ZYMO RESEARCH

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INSTRUCTION MANUAL

Quest 5-hmC Detection Kit™

Catalog Nos. **D5410** & **D5411**

Highlights

- Method to distinguish 5-hydroxymethylcytosine in sequence- and locus-specific context within DNA.
- Convenient and reliable *single tube reaction format*.
- DNA is eluted in water or low salt buffer and is suitable for analysis by a variety of downstream applications.

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ZYMO RESEARCH CORP.

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Product Contents

Quest 5-hmC Detection Kit™ (Kit Size)	**D5410** (25 Rxns.)	**D5411** (50 Rxns.)	**Storage Temp.**
5-hmC Glucosyltransferase (2 units/µl)	50 µl	100 µl	-20 °C
10X 5-hmC GT Reaction Buffer	1 ml	1 ml	-20 °C
10X UDPG (Uridine Diphosphoglucose) [1 mM]	600 µl	600 µl	-20 °C
MspI Restriction Enzyme (10 units/µl)	75 µl	150 µl	-20 °C
Quest 5-hmC Control DNA	300 ng	300 ng	-20 °C
Quest qPCR Primers 1 & 2 [20µM]	1 set	1 set	-20 °C
DNA Clean & Concentrator™- 5	50 preps	50 preps	RT
Instruction Manual	1	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.

*RT- Room Temperature

Specifications

- **5-hmC Glucosyltransferase** – 5-hmC Glucosyltransferase is guaranteed for one year at -20°C. Long term storage at -80°C is recommended. Avoid multiple freeze thawing.
- **MspI Restriction Enzyme** – MspI is guaranteed for one year at -20°C. Long term storage at -80°C is recommended. Avoid multiple freeze thawing.
- **Quest 5-hmC Control DNA** – A 90 bp DNA standard used for qPCR. [10 ng/µl] in storage buffer (10 mM Tris-HCl; 0.1 mM EDTA; pH 8.0) supplied in 30 µl volume. See **Appendix I** (pg. 9) for more details.
- **Quest qPCR Primers** – Control primers specific to Quest 5-hmC Control DNA (above) validated for qPCR. See **Appendix I** (pg. 9) for detailed information.

Product Description

Background

5-Hydroxymethylcytosine (^{5hm}C), also known as the "6th base", is a newly discovered epigenetic modification. Although it was first identified in bacteriophages, its role was thought to be limited to protecting the phage genome from host induced restriction endonucleases [1]. Interestingly, 5-hydroxymethylcytosine has recently been found in embryonic stem cells, the brain, as well as numerous other organs [2,3]. Even though its presence has been confirmed, its biological role remains elusive. It has been proposed that 5-hydroxymethylcytosine may play a role in DNA demethylation (5-methylcytosine) or be involved in another layer of gene expression regulation.

To date, studies of 5-hydroxymethylcytosine has been limited to global quantification or characterization studies. The reason being is that the "gold standard" for 5-methylcytosine detection, bisulfite DNA analysis, is unable to effectively distinguish 5-methylcytosine from 5-hydroxymethylcytosine.

Product Overview

The **Quest 5-hmC Detection Kit™** from Zymo Research allows for sequence specific detection of 5-hydroxymethylcytosine within DNA using a simple and efficient reaction setup. Utilizing a robust and highly specific **5-hmC Glucosyltransferase** enzyme, 5-hydroxymethylcytosine in DNA is specifically tagged with a glucose moiety yielding a modified base, glucosyl-5-hydroxymethylcytosine (Figure 1).

