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ZYMO RESEARCH

Epigenetics
Made Simple™

Human Methylated & Non-Methylated DNA Set

Standards for DNA methylation analysis workflows

Highlights

- Ideal positive and negative controls for methylation detection assays
- Utilize as mock samples to optimize methylation analysis workflows
- Provided with validated bisulfite primers to evaluate bisulfite conversion workflows

Catalog Numbers:
D5014, D5014-1, D5014-2

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Human Methylated & Non-Methylated DNA Set	D5014	D5014-1	D5014-2	Storage Temp.
Human HCT116 DKO Non-Methylated DNA	5 µg/20 µl	5 µg/20 µl	-	-20ºC
Human HCT116 DKO Methylated DNA	5 µg/20 µl	-	5 µg/20 µl	-20ºC
DAPK1 Primers	20 µl	-	-	-20ºC

Specifications

Human HCT116 DKO Non-Methylated DNA

- **Source –** DNA purified from HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)].
- **Concentration –** 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Human HCT116 DKO Methylated DNA

- **Source –** DNA purified from HCT116 DKO cells that has been enzymatically methylated by M.SssI methyltransferase.
- **Concentration –** 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

DAPK1 Primers

- **Concentration –** 20 µM each primer in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
- **Primer sequences –**

  DAPK1 Forward Primer:

  5′ – TAGAATTTAGTTAGAGGGTAGTTTAGTA – 3′

  DAPK1 Reverse Primer:

  5′ – AAACRACCAATAAAAACCCTACAAA – 3′

Product 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-Lightning™**, **EZ DNA Methylation-Direct™**, **EZ DNA Methylation-Gold™** or **EZ DNA Methylation™** bisulfite conversion kits from Zymo Research to assess the efficiency of bisulfite conversion of DNA.

The **Human HCT116 DKO Non-methylated DNA** is purified from cells that contain genetic knockouts of both DNA methyltransferase 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 and has been enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[2] and can be used as a positive control for DNA methylation analysis.

Following bisulfite treatment, methylated cytosines remain unconverted (in mammals, cytosine methylation occurs primarily in a CpG context), whereas non-methylated cytosines are converted to uracil and detected as thymine following PCR. The **DAPK1 control primers** amplify methylated, non-methylated, and mixed methylation copies of the death-associated protein kinase 1 (*DAPK1*) 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.

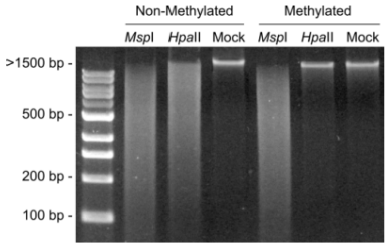

Figure 1. An assay for complete methylation by M.SssI methyltransferase. Digestion of non-methylated and methylated HCT116 DKO DNA with restriction enzymes MspI and HpaII. MspI digests both non-methylated and methylated DNA. HpaII is sensitive to CpG methylation.

[1]Rhee et al. Nature. 416: 552-556 (2002).
[2]Nur et al. J. Bacteriol. 164: 19-24 (1985).

Recommended Usage

The **Human Methylated & Non-methylated DNA** set can be used in a variety of methylation analysis applications including bisulfite and methylation-specific PCR, methylation sensitive high resolution melt analysis, methylation arrays, methylated DNA immunoprecipitation (MeDIP), library preparation, and more.

Protocol

The **Human HCT116 DKO Non-methylated DNA Standards** are highly intact genomic DNA. For best results, it's important to ensure the DNA is completely homogenous and fully in solution before quantification and usage. The following steps are recommended before quantification and usage[1]:

1. Bring the standards to room temperature.
2. Vortex the standards for 10-15 seconds, briefly spin down for 5-10 seconds.
3. Repeat step 2, three times total.
4. Proceed with quantification or usage.

Bisulfite PCR Setup: The following is designed for a 20 µl reaction.

Component	Volume	Final Concentration
DAPK1 Primers[2]	Variable	0.2 to 1.0 µM each
Bisulfite-converted DNA[3]	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
ZymoTaq™ DNA Polymerase[4]	Variable	1-2 units
Nuclease Free Water	Bring reaction to 20 µl	N/A

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.

[1]Standards are quantified using NanoDrop® measurements. If using other methods, variation may be observed.
[2]Alternatively, you may substitute primers of your choice.
[3]Remember to bisulfite-treat the DNA prior to performing PCR.
[4]We recommend using **ZymoTaq™ DNA Polymerase** or other hot-start DNA polymerases for amplification of bisulfite-treated DNA.

