EPIGENTEK
Complete Solutions for Epigenetics

Methylamp™ 96 DNA Modification Kit

Base Catalog # P-1008

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ 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*™ kit is suitable for MS-PCR, real time MS-PCR, methylation sequencing, and pyrosequencing, as well as methylation microarray.

If you use the *Methylamp*™ 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 1 ng-1 μg. For optimal modification, DNA amount should be 100- 200 ng.

Gentaur Europe BVBA
Voortstraat 49,
1910 Kampenhout
BELGIUM
Tel 0032 16 58 90 45
info@gentaur.com

Genprice Inc.
547 Yurok Cir
San Jose, CA 95123,
USA
Phone: (408) 780-0908
genprice@gentaur.com

KIT CONTENTS

Components	96 samples P-1008-1	192 samples P-1008-2
CF1 (DNA Modification Powder)	1 bottle	2 bottles
CF2 (DNA Modification)	8 ml	16 ml
CF3 (Balance Buffer)	0.5 ml	1 ml
CF4 (DNA Carrier)	0.1 ml	0.2 ml
CF5 (Modified DNA Capture)	15 ml	30 ml
CF6 (Modified DNA Elution)	4 ml	8 ml
96-Well PCR Plate	1	2
96-Well Filter Plate	1	2
Receiver Plate	2	4
Optical Adhesive Cover	3	6
User Guide	1	1

.

SHIPPING & STORAGE

Upon receipt, store the kit at room temperatures (15-25°C) away from light, with the exception of CF4. Separately store CF4 at 4°C. All components are stable for 1 year from the date of shipment when stored properly.

One bottle of CF1 can be used for 96 DNA sample treatments. The mixed CF1/CF2/CF3/CF4 solution should be used immediately, unless it is stored at –20°C away from light (for up to one week). Frozen CF1/CF2/CF3/CF4 solution must be thawed at room temperature and vortexed for 2 minutes prior to use.

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%)

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.

Usage Limitation: The *Methylamp*™ kit is for research use only and is not intended for diagnostic or therapeutic application.

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

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 numerous 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 the same.

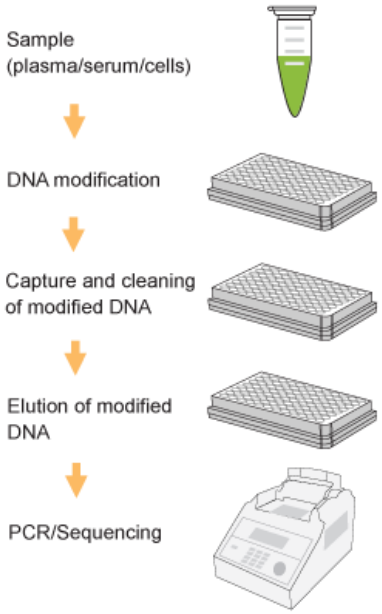
The *Methylamp*™96 DNA Modification Kit uses a unique procedure and composition to modify DNA efficiently. The *Methylamp*™96 DNA Modification Kit has following features:

- Fast procedure, which can be finished within 2 hours and 30 min.
- High throughput is achievable with a 96-well filter format.
- 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 1 ng
- Simple, reliable, and consistent modification conditions.

PRINCIPLE & PROCEDURE

The *Methylamp*™96 DNA Modification Kit contains all reagents required for bisulfite conversion on a DNA sample. DNA is denatured by heating, which allows DNA denaturation and busulfite modificaction 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 enables 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 then can be eluted and stably stored at –20°C for up to 2 months.



Schematic Procedure for Using the *Methylamp*™ 96 DNA Modification Kit

PROTOCOL

Before starting, prepare the following required solutions (not included): 80% and 90% ethanol.

