ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

OneStep qMethyl™ Kit

Catalog No. **D5310**

Highlights

- Single step, real-time PCR procedure for bisulfite-free determination of DNA methylation status.
- Ideal for rapid screening of DNA methylation at specific regions.
- Includes reagents and controls for quantitative detection and reliable performance.

Contents

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

For Research Use Only

Ver. 1.0.7

Product Contents

Reagents provided in this kit are intended for processing 22 individual samples (in duplicate) in one (1) 96-well real-time PCR plate.

***OneStep* qMethyl™ Kit**	**D5310**	**Storage Temperature**
2X Test Reaction PreMix*	0.5 ml	-20ºC*
2X Reference Reaction PreMix*	0.5 ml	-20ºC*
DNase/RNase-free Water	1 ml	Room Temp.
***MGMT* Primers I & II** (10 µM each)	40 µl	-20ºC
Human Methylated & Non-methylated DNA Standards (4 ng/µl each)	40 µl	-20ºC
Instruction Manual	1	-

Note **-** 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.

***Store the Test Reaction PreMix and the Reference Reaction PreMix at -20°C (-80°C for long-term storage) upon arrival.**

The PreMixes in this kit contain SYTO 9® dye for analysis using real-time PCR. The ***OneStep* qMethyl™*-Lite*** product (Cat. No. D5311) *omits this dye. The "Lite" version allows real-time PCR to be performed with other fluorescent dyes or molecular probes of the researcher's choosing.*

Some technologies of the *OneStep* qMethyl™ Kit are patent pending.

Use of SYTO® 9 is provided under an agreement between Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies) and Zymo Research Corporation and the manufacture, use, sale or import of this product is subject to one or more U.S. Patents and corresponding international equivalents. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies), Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300, Fax: (541) 335-0354. SYBRgreen® is a registered trademark of Life Technologies Corporation.

The Polymerase Chain Reaction (PCR) process is covered by U.S. Pat. Nos. 4,683,195 and 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 *OneStep* qMethyl™ Kit. 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.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

Introduction

Epigenetic modifications are regarded as fundamental to the regulation of gene expression. DNA methylation is one such modification that plays crucial roles in widespread biological phenomena including the regulation of gene activity, gene imprinting, embryonic development and X-chromosome inactivation in higher organisms. Current methods used to evaluate DNA methylation including bisulfite sequencing, Methylation Specific PCR (MSP), HPLC, and Methylated DNA Immunoprecipitation (MeDIP) have proven too costly, time consuming, or inappropriate for the investigation of large numbers of regions. The ***OneStep* qMethyl™ Kit** from Zymo Research provides a simple, straightforward, and bisulfite-free procedure for rapid, region-specific DNA methylation assessment. Simply add DNA into the appropriate reaction mix then quantitate via real-time PCR… *OneStep!*

Add DNA

Real-time PCR

Simple *OneStep* DNA Methylation Determination

Unlike conventional qAMP procedures that contain multiple steps, the ***OneStep* qMethyl™** method integrates the workflow into a simple, single step reaction. This minimizes errors that can occur during setup and the likelihood of contamination while allowing for rapid and accurate DNA methylation level detection.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com

Zymo Research also offers technologies for high resolution genome-wide analysis of DNA methylation. Please visit our website for detailed information.

Overview of Procedure

The ***OneStep* qMethyl™ Kit** is used for the detection of region-specific DNA methylation via the selective amplification of methylated cytosines in CpG dinucleotide context. This is accomplished by splitting any DNA to be tested into two parts: a "**Test Reaction**" and a "**Reference Reaction**" (see Figure 1 below). DNA in the **Test Reaction** is digested with Methylation Sensitive Restriction Enzymes (MSREs) while DNA in the **Reference Reaction** is not. The DNA from both samples is then amplified using real-time PCR in the presence of SYTO® 9 fluorescent dye and then quantitated. Cycle threshold (Ct) values for **Test** and **Reference** DNA samples will vary depending on methylation status, with large Ct differences most characteristic of non-methylated DNA.

