ZYMO RESEARCH

Epigenetics
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# Bisulfite Converted Universal Methylated Human DNA Standard

Standards for DNA methylation analysis workflows

## Highlights

- Purified human DNA from that has been enzymatically methylated at all CpG sites and bisulfite converted for direct use in downstream methylation applications.
- Provided primer pair targeting the human MLH1 mismatch repair gene allows for convenient assay quality control.

Catalog Numbers:
D5015

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

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# Product Contents

| Bisulfite Converted Universal Methylated Human DNA Standard | D5015 | Storage Temp. |
|---|---|---|
| Bisulfite Converted Universal Methylated Human DNA Standard | 1 µg/50 µl | -20ºC |
| hMLH1 Primers | 20 µl | -20ºC |

# Specifications

**Bisulfite Converted Universal Methylated Human DNA Standard**

- **Source –** DNA isolated from male human brain tissue.
- **Concentration –** 20 ng/µl in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

**hMLH1 Primers**

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

  **hMLH1 Forward Primer:**

  5′ – GGAGTGAAGGAGGTTACGGGTAAGT – 3′

  **hMLH1 Reverse Primer:**

  5′ – AAAAACGATAAAACCCTATACCTAATCTATC – 3′

# Product Description

The **Bisulfite Converted Universal Methylated Human DNA Standard** contains bisulfite-treated DNA derived from the **Universal Methylated Human DNA Standard**. The supplied DNA has been methylated at all cytosine positions within the CG dinucleotide context using M.SssI methyltransferase1 (EC 2.1.1.37; Figure 1). Following methylation, the DNA was treated with sodium bisulfite according to the protocol described in the **EZ DNA Methylation-Direct™** kit. In this kit the methylated cytosines are unmodified by sodium bisulfite, whereas all non-methylated cytosines are converted to uracil, which are detected as thymine in subsequent PCR.

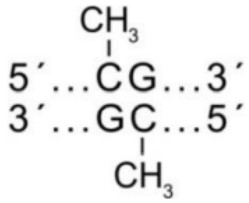


**Figure 1.** M.SssI methyltransferase methylates all cytosine residues in the double stranded CpG context.

The included primer set is designed to amplify a fragment of the human MLH1 mismatch repair gene. The methylated cytosines comprising CG dinucleotides remain unconverted, whereas non-methylated cytosines are converted into uracil and detected as thymine after PCR.

[1]Nur et al. J. Bacteriol. 164: 19-24 (1985).

# Recommended Usage

The **Bisulfite Converted Universal Methylated Human DNA Standard** can be used as a control for bisulfite conversion and downstream analyses such as bisulfite PCR, methylation sequencing applications, and more.

# Protocol

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. Thaw the standard slowly on ice.
2. Mix the standard well, briefly spin down for 5-10 seconds.
3. Repeat Step 2 twice, for three times total.
4. Proceed with quantification or usage.

**Bisulfite PCR Setup**: The following is designed for a 25 µl reaction.

| Component | Volume | Final Concentration |
|---|---|---|
| **hMLH1 Primers**[2] | Variable | 0.2 to 0.8 µM each |
| Bisulfite-converted DNA | 1 µl | 0.8 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 |
| **ZymoTaq™ DNA Polymerase**[3] | Variable | 1-2 units |
| Nuclease Free Water | Bring reaction to 25 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]We recommend using **ZymoTaq™ DNA Polymerase** or other hot-start DNA polymerases for amplification of bisulfite-treated DNA.

# Appendix

**hMLH1 Bisulfite PCR**

The expected PCR amplicon for the **Bisulfite Converted Universal Methylated Human DNA Standard** is 182 bp, corresponding to nucleotide positions 804 to 985 of human MLH1 DNA including the regions (italicized) that hybridize to the primers (GenBank Accession #: U83845).

Expected sequence following PCR amplification (sense strand 5' to 3'). Methylated cytosines (underlined) in the CpG dinucleotide context (bold capital letters) 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.

5′ – *ggagtgaagg aggtta**CG**gg taagt**C**G*ttt
tga**C**G*taga**C*** **G**ttttattag ggt**C**G**C**G**C**G*t* t**C**G*t**C**G*tt**C**G*
ttatatat**C**G* tt**C**G*tagtat t**C**G*tgtttag ttt**C**G*tagtg
g**C**G*tttga**C**G* t**C**G**C**G*tt**C**G**C*** **G**ggtagtta**C*** **G**atgagg**C**G*g
**C**G**atagatta ggtatagggt tttat**C**G**ttt tt – 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<br>E2055 | 50 Rxns.<br>200 Rxns. |
| **Zymo*Taq*™ Premix** | E2003<br>E2004 | 50 Rxns<br>200 Rxns. |
| **EZ DNA Methylation-Lightning™ Kit** | D5030<br>D5031 | 50 Rxns<br>200 Rxns. |
| **EZ DNA Methylation-Direct™ Kit** | D5020<br>D5021 | 50 Rxns<br>200 Rxns. |
| **EZ DNA Methylation™ Kit** | D5001<br>D5002 | 50 Rxns<br>200 Rxns. |
| **EZ DNA Methylation-Gold™ Kit** | D5005<br>D5006 | 50 Rxns<br>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 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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