CUTANA® *E. coli* Spike-in DNA

Catalog No. 18-1401
Lot No. 20120001-26
Pack Size 100 ng

Product 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). CUTANA *E. coli* Spike-in DNA contains sufficient material for 100-200 CUT&RUN samples (high and low abundance targets, respectively).

Formulation:

100 ng lyophilized DNA. *NOTE: May not be visible.

Storage and Stability:

Stable for 2 years at -20°C from date of receipt. After resuspending, aliquots should be stored at -80°C.

Application Notes:

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

Use in CUT&RUN:

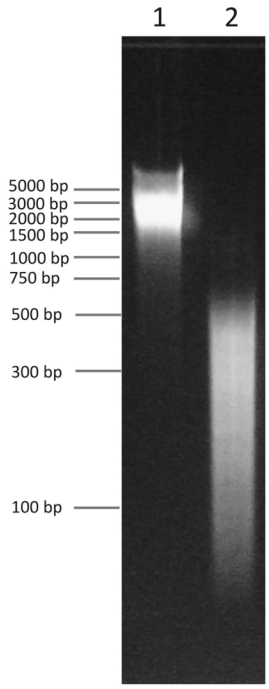
1. Use in combination with CUTANA pAG-MNase for ChIC/CUT&RUN (EpiCypher 15-1016), which has very low levels of *E. coli* DNA.

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 (see protocol: www.epicypher.com/resources/protocols/). Gently vortex to mix, and add to CUT&RUN samples.
**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 (see Table, right).*

3. After sequencing, align reads to the reference genome from the experimental samples (*e.g.* human) as well as to the *E. coli* genome.

4. Normalize data by following recommendations described in the EpiCypher CUTANA CUT&RUN Protocol: www.epicypher.com/resources/protocols/.

References:



DNA Gel Data: Lane 1: gDNA extracted from JM101 *E. coli* cells (500 ng) and 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
0.5 ng	IgG	3,644,233	155,549	4.27%
	H3K4me3	3,121,112	42,210	1.35%
	H3K27me3	5,254,299	8,511	0.16%
1.0 ng	IgG	2,569,291	241,645	9.41%
	H3K4me3	3,127,912	147,565	4.72%
	H3K27me3	9,650,258	22,419	0.23%

CUT&RUN Sequencing Data: CUTANA *E. coli* Spike-in DNA (0.5 and 1.0 ng) was added to CUT&RUN samples using 500,000 K562 cells enriched for a low abundance target (H3K4me3, EpiCypher Catalog No. 13-0041), a high abundance target (H3K27me3, EpiCypher Catalog No. 13-0030) and IgG negative control (EpiCypher Catalog No. 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. Green boxes highlight the recommended *E. coli* DNA spike-in amounts for each target.

This product is for *in vitro* research use only and is not intended for use in humans or animals.