EPIGENTEK
Complete Solutions for Epigenetics

# Methylamp™ Whole Cell Bisulfite Modification Kit

Base Catalog # P-1016

## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ Whole Cell Bisulfite Modification Kit is specifically designed for DNA methylation research using minute amounts of starting materials including cells cultured in 96-well/384 well plates, tissue section samples, microdissection samples, tissue biopsy, and early embryonic cells/oocytes. These materials may not be suitable for DNA isolation prior to bisulfite modification.

The *Methylamp*™ Whole Cell Bisulfite Modification Kit can also be suitable for DNA modification directly using cells or tissues for increasing modified DNA yield, saving time and reducing labor.

Eluted modified DNA by using the *Methylamp*™ Whole Cell Bisulfite Modification Kit is suited for real time MS-PCR. It is also suitable for all techniques currently used for the analysis of DNA methylation; including conventional MS-PCR, bisulfite sequencing, pyrosequencing, and methylation microarray.

If you use the *Methylamp*™ Whole Cell Bisulfite Modification Kit for MSP, the numbers of PCR cycles should be greater than 45. The amount of starting materials for each modification can be 100-20000 cells, or 1 $\mu$g-100 $\mu$g tissues, or 0.2-2 $mm^2$ tissue section samples. For optimal modification, the amount should be 500-5000 cells, or 5-20 $\mu$g tissues, or 0.5-1 $mm^2$ tissue section samples, respectively.

Suitable lab coat, disposable gloves, and eye protection are required when working with the kit.

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## KIT CONTENTS

| Components | 40 samples<br>P-1016-40 | 80 samples<br>P-1016-80 |
|---|---|---|
| W1 (Digestion Powder) | 1 vial | 1 vial |
| W2 (Digestion Solution) | 0.1 ml | 0.2 ml |
| W3 (Cell Collection Buffer) | 1 ml | 2 ml |
| W4 (DNA Modification Powder) | 4 vials | 8 vials |
| W5 (DNA Modification Buffer) | 5 ml | 10 ml |
| W6 (Balance Buffer) | 0.4 ml | 0.8 ml |
| W7 (DNA Binding Buffer) | 14 ml | 28 ml |
| W8 (Modified DNA Elution) | 1 ml | 2 ml |
| F-Spin Column | 40 | 80 |
| F-Collection Tube | 40 | 80 |
| User Guide | 1 | 1 |

## SHIPPING & STORAGE

The kit can be stored at room temperature (20-22°C) away from light (with the exception of component W1 Digestion Powder) for 6 months from the shipping date.

Upon receipt, W1 should be stored at –20°C, or stored at 4°C as soon as it is dissolved in W2 (up to 6 months). Each vial of W4 can be used for 10 sample treatments. The prepared W4/W5/W6 solution should be used immediately, unless it is stored at –20°C away from light (up to one week). Frozen W4/W5/W6 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
- ☐ 0.2 ml PCR tube
- ☐ 15 ml conical tube
- ☐ Ethanol (96-100%)

## GENERAL PRODUCT INFORMATION

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

**Product Warranty:** 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.

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

## A BRIEF OVERVIEW

DNA methylation 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 such as cancer.

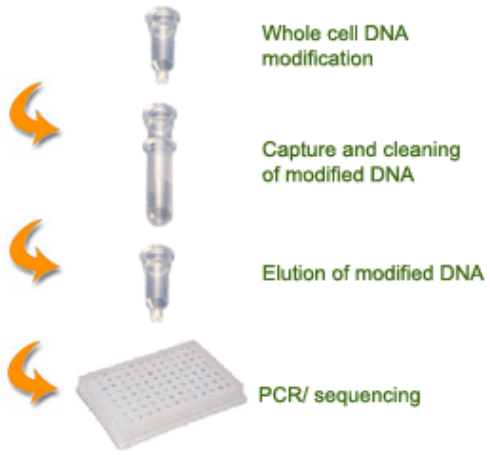
There have been many methods for the detection of DNA methylation. Most of them require a bisulfite-based DNA modification before starting methylation assays such as MSP, sequencing, restriction analysis, and others. The bisulfite-based DNA modification is used to discriminate between cytosine and methylated cytosine, in which bisulfite salt converts cytosine residues to uracil in single-stranded DNA, while methylated cytosine remains the same. All current methods for DNA modification need to use isolated DNA as starting material, which leads to the inability to achieve enough modified DNA in tiny amounts of tissue or cell samples. It is common that only minute amounts of tissue or cell samples can be available in biomedical research, high throughput biomarker/drug screening, and pathological diagnosis. These kinds of samples may include tissue biopsy, microdissection samples, cells contained in body fluids, cells cultured in 96 and 384 well plates, and early embryonic cells/oocytes. Thus, direct DNA modification from whole cells or tissues would give an advantage to efficiently utilize these kinds of samples. It could also generate a greater yield of modified DNA because of avoiding DNA loss caused by DNA isolation/purification prior to bisulfite modification. To address this problem, the *Methylamp*™ Whole Cell Bisulfite Modification Kit provides a useful tool to modify DNA directly from the cells or tissues. The kit has the following features:

