SNAP-CUTANA™ K-MetStat Panel

Catalog No	19-1002	**Pack Size**	50 Reactions
Lot No	25038004-01	**Concentration**	0.6 nM per nucleosome 9.6 nM total nucleosome

DESCRIPTION

The SNAP-CUTANA™ K-MetStat Panel of spike-in controls for CUT&RUN and CUT&Tag offers an all-in-one solution to determine antibody specificity for histone post-translational modifications (PTMs), monitor assay success, and normalize data for quantitative chromatin mapping. The panel contains designer nucleosomes (dNucs) representing 15 different K-methyl PTM states: mono-, di-, and trimethylation at H3K4, H3K9, H3K27, H3K36, & H4K20, as well as an unmodified control (**Figure 1**). Each PTM is represented by two unique DNA-barcoded templates (A and B, for an internal technical replicate). Each dNuc is individually conjugated to paramagnetic beads and pooled into a single panel for convenient one-step spike-in to CUT&RUN and CUT&Tag experiments. The panel is added to ConA-immobilized cells prior to the addition of an antibody targeting a histone lysine methylation state or IgG negative control (see **Application Notes** and **Table 1**). pAG-MNase-mediated release or pAG-Tn5-mediated tagmentation of genomic chromatin and the barcoded dNucs is dependent on the specificity of the antibody used. After sequencing, the relative read count of each spike-in nucleosome barcode provides a quantitative metric of on- vs. off-target recovery (**Figures 4 and 5**) as well as quantitative sample normalization, thereby gauging experimental success, guiding troubleshooting efforts, and enabling reliable cross-sample comparisons.

TECHNICAL INFORMATION

Storage	Store at -20°C. **Lower temperatures can cause freezing and will permanently damage the magnetic beads.** Stable for six months from date of receipt. Pipette to resuspend beads; **DO NOT VORTEX.**
Formulation	A mixture of 16 PTM-defined semi-synthetic nucleosomes conjugated to paramagnetic beads in 10 mM sodium cacodylate pH 7.5, 100 mM NaCl, 1 mM EDTA, 50% glycerol (w/v), 1x Protease Inhibitor Cocktail, 100 µg/mL BSA, 10 mM β-mercaptoethanol.

APPLICATION NOTES

Product Use: Use the SNAP-CUTANA™ K-MetStat Panel for control reactions containing positive (e.g., H3K4me3) and negative (IgG) antibodies, as well as samples with an antibody to any of the 15 lysine methyl states in the K-MetStat Panel. Just before antibody addition, pipette to resuspend beads (do not vortex), then spike in 2 µL per 500k cells for CUT&RUN and 2 µL per 100k cells for CUT&Tag. If using less than the standard number of cells, decrease the amount of SNAP-CUTANA spike-in linearly by preparing a "working stock" dilution of the panel in the appropriate buffer, made fresh on the day of use (**Table 1**). Adjust spike-in volume as needed aiming for the spike-in barcodes to comprise ~1% of the total unique sequencing reads. Expect higher for low abundance targets / negative controls (H3K4me3; ~1-10% / IgG; ~10-20%) and lower for high abundance targets (H3K27me3; 0.1-1%). Table 1 gives recommended dilution amounts for varying numbers of starting cells, but optimization may be required for user-specific conditions.

REFERENCES

[1] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715

APPLICATION NOTES (continued)

Starting # Cells (CUT&RUN)	Starting # Cells (CUT&Tag)	Working Stock Dilution*†	Volume Added to Sample	Final Dilution in Sample
500,000	100,000	Stock	2 µL	1:25
250,000	50,000	1:2	2 µL	1:50
100,000	20,000	1:5	2 µL	1:125
50,000 or less‡	10,000 or less‡	1:10	2 µL	1:250

* Create a working stock dilution of the SNAP-CUTANA Panel in Antibody Buffer for CUT&RUN or Antibody150 Buffer for CUT&Tag.
† Prepare working stock dilution FRESH on the day of the experiment and discard after use.
‡ Lower cell numbers and dilutions are possible, but experimental variation may be likely and require independent optimization.

