5-hmC Glucosyltransferase

Catalog No. 14-1047

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Not for use in diagnostic procedures

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Introduction

Methylation of DNA occurs on the 5 carbon position of cytosine to form 5-methylcytosine (5-mC). This reaction is catalyzed by DNA methyltransferases (DNMT) and occurs primarily in the context of CpG dinucleotides. Patterns of methylated cytosines have been linked to gene silencing, embryonic development, gene imprinting, chromosome stability, and cancer.

5-mC may become hydroxylated by TET enzymes to form 5-hydroxymethylcytosine (5-hmC), which has different roles from 5-mC in gene regulation and biological function. Hence, assays and applications that can sensitively detect and distinguish between 5-mC and 5-hmC modifications are essential to gene methylation studies. The 5-hmC glucosyltransferase is a highly active enzyme that selectively glycosylates pre-existing 5-hmC residues in DNA, and enables global quantification of 5-hmC[1], or detection of 5-hmC in a specific DNA sequence or locus.

Glucosyltransferase Enzymatic Activity

5-hmC glucosyltransferase catalyzes the transfer of a glucose moiety from uridine diphosphoglucose (UDPG) to 5-hmC residues in DNA to form glucosyl-5-hydroxymethylcytosine (glucosyl-5-hmC). 5-hmC Glucosyltransferase *does not* modify cytosines or methylated cytosines (5-mC). Glucosylation of 5-hydroxymethylcytosine can be used for sequence-specific, locus-specific, or global quantification of 5-hydroxymethylcytosine.

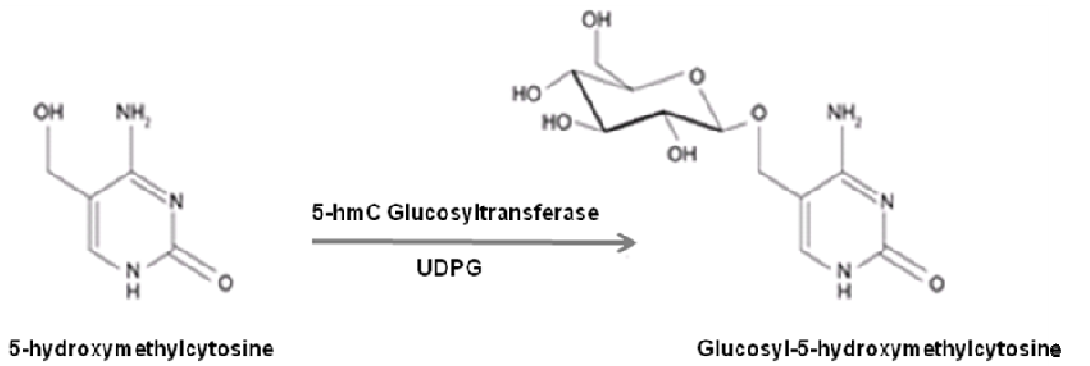

Figure 1: 5-hmC Glucosyltransferase catalyzes the transfer of a glucose moiety from UDPG to 5-hmC.

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Components Provided and Unit Definition

This product contains three reagents necessary for a standard 5-hmC Glucosyltransferase reaction: a highly active 5-hmC Glucosyltransferase enzyme, 10X Glucosyltransferase Reaction Buffer, and 10X Uridine Diphosphoglucose.

Component No.	Description	Quantity
CS211003	5-hmC Glucosyltransferase Enzyme, 2 units/µL Store at -20℃	200 Units
CS211004	10X Glucosyltransferase Reaction Buffer Store at -20℃	1 mL
CS211005	10X UDPG (1 mM Uridine Diphosphoglucose) Store at -20℃	300 µL

5-hmC Glucosyltransferase is presented in a concentration of 2 Units/µL. One unit is defined as the quantity of 5-hmC Glucosyltransferase required to glycosylate 1 µg of 5-hmC DNA Standard (Cat. No. S8005) and prevent GlaI digestion in a reaction incubated for 1 hour at 30℃.

Additional Materials Required

- 1.5 mL microcentrifuge tubes
- Heat Block

Storage

Store at -20℃ for up to 1 year. 5-hmC Glucosyltran sferase can be stored for longer periods at -80℃. Avoid multiple freeze/thaw cycles.

Protocol

A. Important Experimental Guidelines

1. To ensure that 5-hmC DNA substrate is completely glycosylated, the following are recommended:
    a. Use appropriate enzyme unit:DNA ratio; for example, if glycosylating 1 µg of DNA, then use 4 units of 5-hmC Glucosyltransferase.
    b. Extend incubation at 30℃ for longer than 2 hrs.

2. For best performance, and to optimize modification, the following are recommended:
    a. Use appropriate controls for this assay such as 5-hmC DNA Standard (Cat. No. S8005), or perform a "no enzyme" negative control reaction.
    b. Enzymes are sensitive to temperature. Ensure that the 5-hmC Glucosyltransferase enzyme is stored on ice during the preparation steps.

