	19-1002k
ConA Beads	21-1401	H3K4me3 Positive Control Antibody	13-0041k
E. coli Spike-in DNA	18-1401	Rabbit IgG Negative Control Antibody	13-0042k
Bead Activation Buffer	21-1001	pAG-MNase	15-1016

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 4**). Stable for 6 months upon date of receipt.

Instructions for Use See User Manual corresponding to Kit Version 4. This kit is not compatible with previous user manuals. **Version 4 contains SPRI magnetic beads for DNA clean up, while previous versions used DNA spin columns**.

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 (EpiCypher 13-0055), or 0.125 µg of CTCF (EpiCypher 13-2014) antibodies in duplicate. 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). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 3.5 million reads (IgG Rep 1), 3.8 million reads (IgG Rep 2), 4.7 million reads (H3K4me3 Rep 1), 6.9 million reads (H3K4me3 Rep 2), 6.6 million reads (H3K27me3 Rep 1), 4.7 million reads (H3K27me3 Rep 2), 3.9 million reads (CTCF Rep 1) and 4.6 million reads (CTCF 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.

VALIDATION DATA

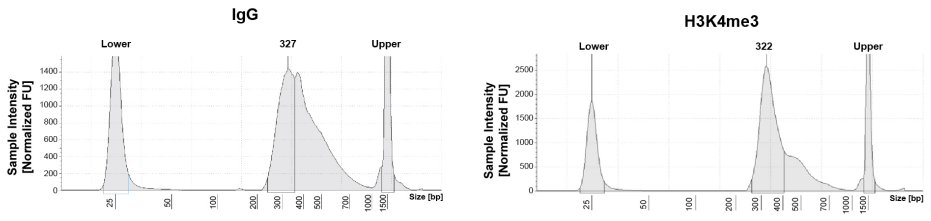

FIGURE 1 CUT&RUN DNA fragment size distribution analysis. CUT&RUN was performed as described above. Library DNA was analyzed by Agilent TapeStation®. This analysis confirmed that mononucleosomes were predominantly enriched in CUT&RUN (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters).

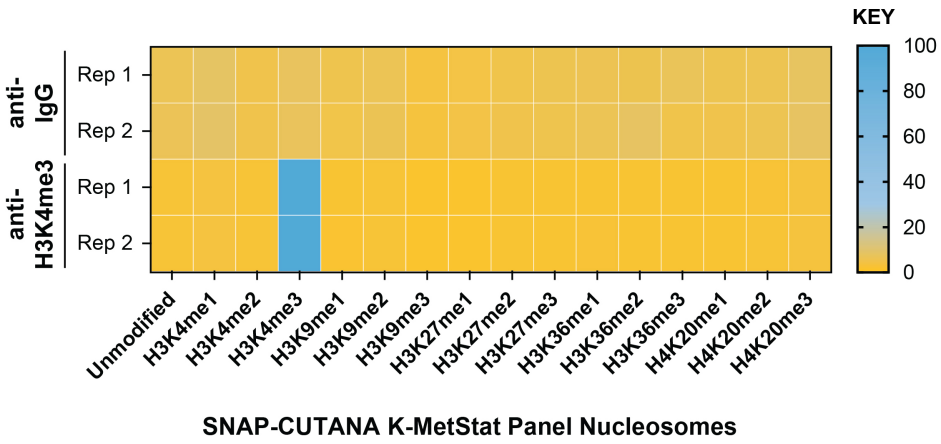

FIGURE 2 SNAP-CUTANA™ K-MetStat Spike-in controls. DNA-barcoded designer nucleosomes (dNucs) representing 16 K-methyl PTMs: mono-, di-, and tri-methylation at H3K4, H3K9, H3K27, H3K36, and H4K20, as well as unmodified control, were spiked into CUT&RUN reactions prior to the addition of antibodies (IgG, 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).

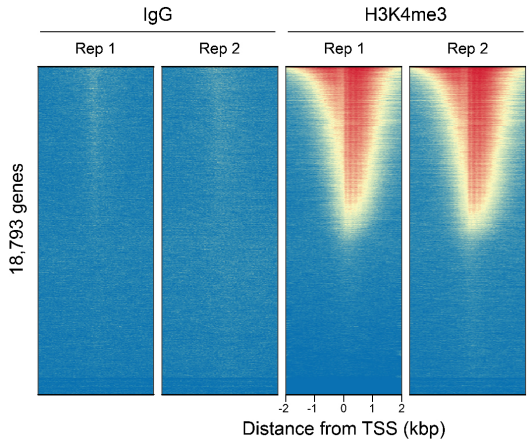

FIGURE 3 CUT&RUN genome-wide heatmaps. CUT&RUN was performed as described above. Heatmaps show two replicates ("Rep") of IgG (left) and H3K4me3 (right) kit control antibodies in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes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

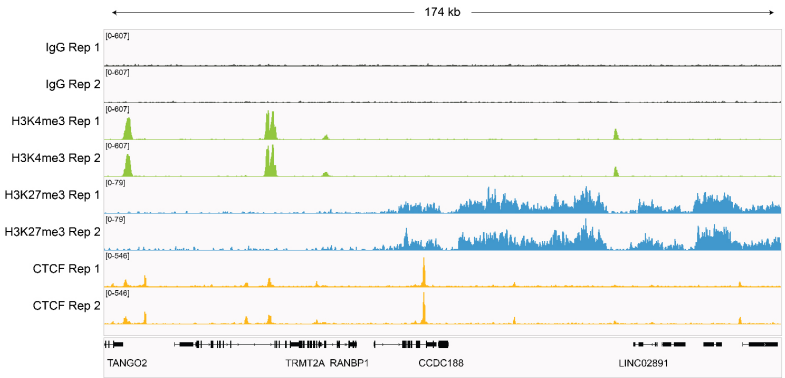

FIGURE 4 Representative gene browser tracks. CUT&RUN was performed as described above. A representative 174 kb window at the TRMT2A gene is shown for two replicates ("Rep") of IgG and H3K4me3 kit control antibodies. Representative tracks are also shown for antibodies to H3K27me3 and the transcription 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).