CUTANA™ CUT&Tag Kit with Primer Set 2

Catalog No	14-1103	**Pack Size**	48 Reactions
Lot No	23046001-81	**Kit Version**	v1

DESCRIPTION

The CUTANA™ CUT&Tag Kit offers a comprehensive solution for ultra-sensitive mapping of histone post-translational modifications (PTMs). This kit uses an exclusive Direct-to-PCR strategy to go from cells to PCR amplified sequencing libraries in one tube, bypassing traditional library prep and minimizing sample loss. The protocol is also designed for compatibility with multi-channel pipetting for increased throughput and reproducibility. Positive (H3K27me3) and negative (IgG) control antibodies are paired with the SNAP-CUTANA™ K-MetStat Panel of nucleosome spike-in controls (**Figure 2**) to continuously monitor workflows and guide troubleshooting.

The recommended input for CUT&Tag is 100,000 native nuclei per reaction. Comparable data can be generated down to 10,000 nuclei, and the protocol is also validated for whole cells, cryopreserved samples, and lightly cross-linked nuclei or cells. CUT&Tag provides robust profiling for histone PTMs. For chromatin-associated proteins (e.g. transcription factors), CUTANA™ CUT&RUN is recommended (EpiCypher 14-1048, EpiCypher 14-1001).

KIT CONTENTS

Item	Cat. No.	Item	Cat. No.
8-strip Tubes	10-0009t	Pre-Nuclear Extraction Buffer	21-1021
0.5 M EDTA	21-1014	Pre-Wash Buffer	21-1020
4.5 M NaCl	21-1013	5% Digitonin	21-1023
1 M $MgCl_2$	21-1015	1 M Spermidine	21-1024
TAPS Buffer	21-1016	SNAP-CUTANA K-MetStat Panel	19-1002t
SDS Release Buffer	21-1017	Rabbit IgG Negative Control Antibody	13-0042t
SDS Quench Buffer	21-1018	H3K27me3 Positive Control Antibody	13-0055t
SPRIselect reagent from Beckman Coulter, Inc.	21-1404	Anti-Rabbit Secondary Antibody	13-0047
0.1X TE Buffer	21-1019	pAG-Tn5	15-1017
ConA Beads	21-1401	Non-Hot Start 2X PCR Master Mix	15-1018
Bead Activation Buffer	21-1022		
Multiplexing Primers	This kit includes combinatorial dual indices for multiplexed sequencing of up to 48 reactions. Pair with EpiCypher 14-1102 for multiplexing up to 96 reactions.		

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

Instructions for Use See User Manual corresponding to Kit Version 1

REFERENCES

VALIDATION DATA

CUT&Tag Methods

CUT&Tag was performed using the CUTANA™ CUT&Tag Kit starting with 100k K562 cells and 0.5 µg of either IgG (EpiCypher 13-0042t), H3K27me3 (EpiCypher 13-0055t), H3K4me1 (EpiCypher 13-0057), or H3K4me3 (EpiCypher 13-0041*) antibodies. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 6.3/4.7 million reads (IgG Rep 1/Rep 2), 4.1/4.6 million reads (H3K27me3 Rep 1/Rep 2), 4.7/5.0 million reads (H3K4me1 Rep 1/Rep 2), and 5.0/4.5 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.

*EpiCypher 13-0041 does not currently meet our "SNAP-Certified in CUT&Tag" efficiency standards for robust profiling down to 10k nuclei, but is specific at 100k nuclei.

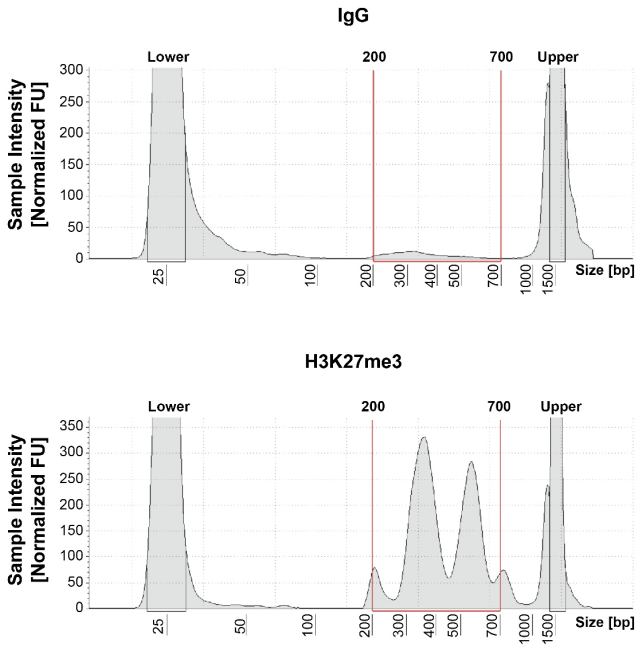

FIGURE 1 CUT&Tag DNA fragment size distribution analysis. CUT&Tag was performed as described above. Library DNA was analyzed by Agilent TapeStation®, which confirmed that mononucleosomes were predominantly enriched in CUT&Tag (peak between 300-400 bp). Peak between 500-700 bp represents dinucleosomes.