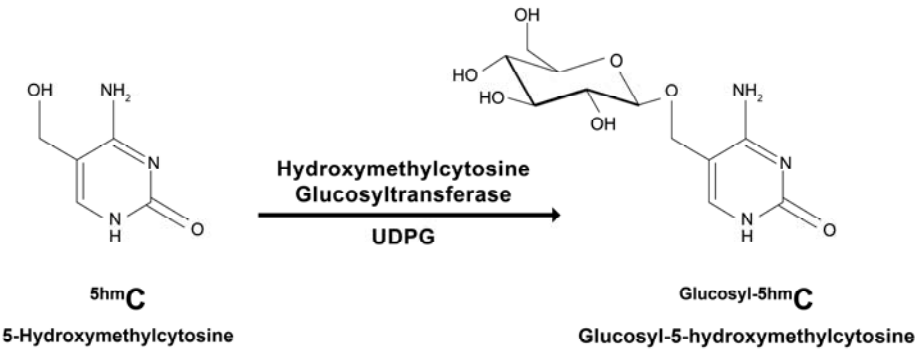


Figure 1: 5-hmC Glucosyltransferase transfers a glucose moiety from uridine diphosphoglucose (UDPG) onto preexisting 5-hydroxymethylcytosines within DNA.

After glucosylation of 5-hydroxymethylcytosine, digestion of DNA with "glucosyl-5-hydroxymethylcytosine sensitive" restriction endonucleases (GSREs) allow differentiation of 5-methylcytosine from 5-hydroxymethylcytosine according to the context of a GSRE's recognition sequence (see Table 1). GSREs can efficiently digest DNA when cytosine, 5-methylcytosine, or 5-hydroxymethylcytosine is within their recognition sequence. However, if 5-hydroxymethylcytosine is glucosylated (i.e., glucosyl-5-hydroxymethylcytosine), GSREs can no longer digest the DNA (Figure 3). Exploitation of this sensitivity to glucosyl-5-hydroxymethylcytosine facilitates the effective detection 5-hydroxymethylcytosine using a number of downstream applications (e.g., qPCR, NextGen Sequencing, Southern blotting, array, etc.). QPCR is recommended to assess locus-specific context of 5-hydroxymethylcytosine within the DNA (see Protocol, "Step 4 – Locus-specific 5-hmC Detection via qPCR", pgs. 6-8).

References:

1. Wyatt GR, Cohen SS (1952) . A new pyrimidine base from bacteriophage nucleic acids. Nature 170 (4338) 1072-3, 1952.

2. Riaucionis S., Heintz N., (2009). The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324 (5929): 929-30.

3. Tahiliani M., et al. (2009). "Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1". Science 324 (5929): 930–35

4. Huang Y., et al. (2010). "The behaviour of 5-hydroxymethylcytosine in bisulfite sequencing". PLoS One. 2010 Jan 26;5(1)

