Universal Methylated DNA Standard & Control Primers

Cat. Nos. D5010

Storage: -20 °C

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

Product Information

Product Contents:

	Cat. # D5010	Storage Temp.
Universal Methylated DNA Standard	100 pg/20 µl*	-20 °C
Methyl Primers	20 µl	-20 °C

*Also contains 5 µg/20 µl salmon sperm DNA as a carrier

Description:

The **Universal Methylated DNA Standard** includes enzymatically methylated DNA together with a specially-designed primer set to be used in conjunction with Zymo Research Corporation's **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, and **EZ DNA Methylation-Direct™** kits to assess the efficiency of bisulfite-mediated conversion of DNA. Central to this, is the pUC19 DNA that was isolated from a methylation-negative strain of bacteria (Dam⁻, Dcm⁻) prior to its enzymatic modification with M.SssI methyltransferase[1] (EC 2.1.1.37). The DNA is methylated at cytosine positions comprising CG dinucleotides (Figure 1).

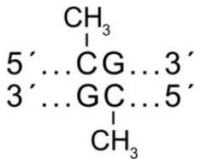

Figure 1. M.SssI methytransferase methylates all cytosine residues in the double-stranded CpG context.

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

References:

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

Protocol:

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

1. PCR Setup:

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

Component	Volume	Final Conc.
hMLH1 primer I*	Variable	0.2 to 0.8 µM
hMLH1 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.

2. Recommended Thermocycler Conditions:

A. 95 °C, 10 minutes
B. 95 °C, 30 seconds
C. 59 °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. Universal Methylated DNA Standard, 20 µl.
Source: DNA purified from pUC19 DNA [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
Concentration: 5 pg/µl of universal methylated pUC19 DNA and 250 ng/µl of salmon sperm DNA as a carrier in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
Storage: -20 °C

II. Control 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:

Methyl Primer I; contains an XhoI site (**bold**) for cloning:

5´ - CT**CTCGAG**AAAATATCGTATTAGGCGTTATTCGTT - 3´

Methyl Primer II; contains a BamHI site (**bold**) for cloning.

5´ - CG**GGATCC**AACCGCCTCTCCCCGCGCGTTAACCG - 3´

Appendix:

The expected PCR amplicon for the Universal Methylated DNA Standard is 466 bp, corresponding to nucleotide positions 221 to 670 of the pUC19 sequence, including the regions (italicized) that hybridize to the primers (an additional 16 bp is added to the primer sequence for cloning purposes).

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:

```
221   aaaatacCGc atcaggCGcc attCGccatt caggctgCGc
261   aactgttggg aagggCGatC GgtgCGggcc tcttCGctat
301   taCGccagct ggCGaaaggg ggatgtgctg caaggCGatt
341   aagttgggta aCGccagggt tttcccagtc aCGaCGttgt
381   aaaaCGaCGg ccagtgaatt CGagctCGgt accCGgggat
421   cctctagagt CGacctgcag gcatgcaagc ttggCGtaat
461   catggtcata 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 ---------- ---------- ----------
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Appendix (continued…):

Expected sequence of above DNA following bisulfite treatment. 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 uracil and detected as thymine after PCR.

```
221   aaaatatCGt attaggCGtt attCGttatt taggttgCGt
261   aattgttggg aagggCGatC GgtgCGggtt ttttCGttat
301   taCGttagtt ggCGaaaggg ggatgtgttg taaggCGatt
341   aagttgggta aCGttagggt ttttttagtt aCGaCGttgt
381   aaaaCGaCGg ttagtgaatt CGagttCGgt attCGgggat
421   tttttagagt CGatttgtag gtatgtaagt ttggCGtaat
461   tatggttata gttgtttttt gtgtgaaatt gttattCGtt
501   tataatttta tataatataC GagtCGgaag tataaagtgt
541   aaagtttggg gtgtttaatg agtgagttaa tttatattaa
581   ttgCGttgCG tttattgttC Gtttttttagt CGggaaattt
621   gtCGtgttag ttgtattaat gaatCGgtta aCGCGCGggg
661   agaggCGgtt ---------- ---------- ----------
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Also Available:

Product Name	Size	Catalog number
EZ DNA Methylation™ Kit	50 200 2 x 96 2 x 96	D5001 D5002 D5003 D5004
EZ DNA Methylation-Gold™ Kit	50 200 2 x 96 2 x 96	D5005 D5006 D5007 D5008
EZ DNA Methylation-Direct™ Kit	50 200 2 x 96 2 x 96	D5020 D5021 D5022 D5023
EZ DNA Methylation-Startup™ Kit	1 Kit	D5024
EZ Bisulfite DNA Clean-up Kit™	50 200 2 x 96 2 x 96	D5025 D5026 D5027 D5028
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human HCT116 DKO Methylation Standards	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
ChIP DNA Clean & Concentrator™	50 50	D5201 D5205
Methylated-DNA IP Kit	10	D5101
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
Zymo*Taq*™ DNA Polymerase	50 200	E2001 E2002
Zymo*Taq*™ PreMix (2X concentrated)	50 200	E2003 E2004
CpG Methylase (M.SssI)	200 units 400 units	E2010 E2011

Trademarks and Disclaimers:

This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. 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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