Table 1: Recommended SNAP-CUTANA Spike-in dilution for CUT&RUN / CUT&Tag reactions of varying starting cell number.

Data Analysis: Perform paired-end sequencing for a minimum of 50 bases. The Widom 601 DNA [1] and DNA barcodes are distinct from human, mouse, fly, and yeast genomes such that they can be readily distinguished from sample chromatin. A shell script (.sh file extension) for spike-in alignment and an excel template for heatmap generation are available at epicypher.com/19-1002. The shell script can be opened with any basic text editor program and contains detailed instructions hashed (#) at the beginning of the document. Make sure to copy and paste the R1 & R2 echo loop so there is a set for each sample being analyzed.

VALIDATION DATA

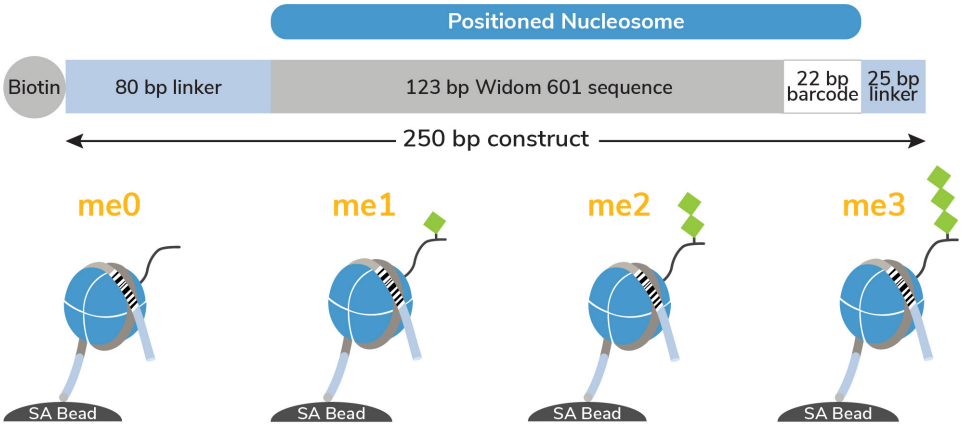

FIGURE 1 Schematic of SNAP-CUTANA™ Spike-in controls. The K-MetStat Panel controls for 15 widely studied methyl states (see **Description**) and an unmodified control (me0), with each of the 16 octamers wrapped with two uniquely barcoded DNA templates (A and B). Each 250 bp DNA template contains a 123 bp 601 nucleosome positioning sequence (gray) [1], a unique 22 bp DNA-barcode (white; 32 barcodes total), and a 5' biotin-TEG. The 5' and 3' linkers (blue) are compatible with cleavage by pAG-MNase (EpiCypher 14-1048, 15-1016) during CUT&RUN as well as tagmentation by pAG-Tn5 (EpiCypher 15-1017) during CUT&Tag. The dNucs are individually pre-conjugated to paramagnetic beads and pooled for convenient use.