5. Szwagierczak A., et al. (2010), "Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA" Nucleic Acids Res.

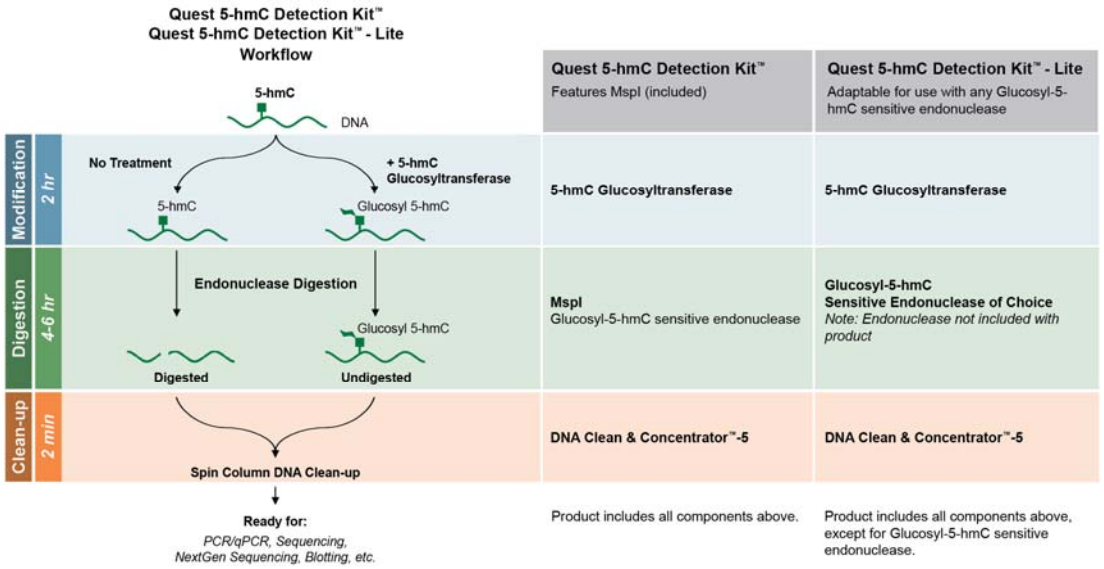


Figure 2: Quest 5-hmC Detection Kit™ and Quest 5-hmC Detection Kit™- Lite Workflows
Treatment of DNA containing ^{5hm}C with 5-hmC Glucosyltransferase specifically adds a glucose moiety yielding glucosyl-5-hydroxymethylcytosine. Subsequent digestion with GSREs (see Table 1 below) will specifically cleave DNA having cytosine, 5-methylcytosine, or 5-hydroxymethylcytosine in the recognition sequence. Glucosyl-5-hydroxymethylcytosine in the DNA will remain uncleaved.

MspI is included in the Quest 5-hmC Detection Kit™. MspI is a GSRE that cleaves DNA when cytosine, 5-methylcytosine, or 5-hydroxymethylcytosine is located at the inner C position (underlined) within its recognition sequence (i.e., C**C**GG). Glucosylation at this position (i.e., glucosyl-5-hydroxymethylcytosine) will inhibit cleavage by the enzyme.

Table 1: $^{Glucosyl\text{-}5hm}$C Sensitive Restriction Endonucleases - GSREs

GSRE	Recognition Sequence
MspI (included w/ this kit)	C<u>C</u>GG
GlaI	GCGC
Csp6I	GTAC
HaeIII	GGC<u>C</u>
Taq$^{\alpha}$I	TCGA
MboI	GATC
McrBC	$R^mC(N_{40\text{-}3000})R^mC$

* ***Cat. Nos. D5415*** & ***D5416 - Quest 5-hmC Detection Kit™- Lite*** can be used with any GSRE of your choice for greater coverage and detection in CpG and non-CpG contexts (includes all reagents except for the GSRE).

** Detection of 5-hmC with MspI and HaeIII is <u>only</u> at the indicated "C" position (underlined and italicized).*

** Taq$^{\alpha}$I and MboI display incomplete sensitivity to $^{Glucosyl\text{-}5hm}$C. Titration of the enzyme amount and incubation time will be required to yield optimal results.*

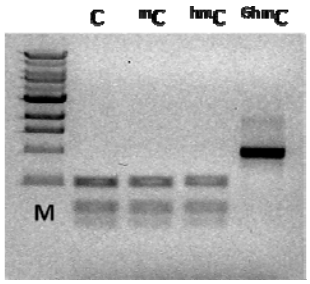


Figure 3: 5-hmC Glucosyltransferase inhibits cleavage of ^{5hm}C DNA by the GSRE Csp6I.
DNA with all cytosines as: unmodified (C) [Cat No. D5405-1], 5-methylcytosine (^{m}C) [Cat No. D5405-2], 5-hydroxymethylcytosine (^{hm}C) [Cat No. D5405-3], or glucosyl-5-hydroxymethylcytosine (^{Ghm}C) was digested with 4 units of Csp6I for 2 hours at 37°C and resolved in a 0.8% w/v agarose/TAE/EtBr gel. "M" is 1kb DNA ladder (Zymo Research Corp.).

Analysis of ^{5hm}C Sites

After processing DNA with the Quest 5-hmC Detection Kit™, subsequent analysis of ^{5hm}C sites is achieved with qPCR, ultra-deep sequencing, Southern blotting, or microarray.

