CUTANA™ *E. coli* Spike-in DNA

Catalog No	18-1401	**Pack Size**	100 ng
Lot No	25342007-02		

DESCRIPTION

Fragmented DNA derived from *Escherichia coli* (*E. coli*) can be used as a spike-in control for experimental normalization in Cleavage Under Targets and Release Using Nuclease (CUT&RUN). CUT&RUN normalization was originally performed with carryover *E. coli* DNA from MNase preparation [1], however pure preps of enzyme require post hoc DNA spike-in. CUTANA™ *E. coli* Spike-in DNA contains sufficient material for 100-200 CUT&RUN reactions (range for high and low abundance targets).

TECHNICAL INFORMATION

Storage	Store at 4°C. After resuspending, aliquots should be stored at -20°C.
Formulation	100 ng lyophilized DNA. *NOTE: May not be visible

APPLICATION NOTES

Prior to opening, pellet DNA by quickly spinning in a benchtop microfuge. Reconstitute in 200 µL DNase free water (0.5 ng/µL). Vortex tube on all sides to ensure complete resuspension. Quick spin in benchtop microfuge prior to use.

Use in CUT&RUN:
See full protocol: www.epicypher.com/protocols
1. Use with CUTANA™ pAG-MNase for ChIC/CUT&RUN (EpiCypher 15-1016), which has very low *E. coli* DNA carryover.
2. Add 1-2 µL* (0.5-1 ng) *E. coli* Spike-in DNA directly to the Stop Buffer, which quenches calcium-mediated pAG-MNase DNA digestion. Gently vortex to mix, and add to reactions.
***NOTE:** Based on target abundance and antibody efficiency, the amount of E. coli DNA may need to be adjusted. Aim for spike-in DNA to comprise ~1% (0.2 - 5%) of total sequencing reads in on-target reactions (H3K4me3, H3K27me3)* (**Figure 2**).
3. After sequencing, align reads to the reference genome of experimental samples (e.g. human) as well as to *E. coli* genome.
4. Normalize data by following recommendations in the CUT&RUN Protocol: www.epicypher.com/protocols.

REFERENCES

[1] Meers et al. *Elife* (2019). PMID: 31232687

VALIDATION DATA

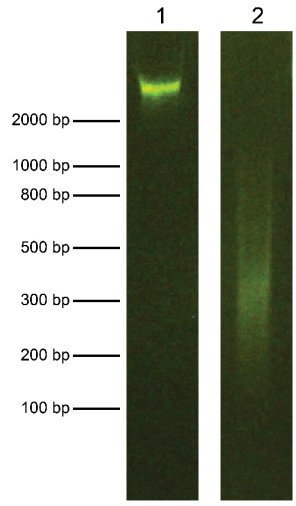

FIGURE 1 **DNA gel data.** *E. coli* fragment size distribution. **Lane 1:** gDNA extracted from JM101 *E. coli* cells (200 ng). **Lane 2:** Digested and purified CUTANA™ *E. coli* Spike-in DNA (200 ng) resolved via 2% E-Gel™ EX Agarose Gel. All lanes resolved on a single gel. Migration positions of DNA molecular weight markers are indicated.

***E. coli* Spike-in DNA**	**Target**	**Total Reads**	***E. coli* Reads**	***%E. coli* Reads**
1 ng	IgG	6,777,094	1,660,423	24.5
	H3K4me3	9,673,386	334,597	3.46
	H3K27me3	9,837,020	32,008	0.33

FIGURE 2 **CUT&RUN sequencing data.** CUTANA™ *E. coli* Spike-in DNA (1.0 ng) was added to CUT&RUN reactions using 500,000 K562 cells enriched for a low abundance target (H3K4me3, EpiCypher 13-0060), a high abundance target (H3K27me3, EpiCypher 13-0055), and an IgG negative control (EpiCypher 13-0042). Total numbers of paired-end sequencing reads, reads aligned to *E. coli*, and percentage of total sequencing reads aligned to *E. coli* spike-in DNA are shown. **NOTE: Spike-in DNA amount should be optimized by the end user with the goal of *E. coli* DNA comprising ~1% (0.2 - 5%) of total sequencing reads in on-target reactions (H3K4me3, H3K27me3).**