CUTANA™ Multiomic CUT&RUN Controls Set

Catalog No	14-1802	**Pack Size**	1 Set
Lot No	24223001-81	**Version**	v1

DESCRIPTION

The CUTANA™ Multiomic CUT&RUN Controls Set is a specialized set of controls designed to be paired with the CUTANA™ ChIC/CUT&RUN Kit (EpiCypher 14-1048) to enable direct, simultaneous analysis of DNA methylation and chromatin proteins in a single workflow. Following isolation of chromatin protein-bound DNA in CUT&RUN, DNA fragments are prepared for sequencing with a cytosine conversion strategy such as Enzymatic Methyl-seq (NEB® EM-seq™, preferred) or bisulfite sequencing to provide base-pair resolution of 5-methylcytosine (5mC). This set includes the essential controls for CUT&RUN followed by EM-seq (CUT&RUN-EM). H3K36me3 antibody is a positive control that enriches DNA associated with active gene bodies, which contain high levels of DNA methylation. H3K36me3 antibody complements the antibodies included in the CUT&RUN kit (H3K4me3, which enriches unmethylated promoters, and IgG, which serves as a negative control) to validate the technical success of the experimental workflow. Pre-fragmented methylated pUC19 and unmethylated Lambda DNAs serve as controls in EM-seq to assess conversion efficiency of unmethylated cytosines.

This controls set is a tailored solution to unlock the capacity of CUT&RUN to generate multiomic insights and investigate crosstalk between DNA methylation and chromatin proteins.

SET CONTENTS

Item	Cat No	Qty	Item	Cat No	Qty
H3K36me3 Antibody	13-0058-03	8 rxn	Methylated pUC19 Frag. Ctrl DNA	18-8001-05	20 µL
0.1X TE Buffer	21-1025-05	2 x 2 mL	Unmethylated Lambda Frag. Ctrl DNA	18-8002-05	20 µL

RECOMMENDED ACCESSORY PRODUCTS

Item	Cat No	Item	Cat No
CUTANA™ ChIC/CUT&RUN Kit	14-1048	NEBNext® Enzymatic Methyl-seq v2 Kit	NEB E8015

TECHNICAL INFORMATION

Storage OPEN SET IMMEDIATELY and store components at room temperature, 4°C, and -20°C as indicated. Stable for 6 months upon date of receipt.

Instructions for Use See User Manual corresponding to Controls Set Version 1.

VALIDATION DATA

CUT&RUN-EM Methods CUT&RUN-EM was performed using the CUTANA™ Multiomic CUT&RUN Controls Set and the CUTANA™ ChIC/CUT&RUN Kit (EpiCypher 14-1048) starting with 500k K562 cells and 0.5 µg of IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0060), or H3K36me3 (EpiCypher 13-0058) antibodies. Library preparation was performed with 1 ng of DNA using the NEBNext® Enzymatic Methyl-seq v2 Kit (NEB E8015). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 31.5 million reads (IgG), 21.3 million reads (H3K4me3), and 20.6 million reads (H3K36me3). Data were aligned to the hg38 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions. Validation data are representative where noted. Refer to the product page for 13-0058 for the lot-specific validation approach for H3K36me3 antibody.

VALIDATION DATA

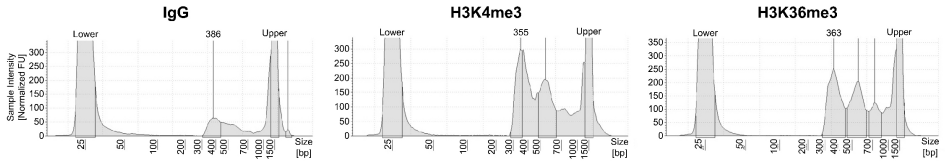

FIGURE 1 Representative CUT&RUN-EM DNA fragment size distribution analysis. CUT&RUN-EM was performed as described above. Library DNA was analyzed by Agilent TapeStation®. This analysis confirmed that mononucleosomes were predominantly enriched in CUT&RUN-EM (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters). High molecular weight nucleosome laddering is expected with EM-seq and does not affect sequencing quality.