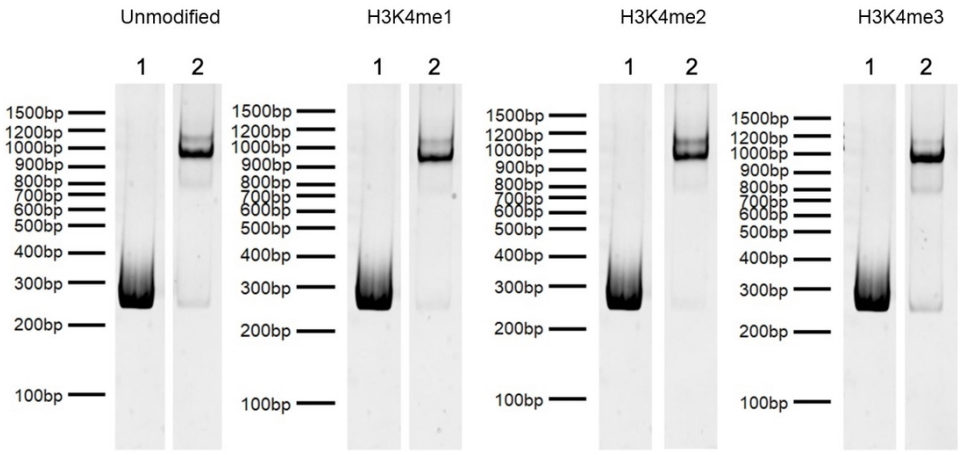

FIGURE 2 DNA gel representative data (not lot-specific). Representative images for SNAP-CUTANA K-MetStat nucleosomes resolved by native PAGE and stained with ethidium bromide to confirm intact nucleosome assembly with minimal free DNA. **Lane 1:** Free 250 bp DNA used in nucleosome assembly (100 ng). **Lane 2:** Intact nucleosomes (400 ng). Comparable experiments were performed for the entire SNAP-CUTANA K-MetStat Panel.

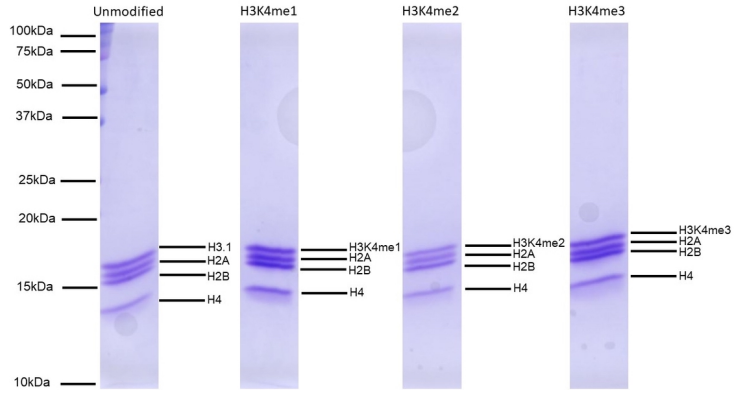

FIGURE 3 Protein gel representative data (not lot-specific). Representative Coomassie stained PAGE gels of SNAP-CUTANA K-MetStat dNucs (1 µg each) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3, and H4) are indicated. Comparable experiments were performed for the entire SNAP-CUTANA K-MetStat Panel.

