



ZYMO RESEARCH



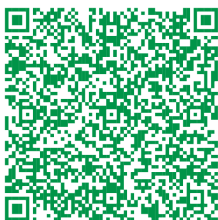
Human Methylated & Non-Methylated DNA Set

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	01
Specifications	01
Product Description	02
Recommended Usage	03
Bisulfite PCR	03
Appendices	04
Ordering Information	05
Notes	06
Guarantee	09

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	-	-20°C
Human HCT116 DKO Methylated DNA	5 µg/20 µl	-	5 µg/20 µl	-20°C
DAPK1 Control Primers	20 µl	-	-	-20°C

Specifications

Human Methylated and Non-Methylated DNA

- **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).

DAPK1 Control 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' – AAACRACCAATAAAAAACCCTACAAA – 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 HCT 116 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 methyltransferase² 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.

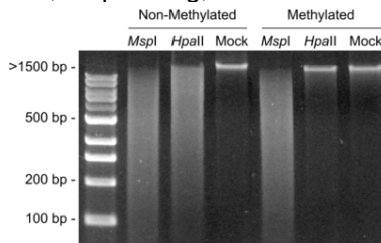


Figure 1. An assay for complete methylation by M.SssI methyltransferase. Digestion of non-methylated and methylated HCT116 DKO DNA with restriction enzymes MspI and HpaII. MspI digests both non-methylated and methylated DNA. HpaII is sensitive to CpG methylation.

¹Rhee et al. Nature. 416: 552-556 (2002).

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

Bisulfite PCR

PCR Setup: The following setup is designed for a 20 μ l reaction volume.

Component	Volume	Final Concentration
DAPK1 Primers ¹	Variable	0.2 to 1.0 μ M each
Bisulfite-converted DNA ²	2 μ l	Up to 20 ng/ μ l
10 mM dNTP mix	0.4 μ l	0.2 mM each dNTP
Standard PCR Buffer	Variable	1x
MgCl ₂ or MgSO ₄	Variable	1-4 mM, if needed
ZymoTaq DNA Polymerase ³	Variable	1-2 units
Nuclease Free Water	Bring reaction to 20 μ l	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

¹Alternatively, you may substitute primers of your choice.

²Remember to bisulfite-treat the DNA prior to performing PCR.

³We recommend using **ZymoTaq™ DNA polymerase** or other hotstart DNA polymerases for amplification of bisulfite-treated DNA.

Appendices

DAPK1 Bisulfite PCR

The expected PCR amplicon for the Human HCT116 DKO Non-methylated and Methylated DNA Standards is 268 bp.

Original sequence of the DAPK1 fragment for bisulfite treatment 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' -   tagaaccag  tcagagggca  gcttagcaat  gtgtcacagg
tggggCGcccC  GCGttcCGgg  CGgaCGcact  ggctcccCGg  cCGgCGtggg
tgtggggCGa  gtgggtgtgt  gCGgggtgtg  CGCGgtagag  CGCGccagCG
agccCGgagC  GCGgagctgg  gaggagcagC  GagCGcCGCG  cagaaccCGc
agCGcCGgcc  tggcagggca  gctCGgaggt  gggtgggcCG  CGcCGccagc
cCGtttcag  ggtccccatt  ggcCGcct - 3'
```

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.

```
5' -   tagaatttag  ttagagggta  gtttagtaat  gTGttatagg
TGgggTGttT  GTGtttTGgg  TGgaTGtatt  ggtttttTGg  tTGgTGTGgg
TGTGgggTGa  gTGggTGTGt  gTGgggTGTG  TGTGgtagag  TGTGttagTG
agttTGgagT  GTGgagtTGg  gaggagtagT  GagTGtTGTG  tagaattTGt
agTGtTGgtt  TGgtagggta  gttTGgaggt  gggTGggtTG  TGtTGttagt
tTGttTGtag  ggtttttatt  ggtTGttt - 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
tggggCGttC  GCGtttCGgg  CGgaCGtatt  ggtttttCGg  tCGgCGtggg
tgtggggCGa  gtgggtgtgt  gCGgggtgtg  CGCGgtagag  CGCGttagCG
agttCGgagC  GCGgagttgg  gaggagtagC  GagCGtCGCG  tagaattCGt
agCGtCGgtt  tggtagggta  gttCGgaggt  gggtggggtCG  CGtCGttagt
tCGtttag  ggtttttatt  ggtCGttt - 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
ZymoTaq™ qPCR Premix	E2054 E2055	50 Rxns. 200 Rxns.
ZymoTaq™ 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.



100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

™ Trademarks of Zymo Research Corporation

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.



ZYMO RESEARCH

The **BEAUTY** of **SCIENCE** is to Make Things **SIMPLE**®



tech@zymoresearch.com



www.zymoresearch.com



Toll Free: (888) 882-9682