Methylamp™ DNA Modification Kit

Base Catalog # P-1001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

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

The *Methylamp*™ 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*™ 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.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Components	40 Reactions P-1001-1	80 Reactions P-1001-2
R1 (DNA Denature)	0.25 ml	0.5 ml
R2 (DNA Modification Powder)	4 vials	8 vials
R3 (DNA Modification)	5 ml	10 ml
R4 (Modified DNA Capture)	14 ml	28 ml
R5 (Modified DNA Cleaning)	3 ml	6 ml
R6 (Modified DNA Elution)	1 ml	2 ml
F-Spin Column	40	80
F-Collection Tube	40	80

SHIPPING & STORAGE

The *Methylamp*™ DNA Modification Kit is shipped at room temperature (15-25°C). Upon arrival, the kit can be stored at room temperature (15-25°C) away from light for 6 months. Each vial of R2 can be used for 10 DNA sample treatments. The prepared R1/R2/R3 solution should be used immediately, unless it is stored at –20°C away from light (for up to two weeks). Frozen R1/R2/R3 solution must be thawed at room temperature and vortexed for 2 minutes prior to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Waterbath or heat block
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Ethanol (96-100%)

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 Limitations: The *Methylamp*™ DNA Modification Kit is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

Epigenetic inactivation of genes plays a critical role in many important human diseases, especially cancer. The 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 cytosine. 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, where 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 the same.

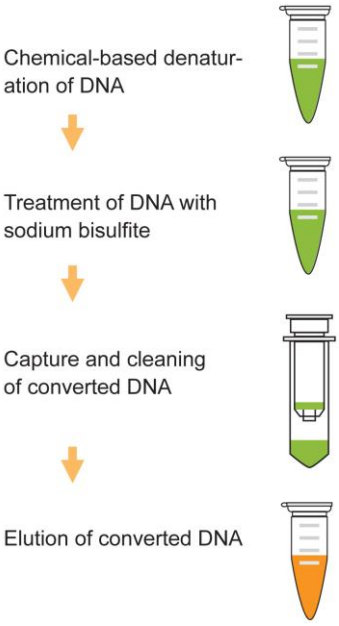
The *Methylamp*™ DNA Modification Kit uses a unique procedure and composition to modify DNA efficiently. The kit has the following features:

- The fastest procedure available, which can be finished within 1 hour and 55 minutes with consistent reaction conditions.
- 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.

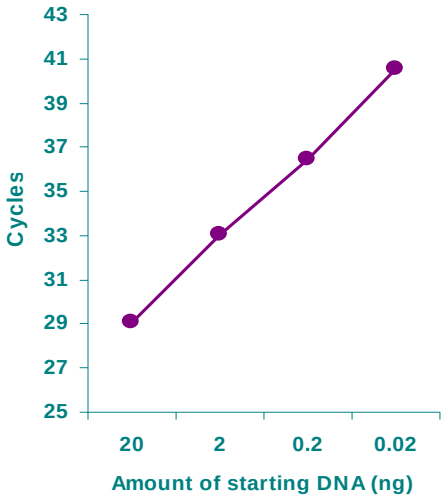
PRINCIPLE & PROCEDURE

The *Methylamp*™ DNA Modification Kit contains all reagents required for bisulfite conversion on a DNA sample. DNA is chemically denatured to allow bisulfite reagent to react 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 enables DNA to bind tightly to the column filter, thus clean DNA 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*™ DNA Modification Kit



The different amounts of DNA isolated from a serum sample were chemically modified using the *Methylamp*™ DNA Modification Kit. Real time PCR was performed by using a pair of primers and a probe designed to amplify both methylated and unmethylated alleles of β-actin.

PROTOCOL

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

Before starting, add 7 ml (for P-1001-1) or 15 ml (for P-1001-2) of 100% Ethanol to R5 to make the *final cleaning buffer*. Prepare the 90% Ethanol.

1. Add DNA sample and distilled water into a vial with total volume of 24 µl and mix well. Add 1 µl of **R1** to the above sample. Mix well and incubate the sample at 37°C for 10 minutes.

2. Add 1.1 ml of **R3** to 1 vial of **R2**. Vortex until solution is clear or saturated (about 2 minutes). Add 40 µl of **R1** to the solution, lightly vortex.

3. Add 125 µl of this mixed **R1/R2/R3 solution** to the sample. Vortex and incubate at 65°C for 90 minutes.

4. Place a spin column into a 2 ml collection tube. Add 300 µl of **R4** to the sample; mix, and transfer to the column. 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.

5. Add 200 µl of **R5 solution** (*final cleaning buffer*) to the column, and centrifuge at 12,000 rpm for 15 seconds.

6. Add 10 µl of **R1** to 1.1 ml of *90% ethanol*, and mix. Add 50 µl of the mixed **R1/ethanol solution** to the column; let it sit for 8 minutes at room temperature, then centrifuge at 12,000 rpm for 15 seconds.

7. Add 200 µl of *90% ethanol* to the column, centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough. Replace the column to the collection tube. Add 200 µl of *90% ethanol* to the column again and centrifuge at 12,000 rpm for 35 seconds.

8. Place the column in a new 1.5 ml vial. Add 8-18 µl of **R6** 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 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 **R1** is added into the sample.
5. Incorrect temperature of bisulfite reaction.	Ensure that temperature is at 65°C.
6. Bisulfite reaction components are not mixed correctly.	Ensure that each component is added correctly.
7. Insufficient cleaning.	Ensure that sufficient **R1** is added into 90% ethanol.
8. Incorrect storage of the **R1/R2/R3 solution**.	Ensure the **R1/R2/R3 solution** is stored at –20°C away from light, 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. R4 (Modified DNA Capture) is not added into the sample.	Ensure that R4 is added during step 4.
3. R5 is prepared with 70% ethanol, not with 100% ethanol.	Ensure that the appropriate volume of 100% ethanol is added into R5 before use.
4. DNA cleaning solution is prepared incorrectly at step 6 of the protocol.	Ensure that R1 is added into 90% ethanol.
5. The column is not washed with 90% ethanol.	Ensure that the 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 4 to 8.

Elution Contains Both Unmodified and Modified DNA

1. Amount of DNA used is out of recommended range.	Adjust the amount of starting DNA to the 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 added properly.
2. DNA is degraded.	Check if DNA is degraded prior to or after DNA modification.

RELATED PRODUCTS

P-1002	*Methylamp*™ Coupled DNA Isolation and Modification Kit
P-1008	*Methylamp*™-96 DNA Modification Kit
P-1010	*Methylamp*™ One-Step DNA Modification Kit
P-1011	*Methylamp*™ Universal Methylated DNA Kit
P-1026	*BisulFlash* ™ DNA Modification Kit