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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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ZYMO RESEARCH

THE Epigenetics COMPANY™

EZ DNA Methylation-Startup™ Kit

Quick start to epigenome work

Highlights

- Designed for the first-time user requiring a consolidated product to perform DNA methylation analysis.
- A complete system for DNA methylation detection: DNA bisulfite treatment, robust hot start PCR master mix, and a universally methylated human control with primers.
- Ideal for system setup and calibration for DNA methylation detection and analysis.

Catalog Numbers:
D5024

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

EZ DNA Methylation-Startup™ Kit	D5024 (1 Kit)	Storage Temperature
EZ DNA Methylation-Direct™ Kit* (Cat. No. D5020)	50 Rxns.	Room Temp.
Universal Methylated Human Standard (Cat. No. D5011)	20 µl	-20ºC
hMLH1 Primers	20 µl	-20ºC
2X Zymo*Taq*™ PreMix (Cat. No. E2003)	2 x 625 µl	-20ºC
DNase/RNase-Free H_2O	2 x 1 ml	Room Temp.

*See protocol included in the kit for product contents and storage guidelines.

Specifications

- **Application –** Workflow design allows direct sample input for bisulfite treatment of DNA followed by robust PCR amplification of the “converted” DNA for methylation analysis.

- **Components –** The EZ DNA Methylation-Startup™ Kit is a two component kit: one is the EZ DNA Methylation-Direct™ Kit (D5020) and the other the Universal Methylated Human Standard (D5011) and 2X Zymo*Taq*™ PreMix (E2003).

- **DNA Recovery –** Typical yields of “bisulfite-converted” DNA are between 80-90%.

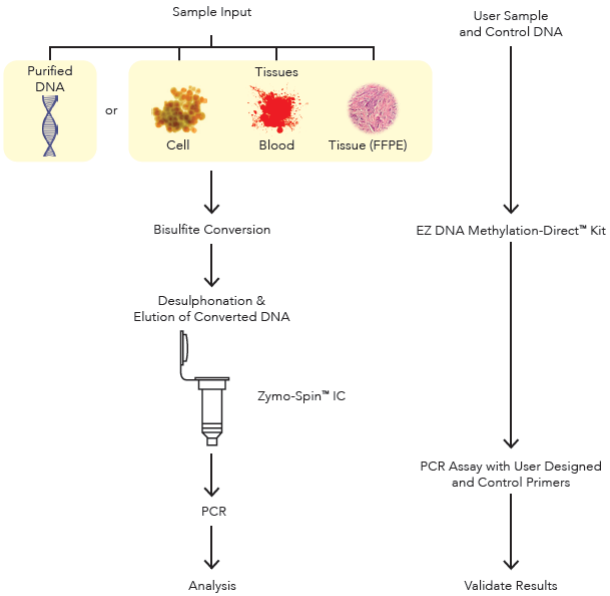
- **Elution –** DNA can be eluted with ≥ 10 µl M-Elution Buffer or water.

DNA Methylation and Bisulfite Treatment

DNA methylation has been shown to play key roles in gene imprinting, embryonic development, X-chromosome gene silencing, cell cycle regulation, and is one of the critical factors in regulating epigenetic control of the genome. In most plants and animals, DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring creating 5-methylcytosine via a methyltransferase enzyme. The majority of DNA methylation in mammals occurs in the context of 5′-CpG-3′ dinucleotides, which can be used as an epigenetic marker in both normal and diseased conditions.

Currently, the most reliable technique for methylation detection has been the bisulfite conversion method. This technique consists of treating DNA with bisulfite, which causes unmethylated cytosines to be “converted” into uracil while methylated cytosines remain unchanged. With the EZ DNA Methylation-Startup™ Kit, purified DNA, blood, or tissue samples can be processed directly. After bisulfite modification, the DNA is then amplified by PCR and the resulting PCR products analyzed either by DNA sequencing or by other methods typically used in determining the methylation status of DNA.

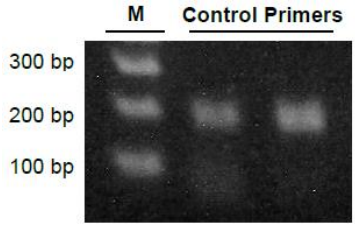
Diagrammed Workflow of the EZ DNA Methylation-Startup™ Kit



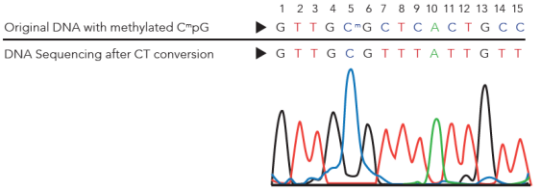
Product Description

Zymo Research's technologies for bisulfite conversion and DNA methylation detection remain the most popular and cited to date. The **EZ DNA Methylation-Startup™ Kit** is designed to include these technologies for the first-time user requiring a consolidated product to perform DNA methylation analysis.