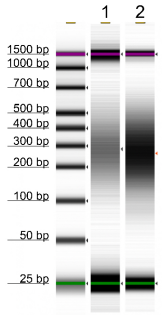

FIGURE 2 Control DNA fragment size. TapeStation confirms fragmentation of pUC19 (Lane 1, 0.2 ng) and Lambda (Lane 2, 4 ng) control DNAs to target size of 200-400 bp.

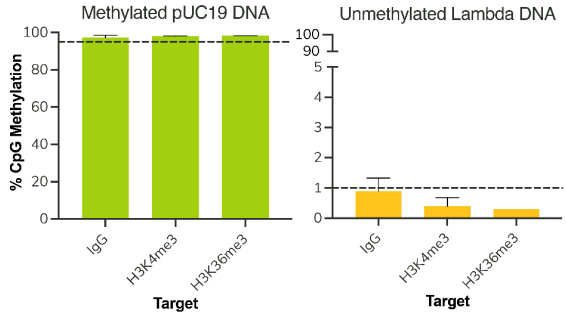

FIGURE 3 Representative EM-seq conversion efficiency. CUT&RUN-EM was performed as described above. Across multiple CUT&RUN targets, methylated pUC19 control DNA shows >95% methylated CpGs, as expected. Unmethylated Lambda control DNA shows <1% DNA methylation, indicating >99% EM-seq conversion efficiency.

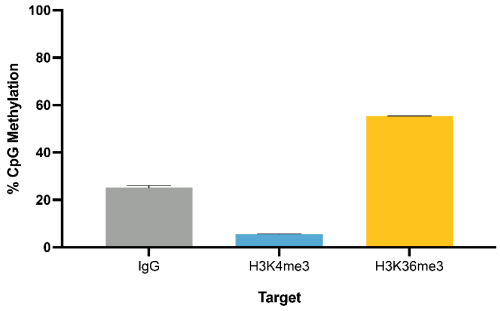

FIGURE 4 Representative percent methylation by target. CUT&RUN-EM was performed as described above. Global levels of DNA methylation associated with each target (IgG, H3K4me3, and H3K36me3) are shown. The H3K36me3 positive control is highly associated with DNA methylation at active gene bodies, while H3K4me3 has very low levels of DNA methylation at active promoters.

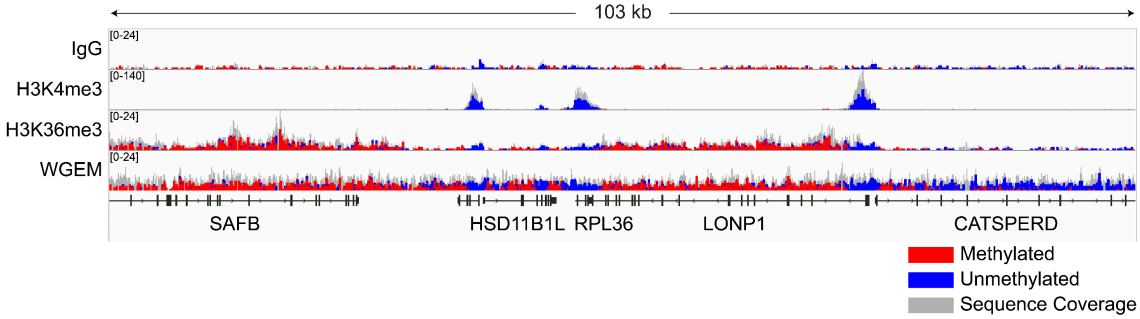

FIGURE 5 Representative gene browser tracks. CUT&RUN-EM was performed as described above. A 103 kb window is shown for IgG, H3K4me3, and H3K36me3. CUT&RUN-EM produced the expected genomic distribution for each chromatin protein, whereby H3K36me3 peaks are highly enriched for methylated DNA (red) in active gene bodies, and H3K4me3 peaks are typically unmethylated DNA (blue) at active promoters. A whole genome EM-seq (WGEM) track showing genome-wide DNA methylation is shown for reference. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).