Methylamp™ One-Step DNA Modification Kit

Base Catalog # P-1010

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ One-Step DNA Modification Kit is very suitable for methylation research using tiny amounts of DNA including that from 96-well plate cultured cells, microdissection sample, paraffin-embedded tissue, plasma/serum sample, body fluid sample, etc.

The *Methylamp*™ One-Step DNA Modification Kit is suitable for MS-PCR, real time MS-PCR, methylation sequencing, and pyrosequencing, as well as methylation microarray.

If you use the *Methylamp*™ One-Step DNA Modification Kit for MSP with tiny amounts of starting DNA, the numbers of PCR cycles should be greater than 45. The amount of DNA for each modification can be 0.1 ng-1 μg. For optimal modification, DNA amount should be 50-200 ng.

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KIT CONTENTS

Components	40 Samples P-1010-1	80 Samples P-1010-2
G1 (DNA Modification Powder)	4 vials	8 vials
G2 (DNA Modification)	5 ml	10 ml
G3 (Balance Solution)	0.2 ml	0.4 ml
G4 (Modified DNA Capture)	14 ml	28 ml
G5 (Modified DNA Cleaning)	3 ml	6 ml
G6 (Modified DNA Elution)	1 ml	2 ml
F-Spin Column	40	80
F-Collection Tube	40	80

SHIPPING & STORAGE

Methylamp™ One-Step DNA Modification Kit can be stored at room temperatures (20-22°C) away from light for up to 6 months. Each vial of G1 can be used for 10 DNA sample treatments. The prepared G1/G2/G3 solution should be used *immediately*, unless it is stored at –20°C away from light (stable for up to one week). Frozen G1/G2/G3 solution must be thawed at room temperature and vortexed for 2 minutes prior to use.

GENERAL PRODUCT INFORMATION

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

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *Methylamp*™ One-Step DNA Modification Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The *Methylamp*™ kits and methods of use contain proprietary technologies by Epigentek. *Methylamp*™ is a trademark of Epigentek Group Inc.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Thermal cycler with heated lid
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and pipette tips

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Ethanol (96-100%)

A BRIEF OVERVIEW

Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. 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. Aberrant DNA methylation is mainly found in 5'-CpG-3' dinucleotides within promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. It is well demonstrated that DNA methylation plays an important role in the regulation of gene expression, tumorigenesis, and other genetic and epigenetic diseases. Thus, detection of methylation in some genes of diseased cells could provide very useful information for discrimination of that disease.

There have been many methods for the detection of DNA methylation. All of these methods require a bisulfite-based DNA modification before starting methylation assays such as MSP, sequencing, restriction analysis, and others. The bisulfite-based DNA modification is used to discriminate between cytosine and methylated cytosine, in which DNA is treated with bisulfite salt to convert cytosine residues to uracil in single-stranded DNA, while methylated cytosine remains same.

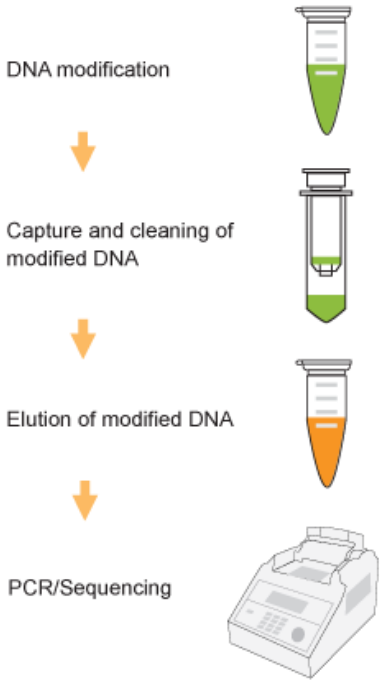
The *Methylamp*™ One-Step DNA Modification Kit is an improved form of our *Methylamp*™ DNA Modification Kit, which eliminates the chemical DNA denaturizing step in DNA modification process and enables cytosine conversion to be simpler and more convenient. The *Methylamp*™ One-Step DNA Modification Kit has the following features:

- The procedure can be finished within 1 hour and 45 minutes.
- Completely converts unmethylated cytosine into uracil: modified DNA > 99.98%
- The lowest degradation of DNA in the modification process: more than 90% of DNA loss can be prevented.
- The lowest requirement of starting DNA for modification: only 50 pg or 20 cells.
- Simple, reliable, and consistent modification conditions.

