Human Methylated & Non-methylated DNA Set

Cat. Nos. D5014, D5014-1, & D5014-2

Storage: -20 °C

Product Information

Highlights:

- Purified, non-methylated and methylated human DKO DNA is ideal for use as negative and positive controls, respectively, for many methylation detection applications.
- Control primers are designed to amplify non-methylated, methylated, and mixed methylation copies of the death-associated protein kinase 1 gene (DAPK1) following bisulfite conversion.

Product Contents:

	Cat. # D5014	Cat. # D5014-1	Cat. # D5014-2	Storage Temp.
Human HCT116 DKO Non-methylated DNA	5 µg/20 µl	5 µg/20 µl	--	-20 °C
Human HCT116 DKO Methylated DNA	5 µg/20 µl	--	5 µg/20 µl	-20 °C
DAPK1 Primers	20 µl	--	--	-20 °C

Description:

The **Human Methylated & Non-methylated DNA Set** consists of two control DNAs (non-methylated and methylated) along with a set of specifically designed primers that can be used in conjunction with the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, **EZ DNA Methylation-Direct™** and **EZ DNA Methylation-Lightning™** kits from Zymo Research to assess the efficiency of bisulfite-mediated conversion of DNA.

The **Human HCT116 DKO Non-methylated DNA** is purified from cells that contain genetic knockouts of both DNA methyltransferases DNMT1 (-/-) and DNMT3b (-/-)[1]. The DNA derived from HCT116 DKO cells has a low level of DNA methylation and can be used as a control for DNA methylation analysis (Figure 1). The **Human HCT116 DKO Methylated DNA** is purified HCT116 DKO DNA that has been enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[2] (EC 2.1.1.37; Figure 2) and can be used as a positive control for DNA methylation analysis.

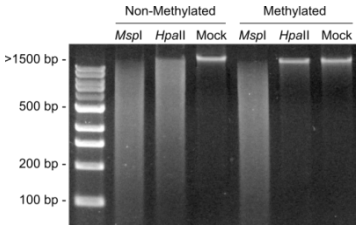

Figure 1. An assay for complete methylation by M.SssI methytransferase. Digestion of non-methylated and methylated HCT116 DKO DNA with restriction enzymes MspI and HpaII. MspI digests both non-methylated and methylated DNA. HpaII is sensitive to CpG methylation.

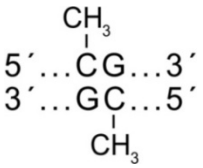

Figure 2. M.SssI methytransferase methylates all cytosine residues in the double-stranded CpG context.

Methylated cytosines comprising CG dinucleotides within DNA remain unconverted following bisulfite treatment, whereas non-methylated cytosines are converted to uracil and detected as thymine following PCR. The control primers, DAPK1 primer I and DAPK1 primer II amplify methylated, non-methylated, and mixed methylation copies of the death-associated protein kinase 1 gene and are intended for use after bisulfite conversion of the control DNA. Recovered DNA is ideal for many applications including downstream analyses such as PCR, restriction endonuclease digestion, sequencing, etc.

References:

1. Rhee *et al.* Nature. 416: 552-556 (2002).
2. 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 20 µl total reaction volume:

Component	Volume	Final Conc.
DAPK1 primers*	Variable	0.2 to 1.0 µM each
Bisulfite-converted DNA**	2 µl	up to 20 ng/µl
10 mM dNTP mix	0.4 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 20 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

Product Specifications:

I. Human HCT116 DKO Non-methylated DNA, 5 µg/20 µl.

Source: DNA purified from HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)].
Concentration: 250 ng/µl in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Storage: -20 °C

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Product Specifications (continued…):

II. Human HCT116 DKO Methylated DNA, 5 µg/20 µl.

Source: DNA purified from HCT116 DKO cells [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
Concentration: 250 ng/µl in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Storage: -20 °C

III. 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:

DAPK1 Primer I:

5' - ATTGGGAAGGTTAAGGYGGAGGGAAATTTGGT - 3'

DAPK1 Primer II:

5' - CCCCAAACRAAACAATCCCCAAAACCACATTCCTA - 3'

Appendix:

The expected PCR amplicon for the Human HCT116 DKO Non-methylated DNA Standard is 274 bp and corresponds to the region 867 to 594 nucleotides upstream from the start of the DAPK1 coding sequence, including the regions (italicized) that hybridize to the primers (GenBank Accession # NM_004938).

Original sequence of the DAPK1 fragment for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capitol letters) are non-methylated in HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)] or methylated enzymatically by M.SssI methyltransferase:

```
-867   actgggaagg ccaaggCGga gggaaacttg gcttCGggga
-827   gaagtgCGat CGcagcCGgg aggcttcccc agcccCGCGg
-787   gcCGggtgag aacaggtggC GcCGgccCGa ccaggCGctt
-747   tgtgtCGggg CGCGaggatc tggagCGaac tgctgCGcct
-707   CGgtgggcCG ctcccttccc tcccttgctc cccCGggCGg
-667   cCGcaCGcCG ggtCGgcCGg gtaaCGgaga gggagtCGcc
-627   aggaatgtgg ctctggggac tgcctCGctC Gggg------
```

Expected sequence of the above DNA following bisulfite treatment:

Human HCT116 DKO Non-methylated DNA. Below is the expected sequence for the Human HCT116 DKO Non-methylated DNA (sense strand). During treatment with sodium bisulfite, non-methylated cytosines are converted into uracils, which are later detected as thymines after PCR.

```
-867   attgggaagg ttaaggTGga gggaaatttg gtttTGggga
-827   gaagtgTGat TGtagtTGgg aggtttttt agtttTGTGg
-787   gtTGggtgag aataggtggT GtTGgttTGa ttaggTGttt
-747   tgtgtTGggg TGTGaggatt tggagTGaat tgttgTGttt
-707   TGgtgggtTG tttttttttt tttttgttt tttTGggTGg
-667   tTGtaTGtTG ggtTGgtTGg gtaaTGgaga gggagtTGtt
-627   aggaatgtgg ttttggggat tgtttTGttT Gggg------
```

Human HCT116 DKO Methylated DNA. Below is the expected sequence for the Human HCT116 DKO Methylated 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.

```
-867   attgggaagg ttaaggCGga gggaaatttg gtttCGggga
-827   gaagtgCGat CGtagtCGgg aggtttttt agtttCGCGg
-787   gtCGggtgag aataggtggC GtCGgttCGa ttaggCGttt
-747   tgtgtCGggg CGCGaggatt tggagCGaat tgttgCGttt
-707   CGgtgggtCG tttttttttt tttttgttt tttCGggCGg
-667   tCGtaCGtCG ggtCGgtCGg gtaaCGgaga gggagtCGtt
-627   aggaatgtgg ttttggggat tgtttCGttC Gggg------
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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 – Lightning™ Kit	50 200 2 x 96 2 x 96	D5030 D5031 D5033 D5034
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 Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
E. coli Non-methylated Genomic DNA	5 µg	D5016
Methylated-DNA IP Kit	10	D5101
ChIP DNA Clean & Concentrator™	50 50	D5201 D5205
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 DKO technology is licensed from The Johns Hopkins University.

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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