Methylated & Non-methylated pUC19 DNA Set

ZYMO RESEARCH
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Cat. Nos. D5017

Storage: -20 °C

Product Information

Product Contents:

	Cat. # D5017	Storage Temp.
Methylated pUC19 DNA	20 ng/20 µl	-20 °C
Non-methylated pUC19 DNA	20 ng/20 µl	-20 °C
pUC19MN Primers	20 µl	-20 °C

Description:

The **Methylated & Non-methylated pUC19 DNA Set** consists of two control DNAs (methylated and non-methylated) along with a set of specifically designed primers that can be used in conjunction with the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, and **EZ DNA Methylation-Direct™** kits from Zymo Research to assess the efficiency of bisulfite-mediated conversion of DNA. These plasmids can be used in conjunction with genomic DNAs to provide internal controls to assess bisulfite conversion efficiency or to produce known mixtures of methylated and non-methylated DNA for assay calibration.

The **Non-Methylated pUC19 DNA** is pUC19 that was isolated from a methylation-negative strain of bacteria (Dam⁻, Dcm⁻) and can be used as a negative control for DNA methylation analysis. The **Methylated pUC19 DNA** is pUC19 that has been isolated from the same strain and has been enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[2] (EC 2.1.1.37; Figure 1) and can be used as a positive control for DNA methylation analysis.

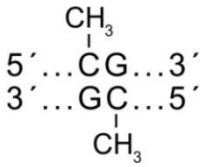

Figure 1. M.SssI methytransferase methylates all cytosine residues in the double-stranded CpG context.

The primer set herein has been designed to amplify a fragment of the supplied pUC19 DNAs following bisulfite treatment. The methylated cytosines, comprising CG dinucleotides in the **Methylated pUC19 DNA** remain unconverted following bisulfite treatment, whereas non-methylated cytosines are converted into uracil and detected as thymine after PCR. The supplied pUC19 DNA has been linearized at position 2177 using ScaI endonuclease.

References:

1. Nur *et al.* J. Bacteriol. 164: 19-24 (1985).

Protocol:

1. Bisulfite Conversion:

For most applications 5-50 pg of plasmid may be used as an internal control for reactions containing 250 ng to 2 µg of genomic DNA. Refer to the kit specifications for setup of the bisulfite conversion reaction.

2. PCR Setup:

Note: We recommend using ZymoTaq™ DNA polymerase or other hot-start DNA polymerases for amplification of bisulfite-treated DNA.

The following setup is designed for a 25 µl total reaction volume:

Component	Volume	Final Conc.
pUC19MN Primer I*	Variable	0.2 to 0.8 µM
pUC19MN Primer II*	Variable	0.2 to 0.8 µM
Bisulfite-converted DNA**	1 µl	up to 0.25 pg/µl
10 mM dNTP mix	0.5 µl	0.2 mM each dNTP
Standard PCR buffer	Variable	1x
$MgCl_2$ or $MgSO_4$	Variable	1-4 mM, if needed
Zymo*Taq*™ DNA Polymerase *(or other Hot-start DNA polymerase)*	Variable	1 to 2 units
Add water to 25 µl		

* Alternatively, you may substitute primers of your choice.
** Remember to bisulfite-treat the DNA prior to performing PCR.

3. Recommended Thermocycler Conditions:

   A. 95 °C, 10 minutes
   B. 95 °C, 30 seconds
   C. 57 °C, 30 to 60 seconds
   D. 72 °C, 60 seconds
   E. Repeat steps B through D an additional 29 to 39 times depending on the polymerase used.
   F. 72 °C, 7 minutes
   G. 4 °C

The PCR amplicon can now be used directly for sequencing analysis or cloning.

Product Specifications:

I. Methylated pUC19 DNA, 20 µl.
Source: pUC19 plasmid purified from Dam⁻, Dcm⁻ E. coli [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
Concentration: 1 ng/µl of Methylated pUC19 DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
Storage: -20 °C

II. Non-methylated pUC19 DNA, 20 µl.
Source: pUC19 plasmid purified from Dam⁻, Dcm⁻ E. coli.
Concentration: 1 ng/µl of Non-methylated pUC19 DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
Storage: -20 °C

III. pUC19MN Primers.
Concentration: 20 µM each primer in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Volume: 20 µl of mixed primers
Storage: -20 °C
Sequence:

pUC19MN Primer I:

5´ - GGTTATAGTTGTTTTTTGTGTGAAATTGTTATT - 3´

pUC19MN Primer II:

5´ - CTAACCTTTTACTCACATATTCTTTCCTAC - 3´

Continued on reverse side...

Appendix:

The expected PCR amplicon for both the methylated and non-methylated DNA is 362 bp, corresponding to nucleotide positions 464 to 825 of the pUC19 sequence, including the regions (italicized) that hybridize to the primers.

Original sequence of pUC19 for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capitol letters) are methylated enzymatically by M.SssI methyltransferase:

```
461  ---GGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT
501  CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT
541  AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA
581  TTGCGTTGCG CTCACTGCCC GCTTTCCAGT CGGGAAACCT
621  GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG
661  AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC
701  TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC
741  GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA
781  GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG
821  GCCAG
```

Expected sequence of above DNA following bisulfite treatment.