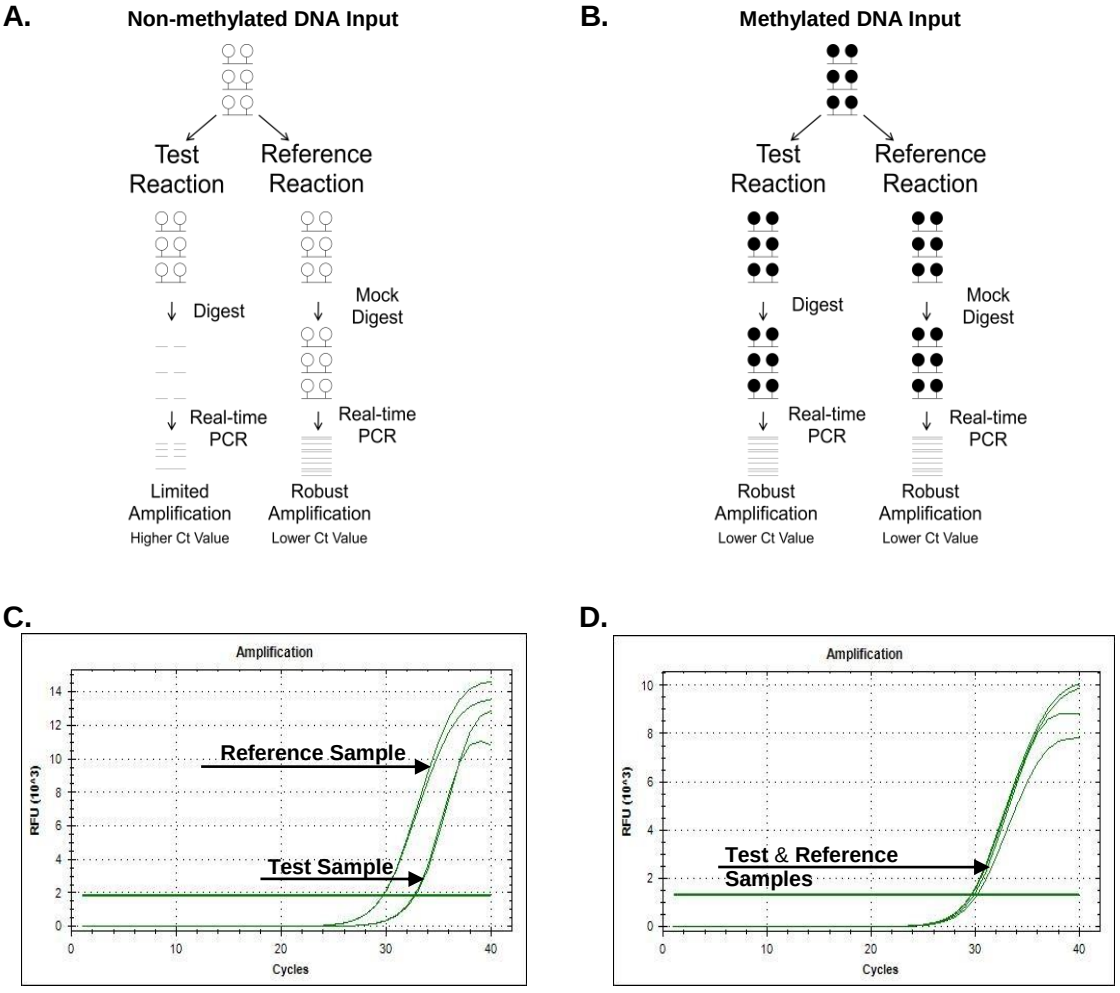


Figure 1. Schematics A and B (above) illustrate the sample workflow of Non-methylated DNA and Methylated DNAs. In both cases the DNA is divided in two parts; a **Test Reaction** and a **Reference Reaction**. **Test Reaction** samples are MSRE digested while the **Reference Reaction** samples are not (mock digested). Following digestion, DNA from both samples is used for real-time PCR. The white lollipops in the image represent unmethylated cytosines and black lollipops methylated cytosines in CpG dinucleotide context. Following real-time PCR, amplification plots (C and D) demonstrate non-methylated DNA exhibits large differences in the Ct values for **Test** and **Reference Reactions** (C), while highly methylated DNA samples exhibit little difference (D). These data are **Test** as the basis for methylation determinations. Details on page 7

Considerations for Experimental Design

A. DNA Quality

Input DNA processed using the ***OneStep* qMethyl™** procedure should be high quality and suitable for use in restriction enzyme (i.e., MSRE) digestion. If input sample purity is in question, it is recommended to use the **Genomic DNA Clean & Concentrator ™** (Cat. Nos. D4010, D4011, Zymo Research Corp.) For FFPE samples, we recommend the **ZR FFPE DNA MiniPrep™** (Cat Nos. D3065, D3066, Zymo Research Corp.) for purifying high quality, intact dsDNA.

B. Input DNA and Reaction Volume

Each reaction mixture for the ***OneStep* qMethyl™** procedure (page 5) is optimized for 20 ng input DNA. For each sample, 5 µl DNA (4 ng/µl) should be added to bring the final reaction volume to 20 µl (i.e., 1 ng/µl final concentration). Input DNA should be diluted in water or "modified" TE containing a low concentration of EDTA (*see sidebar*).

"Modified" TE Buffer: (10 mM Tris pH 8.0-8.5. 0.1 mM EDTA)

C. Duplicate Sampling

Precise Ct value determination is critical for accurate quantification of DNA methylation by real-time PCR using the ***OneStep* qMethyl™** procedure. Therefore, it is recommended to set up each **Test Reaction** and **Reference Reaction** measurement in duplicate to ensure accurate, non-biased data collection.

D. Primer Design and PCR Amplicons