1. Add 7.3 ml of **CF2** to 1 bottle of **CF1**. Vortex until solution is clear or saturated. Add 250 µl of **CF3** and 90 µl of **CF4** to the solution. Lightly vortex.
2. Add 75 µl of the mixed **CF1/CF2/CF3/CF4 solution** into each well of 96-well PCR plate followed by adding 5-10 µl of DNA sample (100 ng to 500ng) to the solution. Cover the plate with optical adhesive cover and place the plate in a thermal cycler with a program of 98°C for 6 min followed by 65°C for 90 min.
   Alternative option: *Dilute **CF3** with distilled water at 1:10 ratio. Add 5 µl of the diluted **CF3** into each well of the 96-well PCR plate followed by adding 7 µl of DNA sample (100 ng to 1 µg). Cover the plate with optical adhesive cover and incubate the plate at 37°C for 10 min. Add 75 µl of the mixed **CF1/CF2/CF3/CF4 solution** into each well of the plate and incubate the sample at 65°C for 90 min.*
3. Place the 96-well filter plate on the receive plate. Add 150 µl of **CF5** to the samples, mix and transfer the mixed solution to each well of the filter plate. Cover the filter plate with the optical adhesive cover and use tape to hold the filter plate and the receive plate together. Centrifuge at 3000 rpm for 8 min in a centrifuge with microtiter carrier. Be sure the centrifuge is balanced.
4. Remove the filter plate and discard the flowthrough. Replace filter plate to the receiver plate. Add 250 µl of 80% ethanol into each well, and centrifuge at 3000 rpm for 5 min. Remove the filter plate and discard the flowthrough. Replace filter plate to the receiver plate. Add 250 µl of 80%

ethanol into each well, and centrifuge at 3000 rpm for 5 min again. Remove the filter plate and discard the flowthrough.

5. Add 100 µl of CF3 to 10 ml of 90% ethanol, mix. Add 100 µl of the mixed CF3/ethanol to each well and sit for 10 min at room temperature, then centrifuge at 3000 rpm for 5 min.
6. Add 200 µl of 90% ethanol to each well, and centrifuge at 3000 rpm for 5 min. Discard the flowthrough. Add 200 µl of 90% ethanol, and centrifuge at 3000 rpm for 5 min again.
7. Discard the flowthrough and centrifuge at 3000 rpm for 3 min to eliminate residual ethanol.
8. Place the filter plate on a new receiver plate. Add 40 µl of CF6 directly to the filter in each well and sit for 1-2 min at room temperature, then centrifuge at 3000 rpm for 5 min to elute modified DNA to the receive plate. Cover the plate with optical adhesive cover sheet.

Modified DNA is now ready for methylation amplification, or wrap the plate with plastic wrap and store it 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 min.
4. Insufficient DNA denaturation.	Ensure that sufficient CF3 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 CF3 is added into 90% ethanol.
8. Incorrect storage of CF1/CF2/CF3/CF4 mix.	Ensure that CF1/CF2/CF3/CF4 mix is stored at –20°C and not for more than 2 weeks.

Elution Contains No or Little DNA

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

2. Buffer CF5 (DNA capture) is not added into the sample.	Ensure that CF5 is added.
3. DNA cleaning solution is prepared incorrectly at Step 5 of the protocol.	Ensure that CF3 is added into 90% ethanol.
4. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
5. Sample is not completely passed through the filter.	Purify DNA before modification and increase centrifuge time to 1 min at step 3-8.

Elution Contains Both Unmodified and Modified DNA

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

Poor Methylation Specific-PCR Products

1. PCR components are not sufficiently added.	Check if all PCR components were added.
2. Poor DNA template quality.	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 & Modification Kit
P-1010	*Methylamp*™ One-Step DNA Modification Kit
P-1011	*Methylamp*™ Universal Methylated DNA Kit
P-1034	*MethyFlash*™ Methylated DNA Quantification Kit (Colorimetric)

Gentaur
Gentaur Europe BVBA
Voortstraat 49,
1910 Kampenhout
BELGIUM
Tel 0032 16 58 90 45
info@gentaur.com

Genprice
Genprice Inc.
547 Yurok Cir
San Jose, CA 95123,
USA
Phone: (408) 780-0908
genprice@gentaur.com