EPIGENTEK

Version 2.0911

Methylamp™ Global DNA Methylation Quantification Ultra Kit

Catalog No. P-1014B

User Guide*

*Always use the most updated User Guide included in your current order.

Epigentek Group Inc.
760 Parkside Avenue
Brooklyn, NY 11226
Tel: 1 877-374-4368
Fax: 1 718-484-3956

www . EPIGENTEK . com

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Epigentek Group Inc.
760 Parkside Avenue
Brooklyn, NY 11226
Tel: 1 877 374-4368
Fax: 1 718-484-3956
info@epigentek.com

INTRODUCTION

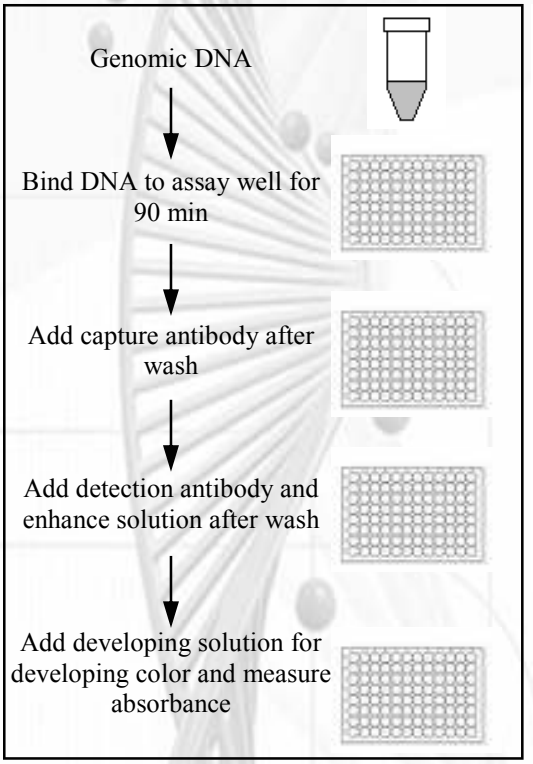
Epigenetic alterations of genomic DNA play a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Region-specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. Global DNA hypomethylation is likely caused by methyl-deficiency due to variety of environmental influences, and has been proposed as a molecular marker in multiple biological processes such as cancer. It is well demonstrated that the decrease in global DNA methylation is one of the most important characteristics of cancer. Thus, the quantification of global methylation in cancer cells could provide very useful information for detection and analysis of this disease.

For nearly two years, Epigentek has been providing the *Methylamp*™ Global DNA Methylation Quantification Kit which uses a unique procedure to quantify global DNA methylation. We have now added an upgraded form, *Methylamp*™ Global DNA Methylation Quantification Ultra Kit. This new version has the following advantages compared to the previous version:

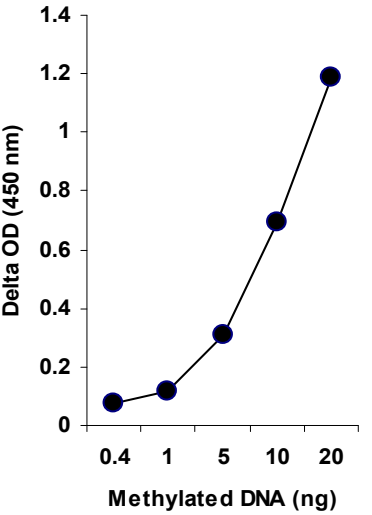
- Shorter procedure, which can be finished within < 4 hours.
- Higher sensitivity, of which detection limitation can be as low as 0.2 ng of methylated DNA, representing approximately a 5 fold increase in sensitivity.
- Universal positive control, which is suitable for quantifying methylated DNA from any species.

PRINCIPLE AND PROCEDURE

The *Methylamp*™ Global DNA Methylation Quantification Ultra Kit contains all reagents required for quantification of global DNA methylation. In this assay, DNA is immobilized to the strip well specifically coated with DNA affinity substance. The methylated fraction of DNA can be recognized by 5-methylcytosine antibody and quantified through an ELISA-like reaction. The amount of methylated DNA is proportional to the OD intensity.