Primers should span a DNA region that is 120 bp to 350 bp and contains at least two (2) MSRE sites. *See* **Appendix I** (page 8) for a list of the MSREs featured in the ***OneStep* qMethyl™ Kit**. Primers can be designed using conventional procedures.

E. Human Methylated & Non-methylated DNA Standards

It is critical to include the **Human Methylated & Non-methylated DNA Standards** (in duplicate) with the control *MGMT* primers to validate the accuracy of the ***OneStep* qMethyl™** procedure for determining methylation percentage.

Refer to **Appendix III** for detailed information regarding the **Methylated** and **Non-methylated Human DNA Standard** and ***MGMT*** primers.

Notes:

[1]*See* **Appendix II** to use the ***OneStep* qMethyl™-PCR** procedure for simultaneous methylation level determination in multiple regions.

[2]Alternatively, if not performed in duplicate, 44 individual DNA samples can be processed.

[3]Enough of the Human Methylated & Non-methylated DNA Standards is provided for a single run, 96-well analysis.

[4]The final concentration of $MgCl_2$ in the **Reference Reaction** & **Test Reaction** mixtures is 3.75 mM. The final concentrations of primers will need to be optimized. Final concentrations shown here are at 500 nM. Final primer concentrations range between 250 nM to 800nM.

Protocol

The following protocol illustrates the use of the ***OneStep* qMethyl™** procedure for DNA methylation detection at a single region[1]. This allows for the processing of up to 22 DNA samples (in duplicate)[2] using a 96-well real-time PCR plate. The provided **Human Methylated & Non-methylated DNA Standards**[3] should be processed along with the samples for the purpose of validating the combined MSRE digestion/real-time PCR step (*see* **Appendix III**, page 10). The following should serve as a guideline when setting up your own experiment. *The format is also compatible with thin-walled PCR tubes and tube strips.*

I. Preparation of Test Reaction and Reference Reaction Mixtures[4].

Protocol for Methylation Detection Levels for Multiple DNA Samples

1. For each DNA sample (and Standard) to be analyzed, setup (on ice) both a **Test Reaction** and a **Reference Reaction** mixture.