Methylated pUC19 DNA: Below is the expected sequence for the Methylated pUC19 DNA after bisulfite conversion and PCR (sense strand). Methylated cytosines in the CpG dinucleotide context remain unconverted following bisulfite treatment, whereas non-methylated cytosines, or cytosines not in the CpG context, are converted to uracils and detected as thymines after PCR..

```
461  ---GGTTATA GTTGTTTTTT GTGTGAAATT GTTATTCGTT
501  TATAATTTTA TATAATATAC GAGTCGGAAG TATAAAGTGT
541  AAAGTTTGGG GTGTTTAATG AGTGAGTTAA TTTATATTAA
581  TTGCGTTGCG TTTATTGTTC GTTTTTTAGT CGGGAAATTT
621  GTCGTGTTAG TTGTATTAAT GAATCGGTTA ACGCGCGGGG
661  AGAGGCGGTT TGCGTATTGG GCGTTTTTTC GTTTTTTCGT
701  TTATTGATTC GTTGCGTTCG GTCGTTCGGT TGCGGCGAGC
741  GGTATTAGTT TATTTAAAGG CGGTAATACG GTTATTTATA
781  GAATTAGGGG ATAACGTAGG AAAGAATATG TGAGTAAAAG
821  GTTAG
```

Non-methylated pUC19 DNA: Below is the expected sequence for the Non-methylated pUC19 DNA after bisulfite conversion and PCR (sense strand). During treatment with sodium bisulfite, non-methylated cytosines are converted into uracils, which are later detected as thymines after PCR.

```
461  ---GGTTATA GTTGTTTTTT GTGTGAAATT GTTATTTGTT
501  TATAATTTTA TATAATATAT GAGTTGGAAG TATAAAGTGT
541  AAAGTTTGGG GTGTTTAATG AGTGAGTTAA TTTATATTAA
581  TTGTGTTGTG TTTATTGTTT GTTTTTTAGT TGGGAAATTT
621  GTTGTGTTAG TTGTATTAAT GAATTGGTTA ATGTGTGTGG
661  AGAGGTGGTT TGTGTATTGG GTGTTTTTTT GTTTTTTTGT
701  TTATTGATTT GTTGTGTTTG GTTGTTTGGT TGTGGTGAGT
741  GGTATTAGTT TATTTAAAGG TGGTAATATG GTTATTTATA
781  GAATTAGGGG ATAATGTAGG AAAGAATATG TGAGTAAAAG
821  GTTAG
```

Also Available:

Product Name	Size	Cat. No.
BISULFITE TREATMENT OF DNA		
EZ DNA Methylation™ Kit	50 rxns. 200 rxns. 2 x 96 rxns. 2 x 96 rxns.	D5001 D5002 D5003 D5004
EZ DNA Methylation-Gold™ Kit	50 rxns. 200 rxns. 2 x 96 rxns. 2 x 96 rxns.	D5005 D5006 D5007 D5008
EZ DNA Methylation-Direct™ Kit	50 rxns. 200 rxns. 2 x 96 rxns. 2 x 96 rxns.	D5020 D5021 D5022 D5023
EZ DNA Methylation-Startup™ Kit	50 rxns.	D5024
EZ Bisulfite DNA Clean-up Kit™	50 preps. 200 preps. 2 x 96 preps. 2 x 96 preps.	D5025 D5026 D5027 D5028
METHYLATED/NON-METHYLATED DNA STANDARDS		
Universal Methylated DNA Standard	1 set	D5010
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human Methylated and Non-methylated DNA Set	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
E. coli Non-methylated Genomic DNA	5 µg	D5016
AMPLIFICATION OF BISULFITE CONVERTED DNA		
Zymo*Taq*™ DNA Polymerase	50 rxns. 200 rxns.	E2001 E2002
Zymo*Taq*™ PreMix (2X concentrated)	50 rxns. 200 rxns.	E2003 E2004
QUANTITATIVE DETECTION OF METHYLATED DNA		
EZ qPCR/HRM MasterMix	100 rxns. 400 rxns.	E2005 E2006
EZ DNA Methylation-Direct™ qPCR/HRM Kit	100 rxns.	D5300
ANTIBODIES & IMMUNOPRECIPITATION		
Methylated-DNA IP Kit	10 preps.	D5101
ChIP DNA Clean & Concentrator™	50 preps. 50 preps.	D5201 D5205
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
METHYLTRANSFERASES		
CpG Methylase (M.SssI)	200 U 400 U	E2010 E2011
GpC Methylase (M.CviPI)	200 U 1000 U	E2014 E2015
DNA FRAGMENTATION		
DNA Degradase™	500 U 2000 U	E2016 E2017
DNA Degradase Plus™	250 U 1000 U	E2020 E2021
DNA Shearase™	50 U 200 U 50 U & DCC™ 200 U & DCC™	E2018-50 E2018-200 E2019-50 E2019-200
NUCLEOSOME MAPPING		
EZ Nucleosomal DNA Prep Kit	20 preps	D5220
5-HYDROXYMETHYLCYTOSINE		
5-Hydroxymethylcytosine DNA	5 µg	D5400
5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	1 set	D5405

Trademarks and Disclaimers:

This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. It is not intended for use in diagnostic procedures. Follow the safety guidelines and rules enacted by your research institution or facility.

The Polymerase Chain Reaction (PCR) process is covered by U.S. Patent: #4,683,195; 4,683,202 assigned to Hoffmann-La Roche. Patents pending in other countries. No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of Zymo Research's products. Further information on purchasing licenses to practice the PCR process can be obtained from the director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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