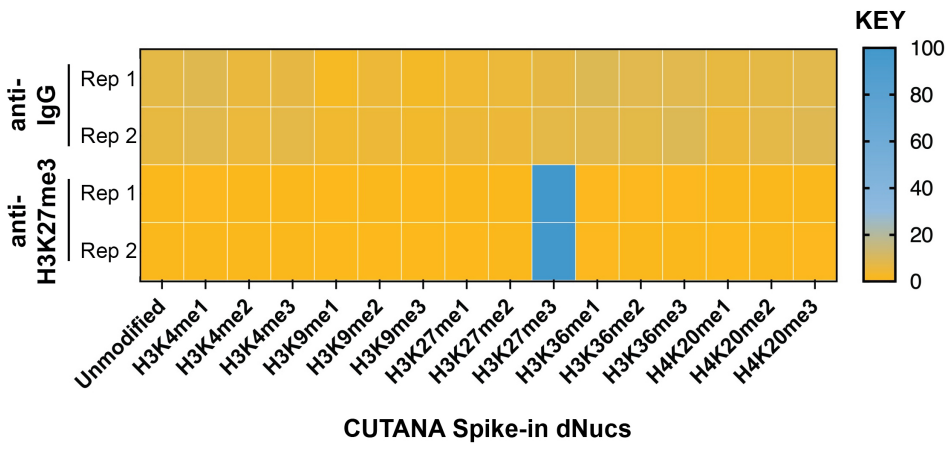

FIGURE 2 SNAP-CUTANA™ K-MetStat Spike-in controls. DNA-barcoded designer nucleosomes (dNucs) representing 16 different K-methyl PTM states: mono-, di-, and tri-methylation at H3K4, H3K9, H3K27, H3K36, and H4K20, as well as unmodified control, were spiked into CUT&Tag samples prior to the addition of the control antibodies provided with the kit (IgG, H3K27me3). After sequencing, instances of each spike-in barcode recovered in the CUT&Tag reactions were counted and normalized from raw fastq files using the shell script and analysis excel sheet available on the spike-in product page (epicypher.com/19-1002). Barcodes for IgG (top; normalized to the sum of total reads) and H3K27me3 (bottom; normalized to on-target) antibodies provided with this kit are shown. The spike-ins confirmed optimal experimental conditions (H3K27me3 antibody specifically recovered the target dNuc, while IgG showed no preferential enrichment).

VALIDATION DATA

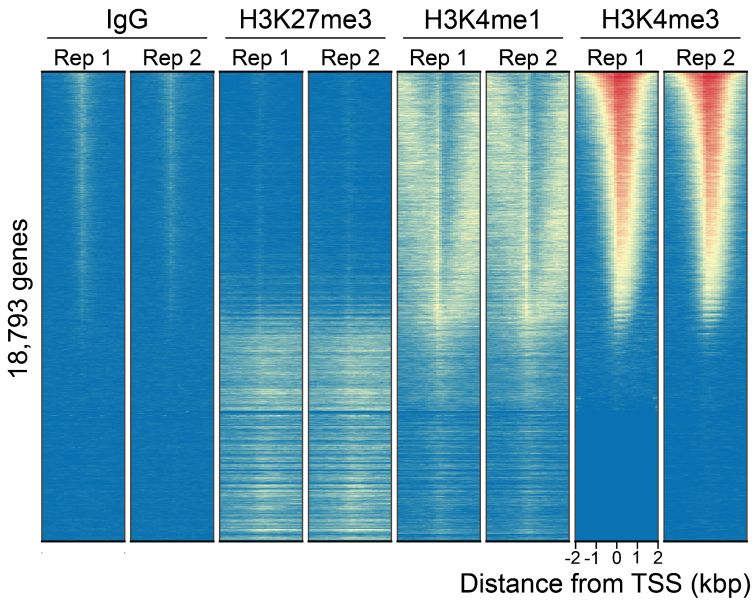

FIGURE 3 CUT&Tag genome-wide heatmaps. CUT&Tag was performed as described above. Heatmaps show two replicates ("Rep") of IgG, H3K27me3, H3K4me1, and H3K4me3 antibodies in aligned rows ranked by intensity (top to bottom) relative to the H3K4me3 Rep 1 reaction. High, medium, and low intensity are shown in red, yellow, and blue, respectively. Antibodies to histone PTMs showed expected enrichment patterns and high reproducibility. H3K4me3, a marker of active transcription localized to TSSs shows oppositional enrichment to H3K27me3 (a marker of repressive chromatin), while H3K4me1 signal flanks TSSs. IgG shows low background enrichment.

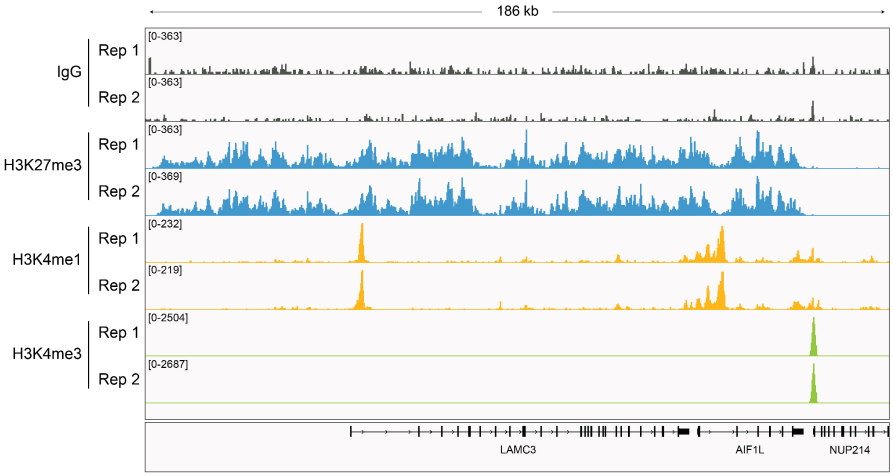

FIGURE 4 Representative gene browser tracks. CUT&Tag was performed as described above. A representative 186 kb window at the LAMC3 gene is shown for two replicates ("Rep") of IgG and H3K27me3 kit control antibodies. Representative tracks are also shown for two replicates of H3K4me1 and H3K4me3 antibodies. The CUT&Tag kit produced the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).