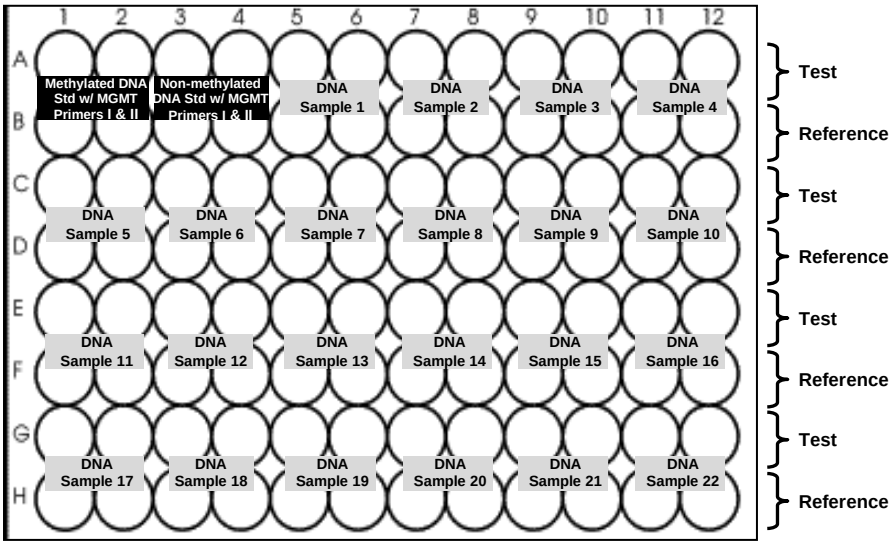
Test Reaction (per well)

10 µl	**2X Test Reaction PreMix** (contains MSREs)
2 µl	10 µM ***MGMT* Primer I & II**
3 µl	**DNase/RNase-free Water**
15 µl	Total Volume

Reference Reaction (per well)

10 µl	**2X Reference Reaction PreMix**
2 µl	10 µM ***MGMT* Primer I & II**
3 µl	**DNase/RNase-free Water**
15 µl	Total Volume

Make a **Test** and **Reference Reaction** master mix to accommodate the 22 DNA samples and the **Methylated & Non-methylated DNA Standards**. Add 15 µl of **Test** and **Reference Reaction** master mix to the wells of a 96-well real-time PCR plate (not provided) according to the diagram below.



2. Add 5 µl (20 ng) of the appropriate DNA sample to those designated wells (*in duplicate*) as shown in the diagram.

3. Seal the plate with sealing tape or a similar device[1]. Transfer to a 96-well real-time PCR instrument (e.g., Applied Biosystems 7500 Series, Roche LightCycler™, Bio-Rad CFX96™, Illumina Eco™ Real-Time PCR System, or similar). Proceed with Step II below.

II. *OneStep* MSRE Digestion/Real-Time PCR

Reaction conditions for the combined MSRE Digestion/Real-Time PCR processes have been optimized for direct input, digestion, and real-time amplification of DNA samples. However, annealing temperature and elongation time may need to be optimized depending on the design of the primers. The parameters below are for the supplied ***MGMT***[2] primers and should be used as a guideline when setting up your own experiment. Typically, between 40-45 cycles is recommended for the amplification of most DNA templates.

1. Set the real-time PCR instrument to excitation and emission wavelengths of (~) **465 nm** and **510 nm**, respectively.
   Note: *a setting that is compatible with SYBRgreen® should be compatible with the SYTO 9® dye in this kit*.