Schematic Procedure for Using the *Methylamp*™ Global DNA Methylation Quantification Ultra Kit



Quantification of Methylated DNA

PRODUCT USE INFORMATION

The *Methylamp*™ Global DNA Methylation Quantification Ultra Kit is suitable for detecting global DNA methylation status using genomic DNA isolated from any species such as mammalians, plants, fungi, bacteria and virus in variety of form including cultured cells, fresh and frozen tissues, paraffin-embedded tissue, plasma/serum sample, and body fluid sample, etc.

Epigentek guarantees the performance of all products in the manner described in our product instructions.

Epigentek reserves the right to change or modify any product to enhance its performance and design.

The *Methylamp*™ Global DNA Methylation Quantification Ultra Kit is for research use only and is not intended for diagnostic or therapeutic application.

Methylamp™ is a trademark of Epigentek Group Inc.

The *Methylamp*™ kits and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 samples P-1014B-48	96 samples P-1014B-96
GU1 (10X wash buffer)	15 ml	30 ml
GU2 (DNA binding solution)	1.5 ml	3 ml
GU3 (positive control, 100 µg/ml)**	10 µl	20 µl
GU4 (block solution)	10 ml	20 ml
GU5 (capture antibody, 1000 µg/ml)*	5 µl	8 µl
GU6 (detecting antibody, 400 µg/ml)*	10 µl	20 µl
GU7 (enhance solution) *	10 µl	20 µl
GU8 (developing solution)	5 ml	10 ml
GU9 (stop solution)	3 ml	6 ml
Negative control DNA (50 ng/µl)*	10 µl	20 µl
8 well assay strips (with frame)	6	12
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** Spin the solution down to the bottom before use.*
*** This control is synthesized polynucleotide methylated at every 5-cytosine*

SHIPPING AND STORAGE

Store **GU3, GU6, GU7** and **negative control DNA** at –20°C away from light. Store **GU1, GU4, GU5, GU8** and **8 well assay strips** at 4°C away from light. Store **all other components** at room temperature. The kit is stable for up to 6 months from date of shipment when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

Microplate reader
Pipettes and pipette tips
1.5 ml microcentrifuge tubes

PROTOCOL

1. Prepare DNA by using your own successful method. For your convenience and the best results, Epigentek offers a series of DNA isolation kits which is optimized for extracting DNA from cultured cells, tissues, body fluids, and paraffin sections.
2. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute **GU1** with distilled water (pH 7.2-7.5) at 1:10 ratio (ex: 1 ml of **GU1** + 9 ml distilled water).
3. For samples, add 28 µl of **GU2** solution into the well followed by adding 2 µl (100-200 ng) of sample DNA. For the positive control, dilute **GU3** with **GU2** at the 1:20 ratios (ex: 1 µl of **GU3** + 19 µl of **GU2**). Add 28 µl of **GU2** into the strip well followed by adding 2 µl of the **diluted GU3** solution (10 ng/well). Optionally, dilute **GU3** to 0.2-10 ng/µl , respectively with **GU2.** Add 28 µl of **GU2** into the strip well followed by adding 2 µl of each **diluted GU3** solution to generate a standard curve (4-6 points. Ex: 0.4, 1, 2, 5, 10, and 20 ng/well). For negative control*, add 28 µl of **GU2** followed by adding 2 µl of **negative control DNA** into the wells.
   Shake the plate frame to allow the solution to cover the whole surface of strip well bottom. Incubate the strip wells at 37°C (with no humidity) for 40 min followed by incubating at 60°C (no humidity) for 35-40 min to evaporate the solution and dry the wells.