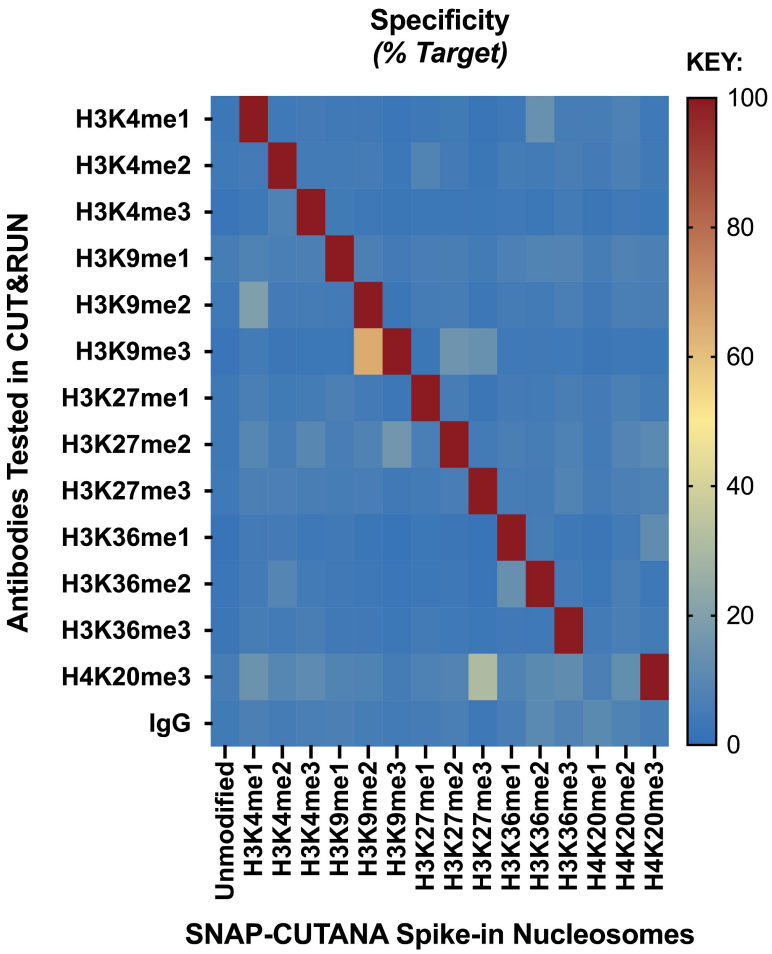

FIGURE 4 Panel identity profiling by CUT&RUN. CUT&RUN was performed on 500k K562 cells utilizing best-in-class identified antibodies to each PTM represented in the SNAP-CUTANA K-MetStat Panel. The CUTANA™ ChIC/CUT&RUN Kit v5 (EpiCypher 14-1048) was used. Prior to antibody addition, 2 µL of the control panel was spiked into each sample. 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). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions. CUT&RUN sequencing reads were aligned to the unique DNA barcodes in the panel and normalized to either on-target (anti-K-methyl PTM) or total counts (IgG). Antibody enrichment of the expected spike-in nucleosome target (red) confirmed the identity and integrity of each member of the panel. CUT&RUN with IgG control antibody showed no preferential enrichment for any particular nucleosome (blue), as expected. No CUT&RUN validated antibodies are available for H4K20me1 or H4K20me2. ***See note below**

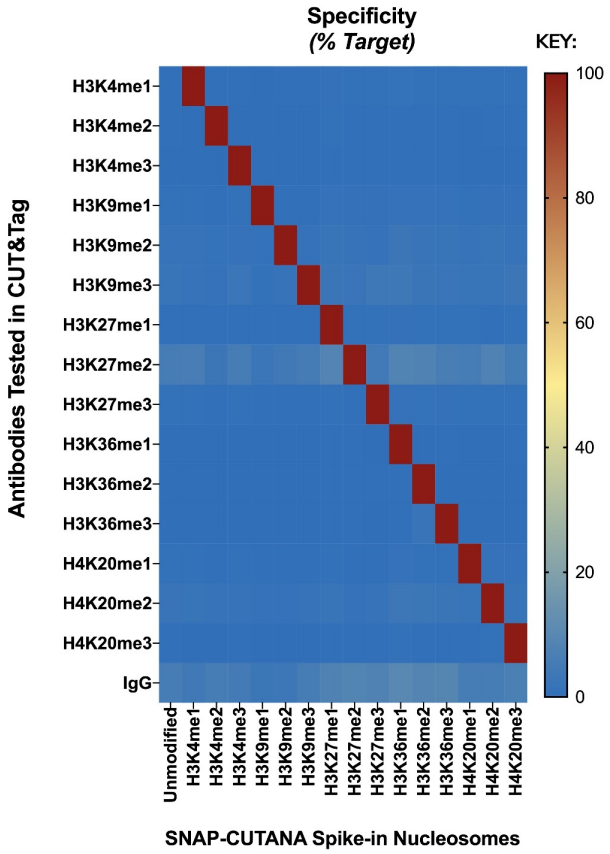

FIGURE 5 Panel identity profiling by CUT&Tag representative data (not lot-specific). CUT&Tag was performed on 100k fresh K562 cells utilizing best-in-class identified antibodies to each PTM represented in the SNAP-CUTANA K-MetStat Panel. Prior to antibody addition, 2 µL of the control panel was spiked into each sample. Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions. CUT&Tag sequencing reads were aligned to the unique DNA barcodes in the panel and normalized to either on-target (anti-K-methyl PTM) or total counts (IgG). Antibody enrichment of the expected spike-in nucleosome target (red) confirmed the identity and integrity of each member of the panel. CUT&Tag with IgG control antibody showed no preferential enrichment for any particular nucleosome (blue), as expected. ***See note below**

Note: While most antibodies used in these experiments show good target specificity in our **standard** CUT&RUN / CUT&Tag conditions, changes to antibody lots and experimental variables (cell number, cell type, experimental treatment, salt concentration, etc.) can have adverse effects on antibody specificity. **Single-point antibody validation under optimal conditions is not a suitable substitute for controlled experiments with spike-ins.** EpiCypher recommends using SNAP spike-in controls to monitor experimental success and accuracy in every possible reaction.