The **EZ DNA Methylation-Startup™ Kit** provides the necessary technologies required for complete bisulfite-conversion of DNA for PCR and methylation analysis. This kit includes bisulfite conversion reagents that allow for direct sampling of blood, cells, and fresh or FFPE tissues without the prerequisite for upstream DNA purification. However, purified DNA can also be bisulfite treated directly. A fully methylated **Universal Methylated Human DNA Standard** is provided together with a special primer set for PCR to control for and assess conversion efficiency. Finally, a unique "hot start" **Zymo*Taq*™ PreMix** is included for robust amplification of bisulfite-treated DNA.



Efficient PCR amplification of bisulfite-treated DNA for methylation detection. The figure shows amplification of a 182 bp product from bisulfite-treated, **Universal Methylated Human DNA Standard** using hMLH1 primers and **Zymo*Taq*™ PreMix**. In duplicate, equal amounts of bisulfite-treated DNA (**EZ DNA Methylation-Direct™ Kit**) were used for each PCR. Products were separated in a 2.0% (w/v) agarose/TAE/EtBr gel. Also evident from the image is the low occurrence of primer dimerization and non-specific product formation.



DNA sequencing results following bisulfite treatment. DNA with methylated $C^{m}pG$ at nucleotide position #5 was processed using the **EZ DNA Methylation™ Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remained intact while the unmethylated cytosines at positions #7, 9, 11, 14 and 15 were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

Protocol

For reagent preparation and DNA bisulfite treatment details, please see the **EZ DNA Methylation-Direct™ Kit** instructions. The following protocol uses the supplied Universal Methylated Human DNA Standard as an example and should be used as a guideline for your experimental samples.

Section I. Bisulfite Conversion of DNA (please reference the **EZ DNA Methylation-Direct™ Kit** instruction manual as needed)

1. Dilute the DNA in a PCR tube as outlined below…

2 µl	**Universal Methylated Human DNA Standard** (or user supplied sample - see note below)
18 µl	H_2O
20 µl	Total Volume

Note: The maximum input volume of DNA is 20 µl. Alternatively, cells and tissues can be input directly without the prerequisite for DNA purification (reference the **EZ DNA Methylation-Direct™ Kit** protocol).

2. Add 130 µl of freshly prepared **CT Conversion Reagent** to the DNA in the PCR tube. Mix the sample and then centrifuge briefly to ensure no droplets are retained in the cap or the sides of the tube.

3. Proceed with the **EZ DNA Methylation-Direct™ Kit** protocol through to the DNA elution step (*i.e.*, Step 9).

   The eluted DNA will be in **Elution Buffer** (10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA) and can be used for PCR, microarrays, bisulfite sequencing, or other applications.

Section II. PCR Amplification of Bisulfite-Treated DNA

The following PCR conditions have been optimized for amplification of the **Universal Methylated Human DNA Standard** following bisulfite treatment and should be used as a guideline when setting up your own PCR. The control primers (*i.e*. hMLH1) are designed to specifically amplify a 182 bp product. See **Appendix I** (page 7) for detailed information regarding the design and use of the **Universal Methylated Human DNA Standard** and **Control Primers**, see **Appendix II** for general information on bisulfite PCR.

1. Set up the following reaction…

12.5 µl	2X Zymo*Taq*™ PreMix*
2 µl	hMLH1 Primers
2 µl	Bisulfite-Treated **Universal Methylated Human DNA Standard**
8.5 µl	ddH_2O
25 µl	Total Volume

Note: The amount of input DNA in the PCR can be increased or decreased as needed. The final concentration of $MgCl_2$ in the reaction is 1.75 mM. If required, adjust reaction volumes accordingly to optimize the $MgCl_2$, primer, and/or template concentrations.

2. Use the parameters below for amplification of the bisulfite-treated, **Universal Methylated Human DNA Standard**.

Temperature	Time	
95°C	10 minutes	
95°C	30 seconds	35-40 cycles
59°C	30 seconds	
72°C	60 seconds	
72°C	7 minutes	
4°C	"hold"	

For amplification of user provided bisulfite-treated DNA, the annealing temperature and extension time should be adjusted according to the primer *Tm*s and amplicon size, respectively. We recommend using between 35-40 cycles for most templates. Please refer to **Appendix II** for additional PCR and primer design guidelines. The amplified PCR product can be used for gel analysis, TA cloning, restriction endonuclease digestion, microarrays, sequencing, and other downstream molecular applications.

The final concentration of Zymo*Taq*™ DNA Polymerase in the 1X Zymo*Taq*™ PreMix is 2 U/50 µl.

Appendices

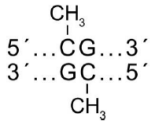
Appendix I: Universal Methylated Human DNA Standard and Control Primers

1. **Universal Methylated Human DNA Standard.**

   Source: DNA derived from a human male. DNA has methylated cytosines (C^5) at all CG dinucleotide positions.

