BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit

Base Catalog # P-1050

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit uses a unique conversion solution and magnetic binding bead clean-up to generate bisulfite-converted DNA in a fast, reliable, and high throughput format. Each kit contains sufficient components for 96 total reactions.

Input DNA: The amount of DNA for each reaction can be 0.1-1 µg. For an optimal reaction, the input DNA amount should be 100 ng. If small amounts (ex: <10 ng) of starting DNA are used, the number of PCR cycles should be greater than 45.

The yield of purified DNA after bisulfite modification depends on the amount of input DNA, nature of DNA, and source of the starting material.

Starting Material: Starting materials may include various tissue or cell samples such as: cultured cells from a flask or microplate, microdissection sample, paraffin-embedded tissue, plasma/serum sample, and body fluid sample, etc.

Precautions: To avoid cross-contamination, the following precautions are necessary for handling samples in tube/vials: Carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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

Component	96 Reactions Cat # P-1050-096	192 reactions Cat # P-1050-192	Storage Upon Receipt
BisulFlash Conversion Mix	4 ml	8 ml	RT
DNA Binding Solution	15 ml	30 ml	RT
Desulfonation Buffer	0.6 ml	1.2 ml	RT
Elution Buffer	2 ml	4 ml	RT
Binding Beads	0.5 ml	1 ml	4˚C
Mag-96 Well Plate	1	2	RT
Adhesive Cover Film	1	2	RT
User Guide	1	1	RT

SHIPPING & STORAGE

The kit is shipped at ambient room temperature.

Upon receipt: Store **Binding Beads** at 4°C; Store other components at room temperature (15-22°C) away from light.

All components of the kit are stable for up to 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Thermal cycler with heated lid*

  **Since the bisulfite reaction is not overlaid with mineral oil, only thermal cyclers with heated lids are suitable for this procedure.*

- ☐ Pipette and pipette tips
- ☐ 0.2 ml PCR tubes and PCR plate
- ☐ Magnetic stand (96-well format)
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 90% ethanol

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply call our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

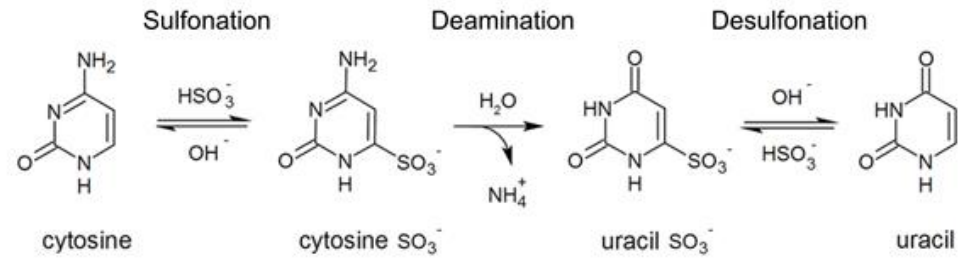
Usage Limitation: The BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit is for research use only and is not intended for diagnostic or therapeutic application.

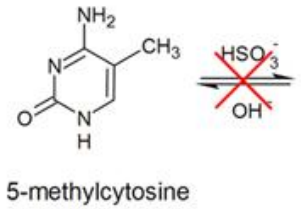
Intellectual Property: The BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

DNA methylation occurs by the covalent addition of a methyl group (CH_3) at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine (5-mC). DNA methylation is essential in regulating gene expression in nearly all biological processes including development, growth, and differentiation. Aberrant DNA methylation is associated with pathogenesis of diseases such as cancer, autoimmune disorders, and schizophrenia. Thus gene/region-specific or genome-wide analysis of DNA methylation or 5-methylcytosine (5-mC) could provide valuable information for discovering epigenetic markers used for disease diagnosis, and potential targets used for therapeutics.

Bisulfite modification of genomic DNA followed by PCR amplification, cloning-sequencing or whole-genome sequencing is currently considered to be the most reliable method in assessing the methylation states of individual cytosine on individual DNA molecules. By treating DNA with bisulfite, cytosine residues are deaminated to uracil while leaving 5-methylcytosine intact:





5-methylcytosine residues are left unchanged during the bisulfite modification process

However, the currently used DNA bisulfite conversion methods are still lacking, especially in speed, convenience and throughput. To address this issue, Epigentek continues to innovate with the development of the BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit. With novel procedures and

optimized components, the kit allows for preparing bisulfite converted DNA in a high throughout format within 1 h and 20 min. The kit has the following advantages and features:

- **Fast** - Complete the entire procedure in as little as 1 hour and 20 min for as many as 96 samples simultaneously, averaging just 50 seconds per sample.
- **Convenient** - Ready-to-use liquid conversion mix is simply added to the DNA samples directly, without need for pre-preparation of the conversion reagent.
- **Streamlined** - Concurrently processes the DNA denaturation and C to T conversion steps without the need for a separate DNA denaturation step.
- **Complete Conversion** - Completely converts unmethylated cytosine into uracil (>99.9%) with negligible inappropriate or error conversion of methylcytosine to thymine (<0.1%).
- **Flexible** - Choice of either (a) manual with one single reaction each time; or (b) high throughput with 96 reactions each time can be used, making the assay flexible.
- **Robust** - Simple, reliable, and consistent reaction conditions with an easy-to-follow protocol and high yield.

