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Epigenetics
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Universal Methylated Human DNA Standard

Standards for DNA methylation analysis workflows

Highlights

- Purified, methylated human DNA for use as a positive control to assess bisulfite conversion efficiency.
- All cytosines within a CpG dinucleotide context have been enzymatically methylated by M.SssI methyltransferase.
- Provided primer pair targeting the human MLH1 mismatch repair gene allows for convenient assay quality control.

Catalog Numbers:
D5011

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

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Product Contents

Universal Methylated Human DNA Standard	D5011	Storage Temp.
Universal Methylated Human DNA Standard	5 µg/20 µl	-20ºC
hMLH1 Primers	20 µl	-20ºC

Specifications

Universal Methylated Human DNA Standard

- **Source –** DNA isolated from male human brain tissue.
- **Concentration –** 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Confirmed via NanoDrop quantification.

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 **Universal Methylated Human DNA Standard** includes enzymatically methylated DNA together with a specially designed primer set to 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 supplied DNA was isolated from a male human source, and is enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[1] (EC 2.1.1.37; Figure 1).

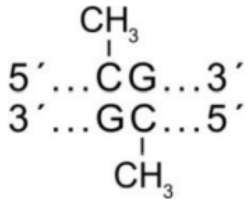

Figure 1. M.SssI methyltransferase methylates all cytosine residues in the double stranded CpG context.

The included primer set is designed to amplify a fragment of the human MLH1 mismatch repair gene following bisulfite treatment. The methylated cytosines comprising CG dinucleotides remain unconverted following bisulfite treatment, 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 **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. Bring the standard to room temperature.
2. Vortex the standards for 10-15 seconds, 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[3]	2 µl	Up to 20 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[4]	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]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

hMLH1 Bisulfite PCR

The expected PCR amplicon for the **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).

Original sequence of human MLH1 DNA for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capital letters) are methylated enzymatically by M.SssI methyltransferase:

```
      5′ – ggagtgaagg aggccaCGgg caagtCGccc
tgaCGcagaC GctccaccaG ggcCGCGCGc tCGcCGtcCG
ccacatacCG ctCGtagtat tCGtgctcag cctCGtagtg
gCGcctgaCG tCGCGttCGC GggtagctaC GatgaggCGg
CGacagacca ggcacagggc cccatCGccc tc – 3′
```

Expected sequence of the above PCR amplicon following bisulfite treatment:

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 uracil and detected as thymine after PCR.

```
      5′ – ggagtgaagg aggttaCGgg taagtCGttt
tgaCGtagaC GttttattaG ggtCGCGCGt tCGtCGttCG
ttatatatCG ttCGtagtat tCGtgtttag tttCGtagtg
gCGtttgaCG tCGCGttCGC GggtagttaC GatgaggCGg
CGatagatta ggtatagggt tttatCGttt 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
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.

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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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