Methylamp™ Universal Methylated DNA Kit

Base Catalog # P-1011

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KIT CONTENTS

Components	10 modifications P-1011-1	20 modifications P-1011-2
Methylated Human Genomic DNA*	5 μg (0.1 mg/ml)	10 μg (0.1 mg/ml)
U1 (DNA Modifier Powder)	1 vial (0.7 g)	1 vial (1.4 g)
U2 (DNA Modification Solution)	1.5 ml	3 ml
U3 (Balance Solution)	0.08 ml	0.15 ml
U4 (DNA Binding Buffer)	4 ml	8 ml
U5 (Elution Buffer)	0.5 ml	1 ml
F-Spin Column	10	20
F-Collection Tube	10	20

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

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 the **Methylated Human Genomic DNA** in aliquots at –20°C to avoid multiple freeze-thaws; (2) Store **all other components** at room temperature (15-25°C) away from light. The kit is stable for up to 1 year from date of shipment when stored properly.

Note: The prepared **U1/U2/U3 solution** should be used immediately, unless it is stored at –20°C for no more than 2 weeks.

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 Kit provides a methylation-positive control for methylation studies. The kit includes the enzymatically methylated human genomic DNA and all components for DNA modification. Methylated human DNA needs to be modified before it is used as a positive control in MS-PCR. DNA modification reagents included in the kit are sufficient for modifying 10 µg of methylated human genomic DNA (20 modifications). This product is intended for use with the *Methylamp*™ DNA Modification Kit series. It can also be used with other DNA modification or methylation kits.

PROTOCOL

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

Before starting, prepare the following required solutions (not included): 70% and 90% ethanol.

1. Prepare the **U1/U2/U3 solution**: For each modification, weigh 60 mg of **U1** powder and place it into a 0.5 ml vial. Add 0.1 ml of **U2** and vortex until the solution is clear or saturated. Add 3 µl of **U3** to the solution and lightly vortex.

2. Dilute **U3** with distilled water at a 1:15 ratio. Add 10 µl of the **diluted U3** into a 0.5 ml vial, followed by adding 5 µl (0.5 µg) of **Methylated Human Genomic DNA**. Mix and incubate the sample at 37°C for 10 minutes. Add 100 µl of the mixed **U1/U2/U3 solution** into the vial and incubate the sample at 65°C for 90 minutes.

   Alternative Option: Add 5 µl (0.5 µg) of **Methylated Human Genomic DNA** into 100 µl of the mixed **U1/U2/U3 solution**; mix and place the sample in a thermal cycler with a program of 95°C for 4 minutes, followed by 65°C for 90 minutes.

3. Place a spin column into a 2 ml collection tube. Add 300 µl of **U4** to the sample; mix and transfer the *mixed solution* to the column. 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 *70% ethanol* to the column, and centrifuge at 12,000 rpm for 20 seconds.

5. Add 1 µl of **U3** to 0.1 ml of *90% ethanol* and mix. Add 50 µl of the mixed **U3/ethanol solution** to the column. Let it sit for 8 minutes at room temperature, and then centrifuge at 12,000 rpm for 20 seconds.

6. Add 200 µl of *90% ethanol* to the column, 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. Add 200 µl of *90% ethanol* to the column again, and centrifuge at 12,000 rpm for 35 seconds.

7. Place the column in a new 1.5 ml vial. Add 20-30 µl of **U5** 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. Template contains high GC region or secondary structure.	Increase bisulfite reaction time to 150-180 minutes.
2. Insufficient DNA denaturation.	Ensure that sufficient U3 is added into the sample.
3. Incorrect temperature of bisulfite reaction.	Ensure that temperature is at 65°C.
4. Bisulfite reaction components are not correctly mixed.	Ensure that each component is added correctly.
5. Insufficient DNA cleaning.	Ensure that sufficient U3 is added into 90% ethanol correctly.
6. Incorrect storage of U1/U2/U3 solution.	Ensure that the U1/U2/U3 solution is stored at –20°C for no more than 2 weeks.

Elution Contains Little or No DNA

1. U4 (DNA Binding Buffer), was not added into the sample.	Ensure that U4 is added.
2. U5 (Elution Buffer) is prepared with 70% ethanol, not 100% ethanol.	Ensure that appropriate volume of the 100% ethanol is added into U5 before use.
4. DNA cleaning solution is prepared incorrectly at step 5 of the protocol.	Ensure that U3 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 not sufficiently added.	Check if all PCR components were added.

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-1010	Methylamp™ One-Step DNA Modification Kit
P-1014B	Methylamp™ Global DNA Methylation Quantification Ultra Kit

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