EPIGENTEK
Complete Solutions for Epigenetics

Methylamp™ Coupled DNA Isolation & Modification Kit

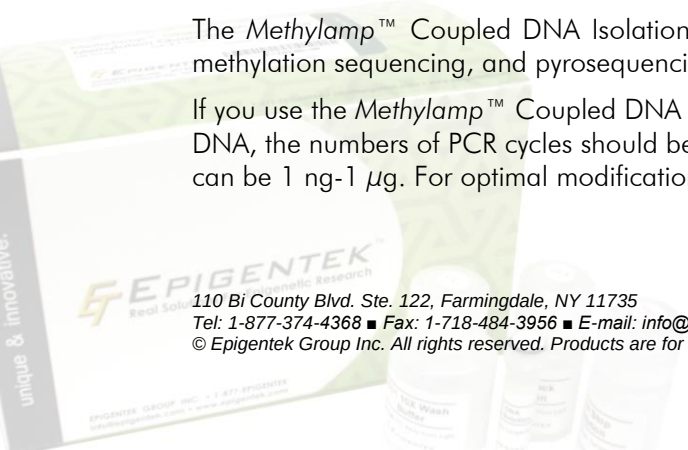
Base Catalog # P-1002

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ Coupled DNA Isolation & 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*™ Coupled DNA Isolation & 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*™ Coupled DNA Isolation & 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 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

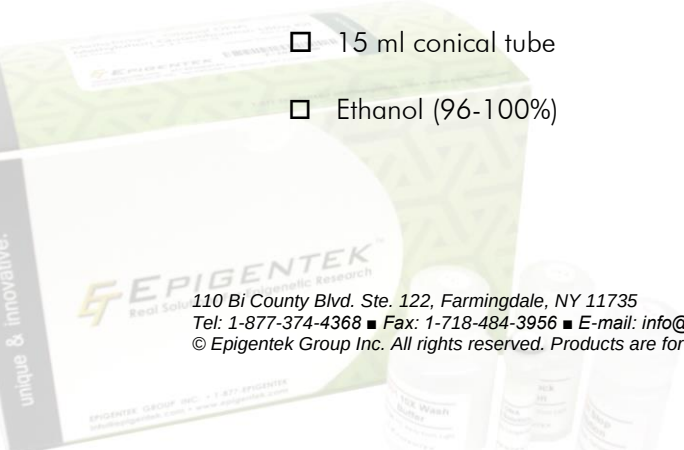
Components	40 samples P-1002-40
MR1 (DNA Digestion Solution)	1 ml
MR2 (DNA Digestion Powder)	1 vial
MR3 (DNA Isolation Buffer)	22 ml
MR4 (DNA Isolation Enhancer)	0.1 ml
MR5 DNA Denature Solution)	0.25 ml
MR6 (DNA Modifier Powder)	4 vials
MR7 (DNA Modifier Solution)	5 ml
MR8 (DNA Binding Buffer)	14 ml
MR9 (DNA Washing Buffer)	3 ml
MR10 (DNA Elution Buffer)	1 ml
1X TE Buffer	16 ml
F-Spin Column	80
F-Collection Tube	80
User Guide	1

SHIPPING & STORAGE

The kit can be stored at room temperature (15-25°C) away from light for 1 year from the date of shipment, with the exception of MR2 and MR4. MR2 should be stored at 0-4°C. MR4 is stable at room temperatures for 2 weeks and at 0-4°C for 1 year. Each vial of MR6 can be used for 10 DNA sample treatments. The prepared MR5/MR6/MR7 solution should be used immediately, unless it is stored at –20°C away from light (stable for up to one week). Frozen MR5/MR6/MR7 solution must be thawed at room temperature and vortexed for 2 minutes prior to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Waterbath or heat block
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tube
- ☐ 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*™ Coupled DNA Isolation & Modification 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.

