Product Manual

Express DNA methylation kit

ENZ-45001-0050

ENZ-45001-0200

Product Manual

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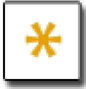
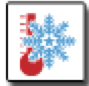
Product Manual



Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTRODUCTION

Cytosine methylation is a naturally occurring base modification, in both prokaryotic and eukaryotic organisms. It involves the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme[1]. In prokaryotes, DNA methylation helps protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA. DNA methylation in higher eukaryotes functions in the regulation/control of gene expression[2].

The majority of DNA methylation in mammals occurs in 5′-CpG-3′ dinucleotides, although other patterns do exist. About 80 percent of all 5′-CpG-3′ dinucleotides in mammalian genomes are found to be methylated, and the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes. It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis[3]. DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis[4] and methylation-sensitive arbitrarily primed PCR[5]. However, the most common techniques used today still rely on bisulfite conversion[6].

DNA treatment with bisulfite chemically modifies non-methylated cytosines into uracil, methylated cytosines remain unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced or processed for Pyrosequencing®. Recent advances in methylation detection also allow the investigation of genome-wide methylation patterns using technologies including array-based methods, reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing[7].

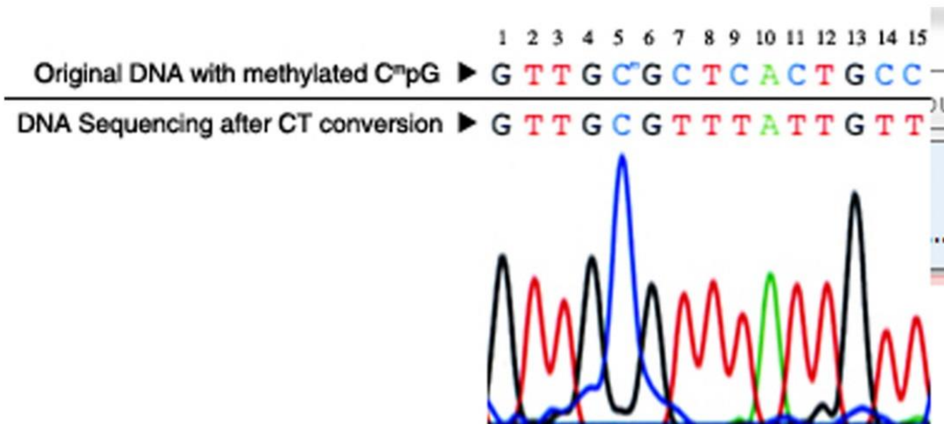

Figure 1 DNA sequencing results following bisulfite treatment. DNA with methylated C at nucleotide position #5 was processed using the **Express DNA Methylation Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remains intact while the unmethylated cytosines at positions #7, 9, 11, 14 and 15 are completely converted into uracil following bisulfite treatment (detected as thymine following PCR).

ASSAY PRINCIPLE

The **Express DNA Methylation Kit** features rapid and reliable bisulfite treatment and conversion of DNA for methylation analysis. Key to the fast workflow is the ready-to-use **Express Conversion Reagent**. Simply add this unique reagent to a DNA sample, wait about an hour, and let the reaction proceed to completion. DNA denaturation and bisulfite conversion processes are combined with added heat to facilitate rapid denaturation. Desulphonation and clean-up of the converted DNA is performed using a unique low-elution spin column. High yield, converted DNA is ideal for PCR, array, bisulfite and next generation sequencing, etc.

- **Conversion Efficiency:** > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- **DNA Recovery:** >80%

MATERIALS PROVIDED

Reagent	ENZ-45001-K050	ENZ-45001-K200	Storage Conditions	Format
Express Conversion Reagent	5 Vials	20 Vials	RT	Liquid
Methylation Binding Buffer	30ml	125ml	RT	Liquid
Methylation Wash Buffer Concentrate	6ml	24ml	RT	Liquid
Methylation Elution Buffer	1ml	4ml	RT	Liquid
IC Column	50 Units	200 Units	RT	N/A
IC Collection Tube	50 Units	200 Units	RT	N/A
L-Desulphonation Reagent	10ml	40mL	RT	Liquid

STORAGE

Store reagents at room temperature conditions.

