Methylamp™ Universal Methylated DNA Preparation Kit

Base Catalog # P-1019

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ Universal Methylated DNA Preparation Kit allows generating methylated DNA using DNA from various sources including genomic DNA, plasmid DNA and oligonucleotides. Double-stranded DNA should be used with this kit.

DNA size can be from 40 bp to full length of genome; DNA quantity can be from 500 ng to 40 μg, optimal at 10 μg. Recovery rate of methylated DNA is greater than 80%.

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KIT CONTENTS

Components	P-1019-1
MA1 (Reaction Buffer)	4 ml
MA2 (Methylation Buffer)	400 µl
MA3 (Adomet, 30 mM)*	50 µl
MA4 (Methylase Mix)*	26 µl
MA5 (Purification Buffer)	4 ml
MA6 (Elution Buffer)	1 ml
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** Spin the solution down to the bottom before use.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C. Upon receipt: (1) Store MA2, MA3, and MA4 at –20°C to avoid multiple freeze-thaws; (2) Store all other components at room temperature (15-25°C). The kit is stable for up to 6 months from the shipment date, when stored properly.

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*™ Universal Methylated DNA Preparation 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.

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 such as methylation-specific PCR (MS-PCR) for the detection of DNA methylation. A methylation-positive control could be required for successful performance of gene methylation studies.

The *Methylamp*™ Universal Methylated DNA Preparation Kit provides a tool for generating methylated DNA at CpG sites as the methylation-positive control used for methylation studies. The kit includes all the components necessary for generating and purifying methylated DNA. The kit is sufficient for methylating 40 µg of DNA. This kit is suitable to be used with the *Methylamp*™ DNA Modification Kit series. It can also be used with other DNA modification or methylation kits.

PROTOCOL

Before starting, prepare the following required solutions (not included): 90% and 100% ethanol.

1. Dilute DNA to 500 ng/µl with DNAse/RNAse free water.

2. Prepare the reaction mix (for 10 µg of DNA) in a 1.5 ml vial:

DNA Solution	20 µl
MA1	61 µl
MA2	10 µl
MA3	2.6 µl
MA4	6 µl (finally added)

   Mix well by pipetting 5-6 times. If DNA concentration is less than 500 ng/µl, you can increase the volume of *DNA solution* (up to 80 µl) and reduce the volume of MA1.

3. Tightly cap the vial and incubate at 37°C (waterbath or thermal cycler) over night.

4. Add 100 µl of MA5 and 800 µl of *100% ethanol* into the vial. Incubate at –20°C for 1-2 hours.

5. Centrifuge at 12,000 rpm for 10 minutes. (At this step, you may see precipitates at the bottom of the vial.)

6. Carefully remove the supernatant. Then add 500 µl of *90% ethanol* into the vial, and centrifuge at 12,000 rpm for 30 seconds.

7. Carefully remove the supernatant. Add 500 µl of *90% ethanol* again into the vial, and centrifuge at 12,000 rpm for 35 seconds. Carefully remove the supernatant as much as possible.

8. Leave the vial open for 10-15 minutes at room temperature to dry. Add 60-70 µl of MA6 into the vial. Pipette the solution several times to dissolve methylated DNA.

9. Store DNA at –20°C (for up to 6 months) or use immediately

RELATED PRODUCTS

P-1011	*Methylamp*™ Universal Methylated DNA Kit
P-1014	*Methylamp*™ Global DNA Methylation Quantification Kit
P-1034	*MethylFlash*™ Methylated DNA Quantification Kit (Colorimetric)