EPIGENTEK

Version 2.0802

Methylamp™ Global DNA Methylation Quantification Kit

Catalog No. P-1014

User Guide*

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

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INTRODUCTION

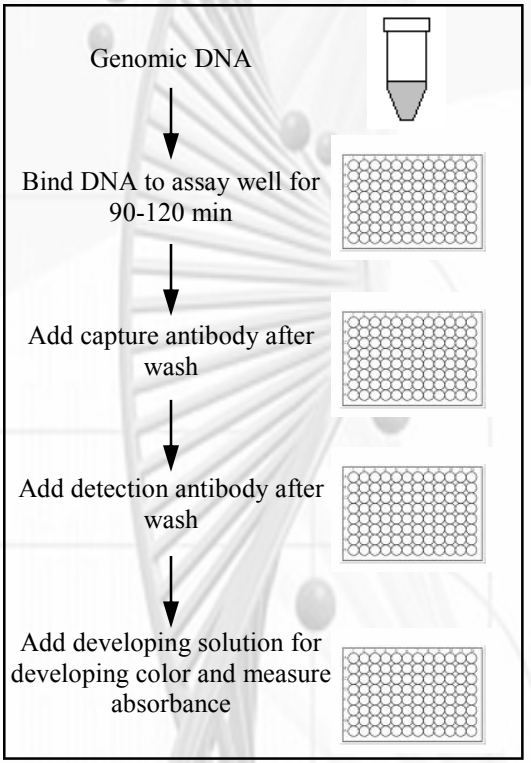
Epigenetic alterations of genomic DNA play a critical role in many important human diseases, especially in cancer. Core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Methylation of CpG islands involves the course 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.

Several methods such as radiolabeled methyl incorporation assay and high-performance separation analysis have been used for quantification of global DNA methylation. However, these methods are time consuming and labor intensive, have low throughput, and/or generate radioactive waste. The *Methylamp*™ Global DNA Methylation Quantification Kit addresses these problems by using a unique procedure to quantify global DNA methylation. The kit has the following features:

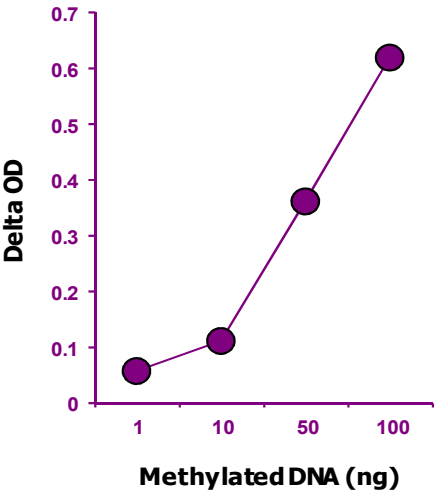
- Very rapid procedure, which can be finished within 4 hours.
- Colorimetric quantification without radioactivity, extraction, and chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The *Methylamp*™ Global DNA Methylation Quantification Kit contains all reagents required for quantification of global DNA methylation. In this assay, DNA is immobilized to the strip well specifically treated to have high affinity to the DNA. The methylated fraction of DNA can be recognized by a 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 Kit



Quantification of Methylated DNA

PRODUCT USE INFORMATION

The *Methylamp*™ Global DNA Methylation Quantification Kit is suitable for detecting global DNA methylation status using genomic DNA isolated from variety of 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 Kit is for research use only and is not intended for diagnostic or therapeutic application.

Methylamp™ is a trademark of Epigentek, Inc.

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

KIT CONTENTS

Components	48 assays P-1014-48	96 assays P-1014-96
GM1 (10X Wash Buffer)	11 ml	22 ml
GM2 (DNA Binding Solution)	1.5 ml	3 ml
GM3 (Methylated DNA Control, 100 µg/ml)*	8 µl	16 µl
GM4 (Block Solution)	10 ml	20 ml
GM5 (Capture Antibody, 1 mg/ml)*	5 µl	10 µl
GM6 (Detecting Antibody, 400 µg/ml)*	10 µl	20 µl
GM7 (Developing Solution)	5 ml	10 ml
GM8 (Stop Solution)	3 ml	6 ml
8-Well Assay Strips (with Frame)	6	12
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**For maximum recovery of the products, centrifuge the original vial after thawing, prior to opening the cap.*

SHIPPING AND STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **GM3** and **GM6** at –20°C away from light; (2) Store **GM1**, **GM4**, **GM5**, **GM7**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature.

Note: Check if wash buffer, **GM1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

All components of the kit are stable for 6 months from the 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 **GM1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of **GM1** + 9 ml distilled water).
3. Adjust sample DNA concentration to 6.6 ng/µl with **GM2** and add 30 µl (200 ng) of *DNA solution* into each well. Alternatively, add 28 µl of **GM2** solution into the well, followed by adding 2 µl (200 ng) of sample DNA. Mix well by pipetting several times. 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 2 hours, followed by incubating at 60°C (no humidity) for 20-30 minutes to evaporate the solution and dry the wells. For the positive control, add 29 µl of **GM2** into the strip well, followed by adding 1 µl of **GM3** solution (100 ng/µl). Mix well by pipetting several times. Optionally, dilute **GM3** to 0.05-3.5 ng/µl with **GM2** and add 30 µl (1.5-100 ng) of the *diluted GM3/GM2 solution* to the wells to generate a standard curve. For the blank, add 30 µl of **GM2** instead of DNA.

   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 minutes at 60°C to dry the well.*

4. Add 150 µl of **GM4** to each dried well. Incubate at 37°C for 30-45 minutes.
5. Aspirate and wash each well with 150 µl of **diluted GM1** three times.
6. Dilute the **GM5** (at a 1:1000 ratio) to 1 µg/ml with **diluted GM1**. Add 50 µl of the **diluted GM5** to each well and incubate at room temperature for 60 min.
7. Aspirate and wash each well with 150 µl of **diluted GM1** four times.

(Continued on Next Page)

8. Dilute **GM6** (at a 1:1000 ratio) with **diluted GM1**. Add 50 µl of **diluted GM6** to each well and incubate at room temperature for 30 minutes.
9. Aspirate and wash each well with 150 µl of **diluted GM1** five times.
10. Add 100 µl of **GM7** to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and the control wells (blue).
11. Add 50 µl of **GM8** to each well and read absorbance on a microplate reader at 450 nm.
12. Calculation of DNA methylation.

For simple calculation, use the following formula:

$$\text{Methylation \%} = \frac{\text{OD (sample – blank)/2}}{\text{OD (positive control – blank)}} \times 100\%$$

Here, the amount of the positive control is 100 ng.

If the sample is treated:

$$\text{Methylation \%} = \frac{\text{OD (treated sample – blank)}}{\text{OD (untreated control – blank)}} \times 100\%$$

For accurate calculation, plot OD value versus amount of **GM3** *and determine the slope as delta OD/ng, then calculate the amount of methylated DNA using the following formula:*

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

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

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 followed correctly.

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 Blank

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Contaminated by DNA.	Ensure the well is not contaminated from adding DNA accidentally or by using DNA contaminated tips.
Overdevelopment.	Decrease the development time at step 10.

ORDERING INFORMATION

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

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.