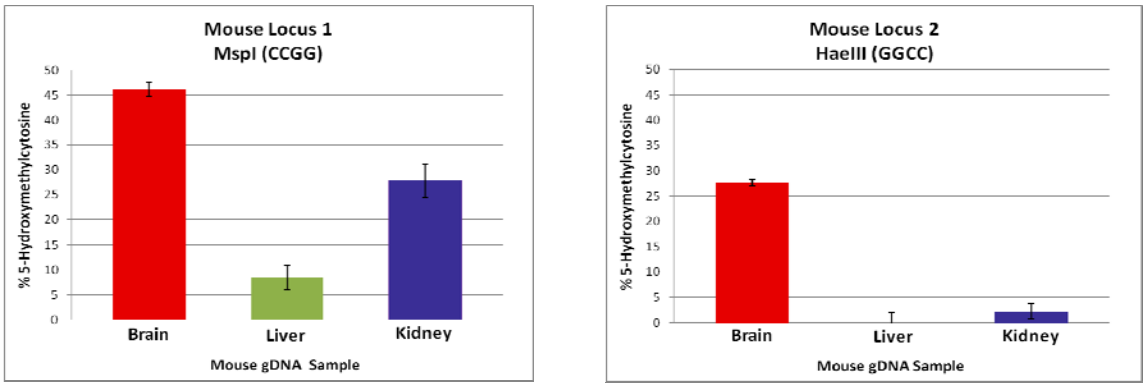
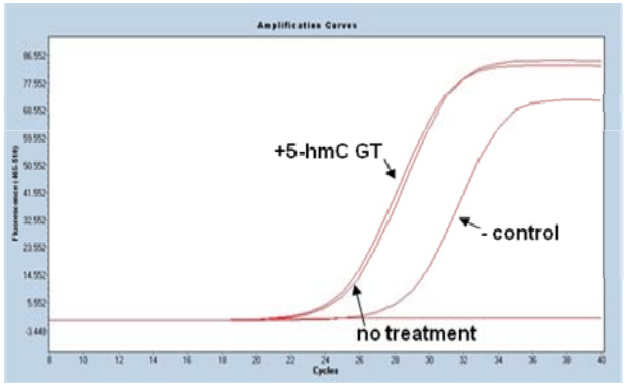


Figure 4: Locus Specific 5-hydroxymethylcytosine Detection
Genomic DNA from **Mouse 5-hmC & 5-mC DNA Set** [Zymo Research Cat. No. D5019] was processed according to Quest 5-hmC Detection Kit™ protocol. qPCR results were quantified as percentage of 5-hydroxymethylcytosine at each loci. 5-hydroxymethylcytosine was detected at Locus 1 and Locus 2 with the use of MspI (CCGG) and HaeIII (GGCC), respectively. Use of HaeIII allows for greater coverage as well as detection of 5-hmC in CpG and non-CpG contexts.

QPCR is recommended for the interrogation of 5-hydroxymethylcytosine at any particular locus (refer to Figure 4 and 5). The relative amplification efficiencies (Cp values) between GSRE-digested, glucosylated DNA (+5-hmC GT) and GSRE-digested unglucosylated DNA (- control) is used to verify the presence of 5-hmC at any particular locus. For example, a DNA template possessing 5-hmC (underlined) at a single MspI site (C**C**GG) was processed according to the protocol (see pg. 6-8). Following (+/-) glucosylation and digestion with MspI, DNA was amplified using qPCR (**Quest*Taq*™ qPCR PreMix**) with primers that flanked the MspI recognition site. The presence of 5-hmC at the MspI site in the DNA results in a higher amplification efficiency for the glucosylated sample (lower Cp value, e.g., +5-hmC GT) relative to the unglucosylated one (higher Cp value, e.g., - control). An unglucosylated, undigested template (e.g., no

Quest*Taq*™ qPCR PreMix (Cat. Nos. E2052 & E2053) has been optimized for the non-biased amplification of cystosine, 5-methylcytosine, and 5-hydroxymethylcytosine, and glucosyl-5-hydroxymethylctosine containing DNA.

treatment) sample DNA establishes the level (i.e., Cp value) representative of complete hydroxymethylation at the interrogation site.



MspI Digestion qPCR

Sample	Cp Value
no treatment	25.1
- control	28.5
+5-hmC Glucosyltransferase	24.9

Figure 5: Detection of 5-hmC by qPCR
DNA template with 5-hmC at the inner C position of the MspI site (C**C**GG) was processed according to protocol (+/- glucosylation and MspI digested). Differences in qPCR amplification efficiencies (Cp values) between "- control" (unglucosylated) and "+5-hmC GT" (glucosylated) samples indicates the presence of 5-hmC. "no treatment" DNA control establishes the level (i.e., Cp value) representative of complete hydroxymethylation at the interrogation site.

