

Universal Methylated DNA Standard & Control Primers

Cat. Nos. D5010

Storage: -20 °C



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 methyltransferase¹ (EC 2.1.1.37). The DNA is methylated at cytosine positions comprising CG dinucleotides (Figure 1).

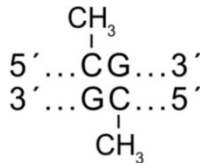


Figure 1. M.SssI methyltransferase methylates all cytosine residues in the double-stranded CpG context.

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 Scal 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
ZymoTaq™ 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:

- 95 °C, 10 minutes
- 95 °C, 30 seconds
- 59 °C, 30 to 60 seconds
- 72 °C, 60 seconds
- Repeat steps B through D an additional 29 to 39 times depending on the polymerase used.
- 72 °C, 7 minutes
- 4 °C

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

Product Specifications:

- 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
- 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' - CTCT**CGAG**AAAAATATCGTATTAGCGGTTATTCGTT - 3'

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

5' - CGGGAT**CCA**ACCGCCTCTCCCGCGCGTTAACCG - 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 capital letters) are methylated enzymatically by M.SssI methyltransferase:

```

221   aaaatacCGc  atcaggCGcc  attCGccatt  caggctgCGc
261   aactgttggg  aagggCGatC  GgtgCGggcc  tcttCGctat
301   taCGcccagct  ggCGaaaaggg  ggatgtgctg  caaggCGatt
341   aagtgggta  aCGccaggt  ttcccagtc  aCGaCGttgt
381   aaaaCGaCGg  ccagtgaatt  CGagctCGgt  accCGgggat
421   cctctagagt  CGacctgcag  gcatgcaagc  ttggCGtaat
461   catggtcata  gctgtttcct  gtgtgaaatt  gttatcCGct
501   cacaattcca  cacaacataC  GagcCGgaag  cataaagtgt
541   aaagcctggg  gtgcctaag  agtgagctaa  ctcacattaa
581   ttgCGttgCG  ctactgccC  Gctttccagt  CGggaaacct
621   gtCGtgccag  ctgcattaa  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   aaaaatCGt   attaggCGtt   attCGttatt   taggttgCGt
261   aattggtggg aagggCGatC GgtgCGggtt   ttttCGttat
301   taCGttagtt   ggCGaaaaggg   ggatggttg   taaggCGatt
341   aagttgggta aCGttagggt   ttttttagtt   aCGaCGttgt
381   aaaaCGaCGg   ttagtgaatt   CGagttCGgt   attCGgggat
421   tttttagagt   CGattttag   gtatgtaagt   ttggCGtaat
461   tatggttata   gttgtttttt   gtgtgaaatt   gttattCGtt
501   tataatttta   tataatataC   GagtCGgaag   tataaagtgt
541   aaagttggg   gtgtttaatg   agtgagttaa   tttatattaa
581   ttgCGttgCG   tttattgttC   Gttttttagt   CGggaaattt
621   gtCGtggttag   ttgtattaat   gaatCGgtta   aCGCGCGggg
661   agaggCGgtt   -----   -----   -----

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Trademarks and Disclaimers:

™ Trademarks of Zymo Research Corporation.

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.

Version 2.0.6

Also Available:

Product Name	Size	Catalog number
EZ DNA Methylation™ Kit	50	D5001
	200	D5002
	2 x 96	D5003
	2 x 96	D5004
EZ DNA Methylation-Gold™ Kit	50	D5005
	200	D5006
	2 x 96	D5007
	2 x 96	D5008
EZ DNA Methylation-Direct™ Kit	50	D5020
	200	D5021
	2 x 96	D5022
	2 x 96	D5023
EZ DNA Methylation-Startup™ Kit	1 Kit	D5024
EZ Bisulfite DNA Clean-up Kit™	50	D5025
	200	D5026
	2 x 96	D5027
	2 x 96	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
<i>E. coli</i> Non-methylated Genomic DNA	5 µg	D5016
ChIP DNA Clean & Concentrator™	50	D5201
	50	D5205
Methylated-DNA IP Kit	10	D5101
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg	A3001-50
	200 µg	A3001-200
Zymo <i>Taq</i> ™ DNA Polymerase	50	E2001
	200	E2002
Zymo <i>Taq</i> ™ PreMix (2X concentrated)	50	E2003
	200	E2004
CpG Methylase (M.SssI)	200 units	E2010
	400 units	E2011