CUTANA™ CUT&Tag Kit with Primer Set 2

Catalog No	14-1102-48s2	**Pack Size**	48 Reactions
Lot No	25175003-01	**Kit Version**	v4

DESCRIPTION

The CUTANA™ CUT&Tag Kit offers a comprehensive solution for ultra-sensitive mapping of histone post-translational modifications (PTMs). In CUT&Tag, antibody-bound chromatin is selectively cleaved with fusion protein pAG-Tn5, which simultaneously adds sequencing adapters. Tagmented fragments bypass traditional library prep with CUTANA™ CUT&Tag Kit's exclusive Direct-to-PCR strategy, enabling users to go from cells to PCR-amplified sequencing libraries in one tube with minimal sample loss. 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).

The CUT&Tag Kit Version 4 (v4) now includes two new Wash Buffer Enhancers, CUTANA™ DNA Purification Beads, and improvements to the CUT&Tag protocol. CUTANA™ DNA Purification Beads are optimized for high yield DNA clean up with precise size selection, while the CUTANA™ Wash Buffer Enhancers reduce clumping and improve bead handling. Protocol improvements amplify sample preservation with changes to nuclei resuspension and refine reaction handling to increase yields for difficult targets. The protocol is also designed for compatibility with multi-channel pipetting for increased throughput and reproducibility. Positive (H3K4me3 and 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. CUTANA™ CUT&Tag Kits are bench-tested, scientist-approved providing users with quality reagents for precision mapping.

KIT CONTENTS

Item	Cat No	Item	Cat No
8-strip Tubes	10-0009t	Pre-Wash Buffer	21-1002t
0.5 M EDTA	21-1006t	5% Digitonin	21-1004t
5 M NaCl	21-1013t	1 M Spermidine	21-1005t
1 M MgCl2	21-1015t	CUTANA™ Wash Buffer Enhancer 1	21-1028t
SNAP-CUTANA™ K-MetStat Panel	19-1002t	CUTANA™ Wash Buffer Enhancer 2	15-1030t
SDS Release Buffer	21-1017t	Rabbit IgG Negative Control Antibody	13-0042t
SDS Quench Buffer	21-1018t	H3K27me3 Positive Control Antibody	13-0055t
CUTANA™ DNA Purification Beads	21-1407t	H3K4me3 Positive Control Antibody	13-0060t
0.1X TE Buffer	21-1025t	Anti-Rabbit Secondary Antibody	13-0047t
ConA Beads	21-1401t	pAG-Tn5	15-1017
Bead Activation Buffer	21-1001t	Non-Hot Start 2X PCR Master Mix	15-1018t
Pre-Nuclei Extraction Buffer	21-1021t		
Multiplexing Primers	This kit includes combinatorial dual indices for multiplexed sequencing of up to 48 reactions. Pair with EpiCypher 14-1102-48s1 or 14-1102-24s3 for multiplexing up to 96 or 72 reactions respectively.		

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 12 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.

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), H3K4me3 (EpiCypher 13-0060t), or H3K27me3 (EpiCypher 13-0055t) antibodies. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 19.7/16.8 million reads (IgG Rep 1/Rep 2), 19.2/17.4 million reads (H3K4me3 Rep 1/Rep 2), and 19.6/21.9 million reads (H3K27me3 Rep 1/Rep 2). Data were aligned to the hg38 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

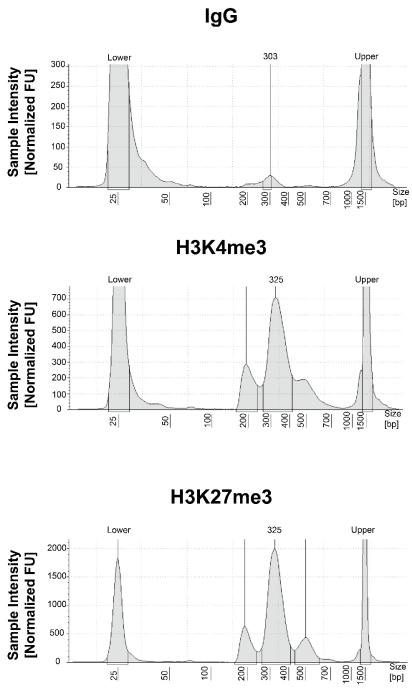

FIGURE 1 CUT&Tag DNA fragment size distribution analysis. CUT&Tag was performed as described above. Library DNA was analyzed by Agilent TapeStation®, which confirmed that mononucleosomes were predominantly enriched in CUT&Tag (peak between 300-400 bp). Peak between 500-700 bp represents dinucleosomes.