Notes:
[1]To ensure the glucosylation reaction is complete, it is recommended that:

a. A higher enzyme to DNA ratio is used (e.g., use 4 units of 5-hmC Glucosyltransferase:1 µg of DNA).

b. Extend incubation times to ≥2 hours at 37°C.

[2]Alternatively, other GSREs (e.g., Csp6I, HaeIII) can be used in lieu of or together with MspI and are compatible with the *single tube reaction format* given in the protocol. GlaI is a methylation dependent GSRE. Test DNA *must* be fully methylated with CpG and GpC DNA methyltransferase *in vitro* prior to cleavage with GlaI. GlaI is compatible with the *single tube reaction format* but cannot be used in conjuction with other GSREs. See product instructions for details.

Protocol

The following protocol describes a streamlined procedure for ^{5hm}C detection in DNA. DNA samples are first modified via glucosylation of ^{5hm}C within DNA and then digested with MspI: performed conveniently in a *single tube reaction format*. A unique low elution spin column is featured for clean-up of ultra-pure DNA which is then used in qPCR for locus-specific analysis of ^{5hm}C.

The provided Quest 5-hmC Control DNA is for gauging glucosylation and GSRE reaction efficiencies via qPCR. Processing of the Quest 5-hmC Control DNA with the protocol provided below should result in different amplification efficiencies (Cp values) during qPCR of glucosylated and unglucosylated samples (reference Figure 5, pg. 5).

Importantly, the procedure is also compatible with other GSREs (please see Table 1, pg. 3 & **Appendix III**, pg. 10), and other downstream molecular analysis procedures including NextGen sequencing, Southern blotting, array, etc.

Step 1 – Glucosylation Reaction[1]

Divide equal amounts of each DNA to be tested into each of the two separate reaction setups (**A** & **B**) below. Reaction mixtures containing Quest 5-hmC Control DNA should be set up in parallel (separately) to test DNAs.

A. + Glucosylation Reaction Setup

Test DNA [100-500 ng] or Quest 5-hmC Control DNA [20-50 ng]	x µl
10X 5-hmC GT Reaction Buffer	5 µl
10X UDPG [1mM]	5 µl
5-hmC GT Enzyme (2 units/µl)	2 µl
ddH2O	x µl
Total	**50 µl**

B. - Glucosylation Reaction Setup

Test DNA [100-500 ng] or Quest 5-hmC Control DNA [20-50 ng]	x µl
10X 5-hmC GT Reaction Buffer	5 µl
10X UDPG [1mM]	5 µl
5-hmC GT Enzyme (2 units/µl)	--
ddH2O	x µl
Total	**50 µl**

Incubate reaction mixtures with DNA at 37°C for ≥ 2 hours.

Step 2 – Digestion w/ MspI[2] (provided)

Following the incubation above, add 30 units of MspI enzyme directly to each (+Glucosylation and -Glucosylation) reaction mixture. Incubate at 37°C for ≥ 4 hours.

Note: *Extended digestion times and/or additional MspI may be necessary for complete digestion of DNA which is important for subsequent analysis.*

Step 3 – Spin Column Clean-up

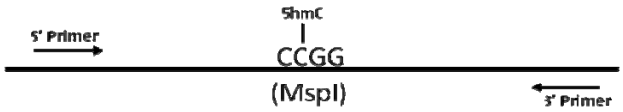
Add a 5:1 ratio of DNA Binding Buffer to the reaction mixtures (e.g., 250 µl DNA Binding Buffer to each 50 µl reaction mixture) and then proceed directly to **Step 2** in the "Protocol" section of the included **DNA Clean & Concentrator™- 5 kit** (Cat No. D4003).

Following elution of the DNA, qPCR is recommended for locus-specific 5-hmC analysis (see Step 4 below).

