Human WGA Methylated & Non-methylated DNA Set

Cat. Nos. D5013, D5013-1, & D5013-2

Storage: -20 °C

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Product Information

Highlights:

- Purified, non-methylated and methylated human WGA (Whole Genome Amplified) DNAs are ideal for use as controls for many methylation detection applications.
- Control primers are designed to amplify non-methylated, methylated, and mixed methylation copies of the Ras association (RalGDS/AF-6) domain family member 1 (RASSF1) gene following bisulfite conversion.

Product Contents:

	Cat. # D5013	Cat. # D5013-1	Cat. # D5013-2	Storage Temp.
Human WGA Non-methylated DNA	5 µg/20 µl	5 µg/20 µl	--	-20 °C
Human WGA Methylated DNA	5 µg/20 µl	--	5 µg/20 µl	-20 °C
RASSF1 Primers	20 µl	--	--	-20 °C

Description:

The **Human WGA 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 WGA Methylated & Non-methylated DNA Set** is generated using phi29 DNA polymerase based whole genome amplification techniques from HCT116 DKO cell line derived genomic DNA (**Human HCT116 DKO Non-methylated DNA**). The **Human WGA Methylated DNA** is **Human WGA Non-methylated DNA** that has been enzymatically methylated at all double-stranded CG dinucleotides using M.SssI methyltransferase[2] (EC 2.1.1.37; Figure 2) and can be used as a positive control for DNA methylation analysis.

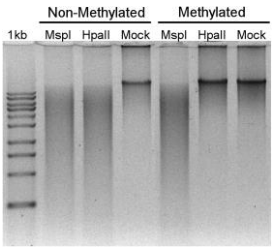

Figure 1. An assay for complete methylation by M.SssI methytransferase. Digestion of non-methylated and methylated WGA DNA with restriction enzymes MspI and HpaII. MspI digests both non-methylated and methylated DNA. HpaII is sensitive to CpG methylation.

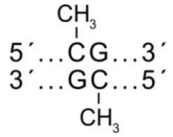

Figure 2. M.SssI methytransferase methylates all cytosine residues in double-stranded CpG context.

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, RASSF1 primer I and RASSF1 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.
RASSF1 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 35 to 45 times depending on the polymerase used.
F. 72 °C, 7 minutes
G. 4 °C

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Product Specifications:

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

Source: Whole genome amplified DNA 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

II. Human WGA Methylated DNA, 5 µg/20 µl.

Source: Whole genome amplified DNA from HCT116 DKO cells [enzymatically methylated by M.SssI methyltransferase].
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:

RASSF1 Primer I:

5' - GGTGGTTAYGGTTAGGGATTAGTTGT - 3'

RASSF1 Primer II:

5' - AACCCCACAATCCCTACACCCAAATTTCCATTA - 3'

Appendix:

The expected PCR amplicon for the Human WGA Non-methylated DNA is 327 bp and corresponds to the region 4962 to 5288 nucleotides downstream from the start of the RASSF1 coding sequence on the reverse strand, including the regions (italicized) that hybridize to the primers (GenBank Accession # NG_023270).

Original sequence of the RASSF1 fragment for bisulfite treatment and PCR amplification (anti-sense strand 5' to 3'). The cytosines in the CpG dinucleotide context (bold, underlined) are methylated enzymatically by M.SssI methyltransferase or not methylated in the non-methylated DNA.

```
5288  GGTGGCCACG   GCCAGGGACC   AGCTGCCGTG   TGGGGTTGCA
5248  CGCGGTGCCC   CGCGCGATGC   GCAGCGCGTT   GGCACGCTCC
5208  AGCCGGGTGC   GGCCCTTCCC   AGCGCGCCCA   GCGGGTGCCA
5168  GCTCCCGCAG   CTCAATGAGC   TCAGGCTCCC   CCGACATGGC
5128  CCGGTTGGGC   CCGTGCTTCG   CTGGCTTTGG   GCGCTAGCAA
5088  GCGCGGGCCG   GGCGGGGCCA   CAGGGCGGGC   CCCGACTTCA
5048  GCGCCTCCCC   CAGGATCCAG   ACTGGGCGGC   GGGAAGGAGC
5008  TGAGGAGAGC   CGCGCAATGG   AAACCTGGGT   GCAGGGACTG
4968  TGGGGTT
```

Expected sequence of the above DNA following bisulfite treatment:

Human WGA Non-methylated DNA. Below is the expected sequence for the Human WGA Non-methylated DNA after bisulfite conversion and PCR. During treatment with sodium bisulfite, non-methylated cytosines are converted into uracils, which are detected as thymines after PCR.

```
5288  GGTGGTTATG   GTTAGGGATT   AGTTGTTGTG   TGGGGTTGTA
5248  TGTGGTGTTT   TGTGTGATGT   GTAGTGTGTT   GGTATGTTTT
5208  AGTTGGGTGT   GGTTTTTTTT   AGTGTGTTTA   GTGGGTGTTA
5168  GTTTTTGTAG   TTTAATGAGT   TTAGGTTTTT   TTGATATGGT
5128  TTGGTTGGGT   TTGTGTTTTG   TTGGTTTTGG   GTGTTAGTAA
5088  GTGTGGGGTG   GGTGGGGTTA   TAGGGTGGGT   TTTGATTTTA
5048  GTGTTTTTTT   TAGGATTTAG   ATTGGGTGGT   GGGAAGGAGT
5008  TGAGGAGAGT   TGTGTAATGG   AAATTTGGGT   GTAGGGATTG
4968  TGGGGTT
```

Human WGA Methylated DNA. Below is the expected sequence for the Human WGA Methylated DNA after bisulfite conversion and PCR. 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.

```
5288  GGTGGTTACG   GTTAGGGATT   AGTTGTCGTG   TGGGGTTGTA
5248  CGCGGTGTTT   CGCGCGATGC   GTAGCGCGTT   GGTACGTTTT
5208  AGTCGGGTGC   GGTTTTTTTT   AGCGCGTTTA   GCGGGTGTTA
5168  GTTTTCGTAG   TTTAATGAGT   TTAGGTTTTT   TCGATATGGT
5128  TCGGTTGGGT   TCGTGTTTCG   TTGGTTTTGG   GCGTTAGTAA
5088  GCGCGGGTCG   GGCGGGGTTA   TAGGGCGGGT   TTCGATTTTA
5048  GCGTTTTTTT   TAGGATTTAG   ATTGGGCGGC   GGGAAGGAGT
5008  TGAGGAGAGT   CGCGTAATGG   AAATTTGGGT   GTAGGGATTG
4968  TGGGGTT
```

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 DNA Methylation-Lightning™ Kit	50 200 2 x 96 2 x 96	D5030 D5031 D5032 D5033
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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