Methylamp™ Methylated DNA Capture Kit

Base Catalog # P-1015

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *Methylamp*™ Methylated DNA Capture Kit can be used for enriching methylated DNA from a broad range of species including human, rat, and mouse tissues.

The *Methylamp*™ Methylated DNA Capture Kit is suitable for combining the specificity of enriched methylated DNA with qualitative and quantitative PCR, and southern blot as well as DNA microarray.

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KIT CONTENTS

Components	24 reactions P-1015-24	48 reactions P-1015-48
MC1 (Antibody Buffer)	8 ml	16 ml
MC2 (Reaction Buffer)	4 ml	8 ml
MC3 (Wash Buffer)	16 ml	2 x 16 ml
MC4 (DNA Release Buffer)	2 ml	4 ml
MC5 (Binding Buffer)	5 ml	8 ml
MC6 (Elution Buffer)	0.6 ml	1.2 ml
Non-immune IgG (1 mg/ml)*	10 µl	20 µl
Proteinase K (10 mg/ml)*	25 µl	50 µl
Anti-5-Methylcytosine (1 mg/ml)*	25 µl	50 µl
8-Well Assay Strips (with Frame)	3	6
8-Well Strip Caps	3	6
F-Spin Column	30	50
F-Collection Tube	30	50

**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 MC1, MC3, Non-immune IgG, Anti-5-Methylcytosine, Proteinase K, and 8-Well Assay Strips at 4°C; (2) Store all other components at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, MC3, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Sonicator
- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ TE buffer (pH 8.0)
- ☐ Ethanol (96-100%)

GENERAL PRODUCT INFORMATION

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

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. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

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

A BRIEF OVERVIEW

A core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation, where 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. Region-specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells. Global DNA hypomethylation is likely caused by methyl-deficiency due to variety of environmental influences. It has been demonstrated that alterations in DNA methylation are associated with many diseases, and especially with cancer.

Highly specific capture of methylated DNA should provide an advantage for convenient and comprehensive identification of methylation status of normal and diseased cells, such as cancer cells, that may lead to the development of new diagnostic and therapeutic methods in cancer. Several methods have been used for enriching methylated DNA such as agarose beads-based methylated DNA capture. However, these methods so far are considerably time consuming, labor intensive, and have low throughput.

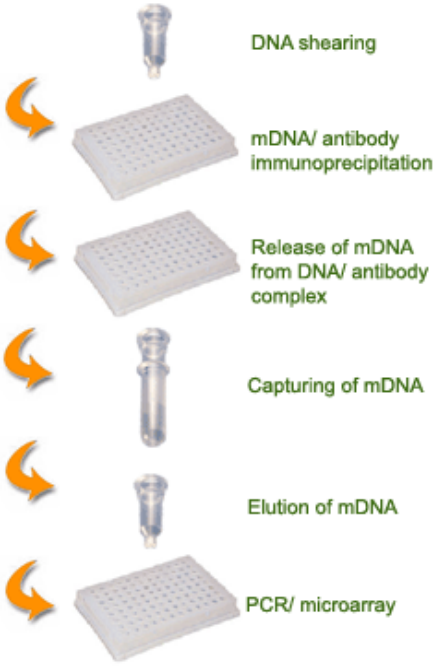
The *Methylamp*™ Methylated DNA Capture Kits use proprietary and unique procedure and compositions to enrich methylated DNA. In the assay, an antibody specific to methyl cytosine is used to capture methylated genomic DNA. The enriched methylated fractions can then be used for a standard DNA detection. The *Methylamp*™ Methylated DNA Capture Kit has the following features:

- Highly efficient enrichment of methylated DNA: > 98%.
- The fastest procedure available, which can be finished within 3 hours.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Columns for DNA purification are included: save time and reduce labor.

- Compatible with all DNA amplification-based approaches.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *Methylamp*™ Methylated DNA Capture Kit contains all reagents required for carrying out a successful capture of methylated DNA from a DNA sample. Particularly, this kit includes a ChIP-grade 5-methylcytosine antibody and a negative control Non-immune IgG. DNA is sheared, added into the microwell, and captured by the antibody. DNA is released from the antibody-captured methylated DNA complex, and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.