Step 4 – Locus-specific 5-hmC Detection via qPCR

Overview:

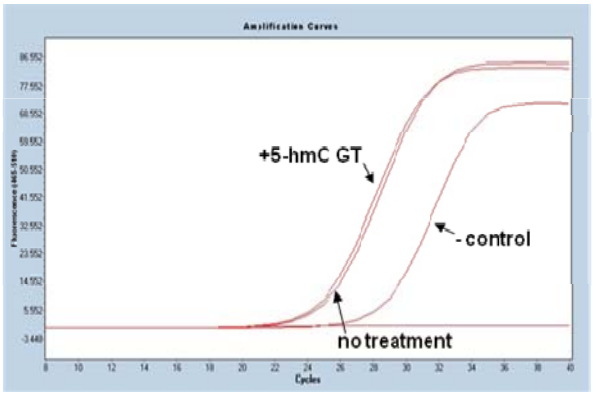
For effective analysis of 5-hmC by qPCR, primers must be designed that flank the GSRE recognition site (interrogation site) of interest (see figure below).



Also, for each locus, a proper control will need to be included for accurate detection of 5-hmC by qPCR (see Figure 6, below). This can be achieved by adding test DNA to the qPCR that has not been processed according to steps 1-3 (above).

Note: Other procedures can be used instead of qPCR for 5-hmC site interrogation including NextGen sequencing, Southern blotting, and array.

This "no treatment" DNA will establish the level (i.e., Cp value) that is representative of complete hydroxymethylation at the interrogation site. Conversely, the "- control" (unglucosylated sample, Step 1, reaction **B**, pg. 6) will indicate a level of no hydroxymethylation at the site. The "+5-hmC Glucosyltransferase" (glucosylated sample, Step 1, reaction A) Cp value should be somewhere between the two, i.e., "no treatment" and "- control" depending on the extent of hydroxymethylation at the interrogation site. *Importantly, it should be noted that GSRE digested unglucoslyated DNA (i.e., "- control") will always yield a signal albeit at a higher Cp value than the other samples.*



MspI Digestion qPCR

Sample	Cp Value
no treatment	25.1
- control	28.5
+5-hmC Glucosyltransferase	24.9

Figure 6: Detection of 5-hmC by qPCR

qPCR:

The parameters below are suggested for qPCR using Quest*Taq*™ qPCR PreMix (Cat. Nos. E2052 & E2053) (see considerations below) and should be used as a guideline when setting up your own experiment. Typically, ~40 cycles are recommended for the amplification/analysis of most DNA templates.

Suggested Reaction Setup

Test DNA [10-50 ng] or Quest 5-hmC Control DNA [2-20 pg]	x µl
2X Quest*Taq*™ qPCR PreMix*	10 µl
Primer 1 [20µM]	0.4 µl
Primer 2 [20µM]	0.4 µl
ddH2O	x µl
Total	**20 µl**

*2X Quest*Taq*™ qPCR PreMix contains Quest*Taq*™ DNA polymerase, dNTPs, Syto® 9 dye, and reaction buffer. Syto® 9 dye has excitation/emission maxima ~480 nm/520 nm, respectively.

1. Set the qPCR instrument (e.g., Applied Biosystems 7500 Series, Roche LightCycler™, Bio-Rad CFX96™, Illumina Eco™ Real-Time PCR System, or similar) to excitation and emission wavelengths of (~) **465 nm** and **510 nm**, respectively.

2. Perform qPCR using the following parameters**.**

Step 1 - Denaturation	95°C	1 min
Step 2 - Denaturation	95°C	30 sec
Step 3 - Annealing	50°C*	15 sec
Step 4 - Elongation	72°C	15 sec*
Step 5 - Cycling	Go to Step 2	40X
Step 6 - Final Elongation	72°C	5 min

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

Considerations for qPCR:
DNA polymerases can exhibit biases to epigenetically-modified DNA templates including those that contain 5-methylcytosine, 5-hydroxymethylcytosine, or glucosyl-5-hydroxymethylctosine. Quest*Taq*™ from Zymo Research has been optimized for the *non-biased* amplification of epigenetically modified DNA. Quest*Taq*™ is not included with this kit and must be purchased separately either as **Quest*Taq*™ qPCR PreMix** (Cat. Nos. E2052 & E2053) or as **Quest*Taq*™ PreMix** (Cat. Nos. E2050 & E2051).

