

## SNAP-CUTANA™ DYKDDDDK Tag Panel

<b>Catalog No</b>	19-5001	<b>Pack Size</b>	50 Reactions
<b>Lot No</b>	24026001-81	<b>Concentration</b>	0.6 nM per nucleosome 1.2 nM total nucleosome

### DESCRIPTION

The SNAP-CUTANA™ DYKDDDDK Tag Panel of spike-in controls for CUT&RUN offers an in-assay control to validate anti-FLAG®\* antibodies and confirm the success of CUT&RUN reactions involving FLAG epitope-tagged chromatin proteins. This essential positive control guides troubleshooting to differentiate problems with FLAG epitope-tagging (including transgene expression, chromatin binding of the tagged protein, solvent accessibility of the tag, etc.) from technical failures in the CUT&RUN workflow. The panel consists of two nucleosomes containing unmodified histone H3 or 3xDYKDDDDK-H3 fusion, each wrapped with two uniquely barcoded DNA templates (A and B, for an internal technical replicate). The nucleosomes are individually conjugated to paramagnetic beads and pooled into a single panel for convenient one-step spike-in to CUT&RUN reactions. The panel is added alongside ConA-immobilized cells just prior to the addition of anti-FLAG or IgG negative control antibodies (see **Application Notes** and **Table 1**). The release of genomic chromatin and the barcoded nucleosomes by pAG-MNase is dependent on the specificity of the antibody used. After sequencing, the relative read count of recovered DYKDDDDK vs. unmodified nucleosomes provides a quantitative metric of on- vs. off-target recovery (**Figure 2**), thereby gauging experimental success and guiding troubleshooting efforts.

### TECHNICAL INFORMATION

<b>Storage</b>	Store at -20°C. <b>Lower temperatures can cause freezing and will permanently damage the magnetic beads.</b> Stable for six months from date of receipt.
<b>Formulation</b>	To resuspend beads, gently mix to an even suspension by pipetting; <b>DO NOT VORTEX.</b> A mixture of two 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

Number of cells	Panel dilution	Volume per reaction
500,000	Use stock	2 µL
250,000	1:2	2 µL
100,000	1:5	2 µL
50,000 or fewer	1:10	2 µL

**Table 1:** Recommended SNAP-CUTANA™ DYKDDDDK Tag Panel Spike-in dilution for CUT&RUN reactions of varying starting cell number. **Product Use:** See the most recent CUTANA™ CUT&RUN protocol and SNAP-CUTANA™ Spike-in User Guide ([epicypher.com/protocols](http://epicypher.com/protocols)) for detailed information on workflow integration, expected results, data analysis, and troubleshooting. In brief, use the SNAP-CUTANA™ DYKDDDDK Tag Panel for reactions containing FLAG and IgG antibodies. Just before antibody addition in CUT&RUN, gently pipette to resuspend beads (do not vortex), then spike in 2 µL per 500k cells. 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 Antibody Buffer, made fresh 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. Table 1 gives recommended dilution amounts for varying numbers of starting cells, but optimization may be required for user-specific conditions.

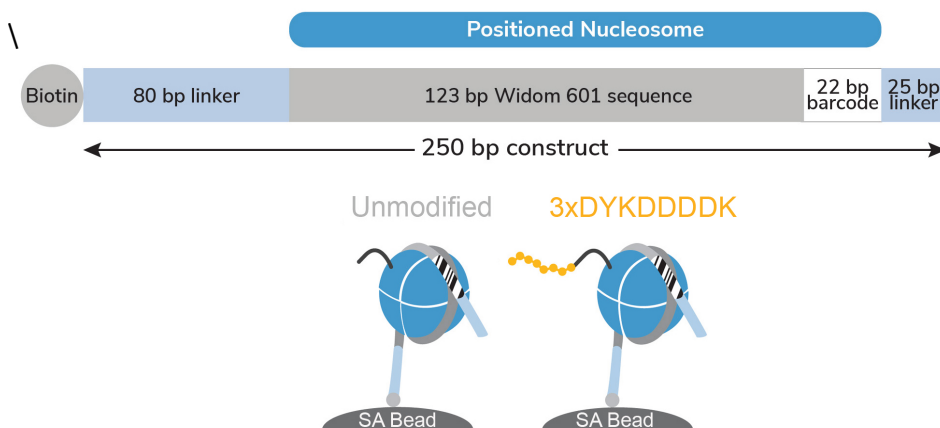
## APPLICATION NOTES (continued)

**Data Analysis:** Detailed instructions are in the SNAP-CUTANA™ Spike-in User Guide ([epicypher.com/protocols](http://epicypher.com/protocols)). Perform paired-end sequencing for a minimum of 50 bases. The Widom 601 DNA 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/19-5001](http://epicypher.com/19-5001). 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 reaction being analyzed.