- Fast results: streamlined 3 hour procedure from cells/tissues to modified DNA.
- Completely converts unmethylated cytosine into uracil: modified DNA >99.5%.
- The lowest degradation of DNA in the modification process: more than 90% of DNA loss can be prevented with unique DNA protecting buffer.

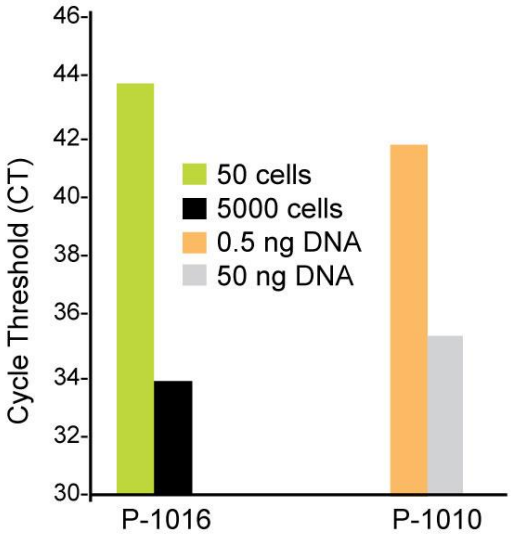
## PRINCIPLE & PROCEDURE

The *Methylamp*™ Whole Cell Bisulfite Modification Kit contains all reagents required for bisulfite conversion directly on a cell or tissue sample. The kit allows DNA to be isolated from cells or tissues, denatured and bisulfite modified simultaneously in the same tube with the specific reaction buffer under the thermodynamic condition. In the modification process, 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 can then be eluted and stably stored at –20°C for up to 2 months.




Schematic Procedure for Using the *Methylamp*™ Whole Cell Bisulfite Kit




The different amounts of MCF-7 cells or DNA isolated from MCF-7 cells were modified using the *Methylamp*T Whole Cell Bisulfite Modification Kit or *Methylamp*T One-Step DNA modification kit, respectively. 10 µl of modified DNA were eluted and 2 µl of elution were used in real time PCR. 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, prepare the following required solution (not included): 70% ethanol; 90% ethanol; and 100% isoprapanol.**

1. Add 50 µl of **W2** to **W1** (for P-1016-40) or add 100 µl of **W2** to **W1** (for P-1016-80) to create the **W1/W2 solution**. Vortex until solution is clear.
2. Collect the samples:
   **For adhesive cultures**, cells are detached by trypsinization and collected by centrifugation. Add 10 µl of **W3** to re-suspend the cells and transfer into a 0.2 ml PCR tube.
   **For body fluids**, such as cerebro-spinal fluid, ascite, saliva, and urine, cells are simply collected by centrifugation. Add 10 µl of **W3** to re-suspend the cells and transfer into a 0.2 ml PCR tube.
   **For tissue biopsy**, add 10 µl of **W3** into a 0.2 ml PCR tube and then add the sample to the PCR tube containing **W3**.
   **For early embryonic cells or oocytes**, add 10 µl of **W3** into a 0.2 ml PCR tube and then directly collect the cells into the PCR tube containing **W3**.

**For tissues from fresh sections**, add 10 µl of **W3** into a 0.2 ml PCR tube. Remove the tissue area you need from slide (0.2-2 $mm^2$, which represent about 200-2000 cells, assuming the section is 5 µm thick) and add it into the PCR tube containing **W3**.

**For tissues from formalin-fixed, paraffin-embedded tissue sections**, remove the paraffin first with deparaffin reagents according to your own successful protocols, or according to the following procedures:

(1) Drop the slide into 100% xylene at room temperature for 5 minutes. Repeat once with new xylene.

(2) Drop the slide into 100% ethanol, 95% ethanol, and 70% ethanol for 5 minutes each. Air dry the slide. Cut the tissue area you need from the slide (0.2-2 $mm^2$) and add it into the PCR tube containing 10 µl of **W3**.

**For microdissection samples from fresh or frozen tissue sections**, add 3-5 µl of **W3** into the center of the cap of a 0.5 ml PCR tube. Place the cap on the tissue to be microdissected and capture cells (100-1000 cells) into the cap. Remove the cap and place it back to the PCR tube containing 10 µl of **W3**. (**Note**: *if cap is dried, add an additional 3-5 µl of* **W3** *into the cap before placing it back to the PCR tube*). Centrifuge at 12,000 rpm for 30 seconds to move the cells down to the solution in the tube. Transfer the solution containing cells into a new 0.2 ml PCR tube.

