EpiCypher®

CUTANA™ Stop Buffer

Catalog No	21-1003	**Pack Size**	48 Reactions
Lot No	24164001-81	**Applications**	CUT&RUN

DESCRIPTION

CUTANA™ Stop Buffer is used in CUT&RUN to terminate pAG-MNase activity and prevent over-digestion of released DNA fragments. The Stop Buffer is added following pAG-MNase incubation, and halts enzyme activity by chelating free calcium ions.

TECHNICAL INFORMATION

Storage Stable for 12 months at 4°C from date of receipt.

Formulation 340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 50 µg/mL RNase A, 50 µg/mL glycogen.

Instructions for Use CUTANA™ Stop Buffer includes sufficient buffer for 48 reactions using the CUTANA™ CUT&RUN protocol (www.epicypher.com/protocols).

RECOMMENDED COMPANION PRODUCTS

Item	CAT
CUTANA™ CUT&RUN Kit	14-1048 / 14-1048-24
CUTANA™ CUT&RUN Library Prep Kit	14-1001 / 14-1002
CUTANA™ pAG-MNase	15-1016 / 15-1116
CUTANA™ ConA Beads	21-1401 / 21-1411
CUTANA™ Nuclei Extraction Buffer	21-1026
CUTANA™ Bead Activation Buffer	21-1001
CUTANA™ 5% Digitonin	21-1004
CUTANA™ *E. coli* Spike-in DNA	18-1401
Magnetic Separation Rack (0.2 mL / 1.5 mL tubes)	10-0008 / 10-0012
8-strip 0.2 mL Tubes	10-0009

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 IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0060), 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 5.5/18.8 million reads (IgG Rep 1/Rep 2), 14.2/17.0 million reads (H3K4me3 Rep 1/Rep 2), 24.7/18.1 million reads (H3K27me3 Rep 1/Rep 2), and 8.6/5.5 million reads (CTCF Rep 1/Rep 2). Data were aligned to the T2T-CHM13v2.0 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

VALIDATION DATA

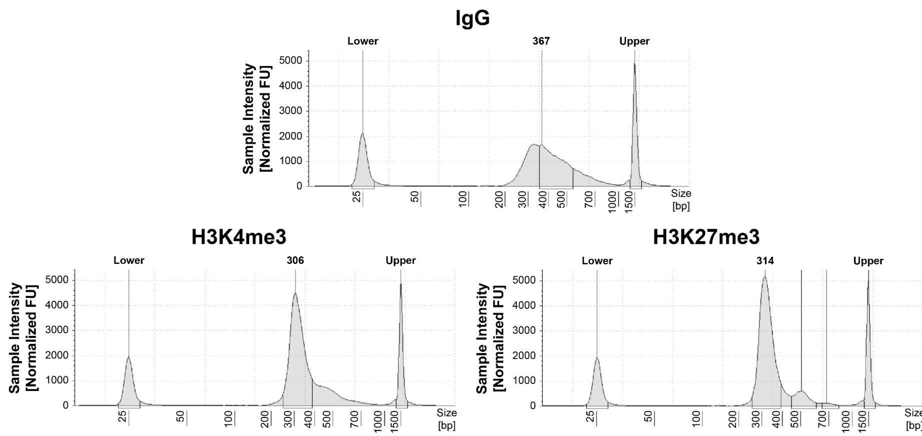

FIGURE 1 CUT&RUN DNA fragment size distribution analysis. CUT&RUN was performed as described above. Library DNA was analyzed by Agilent TapeStation®. This analysis confirmed that mononucleosomes were predominantly enriched in CUT&RUN (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters).

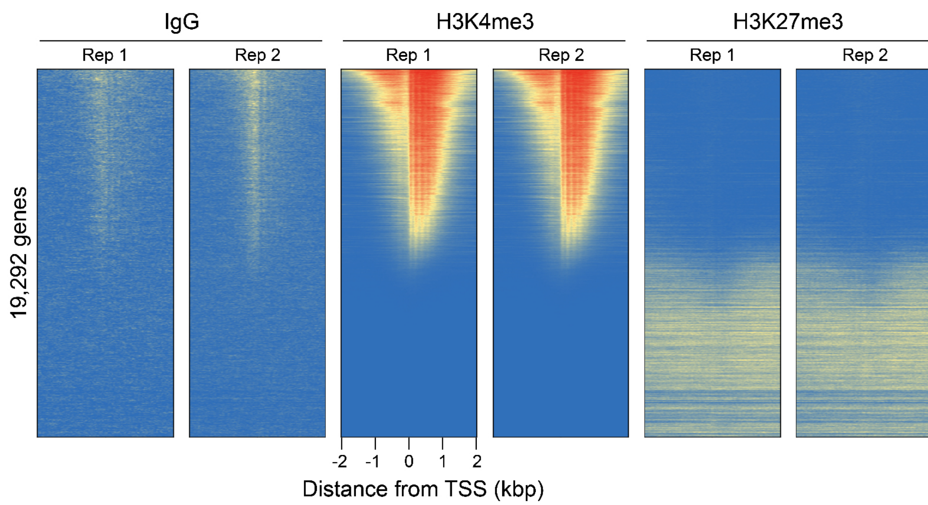

FIGURE 2 CUT&RUN genome-wide heatmaps. CUT&RUN was performed as described above. Heatmaps show two replicates ("Rep") of IgG, H3K4me3, and H3K27me3 control antibodies in aligned rows ranked by intensity (top to bottom) relative to the H3K4me3 Rep 1 reaction and colored such that red indicates high localized enrichment and blue denotes background signal.

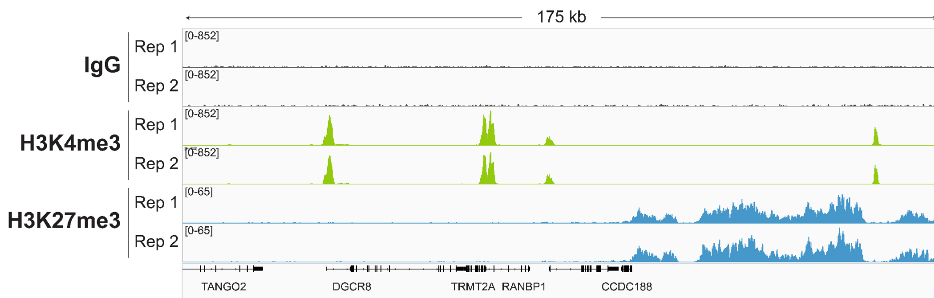

FIGURE 3 Representative gene browser tracks. CUT&RUN was performed as described above. A representative 175 kb window at the TRMT2A gene is shown for two replicates ("Rep") of IgG, H3K4me3, and H3K27me3 control antibodies. Tracks show the expected genomic distribution for each target. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).