   Note: *The non-evaporated solution may be gathered along the edges at the bottom of the well. Make sure the well is completely dry by slightly tilting the well and aspirating against the edge with a P-10 or P-20 pipette. If there is still residue solution, extend incubation time for an additional 5-10 min at 60°C to dry the well.*

   **Required for background, cannot be substituted with DNA-less control.*

4. Add 150 µl of **GU4** to each dried well. Incubate at 37°C for 30 min.
5. Aspirate and wash each well with 150 µl of the **diluted GU1** three times.
6. Dilute the **GU5** (at 1:1000 ratio) to 1 µg/ml with the **diluted GU1**. Add 50 µl of diluted **GU5** to each well and incubate at room temperature for 60 min.
7. Aspirate and wash each well with 150 µl of the **diluted GU1** four times.
8. Dilute the **GU6** (at 1:5000-1:10,000 ratio) with the **diluted GU1.** Add 50 µl of diluted **GU6** to each well and incubate at room temperature for 30 min.
9. Aspirate and wash each well with 150 µl of the **diluted GU1** five times.

10. Dilute the **GU7** (at 1:5000-1:10,000 ratio) with the **diluted GU1**. Add 50 µl of diluted **GU7** to each well and incubate at room temperature for 30 min.
11. Aspirate and wash each well with 150 µl of the **diluted GU1** five times.
12. Add 100 µl of **GU8** to each well and incubate at room temperature for **1-5** min away from light. Monitor color development in the sample and the control well (blue).
13. Add 50 µl of **GU9** to each well to stop enzyme reaction when color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 min.
14. Calculation of DNA methylation.

For simple calculation, use the following formula:

$$\text{Methylation \%} = \frac{\text{OD (sample-NC*)/X}}{\text{OD (positive control– NC) x10}} \times 100\%$$

Here, the amount of the positive control is 10 ng and sample DNA is 100 ng.

*X is GC content of any species DNA. *NC: negative control.*

For example: GC contents is 41% for human genomic DNA , 42% for mouse and rat, 35% for A thaliana, 38% for yeast, respectively.

*For accurate calculation, plot O.D value versus amount of **GU3** and determine the slope as O.D/ng, then calculate the amount of methylated DNA using the following formula:*

$$\text{Methyl DNA (ng)} = \frac{\text{OD (sample – NC)}}{\text{slope}}$$

$$\text{Methylation \%} = \frac{\text{Methyl DNA amount / X}}{\text{Sample DNA amount added}} \times 100\%$$

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.	Check if reagents are added in order and if some steps of the procedure are omitted by mistake.
The well is not completely dried.	Ensure the well is incubated with no humidity and dry before adding block buffer.
The well is incorrectly washed before DNA coating.	Ensure the well is not washed before adding control DNA.
Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol are correctly followed.

No Signal or Very Weak Signal for Only the Positive Control

The positive control DNA is insufficiently added to the well.	Mix the control DNA thoroughly before adding to the well. Ensure sufficient amount of control DNA is added.
The control DNA is degraded due to incorrect storage.	Ensure the control DNA is properly stored according to storage instructions and not expired.

High Background Present for the Negative Control

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Contaminated by sample or positive control DNA	Ensure the well is not contaminated from adding sample or positive control DNA accidentally .
No or insufficient blocking.	Ensure the well is properly blocked with GU4.
Overdevelopment.	Decrease the development time at step 12.

ORDERING INFORMATION

Products	Size	Cat. No.
Methylamp™ Global DNA Methylation Quantification Ultra Kit	48 samples 96 samples	P-1014B-48 P-1014B-96

Available Related Products	Cat. No.
Methylamp™ DNA Modification Kit	P-1001
Methylamp™ Coupled DNA Isolation and Modification Kit	P-1002
Methylamp™-96 DNA Modification Kit	P-1008
Methylamp™ One-Step DNA Modification Kit	P-1010
Methylamp™ Universal Methylated DNA Kit	P-1011

Need more components? You can also order parts separately by calling 1-877-374-4368 or e-mailing sales@epigentek.com.

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