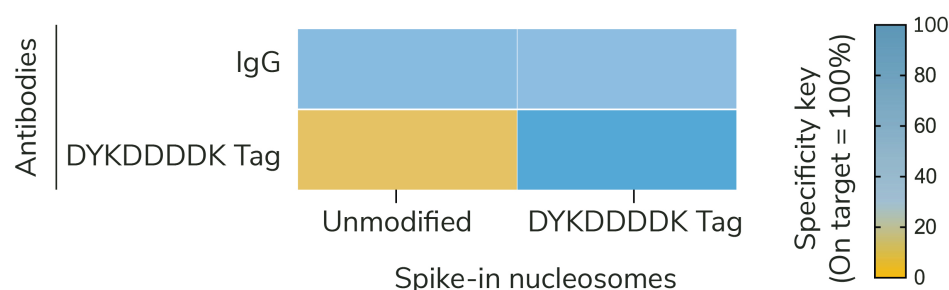
## VALIDATION DATA

### CUT&RUN Methods

CUT&RUN was performed on 500k MDA-MB-231 native cells stably expressing 3xFLAG-tagged GATA3 [1]\*\* using the CUTANA™ ChIC/CUT&RUN Kit v3 (EpiCypher 14-1048). SNAP-CUTANA™ DYKDDDDK Tag Panel was added just prior to the addition of either FLAG Tag (0.05 µg; EpiCypher 13-2031) or IgG negative control (0.5 µg; EpiCypher 13-0042) antibodies. 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.

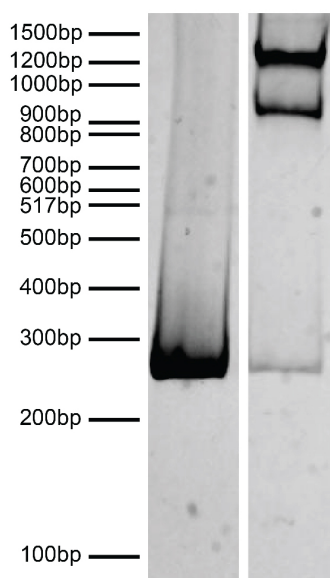


**FIGURE 1 Schematic of SNAP-CUTANA™ DYKDDDDK Tag Panel.** The DYKDDDDK Tag Panel contains two nucleosomes - one has an H3 tail fusion to a 3xDYKDDDDK Tag epitope and one is an unmodified control. Both octamers are wrapped with two uniquely barcoded DNA templates (A and B). Each 250 bp DNA template contains a 123 bp 601 nucleosome positioning sequence (gray) [2], a unique 22 bp DNA-barcode (white; 4 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. The nucleosomes are individually pre-conjugated to paramagnetic beads and pooled for convenient use.

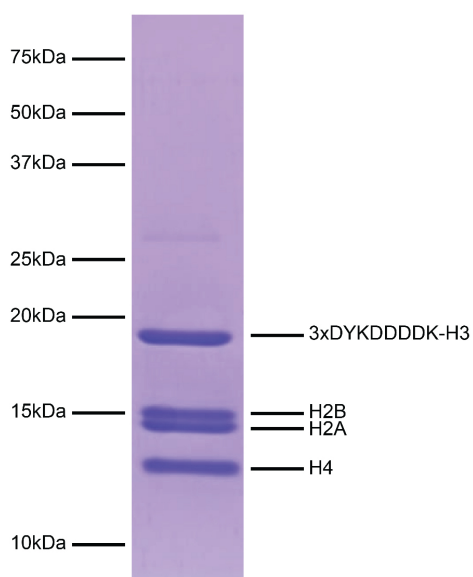


**FIGURE 2 SNAP-CUTANA™ DYKDDDDK Tag Panel provides an in-assay control for CUT&RUN reactions targeting FLAG-tagged proteins.** CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the SNAP-CUTANA™ DYKDDDDK Tag Panel. Data are expressed as a percent relative to on-target recovery (DYKDDDDK Tag set to 100%) or total counts (IgG). IgG antibody results demonstrate equal loading of unmodified and epitope nucleosomes in the panel. DYKDDDDK Tag antibody results show selective enrichment of the DYKDDDDK Tag spike-in nucleosomes, validating all CUT&RUN steps, including DYKDDDDK antibody binding, pAG-MNase cleavage, and wash conditions.

## VALIDATION DATA



**FIGURE 3 DNA gel data.** Nucleosomes in the SNAP-CUTANA DYKDDDDK Tag Panel were resolved via native PAGE and stained with ethidium bromide to confirm intact nucleosome assembly. **Lane 1:** Free 250 bp DNA used in nucleosome assembly (100 ng). **Lane 2:** Intact nucleosomes (400 ng).



**FIGURE 4 Protein gel data.** Coomassie stained SDS-PAGE gel of the nucleosome containing an 3xDYKDDDDK-H3 fusion (1  $\mu$ g) in the SNAP-CUTANA DYKDDDDK Tag Panel demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, 3xDYKDDDDK-H3, and H4) are indicated.

## REFERENCES

[1] Takaku et al. *Genome Biol.* (2016). PMID: 26922637

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

\*FLAG® is a registered trademark of Merck KGaA, Darmstadt, Germany and ANTI-FLAG is a trademark of Sigma-Aldrich Co. LLC.

\*\*Thanks to Dr. Takaku (UND) for 3xFLAG-GATA3-3xHA MDA-MB-231 cells.