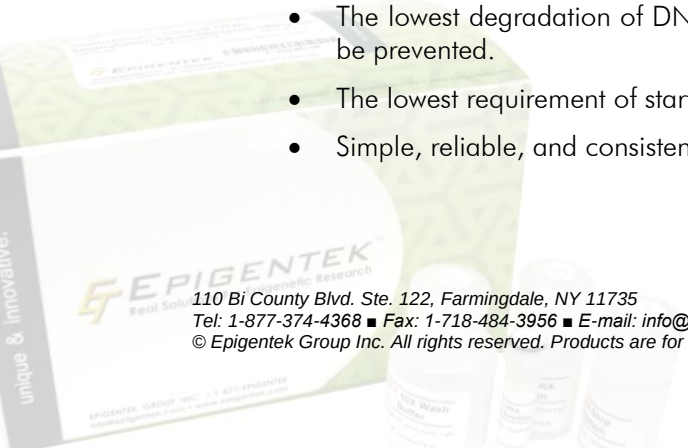
A BRIEF OVERVIEW

Epigenetic inactivation of genes play a critical role in many important human diseases, especially in cancer. A core mechanism for the 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. It is well demonstrated that DNA methylation play 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, and 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 a single-stranded DNA, while methylated cytosine remains same.

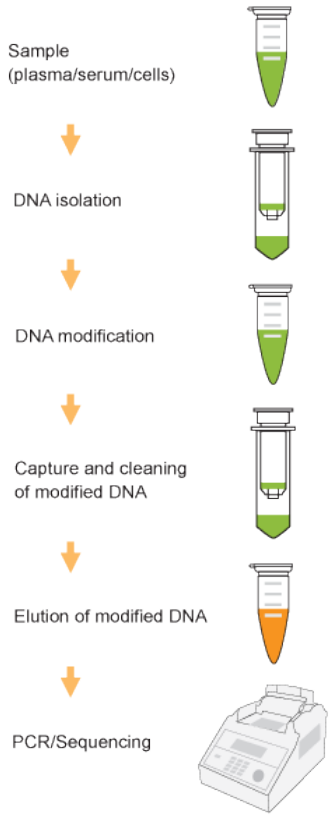
The *Methylamp*™ Coupled DNA Isolation & Modification Kit uses a unique procedure and composition to isolate and modify DNA efficiently. The kit has the following features:

- Fast procedure, which can be finished within 2 hours and 20 minutes.
- High efficiency of DNA isolation from multiple resources of biological material containing tiny amounts of DNA (as low as 1 ng), especially from serum, plasma, and body fluids.
- Completely converts unmethylated cytosine into uracil: modified DNA > 99.9%
- 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*™ Coupled DNA Isolation & Modification Kit contains all reagents required for DNA isolation and bisulfite conversion. DNA is isolated and 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 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*™ Coupled DNA Isolation & Modification Kit

PROTOCOL

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

Before starting, add 7 ml of 100% ethanol to MR9 to make the final wash buffer. Prepare the 90% and 70% ethanol.

Plasma/Serum Sample:

1. Add 1 ml of **MR1** to **MR2**. Vortex until solution is clear. Add 500 µl of **MR3** and then 20 µl of the mixed **MR1/MR2 solution** to 500 µl of plasma/serum or body fluid cell suspension. Mix well and incubate at 65°C for 10 minutes. Meanwhile, place a spin column into a 2 ml collection tube.

Adhesive Cells:

1. Remove culture medium and wash cells with PBS once. Add 100 µl of **1X TE Buffer** to each well for the 96-well plate, or 200 µl of **1X TE Buffer** for the 24-well plate, or 400 µl of **1X TE Buffer** for the 6-well plate. Incubate for 5 minutes. Add equal amount of **MR3** to each well, mix, and pipet up and down several times to break cells. Transfer cell solution to a 1.5 ml vial.
2. Add 1 ml of **MR1** to **MR2**. Vortex until solution is clear. Add 2 µl of mixed **MR1/MR2 solution** per every 100 µl of cell solution. Incubate at 65°C for 10 minutes. Meanwhile, place a spin column into a 2 ml collection tube.