2. Perform real-time PCR using the following parameters.

	Temperature	Time	
MSRE Digestion*	37°C	2 hours	
Initial Denaturation	95°C	10 minutes	
Denaturation	95°C	30 seconds	40-45 cycles
Annealing**	54°C	60 seconds	
Extension	72°C	60 seconds	
Final Extension[3]	72°C	7 minutes	
Hold	4°C	> 5 minutes	

* If necessary, the MSRE Digestion can be performed at 37ºC using an incubator or similar device.

**The annealing temperature and extension time with may vary with user designed primers and the size of the amplicon. Therefore, it may be necessary to adjust and optimize these conditions.

Notes:

[1]To eliminate bubbles that may be present within the wells, spin down the plate prior to conducting real-time PCR (Step II).

[2]Refer to **Appendix III** for detailed information regarding the **Methylated** and **Non-methylated Human DNA Standard** and ***MGMT*** primers.

[3]A melt analysis can be performed after Final Extension prior to the Hold Step. The melt analysis is recommended for the detection of non-specific product and primer dimer formation.

Note:

[1]Visit our website for the ***OneStep* qMethyl™ Calculator** for convenient data analysis at:

www.zymoresearch.com/qmethylcalc

III. Data Analysis[1]

The methylation level for any amplified region can be determined using the following equation:

$$\text{Percent Methylation} = 100 \times 2^{-\Delta Ct}$$

where ΔCt = the average Ct value from the **Test Reaction** minus the average Ct values from the **Reference Reaction**

Example:

The table (below) represents actual real-time PCR data from the ***OneStep* qMethyl™** procedure using the **Human Non-methylated DNA Standard** and ***MGMT*** primers (performed in duplicate).

Ct values of **Test Reaction**	Ct values of **Reference Reaction**
33.83	29.76
33.81	29.81

To determine the methylation level of the **Human Non-methylated DNA standard,**

1. Calculate the average Ct values for **Test** and **Reference Reactions**.

Average Ct value of **Test Reaction**	Average Ct value of **Reference Reaction**
33.82	29.79

2. Determine the ΔCt by subtracting the average Ct value of **Reference Reaction** from the average Ct value of **Test Reaction.**

$$\Delta Ct = 33.82 - 29.79 = 4.03$$

3. Substitute the ΔCt value into the equation: $100 \times 2^{-\Delta Ct}$

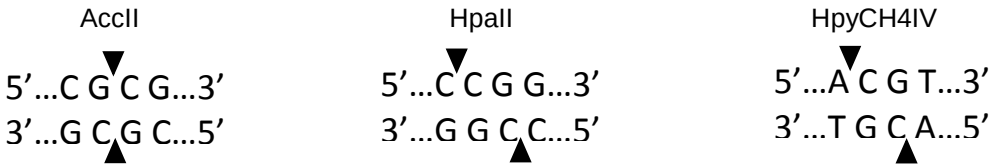
$$100 \times 2^{-4.03} = 6\%$$

Using this equation, the methylation level of the **Human Non-methylated DNA Standard** is determined to be 6 % at the region spanned by the *MGMT* primers. The actual value is ~5% as determined by bisulfite sequencing methods. Due to the low background level of methylation in Zymo's non-methylated standard cell line, the results of the calculation are within the expected limit of methylation detection (**Appendix III, page 10**).

Appendix I Methylation Sensitive Restriction Enzymes (MSREs) Featured in the *OneStep* qMethyl™ Procedure

It is important to consider the consensus sequences of those MSREs (below) when designing primers to any particular DNA region, since any particular region should contain at least two (2) MSRE sites for accurate methylation assessment using the ***OneStep* qMethyl™** procedure.