   Concentration: 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

   Methylation Site:



2. **Control Primers**
   hMLH1 Control Primers are supplied at a concentration of 20 µM (each) in 20 µl TE buffer. The expected PCR amplicon is **182 bp**, corresponding to nucleotide positions **804 - 986** of the hMLH1 DNA sequence that includes the regions spanned by the primers (see below).

   hMLH1 Primer I (sense)
   5′-GGAGTGAAGGAGGTTACGGGTAAGT-3′

   hMLH1 Primer II (antisense)
   5′-AAAAACGATAAAACCCTATACCTAATCTATC-3′

Original sequence of the human MLH1 DNA fragment for bisulfite treatment and PCR amplification (sense strand 5′ to 3′). All cytosines in CG dinucleotides (bold) are methylated. Numbers correlate to the nucleotides from the human MLH1 DNA 5' flanking region (GenBank Accession #U83845). Observed CpG/ Expected CpG is > 1 with a 68% total GC content.

```
781 ---------- ---------- ---ggagtga aggaggccaC Gggcaagt CG ccctgaCGca
841 gaCGctccac cagggcCGCG CGctCGcCGt cCGccacata cCGctCGtag tattCGtgct
901 cagcctCGta gtggCGcctg aCGtCGCGtt CGCGggtagc taCGatgagg CGgCGacaga
961 ccaggcacag ggccccatCG ccctc
```

Expected Sequence of PCR amplified product following bisulfite conversion (sense strand 5′ to 3′).

```
781 ---------- ---------- ---ggagtga aggaggttaC GggtaagtcG ttttgaCGta
841 gaCGttttat tagggtCGCG CGttCGtCGt tCGttatata tCGttCGtag tattCGtgtt
901 tagtttCGta gtggCGtttg aCGtCGCGtt CGCGggtagt taCGatgagg CGgCGataga
961 ttaggtatag ggttttatCG ttttt
```

Appendix II: Bisulfite Conversion and PCR Optimization

1. **Bisulfite Conversion of Double Stranded DNA Templates**. The following illustrates what occurs to a DNA template during bisulfite conversion.

   Template:
   A: 5′-GACCGTTCCAGGTCCAGCAGTGCGCT-3′
   B: 3′-CTGGCAAGGTCCAGGTCGTCACGCGA-5′

   Bisulfite Converted:
   A: 5′-GATCGTTTTAGGTTTAGTAGTGCGTT-3′

   B: 3′-TTGGCAAGGTTTAGGTTGTTATGCGA-5′

   Note: Methylated “C” is underlined in the examples.

   Note: Following bisulfite conversion, the strands are no longer complementary.

2. **PCR Primer Design.** Generally, primers 26 to 32 bases are required for amplification of bisulfite converted DNA. In general, all Cs should be treated as Ts for primer design purposes, unless they are in a CpG context. See example below.

   Bisulfite Converted: **A:** 5′-GATCGTTTTAGGTTTAGTAGTGCGTT-3′
   Primers: Reverse: 3′-ATCATCACRCAA-5′ **R= G/A**
   : Forward: 5′-GATYGTTTTAGGT-3′ **Y= C/T**

   Note: Only one strand (**A**) is amplified by a given primer set. Only the reverse primer binds to the converted DNA, the forward primer will bind the strand generated by the reverse primer. If the primer contains CpG dinucleotides with uncertain methylation status, then mixed bases with C and T (or G and A) can be used. Usually, there should be no more than one mixed position per primer and it should be located toward the 5’ end of the primer. It is not recommended to have mixed bases located at the 3’ end of the primer.

   Zymo Research provides primer design assistance with its Bisulfite Primer Seeker Program, available at:

   www.zymoresearch.com/tools/bisulfite-primer-seeker

3. **Amount of DNA Required for Bisulfite Conversion**. The minimal amount of human or mouse genomic DNA required for bisulfite treatment and subsequent PCR amplification is 100 pg. The optimal amount of DNA per bisulfite treatment is 200 to 500 ng. Although, up to 2 µg of DNA can be processed, it should be noted that high input levels of DNA may result in incomplete bisulfite conversion for some GC-rich regions.

4. **PCR Conditions**. Usually, 35 to 40 cycles are required for successful PCR amplification of bisulfite converted DNA. Optimal amplicon size should be between 150-300 bp; however larger amplicons (up to 1 kb) can be generated by optimizing the PCR conditions. Annealing temperatures between 55-60°C typically work well.

   As most non-methylated cytosine residues are converted into uracil, the bisulfite-treated DNA usually is AT-rich and has low GC composition. Non-specific PCR amplification is relatively common with bisulfite treated DNA due to its AT-rich nature. PCR using "hot start" polymerases is strongly recommended for the amplification of bisulfite-treated DNA.

5. **Quantifying Bisulfite Treated DNA**. Following bisulfite treatment of genomic DNA, the original base-pairing no longer exists since non-methylated cytosine residues are converted into uracil. Recovered DNA is typically A, U, and T-rich and is single stranded with limited non-specific base-pairing at room temperature. The absorption coefficient at 260 nm resembles that of RNA. Use a value of 40 µg/ml for Ab_{260} = 1.0 when determining the concentration of the recovered bisulfite-treated DNA.

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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.

EZ DNA Methylation-Direct™ Kit technologies are patent pending.

Use of Methylation Specific PCR (MSP) is protected by US Patents 5,786,146 & 6,017,704 & 6,200,756 & 6,265,171 and International Patent WO 97/46705. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product.

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