Body Fluids:

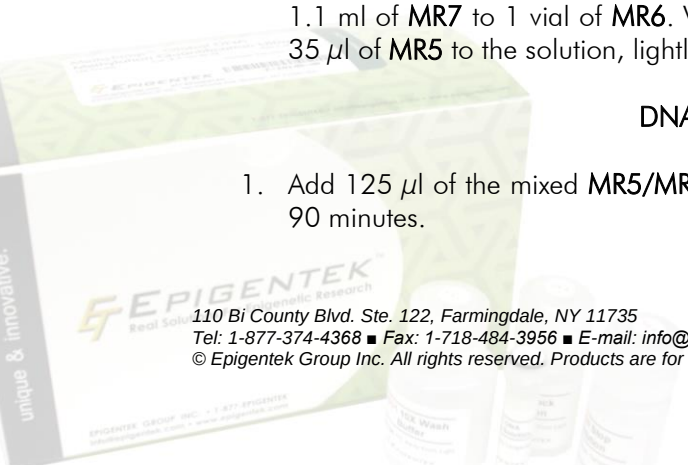
1. For *clear body fluids* such as cerebro-spinal fluid, ascite, saliva, and urine, collect the samples into 15 ml conical tube and centrifuge at 2000 rpm for 10 minutes to pellet cells. Remove supernatant. Add 10 ml of PBS and centrifuge at 1000 rpm for 5 minutes. Remove supernatant. For *sputum or mucoid samples*, use the special protocol such as DTT-sputolysin (See manufacturer's instruction) to collect cells.
2. Add 200 µl of **1X TE Buffer** to the cell pellet, vortex and incubate at room temperature for 5 minutes. Add equal amount of **MR3** to the sample and transfer to a 1.5 ml vial.
3. Add 1 ml of **MR1** to **MR2**. Vortex until solution is clear. Add 2 µl of mixed **MR1/MR2 solution** per every 100 µl of cell solution. Incubate at 65°C for 10 minutes. Meanwhile, place a spin column into a 2 ml collection tube.

DNA Isolation

1. Add 2 µl of **MR4** to the above mixture. Mix by inverting vial. Transfer all or maximum 500 µl of mixture to the column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flowthrough. Replace the column to the collection tube and transfer remaining volume of mixture to the column. Centrifuge again at 12,000 rpm for 30 seconds. Discard the flowthrough and replace the column to the collection tube.
2. Add 300 µl of *70% ethanol* to the column and centrifuge at 12,000 rpm for 20 seconds. Discard the flowthrough and replace the column to the collection tube. Add 300 µl of *90% ethanol* to the column and centrifuge at 12,000 rpm for 40 seconds.
3. Place the column in a new 1.5 ml vial. Add 24 µl of RNA-free water to the column; centrifuge at 12,000 rpm for 20 seconds to elute DNA. Add 1 µl of **MR5** to 24 µl of eluted DNA. Mix well and incubate the sample at 37°C for 10 minutes. Meanwhile, prepare modification solution by adding 1.1 ml of **MR7** to 1 vial of **MR6**. Vortex until solution is clear or saturated (about 2 minutes). Add 35 µl of **MR5** to the solution, lightly vortex.

DNA Modification and Purification

1. Add 125 µl of the mixed **MR5/MR6/MR7 solution** to the sample. Vortex and incubate at 65°C for 90 minutes.



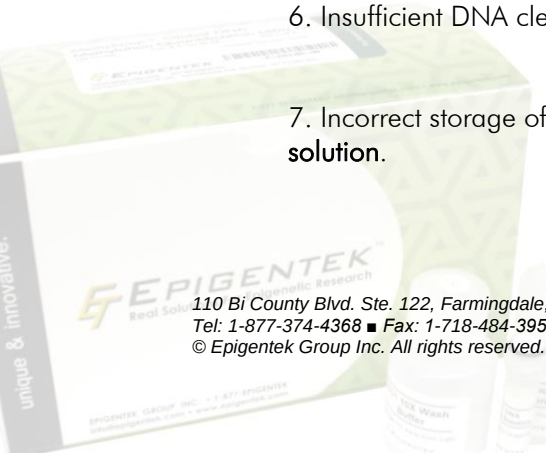
2. Place a spin column into a 2 ml collection tube. Add 300 µl of **MR8** to the sample, mix and transfer to the column. Centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough. Replace column to the collection tube.
3. Add 200 µl of **MR9 solution** (*final wash buffer*) to the column, and centrifuge at 12,000 rpm for 15 seconds.
4. Add 10 µl of **MR5** to 1.1 ml of *90% ethanol*, and mix. Add 50 µl of the mixed **MR5/ethanol solution** to the column; allow it to sit for 8 minutes at room temperature, then centrifuge at 12,000 rpm for 15 seconds.
5. 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 column to the collection tube. Add 200 µl of *90% ethanol* to the column again and centrifuge at 12,000 rpm for 35 seconds.
6. Place the column in a new 1.5 ml vial. Add 8-18 µl of **MR10** 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. The modified DNA can be added at a 1:10 ratio for each DNA amplification reaction.