PRINCIPLE & PROCEDURE

The *Methylamp*™ One-Step DNA Modification Kit contains all reagents required for bisulfite conversion on a DNA sample. DNA is denatured by heating, which allows DNA denaturation and bisulfite modification to be carried out simultaneously. In the modification process, the bisulfite reagent reacts specifically with single-stranded DNA, thereby deaminating cytosine and creating a uracil residue. The unique DNA protection reagents contained in the modification buffer can prevent the chemical and thermophilic degradation of DNA in the bisulfite treatment. The non-toxic modified DNA capture buffer enable DNA to tightly bind to the column filter, thus DNA

cleaning can be carried out on the column to effectively remove residual sodium bisulfite and salts. Modified DNA can then be eluted and stably stored at –20°C for up to 2 months.



Schematic Procedure for Using the *Methylamp*™ One-Step DNA Modification Kit

ASSAY PROTOCOL

Note: Always cap spin columns before placing them in the microcentrifuge.

Before starting, add 7 ml (for P-1010-1) or 15 ml (for P-1010-2) of 100% ethanol to G5 to make the *final cleaning buffer*. Prepare the 90% ethanol.

1. Add 1.1 ml of **G2** to 1 vial of **G1**. Vortex until solution is clear or saturated (for about 2 minutes). Add 40 µl of **G3** to this solution, and lightly vortex.
2. Add 110 µl of the mixed **G1/G2/G3 solution** to 10 µl of DNA sample (10 ng to 1 µg). Vortex and place the vial in a thermal cycler with a program of 99°C for 6 minutes, followed by 65°C for 90 minutes.
3. Place a spin column into a 2 ml collection tube. Add 300 µl of **G4** to the column, and then transfer the sample (from step 2) to the column containing **G4**. Centrifuge at 12,000 rpm for 20 seconds. Remove the column from the collection tube and discard the flowthrough. Replace column to the collection tube.
4. Add 200 µl of **G5 solution** (*final cleaning buffer*) to the column, and centrifuge at 12,000 rpm for 20 seconds.

5. Add 10 µl of G3 to 1.1 ml of *90% ethanol*, and mix. Add 50 µl of the mixed G3/ethanol solution to the column. Allow it to sit for 8 minutes at room temperature, then centrifuge at 12,000 rpm for 20 seconds.
6. Add 200 µl of *90% ethanol* to the column, and centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough. Replace column to the collection tube. Add 200 µl of *90% ethanol* to the column again, and centrifuge at 12,000 rpm for 40 seconds.
7. Place the column in a new 1.5 ml vial. Add 8-18 µl of G6 directly to the column filter, and centrifuge at 12,000 rpm for 20 seconds to elute modified DNA.

Modified DNA is now ready for methylation amplification or storage at –20°C for up to 2 months.

TROUBLESHOOTING

DNA is Poorly Modified

1. Poor DNA quality (ex: DNA is not purified or fragmented).	Check if the sample DNA 260/280 ratio is between 1.6-1.9 and if DNA is degraded by running gel.
2. Too little DNA (ex: < 50 pg).	Increase starting DNA to recommended amount.
3. Template contains high GC region or secondary structure.	Increase bisulfite reaction time to 150-180 minutes.
4. Insufficient DNA denaturation.	Ensure that sufficient G3 is added into the sample.
5. Thermal cycling condition is incorrect.	Check the thermal cycling condition.
6. Bisulfite reaction components are not correctly mixed.	Ensure that each component is added correctly.
7. Insufficient DNA cleaning.	Ensure that sufficient G3 is added into 90% ethanol.
8. Incorrect storage of G1/G2/G3 solution.	Ensure that G1/G2/G3 solution is stored at -20°C for no more than 2 weeks.

Elution Contains Little or No DNA

1. Poor starting DNA quality (ex: degraded).	Check if DNA is degraded by running gel

2. G4 (Modified DNA Capture) is not added into the sample.	Ensure that G4 is added.
3. G5 is prepared with 70% ethanol, not with 100% ethanol.	Ensure that appropriate volume of 100% ethanol is added into G5 before use.
4. DNA cleaning solution is prepared incorrectly at step 5 of the protocol.	Ensure that G3 is added into 90% ethanol.
5. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
6. Sample is not completely passed through the filter.	Purify DNA before modification and increase centrifuge time to 1 minute at steps 3-7.

Elution Contains Both Unmodified and Modified DNA

1. Amount of DNA used is out of recommended range.	Adjust the amount of starting DNA to recommended range (50-200 ng).
2. Template with high G-C content.	Increase bisulfite reaction time to 150-180 minutes.

Poor Methylation Specific-PCR Products

1. PCR components are incorrectly added.	Check if all PCR components were properly added.
2. DNA is degraded.	Check if DNA is degraded prior to or after DNA modification.

RELATED PRODUCTS

P-1001	Methylamp™ DNA Modification Kit
P-1002	Methylamp™ Coupled DNA Isolation and Modification Kit
P-1008	Methylamp™ 96 DNA Modification Kit
P-1011	Methylamp™ Universal Methylated DNA Kit
P-1014	Methylamp™ Global DNA Methylation Quantification Kit

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