Appendices

I. Quest 5-hmC Control DNA

The Quest 5-hmC Control DNA is provided for gauging glucosylation and GSRE reaction efficiencies via qPCR. Incorporated into the Quest 5-hmC Control DNA are 3 GSRE sites (MspI, HaeIII and Csp6I) which can be used for digestion. The Quest 5-hmC Control DNA is a 90 bp, double stranded DNA that is partially hemi-5-hydroxymethylated. All cytosine residues in the sequence below that are highlighted in grey (i.e., top strand only) are hydroxymethylated.

Sequence of Quest 5-hmC Control DNA:

```
1                                                                          90
caaggatcgctcgcggctcttaGGCCGGtaactgtctgcagctctgagGTACgcatggattgtaggcgccgccctataccttgtctgcct
gttcctagcgagcgccgagaatCCGGCCattgacagacgtcgagactcCATGcgtacctaacatccgcggcgggatatggaacagacgga
```

Note: All cytosines in the highlighted sequence are 5-hydroxymethylcytosine. MspI (CCGG), HaeIII (GGCC) and Csp6I (GTAC) sites are capitalized and underlined. MspI and HaeIII sites overlap.

Sequence of Quest qPCR Primers:

Quest qPCR Primer 1	5′- caaggatcgctcgcggctctta -3′
Quest qPCR Primer 2	5′- aggcagacaaggtatagggcg -3′

II. Glucosylation of 5-hmC in DNA

Use the protocol below to completely glucosylate 5-hmC in DNA (without the GSRE digestion and qPCR analysis steps given in the standard protocol, pgs. 5-8).

Note: *This can be used for the quantification of global ^{5hm}C via isotope labeling using Uridine Diphosphate Glucose [Glucose-^{14}C(U)] PerkinElmer (Ref.5, pg. 2)*

Glucoslyation Reaction Setup

DNA [100-500 ng]	10 µl
10X 5-hmC GT Reaction Buffer	5 µl
10X UDPG [1mM]	5 µl
5-hmC GT Enzyme (2 units/µl)	2 µl
ddH2O	28 µl
Total	**50 µl**

5-hmC in DNA should be fully glucosylated after incubating the reaction at 37°C for ≥ 2 hours. DNA can be recovered using the clean-up procedure in the protocol (pg. 6, Step 3).

III. Digestion of Glucosylated-5-hmC DNA using other GSREs

The Quest workflow is adaptable for use with those GSREs listed in Table 1, pg. 3. For the GSREs MspI, GlaI, Csp6I, and HaeIII, the *single tube reaction format* given in the standard protocol (pgs. 5-8) should be used. However, TaqI, MboI, and McrBC are incompatible with the *single tube reaction format* and the protocol given below should be used with these enzymes instead.

Step 1 – Glucosylation Reaction

Divide equal amounts of each DNA to be tested into each of the two separate reaction setups (**A** & **B**) below. Reaction mixtures containing Quest 5-hmC Control DNA should be set up in parallel (separately) to test DNAs.

A. + Glucoslyation Reaction Setup

Test DNA [100-500 ng] or Quest 5-hmC Control DNA [20-50 ng]	x µl
10X 5-hmC GT Reaction Buffer	5 µl
10X UDPG [1mM]	5 µl
5-hmC GT Enzyme (2 units/µl)	2 µl
ddH2O	x µl
Total	**50 µl**

B. Glucoslyation Reaction Setup

Test DNA [100-500 ng] or Quest 5-hmC Control DNA [20-50 ng]	x µl
10X 5-hmC GT Reaction Buffer	5 µl
10X UDPG [1mM]	5 µl
5-hmC GT Enzyme (2 units/µl)	--
ddH2O	x µl
Total	**50 µl**

Incubate reaction mixtures with DNA at 37°C for ≥ 2 hours.

Step 2 – Spin Column Clean-up

Add a 5:1 ratio of DNA Binding Buffer to the reaction mixtures (e.g., 250 µl DNA Binding Buffer to each 50 µl reaction mixture) and then proceed directly to **Step 2** in the "Protocol" section of the included **DNA Clean & Concentrator™- 5 kit** (Cat No. D4003).

