CUTANA™ *E. coli* Spike-in DNA

Catalog No	18-1401	**Pack Size**	100 ng
Lot No	23331005-01		

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	Stable for 2 years at 4°C from date of receipt. 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* (**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

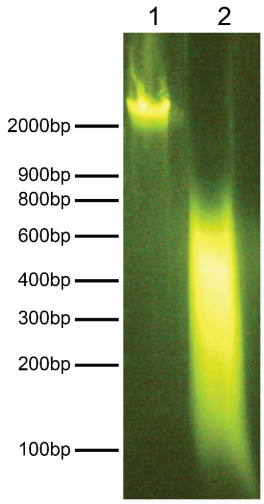

FIGURE 1 DNA gel data. *E. coli* fragment size distribution. **Lane 1:** gDNA extracted from JM101 *E. coli* cells (500 ng). **Lane 2:** Digested and purified CUTANA™ *E. coli* Spike-in DNA (500 ng) resolved via 2% E-Gel™ EX Agarose Gel. Migration positions of DNA molecular weight markers are indicated.

E. coli Spike-in DNA	Target	Total Reads	E. coli Reads	% E. coli Reads
0.1 ng	IgG	7,598,685	415,480	5.47
	H3K4me3	7,825,565	362,192	4.63
	H3K27me3	8,942,144	113,270	1.27

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 13-0041), a high abundance target (H3K27me3, EpiCypher 13-0030) 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.**