Schematic Procedure for Using the *Methylamp*™ Methylated DNA Capture Kit

PROTOCOL

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

Before starting, prepare the following required solution (not included): 90% Ethanol.
Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

1. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C).

2. Add 100 µl of **MC1** to each well and then add the antibodies: 1 µl of **Non-immune IgG** as the negative control, and 1 µl of **Anti-5-Methylcytosine** for the sample. Cover the strip wells with Parafilm M and incubate at room temperature for 60 minutes. Meanwhile, prepare the fragmented DNA as described in the following steps.
3. Add 0.5-1 µg of DNA to each 100 µl of **MC2** (500 µl maximum for each 1.5 ml vial) and shear DNA by sonication. Usually, sonicate 3 pulses of 10-12 seconds each at level 2 using a Branson Microtip probe, followed by 30-40 seconds rest on ice between each pulse. (*The conditions of DNA shearing can be optimized based on sonicator equipment. If desired, remove 5 µl of the sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp.)*
4. Incubate sonicated DNA at 95°C for 2 minutes and immediately place on ice.
5. Remove 5 µl of the sonicated DNA solution to a 0.5 ml vial. Label the vial as "input DNA" and place on ice.
6. Remove the incubated antibody solution and wash the strip wells one time with 150 µl of **MC1** and one time with 150 µl of **MC3** by pipetting in and out.
7. Add 100 µl of the sonicated DNA solution to each well. Cover the strip wells with Parafilm M and incubate at room temperature for 90-120 minutes on an orbital shaker (50-100 rpm).
8. Remove supernatant. Wash the wells three times with 150 µl of **MC3**.
9. Add 1 µl of **Proteinase K** to each 60 µl of **MC4** and mix. Add 60 µl of **MC4** containing Proteinase K to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 60 minutes.
10. Place a spin column into a 2 ml collection tube. Add 100 µl of **MC5** to the column. Add 180 µl of *100% ethanol* to the samples and mix. Transfer the mixed solution to the column containing **MC5**. Centrifuge at 12,000 rpm for 20 seconds.
11. Remove the column and discard the flowthrough. Replace column to the collection tube. Add 200 µl of 90% ethanol to the column and centrifuge at 12,000 rpm for 20 seconds.
12. Remove the column and discard the flowthrough. Replace column to the collection tube and wash the column again with 200 µl of *90% ethanol* at 12,000 rpm for 35 seconds.
13. Place the column in a new 1.5 ml vial. Add 20 µl of **MC6** directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

Methylated DNA is now ready for use or storage at –20°C.

Note: *For PCR positive control (methylation) and negative control (unmethylation), the primers for highly methylated sequences of H19ICR, LAP, or XIST and the primers for unmethylated β-actin or GAPDH sequence could be used, respectively.*
For conventional PCR, the number of PCR cycles may need to be optimized for better PCR results.

References:

Weber M et al: Nature Genetics, 37: 853-862, 2005.

TROUBLESHOOTING

Little or No PCR Products

1. Insufficient starting DNA.	Ensure the amount of starting DNA is sufficient (ex: 1 μg DNA/per reaction).
2. Insufficient/too much sonication.	Follow the protocol instruction for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication.
3. Incorrect temperature/insufficient time for DNA release.	Follow the guidelines in the protocol for appropriate temperature and time.
4. Incorrect PCR conditions.	Check if all PCR components are added. Increase amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction.
5. Incorrect or bad primers.	Ensure the designed primers are specific to the target sequence.
6. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
7. DNA is not completely passed through the filter.	Increase centrifuge time to 1 minute at steps 10-13 of the protocol.

Little or No Amplification Difference Between the Sample and the Negative Control

1. Insufficient wash at each wash step.	Follow the protocol for appropriate wash.
2. Antibody is added into the well for the negative control by mistake.	Ensure antibody is added into the correct well.
3. Too many PCR cycles.	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured generally within the linear PCR amplification phase.

RELATED PRODUCTS

P-1014	*Methylamp*™ Global DNA Methylation Quantification Kit
P-2019	*EpiQuik*™ Methylated DNA Immunoprecipitation Kit
P-2020	*EpiQuik*™ Tissue Methylated DNA Immunoprecipitation Kit