A mix of the following MSREs are present in the **Test Reaction Premix**:



Appendix II Using the *OneStep* qMethyl™ Procedure for Methylation Level Detection for Multiple Regions

The protocol on page 5 illustrates the use of the ***OneStep* qMethyl™** procedure for the evaluation of methylation levels within a *single region* from multiple DNA samples. However, methylation determination within *multiple regions* of a particular DNA sample is often required. For methylation assessment of 22 different regions in a DNA sample, the following example is provided.

1. For each region to be analyzed, setup (on ice) both a **Test Reaction** and **Reference Reaction** mixture[1].

   Protocol for Methylation Detection Levels for Multiple Regions

   Test Reaction (per well)

10 µl	**2X Test Reaction PreMix** (contains MSREs)
5 µl	DNA (or **Methylated & Non-methylated DNA Standard**) (4 ng/µl)
3 µl	**DNase/RNase-free Water**
18 µl	Total Volume

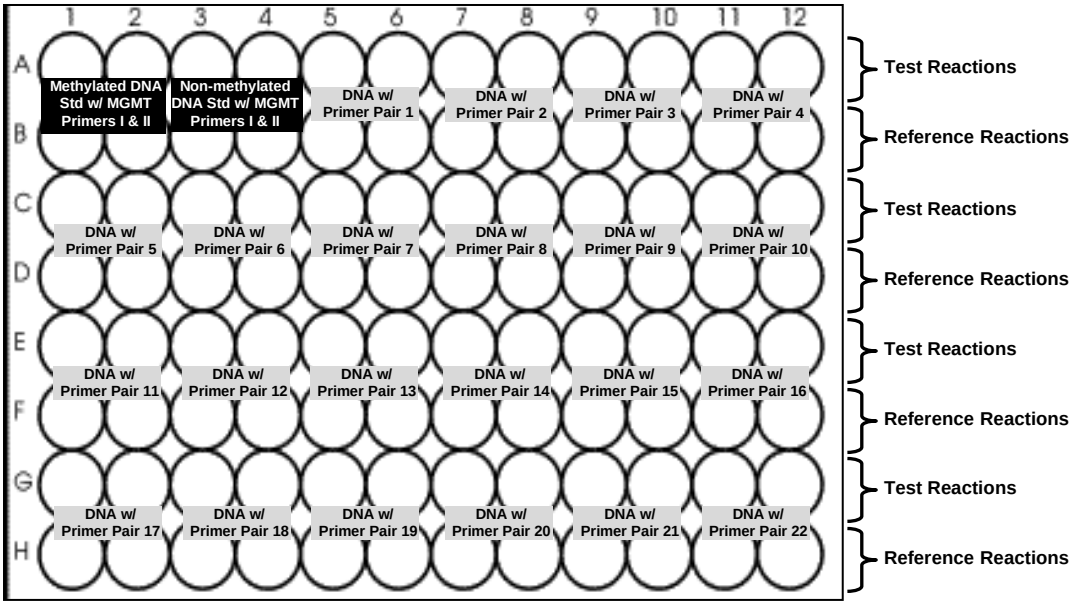
   Reference Reaction (per well)

10 µl	**2X Reference Reaction PreMix** (contains MSREs)
5 µl	DNA (or **Methylated & Non-methylated DNA Standard**) (4 ng/µl)
3 µl	**DNase/RNase-free Water**
18 µl	Total Volume

Note:

[1]The final concentration of $MgCl_2$ in the **Reference Reaction** & **Test Reaction** mixtures is 3.75 mM.

Make a **Test** and **Reference Reaction** master mix to accommodate duplicate sampling of 22 samples and the **Methylated & Non-methylated DNA Standards**. Add 18 µl of **Test** and **Reference Reaction** master mix to the wells of a 96-well real-time PCR plate according to the diagram on the following page.



2. Dilute primers to a final concentration of 10 µM in **DNase/RNase-free Water** and then add 2 µl of the appropriate primers (pre-mixed) to those designated wells (*in duplicate*) as shown in the diagram[1]. Continue with Step 3 in the standard protocol (page 6).