STABILITY

12 months upon receipt

OTHER MATERIALS NEEDED

1. 100% (Absolute) Ethanol
2. PCR tube
3. PCR Instrumentation
4. Calibrated pipette
5. Microtube centrifuge (Counter)
6. -20ºC or -70ºC freezer
7. Designed primers (26-32bp)
8. Prepared DNA (Per bisulfate reaction 200ng-500ng)
9. UV spectrometer
10. Reagents for PCR reaction (e.g. hot start polymerase)

PRECAUTIONS

1. Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately.
2. Interpretation of the results is the sole responsibility of the user.

REAGENT PREPARATION

NOTE: *Reagents should be briefly centrifuged to ensure contents are fully collected.*

Methylation Wash Buffer Concentrate

Add 24ml of 100% ethanol to the 6 ml **Methylation Wash Buffer Concentrate** (ENZ-45001-0050) or 96 ml of 100% ethanol to the 24ml **Methylation Wash Buffer Concentrate** (ENZ-45001-K200) before use.

Express Conversion Reagent

No preparation step is required!

DNA preparation

Use appropriate kit for DNA preparation. Optimal use 200ng-500ng per reaction.

METHODS AND PROCEDURES

The procedure described in this manual assumes that the user is familiar with the basic principles of PCR and with using proper sterile technique.

Overall Procedure

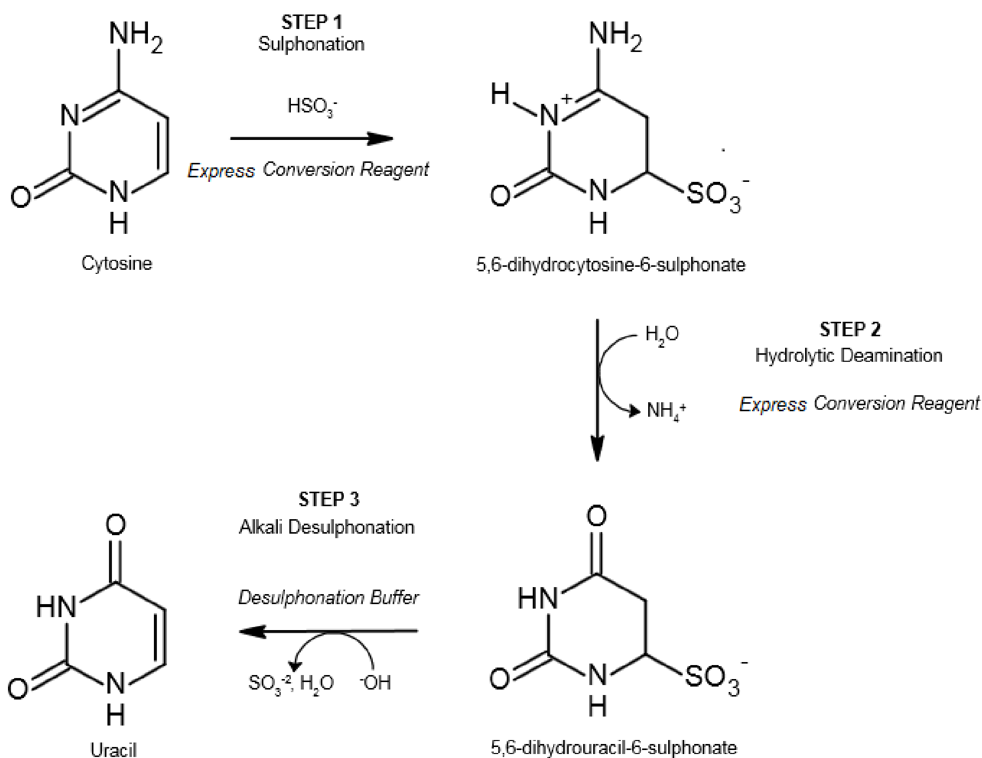

Figure 2 Overview of Bisulfite Conversion. Steps 1 and 2 occur during bisulfite conversion, while Step 3 is performed as the DNA is bound to the column matrix. For the reaction to proceed to completion, it is essential the DNA be fully denatured.

