

## ***E. coli* Non-Methylated Genomic DNA**

Standards for DNA methylation analysis workflows

### Highlights

- Negative control for epigenetic experiments requiring DNA containing zero background levels of cytosine and adenosine methylation.
- *In situ* spike-in control for monitoring bisulfite conversion efficiency in Next-Generation Sequencing experiments.
- Substrate for DNA methylation-sensitive restriction enzyme digestions.

Catalog Numbers:  
D5016



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# Product Contents

<i>E. coli</i> Non-methylated Genomic DNA	D5016	Storage Temp.
<i>E. coli</i> Non-methylated Genomic DNA	5 µg/20 µl	-20°C

## Specifications

- **Source**<sup>1</sup> – *Escherichia coli* strain K-12, substrain MG1655 (Dam<sup>-</sup> and Dcm<sup>-</sup>, ER2925).
- **Concentration** – 250 ng/µl in TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8.0).
- **Genotype**<sup>2</sup> – *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)TetS endA1 rpsL136 dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

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<sup>1</sup>Marinus, M.G. & Morris, N.R., J. Bacteriol. 114: 1143-50 (1973)

<sup>2</sup>For definitions of genes and mutations, please visit the *E. coli* Genetic Stock Center (CGSC) at <https://cgsc.biology.yale.edu/>.

# Product Description

The ***E. coli* Non-methylated Genomic DNA** contains DNA purified from a K-12 *E. coli* strain which is Dam<sup>-</sup> and Dcm<sup>-</sup>. It works perfectly as a negative control for DNA methylation analyses requiring DNA with absolutely no methylation. This standard can also be spiked into or run in parallel with experimental samples during next generation sequencing library prep to monitor bisulfite conversion efficiency and/or workflow performance. If used as an *in-situ* spike-in control, the reference genome of *E. coli* strain K-12 sub-strain MG1655 can be used for alignment and analysis. It can be accessed at the following web address:

[https://www.ncbi.nlm.nih.gov/genome/167?genome\\_assembly\\_id=161521](https://www.ncbi.nlm.nih.gov/genome/167?genome_assembly_id=161521)

## Recommended Usage

The ***E. coli* Non-methylated Genomic DNA** can be used as a control for bisulfite conversion and downstream analyses such as bisulfite PCR, methylation sequencing applications, *in situ* spike in-controls, methylation assay calibration, and more.

## Protocol

The ***E. coli* Non-methylated Genomic DNA** is highly intact genomic DNA. For best results, it's important to ensure the DNA is completely homogenous and fully in solution before quantification and usage. The following steps are recommended before quantification and usage<sup>1</sup>:

1. Bring the standard to room temperature.
2. Vortex the standard for 10-15 seconds, briefly spin down for 5-10 seconds.
3. Repeat Step 2 twice, for three times total.
4. Proceed with quantification or usage.

**Bisulfite Conversion:** For most applications, 0.5-2.0 ng of *E. coli* genomic DNA for every 100 ng of experimental DNA sample may be used as an internal spike-in control for bisulfite conversion reactions. Refer to the kit specifications for setup of the bisulfite conversion reaction.

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<sup>1</sup>Standards are quantified using NanoDrop® measurements. If using other methods, variation may be observed.

# Ordering Information

Product Description	Catalog No.	Size
Human Methylated & Non-methylated DNA Set	D5014	5 µg/20 µl
Human HCT116 DKO Non-Methylated DNA	D5014-1	5 µg/20 µl
Human HCT116 DKO Methylated DNA	D5014-2	5 µg/20 µl
Bisulfite-Converted Universal Methylated Human DNA Standard	D5015	1 µg/50 µl
ZymoTaq™ qPCR Premix	E2054 E2055	50 Rxns. 200 Rxns.
ZymoTaq™ Premix	E2003 E2004	50 Rxns 200 Rxns.
EZ DNA Methylation-Lightning™ Kit	D5030 D5031	50 Rxns 200 Rxns.
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 Rxns 200 Rxns.
EZ DNA Methylation™ Kit	D5001 D5002	50 Rxns 200 Rxns.
EZ DNA Methylation-Gold™ Kit	D5005 D5006	50 Rxns 200 Rxns.

## This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.



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Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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*E. coli* strain ER2925 (Dam<sup>-</sup> and Dcm<sup>-</sup>) used with permission from New England Biolabs.

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