Universal Methylated Human DNA Standard & Control Primers

Cat. Nos. D5011

Storage: -20 °C

ZYMO RESEARCH
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Product Information

Product Contents:

	Cat. # D5012	Storage Temp.
Universal Methylated Human DNA Standard	5 µg/20 µl	-20 °C
hMLH1 Primer I and hMLH1 Primer II	1 of each	-20 °C

Description:

The **Universal Methylated Human 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. The supplied DNA was isolated from a male human source, and is enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[1] (EC 2.1.1.37; Figure 1).

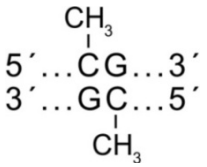

Figure 1. M.SssI methytransferase methylates all cytosine residues in the double-stranded CpG context.

The primer set is designed to amplify a fragment of the human MLH1 mismatch repair gene 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.

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**	2 µl	up to 20 ng/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, 30 seconds
E. Repeat steps B through D an additional 29 to 39 times depending on the polymerase used.
F. 72 °C, 2 minutes
G. 4 °C

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

Product Specifications:

I. Universal Methylated DNA Standard, 5 µg/20 µl.
Source: DNA isolated from male human [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
Concentration: 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
Storage: -20 °C

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

hMLH1 Primer I:

5´ - GGAGTGAAGGAGGTTACGGGTAAGT - 3´

hMLH1 Primer II:

5´ - AAAAACGATAAAACCCTATACCTAATCTATC - 3´

Appendix:

The expected PCR amplicon for the Universal Methylated Human DNA Standard is 182 bp, corresponding to nucleotide positions 804 to 986 of human MLH1 DNA including the regions (italicized) that hybridize to the primers (GenBank Accession #: U83845).

Original sequence of human MLH1 DNA 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:

```
801   ---ggagtga aggaggccaC Ggcaagt CG ccctga CG ca
841   ga CG ctccac cagggc CG CG CG ct CG c CG t c CG ccacata
881   c CG ct CG tag tatt CG tgct cagcct CG ta gtgg CG cctg
921   a CG t CG CG tt CG CG ggtagc ta CG atgagg CG g CG acaga
961   ccaggcacag ggccccat CG ccctc----- ----------
```

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Appendix (Cont'd):

Expected sequence of above PCR amplicon 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.

```
801   ---ggagtga aggaggttaC GggtaagtCG ttttgaCGta
841   gaCGttttat tagggtCGCG CGttCGtCGt tCGttatata
881   tCGttCGtag tattCGtgtt tagtttCGta gtggCGtttg
921   aCGtCGCGtt CGCGggtagt taCGatgagg CGgCGataga
961   ttaggtatag ggttttatCG ttttt----- ----------
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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 DNA Standard	1 set	D5010
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:

™ 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.