TROUBLESHOOTING

DNA is Poorly Modified

1. Poor DNA quality (ex: DNA is not purified or fragmented).	Check if the isolated DNA 260/280 ratio is between 1.6 - 1.9 and if DNA is degraded by running gel.
2. Template contains high GC region or secondary structure.	Increase bisulfite reaction time to 150-180 minutes.
3. Insufficient DNA denaturation.	Ensure that sufficient **MR5** is added into the sample.
4. Incorrect temperature of bisulfite reaction.	Ensure that temperature is at 65°C.
5. Bisulfite reaction components are not correctly mixed.	Ensure that each component is added correctly.
6. Insufficient DNA cleaning.	Ensure that sufficient **MR5** is added into 90% ethanol.
7. Incorrect storage of **MR5/MR6/MR7 solution**.	Ensure that **MR5/MR6/MR7 solution** is stored at –20°C for no more than one week.



Elution Contains Little or No DNA

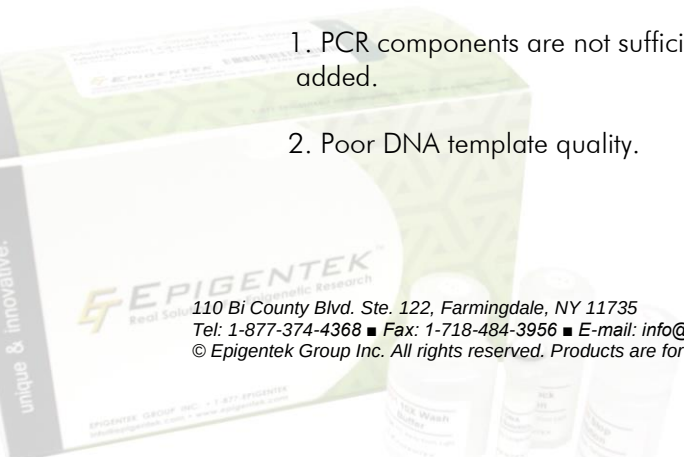
1. Poor starting material quality (Ex: FFPE sample contains fragmented DNA or serum sample contains little DNA).	Check if starting material is good quality.
2. Too little starting material.	Increase starting material.
3. MR4 is not added into the sample.	Ensure that MR4 is added.
4. Buffer MR8 (DNA Binding Buffer) is not added into the sample.	Ensure that MR8 is added.
5. Buffer MR9 is prepared with 70% ethanol not with 100% ethanol.	Ensure that the appropriate volume of of 100% ethanol is added into MR9 before the use.
6. DNA cleaning solution is prepared incorrectly at step 4 of the "DNA Modification and Purification" part of the protocol.	Ensure that MR5 is added into 90% ethanol.
7. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
8. Sample was not completely passed through the filter.	Purify DNA before modification and increase centrifuge time to 1 minute at steps 2-6 of the "DNA Modification and Purification" part of the protocol.

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 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-1008	*Methylamp*™ 96 DNA Modification Kit
P-1010	*Methylamp*™ One-Step DNA Modification Kit
P-1011	*Methylamp*™ Universal Methylated DNA Kit
P-1034	*MethylFlash*™ Methylated DNA Quantification Kit

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