Note:

[1]The final concentration of primers will need to be optimized. Final concentrations shown here are at 500 nM. Final primer concentrations range between 250 nM to 800 nM.

Appendix III ` The Human Methylated & Non-methylated DNA Standards and *MGMT* Primer Set

This kit contains **Human Methylated DNA & Non-methylated DNA Standards** with an *MGMT* primer set for validating the ***OneStep* qMethyl™** system. Enough material is provided for a single run, 96-well analysis. The **Human Methylated DNA** has been enzymatically methylated at all cytosine positions comprising CG dinucleotides using M.*Sss* I methyltransferase. The **Human Non-methylated DNA** was purified from cells containing genetic knockouts of both DNMT1 and DNMT3b DNA methyltransferases and has a low level of DNA methylation (~5%).

Product Specifications

I. Human Non-Methylated DNA Standard
Source: DNA purified from HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)].
Concentration: 4 ng/µl in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Storage: -20 ˚C

II. Human Methylated DNA Standard
Source: DNA purified from HCT116 DKO cells [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
Concentration: 4 ng/µl in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Storage: -20 ˚C

III. *MGMT* Primers Set
Concentration: 10 µM in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
Volume: 20 µl of each primer
Storage: -20 ºC

MGMT Primer I: 5′ - GGT GTG AAA ACT TTG AAG GA - 3′
MGMT Primer II: 5′ - CAC TAT TCA AAT TCC AAC CC - 3′

IV. The expected amplicon for the **Human Methylated** & **Non-methylated DNA Standard** with ***MGMT* I** & **II primers** (underlined) is ~180 bp and contains four (4) MSRE sites (see sequence and diagram below)[1].

```
-1121 gggtgtgaaa actttgaagg aaaccgcgtc aagagcctgg
-1161 ctgattgtta atatcacgtt aactcagagg gccaggatac
-1201 ttgcccagac ccggagtctg cctgcaagta gcagaggaga
-1251 gctggccttg ctctgccgcg tgtctttctt cctgggccct
-1291 ctgtctcggg ttggaatttg aatagtg
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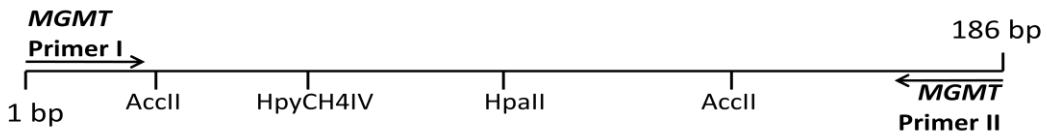

MGMT Region

Note:

[1]At least 2-4 MSRE sites should be contained in an amplicon ranging from 150 to 350 bp long. This size is range is often necessary to obtain the minimum number of MSRE sites.

Ordering Information

Product Description	Catalog No.	Kit Size
***OneStep* qMethyl™ Kit**	D5310	1 x 96 well

For Individual Sale	Catalog No.	Amount(s)
2X Test Reaction PreMix	D5310-1	0.5 ml
2X Reference Reaction PreMix	D5310-2	0.5 ml
DNase/RNase-free Water	W1001-1	1 ml

Epigenetic Products from Zymo Research

THE Epigenetics COMPANY™

Product	Description	Kit Size	Cat No. (Format)
	Bisulfite Kits for DNA Methylation Detection		
EZ DNA Methylation™ Kit	For the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5001** (spin column) **D5002** (spin column) **D5003** (shallow-well plate) **D5004** (deep-well plate)
EZ DNA Methylation-Gold™ Kit	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via heat/chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5005** (spin column) **D5006** (spin column) **D5007** (shallow-well plate) **D5008** (deep-well plate)
EZ DNA Methylation-Direct™ Kit	Features simple and reliable DNA bisulfite conversion directly from blood, tissue (FFPE/LCM), and cells without the prerequisite for DNA purification in as little as 4-6 hrs. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	**D5020** (spin column) **D5021** (spin column) **D5022** (shallow-well plate) **D5023** (deep-well plate)