VALIDATION DATA

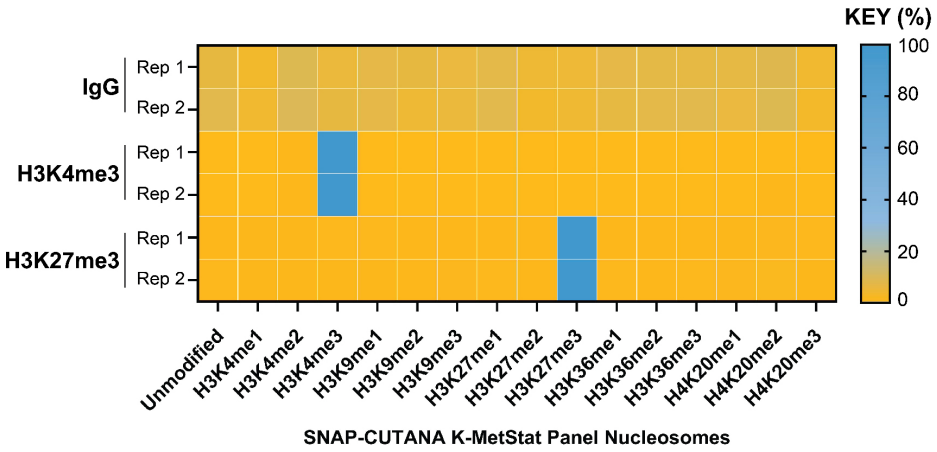

FIGURE 2 SNAP-CUTANA™ Spike-in controls. DNA-barcoded designer nucleosomes (dNucs) harboring distinct K-methyl PTMs were spiked into CUT&Tag reactions prior to addition of control antibodies provided in the kit (IgG, H3K4me3, H3K27me3). Spike-in barcodes were analyzed using the shell script at epicypher.com/19-1002. Barcodes for IgG (top; normalized to total reads), H3K4me3 (middle; normalized to on-target), and H3K27me3 (bottom; normalized to on-target) antibodies are shown. The spike-ins confirmed H3K4me3 and H3K27me3 antibodies specifically recovered the target dNucs, while IgG showed no preferential enrichment.

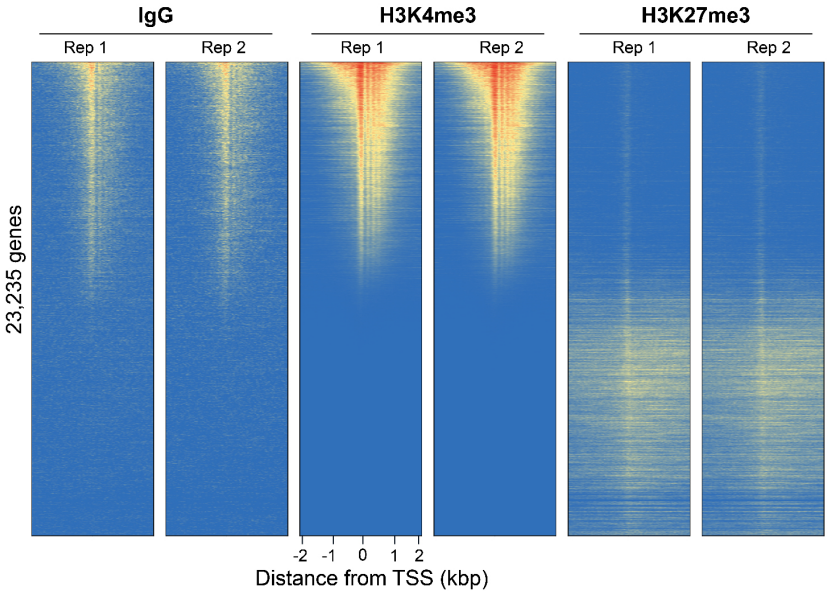

FIGURE 3 CUT&Tag genome-wide heatmaps. CUT&Tag was performed as described above. Heatmaps show two replicates ("Rep") of IgG, H3K4me3, and H3K27me3 antibodies in aligned rows ranked by intensity (top to bottom) relative to the H3K4me3 Rep 1 reaction. High, medium, and low intensity are shown in red,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

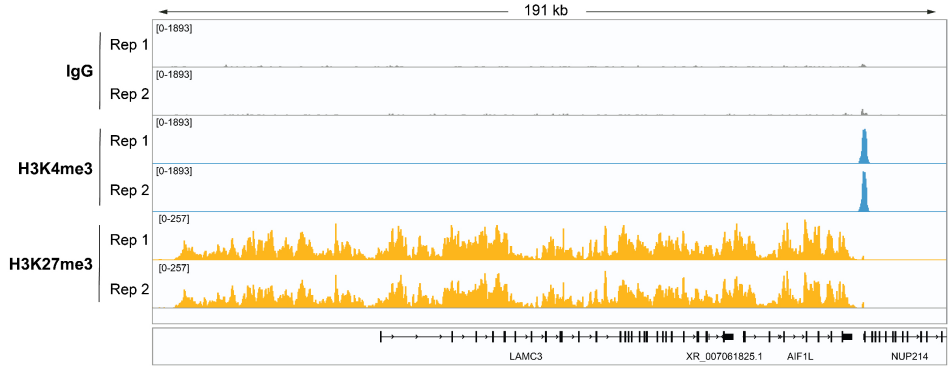

FIGURE 4 Representative gene browser tracks. CUT&Tag was performed as described above. A representative 191 kb window at the LAMC3 gene is shown for two replicates ("Rep") of IgG, H3K4me3, and H3K27me3 kit control antibodies. The CUT&Tag kit produced the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).