B. Standard 5-hmC Glucosyltransferase Reaction Protocol

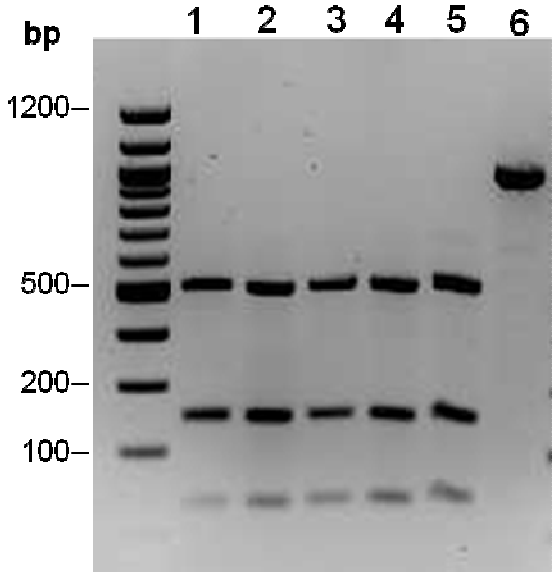
1. Remove the kit components from the freezer, allow to thaw, and store on ice.
2. Add the following volumes of each reagent to your reaction tubes in the order listed.

Reagent	**Volume (µL)**
ddH_2O	28
DNA [10-100ng/µl]	10
10X Glucosyltransferase Reaction Buffer	5
10X UDPG	5
5-hmC GT Enzyme (2 Units/µl)	2
Total	**50**

3. Mix well by gentle pipetting then incubate reactions at 30℃ on a heat block for at least 2 hrs (ca n be left overnight, if necessary).
4. Additional cleanup steps after glycosylation are typically not required. However, cleanup may be necessary before some downstream applications.

Representative Data

Glycosylation of 5-hmC by 5-hmC Glucosyltransferase (bGT) was confirmed by agarose gel analysis of DNA fragments digested by the selective restriction endonuclease CviQ1. CviQ1 cleaves methylated, hydroxymethylated, and unmodified cytosines, but cannot cleave glucosyl-5-hmC.



Lane 1	Unmodified Cytosine -bGT
Lane 2	Unmodified Cytosine +bGT
Lane 3	5'-Methylcytosine -bGT
Lane 4	5'-Methylcytosine + bGT
Lane 5	5'-Hydroxymethylcytosine -bGT
Lane 6	5'-Hydroxymethylcytosine +bGT

Figure 2: 250 ng each of DNA Standard (Cat. No. S8005) containing only 5'-Methylcytosine, 5'-Hydroxymethylcytosine, or unmodified cytosine was subjected to glycosylation for 5 hrs as described in the protocol (see page 3). A control reaction (containing no 5-hmC Glucosyltransferase(bGT)) was also performed for each DNA standard. After glucosylation, 30 Units of the restriction endonuclease CviQ1 was added to each reaction and incubated overnight. DNA from each reaction was then purified and products were analyzed on 2% agarose gel as shown. Lane 1: Cytosine -bGT; Lane 2: Cytosine- +bGT; Lane 3: 5'-Methylcytosine -bGT; Lane 4: 5'-Methylcytosine + bGT; Lane 5: 5'-Hydroxymethylcytosine –bGT; Lane 6: 5'-Hydroxymethylcytosine +bGT.

Kits, Antibodies, and DNA Standards for DNA Methylation Analysis

Description	Catalog No.
CpGenome™ 5-mC and 5-hmC DNA Standard Set	S8005
CpGenome™ Human Methylated and Non-Methylated DNA Standard	S8001
CpGenome™ Human Methylated DNA Standard	S8001M
CpGenome™ Human Non-Methylated DNA Standard	S8001U
CpGenome™ Mouse Methylated DNA Standard	S8000
CpGenome™ Turbo Bisulfite Modification Kit	S7847
CpGenome™ Fast DNA Modification Kit	S7824
CpG MethylQuest™ DNA Isolation Kit	17-10035
CpGenome™ Universal Methylated DNA	S7821
CpGenome™ Universal Unmethylated DNA	S7822
CpG WIZ® Oct-4 Amplification Kit	S7840
CpG WIZ® BRCA1 Amplification Kit (human)	S7830
CpG WIZ® hMLH1 Amplification Kit (human)	S7811
Anti-5-methylcytosine, clone 33D3	MABE146
Anti-5-methylcytosine Mouse mAb (162 33 D3)	NA81-50UG
Anti-5-hydroxymethylcytosine, clone AB3/63.3	MABE176
Anti-5-hydroxymethylcytosine (5hmC), clone HMC 31	MABE251
Anti-DNMT1	07-688
Anti-DNA Methyltransferase 1	AB3429
Anti- Phospho-Dnmt1(Ser714)	07-1594
Anti-DNMT-2 Mouse mAb (102B1259.2)	ST1133-50UG
Anti-DNMT3A2	07-2050
Anti-MeCP2	ABE171
Anti-acetyl-MeCP2 (Lys464)	ABE28
Anti-Kaiso, clone 6F	05-659
Anti-CFP1	ABE211

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References

1. Szwagierczak A., *et al*. (2010). Nucleic Acids Res. 38(19):e181.

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