EZ DNA Methylation-Startup™ Kit	Designed for the first time user requiring a consolidated product to perform DNA methylation analysis. Includes technologies for sample processing, bisulfite treatment of DNA, and PCR amplification of "converted" DNA for methylation analysis.	1 Kit	**D5024**
	Methylated DNA Standards		
Universal Methylated DNA Standard	pUC19 plasmid DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 set	**D5010**
Universal Methylated Human DNA Standard	Human (male) genomic DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 set	**D5011**
Universal Methylated Mouse DNA Standard	Mouse (male) DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 set	**D5012**
	Region-Specific DNA Methylation Screening		
***OneStep* qMethyl™ Kit**	Single step real-time PCR procedure for bisulfite-free determination of DNA methylation status.	1x96 Rxns.	**D5310**
OneStep* qMethyl™-*Lite	Single step real-time PCR procedure for bisulfite-free determination of DNA methylation status. Omits inclusion of fluorescent dye allowing for probe-based detection.	1x96 Rxns.	**D5311**
	Epiquest™ Genomic Services *For more information, visit our webpage or contact us directly*.		
Epiquest™ Next-Gen Sequencing DNA Methylation Analysis Simplify biomarker discovery with our Genome-Wide DNA Methylation Analysis Platform that combines Zymo's well-established bisulfite technologies with Next-Gen sequencing for the most comprehensive DNA methylation analysis service available.			
Global 5-Methylcytosine and 5-Hydroxymethylcytosine Quantitation Implementing an optimized LC/MS protocol, this service yields sensitive differentiation and quantitation of epigenetic markers in a total genomic context, providing rapid and clear snapshots of the epigenomic landscape. Flexible service options allow for direct tissue processing, locus validation, and more.			
	Hydroxymethylation Detection		
Quest 5-hmC Detection Kit™	5-hmC positions in DNA are protected by a glucosyltransferase reaction, preventing restriction by glucosyl-sensitive restriction endonucleases. 5-hmC positions in the treated DNA can then be mapped and quantitated by methods including PCR, qPCR, and IP-seq.	25 Rxns. 50 Rxns. 25 Rxns. 50 Rxns.	**D5410** (spin column) **D5411** (spin column) **D5415** (shallow-well plate) **D5416** (deep-well plate)
QuestTaq™ Premix	Supplied as a 2X master mix, this polymerase is ideal for the unbiased amplification of epigenetically-modified templates, including methylated, hydroxymethylated, and glucosylated DNA. Also available as a master mix for qPCR applications.	50 Rxns. 200 Rxns. 50 Rxns. 200 Rxns.	**E2050** **E2051** **E2052** (for qPCR) **E2053** (for qPCR)
DNA Degradase™ **DNA Degradase Plus™**	Whole genomic DNA can be treated with these enzyme cocktails for processing to individual nucleotides (Degradase™) or nucleosides (Degradase Plus™) for interrogation in chromatographic and spectroscopic methods including TLC, LC/MS, MALDI-TOF, and more.	500 U 2000 U 500 U 2000 U	**E2016** **E2017** **E2020** **E2021**
Anti-5-Hydroxymethylcytosine Polyclonal Antibody	Polyclonal antibody has been engineered to maximize sensitivity to low amounts of hydroxymethylated gDNA while minimizing crossreactivity with unmodified or methylated cytosine residues. The antibody is suitable for use in ELISA, IP, and immunohistochemical labeling.	50 µg 200 µg	**A4001-50** **A4001-200**
Quest 5-hmC ELISA Kit™	Streamlined workflow for both the direct and relative quantitation of 5-hmC, in a global genomic context, with a robust colorimetric readout.	1 plate 2 plates	**D5425** **D5426**