**For microdissection samples from formalin-fixed, paraffin-embedded tissue sections**, remove the paraffin first with deparaffin reagents according to your own successful protocols, or according to the procedures described above. Add 3-5 µl of **W3** into the center of the cap of a 0.5 ml PCR tube. Place the cap on the tissue to be microdissected and capture cells (400-1000 cells) into the cap. Remove the cap and place it back to the PCR tube containing 10 µl of **W3**. (**Note**: *if cap is dried, add additional 3-5 µl of* **W3** *into the cap before placing it back to the PCR tube*). Centrifuge at 12,000 rpm for 30 seconds to move the cells down to the solution in the tube. Transfer the solution containing cells into a new 0.2 ml PCR tube.

3. Add 1 µl of the mixed **W1/W2 solution** to the PCR tubes containing the samples and place the tubes in a thermal cycler with the program of 65°C for 45 minutes. Meanwhile, add 1 ml of **W5** to 1 vial of **W4**, followed by adding 60 µl of **W6** to create the **W4/W4/W6 solution**. Vortex until solution is clear or saturated (about 2 minutes).
4. Add 110 µl of the mixed **W4/W5/W6 solution** to each PCR tube containing the sample. Place the tube in a thermal cycler (with heated lid) and program the thermal cycler as followed:
   99°C for 20 minutes
   65°C for 90 minutes
   99°C for 10 minutes

   *Modified sample can then be held at 25°C in the thermal cycler up to 4 hours without loss of performance*.
5. Place a spin column into a 2 ml collection tube. Add 200 µl of **W7** to the column. Transfer the sample (from step 4) to the column containing **W7**, followed by adding 100 µl of *100% isopropanol* to the column. Sit for 2 minutes at room temperature and 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.
6. Add 200 µl of *70% ethanol* to the column, and centrifuge at 12,000 rpm for 25 seconds.
7. Add 10 µl of **W6** to 1 ml of *90% ethanol* and mix to create the **W6/ethanol solution**. Add 50 µl of the mixed **W6/ethanol solution** (*DNA cleaning solution*) to the column. Sit for 10 minutes at room temperature, then centrifuge at 12,000 rpm for 20 seconds.
8. 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 40 seconds.

9. Place the column in a new 1.5 ml vial. Add 8-18 µl of **W8**, depending on the amount of starting materials, directly to the column filter. 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. Insufficient cell/tissue lysis. | Increase incubation time to 60-90 minutes at 65°C at step 3. |
| 2. Template contains high GC region or secondary structure. | Increase bisulfite reaction time (65°C) to 120 minutes at step 4. |
| 3. Thermal cycling condition is incorrect. | Check if the thermal cycling condition is set according to the protocol. |
| 4. Bisulfite reaction components are not mixed correctly. | Ensure that each component is added correctly. |
| 5. Insufficient DNA cleaning. | Ensure that a sufficient amount of **W6** is added into 90% ethanol. |
| 6. Incorrect storage of **W4/W5/W6 solution**. | Ensure that **W4/W5/W6 solution** is stored at –20°C and for no more than 2 weeks. |

### Elution Contains No or Little DNA

| | |
|---|---|
| 1. Poor starting material quality (Ex: FFPE sample contains fragmented DNA). | Check if starting material is good in quality. |
| 2. Too little starting material (ex: < 50 cells). | Increase starting material. |
| 3. **W7** DNA Binding Buffer is not added into the sample. | Ensure that **W7** is added as described in step 5. |
| 4. DNA cleaning solution is prepared incorrectly at step 7 of the protocol. | Ensure that **W6** is added into 90% ethanol. |
| 5. The column is not washed with | Ensure that wash solution is 90% ethanol. |

90% ethanol.

| | |
|---|---|
| 6. Sample is not completely passed through the filter. | Increase centrifuge time to 1 minute at steps 5-9. |

### Elution Contains Both Unmodified and Modified DNA

| | |
|---|---|
| 1. Amount of cells/tissues used is out of recommended range. | Adjust the amount of starting cells/ tissues to recommended range. |
| 2. Template with high G-C content. | Increase bisulfite reaction time (65°C) to 120 minutes in step 4. |

### 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 & Modification Kit |
| P-1008 | *Methylamp*™-*96* DNA Modification Kit |
| P-1010 | *Methylamp*™ One-Step DNA Modification Kit |
| P-1014 | *Methylamp*™ Global DNA Methylation Quantification Kit |

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