Appendix

DAPK1 Bisulfite PCR

The expected PCR amplicon for the **Human HCT116 DKO Non-methylated and Methylated DNA Standards** is 268 bp. The regions that hybridize to the primers are italicized.

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 capital letters) are non-methylated in HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)] or methylated enzymatically by M.SssI methyltransferase.

```
      5′ – tagaacccag tcagagggca gcttagcaat gtgtcacagg
tgggg**C**G**C**cc**C** G**C**G**C**ttc**C**G**C**gg **C**G**C**ga**C**G**C**cact ggctccc**C**G**C**g c**C**G**C**g**C**G**C**tggg
tgtgggg**C**G**C**a gtgggtgtgt g**C**G**C**gggtgtg **C**G**C**G**C**gtagag **C**G**C**G**C**ccag**C**G**C**
agcc**C**G**C**gag**C** G**C**G**C**gagctgg gaggagcag**C** G**C**ag**C**G**C**c**C**G**C**G**C** cagaacc**C**G**C**c
ag**C**G**C**c**C**G**C**gcc tggcagggca gct**C**G**C**gaggt gggtgggc**C**G**C** **C**G**C**c**C**G**C**ccagc
c**C**G*cttgcag ggtccccatt ggc**C**G**C**cct* – 3′
```

Expected sequence of the above PCR amplicon 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.

```
      5′ – tagaatttag ttagagggta gtttagtaat gtgttatagg
tgggg**T**G**T**tt**T** G**T**G**T**ttt**T**G**T**gg **T**G**T**ga**T**G**T**tatt ggttttt**T**G**T**g t**T**G**T**g**T**G**T**tggg
tgtgggg**T**G**T**a gtgggtgtgt g**T**G**T**gggtgtg **T**G**T**G**T**gtagag **T**G**T**G**T**ttag**T**G**T**
agtt**T**G**T**gag**T** G**T**G**T**gagttgg gaggagtag**T** G**T**ag**T**G**T**t**T**G**T**G**T** tagaatt**T**G**T**t
ag**T**G**T**t**T**G**T**gtt tggtagggta gtt**T**G**T**gaggt gggtgggt**T**G**T** **T**G**T**t**T**G**T**ttagt
t**T**G*tttgtag ggtttttatt ggt**T**G**T**ttt* – 3′
```

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.

```
      5′ – tagaatttag ttagagggta gtttagtaat gtgttatagg
tgggg**C**G**C**tt**C** G**C**G**C**ttt**C**G**C**gg **C**G**C**ga**C**G**C**tatt ggttttt**C**G**C**g t**C**G**C**g**C**G**C**tggg
tgtgggg**C**G**C**a gtgggtgtgt g**C**G**C**gggtgtg **C**G**C**G**C**gtagag **C**G**C**G**C**ttag**C**G**C**
agtt**C**G**C**gag**C** G**C**G**C**gagttgg gaggagtag**C** G**C**ag**C**G**C**t**C**G**C**G**C** tagaatt**C**G**C**t
ag**C**G**C**t**C**G**C**gtt tggtagggta gtt**C**G**C**gaggt gggtgggt**C**G**C** **C**G**C**t**C**G**C**ttagt
t**C**G*tttgtag ggtttttatt ggt**C**G**C**ttt* – 3′
```

Ordering Information

Product Description	Catalog No.	Size
Human Methylated & Non-methylated DNA Set	D5014	5 µg/20 µl
Human HCT116 DKO Non-Methylated DNA	D5014-1	5 µg/20 µl
Human HCT116 DKO Methylated DNA	D5014-2	5 µg/20 µl
Bisulfite-Converted Universal Methylated Human DNA Standard	D5015	1 µg/50 µl
Zymo*Taq*™ qPCR Premix	E2054 E2055	50 Rxns. 200 Rxns.
Zymo*Taq*™ Premix	E2003 E2004	50 Rxns 200 Rxns.
EZ DNA Methylation-Lightning™ Kit	D5030 D5031	50 Rxns 200 Rxns.
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 Rxns 200 Rxns.
EZ DNA Methylation™ Kit	D5001 D5002	50 Rxns 200 Rxns.
EZ DNA Methylation-Gold™ Kit	D5005 D5006	50 Rxns 200 Rxns.

Notes

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Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. 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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