Following elution of the DNA, proceed to "Step 3 – GSRE Digestion" (below).

Step 3 – GSRE Digestion

Eluted DNA from the previous step should be digested with GSRE following supplier's recommended conditions.

Note: *Overdigestion of DNA is recommended. Extended digestion times and/or additional units of GSRE may be necessary for complete digestion of DNA which is important for subsequent analysis.*

Step 4 – Spin Column Clean-up

Add a 5:1 ratio of DNA Binding Buffer to the reaction mixtures (e.g., 250 µl DNA Binding Buffer to each 50 µl reaction mixture) and then proceed directly to **Step 2** in the "Protocol" section of the included DNA Clean & Concentrator™- 5 kit (Cat No. D4003). Following elution of the DNA, qPCR is recommended for locus-specific 5-hmC analysis (see Protocol, Step 4, pg. 6-8).

Ordering Information

Product Description	Catalog No.	Kit Size
Quest 5-hmC Detection Kit™	D5410 D5411	25 Rxns. 50 Rxns.
Quest 5-hmC Detection Kit™-Lite	D5415 D5416	25 Rxns. 50 Rxns.
Quest*Taq*™ PreMix	E2050 E2051	50 Rxns. 200 Rxns.
Quest*Taq*™ qPCR PreMix	E2052 E2053	50 Rxns. 200 Rxns.
Human Matched DNA Set	D5018	2 x 5 µg
Mouse ^{5hm}C & ^{5m}C DNA Set	D5019	4 x 5 µg
5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	D5405	3 x 2 µg
DNA Degradase™	E2016 E2017	500 units 2,000 units
DNA Degradase Plus™	E2020 E2021	250 units 1,000 units
5-hmC Glucosyltransferase	E2026 E2027	100 units 200 units
5-Hydroxymethyl dCTP [100 mM]	D1045	10 µmol
5-Hydroxymethylcytosine dNTP Mix [10 mM]	D1040	2.5 µmol
5-Methyl dCTP [10 mM]	D1035	1 µmol
5-Methylcytosine dNTP Mix [10 mM]	D1030	2.5 µmol

Epigenetics Products From Zymo Research

Product	Description	Prep/Format	Catalog
Bisulfite Kits for DNA Methylation			
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**
EZ Bisulfite DNA Clean-up Kit™	Desulfonation and purification of DNA from any "homebrew" or commercially derived reaction mixture containing bisulfite.	50 Preps. 200 Preps. 2x96 Preps. 2x96 Preps.	**D5025** (spin column) **D5026** (spin column) **D5027** (shallow-well plate) **D5028** (deep-well plate)
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**
Other Epigenetic Products			
Zymo*Taq*™ DNA Polymerase	Zymo*Taq*™ "hot start" DNA Polymerase is specifically designed for the amplification of "difficult" DNA templates including: bisulfite-treated DNA for methylation detection. The product generates specific amplicons with little or no by-product formation. Available either as a single buffer premix or as a polymerase system with components provided separately.	50 Rxns. 200 Rxns. 50 Rxns. 200 Rxns.	**E2001** (system) **E2002** (system) **E2003** (premix) **E2004** (premix)
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	Mouse monoclonal antibody developed to facilitate the differentiation between methylated and non-methylated cytosines in DNA. Can be used in immunoprecipitation-based procedures including Methylated DNA Immunoprecipitation (MeDIP).	50 µg/50 µl 200 µg/200 µl	**A3001-50** **A3001-200**
Methylated-DNA IP Kit	IP with a highly specific anti-5-methylcytosine monoclonal antibody. Designed for the enrichment of 5-methylcytosine-containing DNA from any pool of fragmented genomic DNA for use in genome-wide methylation analysis.	10 Rxns.	**D5101**
ChIP DNA Clean & Concentrator™	Clean and concentrate DNA from any reaction or "crude" preparation in 2 min. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50 Preps. 50 Preps.	**D5201** (uncapped column) **D5205** (capped column)

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