The application protocols included might require optimization

NOTE: *PLEASE READ THE ENTIRE PROCEDURE BEFORE STARTING. Prepare and use appropriate desired controls for each experiment. Prior to beginning experiment, turn on the PCR equipment and set to desired parameters.*

Protocol

1. Add 130µl of **Express Conversion Reagent** to 20µL of a DNA sample in a PCR tube. Mix, then centrifuge briefly to ensure there are no droplets in the cap or sides of the tube.

   Note: If the volume of DNA is less than 20µL, compensate with water.

2. Place the PCR tube in a thermal cycler and perform the following steps:

    a. 98°C for 8 minutes

    b. 54°C for 60 minutes

    c. 4°C for up to 20 hours

    Note: Step c is optional

3. Add 600µl of **Methylation Binding Buffer** to an **IC Column** and place the column into a provided **IC Collection Tube**.

4. Load the sample (from Step 2) into the **IC Column** containing the **Methylation Binding Buffer**. Close the cap and mix by inverting the column several times.

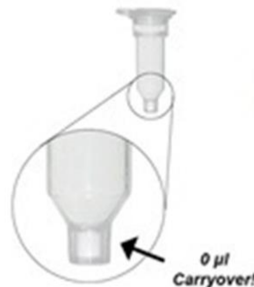

Figure 3. The image above shows the design of the column. This helps facilitate an extremely small elution volumes (≥10µl) without buffer carryover.

5. Centrifuge at full speed (≥ 10,000 x *g*) for 30 seconds. Discard the flow-through.

    Note: The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the column contents by the flow-through.

6. Add 100µL of **Methylation Wash Buffer** to the column. Centrifuge at full speed for 30 seconds. (Please see reagent preparation section for preparation directions)

7. Add 200µL of **L-Desulphonation Reagent** to the column and let stand at room temperature (20-30°C) for 15-20 minutes. After the incubation, centrifuge at full speed for 30 seconds.

8. Add 200µl of **Methylation Wash Buffer** to the column. Centrifuge at full speed for 30 seconds. Repeat this wash step.

9. Place the column into a 1.5ml microcentrifuge tube and add 10µL of **Methylation Elution Buffer** directly to the column matrix. Centrifuge for 30 seconds at full speed to elute the DNA.

   Note: Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.

   The DNA is ready for immediate analysis or can be stored at or below -20°C for later use. For long term storage, store at or below -70°C. We recommend using 1-4µL of eluted DNA for each PCR, however, up to 10µL can be used if necessary. The elution volume can be > 10µl depending on the requirements of your experiments, but small elution volumes will yield higher DNA concentrations.

APPENDIX

A. Bisulfite Conversion of Double Stranded DNA Templates

The following illustrates what occurs to a DNA template during bisulfite conversion.

Template: **A**: 5'-**GACCGTTCCAGGTCCAGCAGTGCGCT**-3'

Bisulfite Converted: **A:** 5'**GATCGTTTTAGGTTTAGTAGTGCGTT**-3'

B: 3'-**TTGGCAAGGTTTAGGTTGTTATGCGA**-5'

B. 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.

Bisulfate Converted: **A:** 5'-**GATCGTTTTAGGTTTAGTAGTGCGTT** 3'

Primers: Reverse: 3'-**ATCATCACRCAA**-5' **R**= **G**/**A**

Forward: 5'-**GATYGTTTTAGGT**-3' **Y**= **C**/**T**

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.

C. 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 100pg. The optimal amount of DNA per bisulfite treatment is 200ng to 500ng. Although, up to 2µg 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.

D. PCR Conditions

Usually, 35 to 40 cycles are required for successful PCR amplification of bisulfite converted DNA. Optimal amplicon size should be between 150-300bp; 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.

E. 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 40µg/ml for Ab_{260} = 1.0 when determining the concentration of the recovered bisulfite-treated DNA.

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NOTES

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