PRINCIPLE & PROCEDURE

The BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit contains all reagents required for a fast bisulfite conversion in a high throughput format. With the unique conversion mix solution, DNA denaturation status is sustained throughout the entire bisulfite conversion process, thereby enabling 100% of unmethylated cytosine to be converted to uracil. Desulphonation and clean-up of the converted DNA is performed using magnetic bead-based binding and separation in a 96-well microplate. High yield, converted DNA can be obtained and used for various downstream applications including PCR, array, and next generation sequencing.

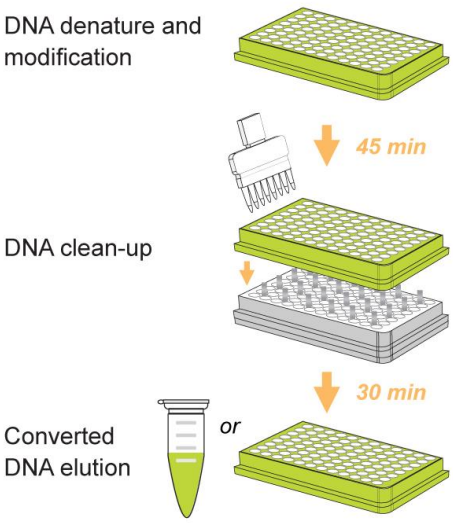


Fig 1. Schematic procedure of the BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit.

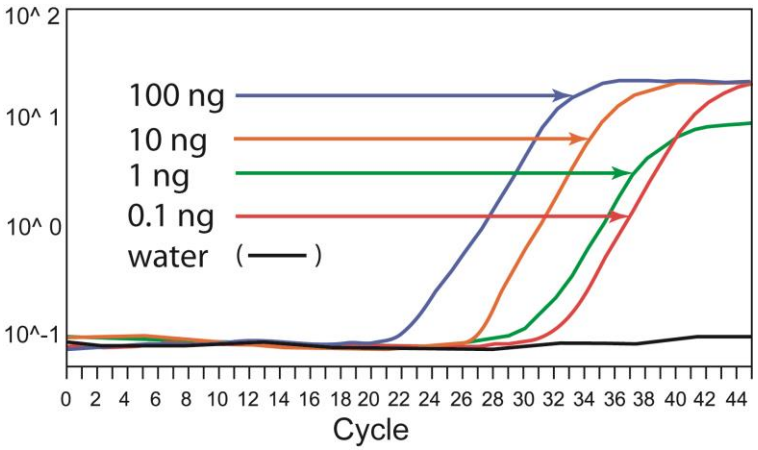


Fig 2. Different amounts of human genomic DNA were converted using the BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit. Converted DNA was amplified using *Methylamp™ MS-qPCR* Fast Kit (Cat. No. P-1028).

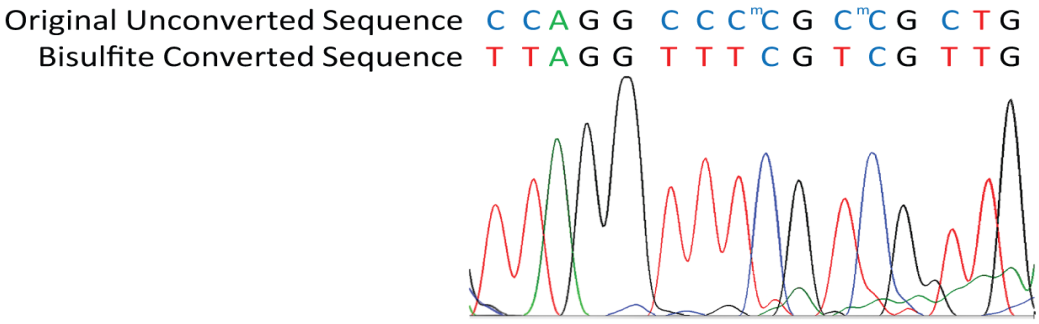


Fig 3. High accuracy of DNA conversion is achieved by the BisulFlash™ DNA Bisulfite Conversion Mag-96 Kit. 100 ng of genomic DNA methylated in all CpG sites by DNA methylases was treated with the kit followed by real time qPCR amplification using primers for multiple promoters containing numerous CpG sites and then directly sequenced. 100% C at non-CpG sites are converted to T and 100% C at CpG sites remain as C.

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input DNA Amount: Optimal DNA amount is 100 ng per reaction. Starting DNA may be in water or in a buffer such as TE.

DNA Isolation: You can use your method of choice for DNA isolation. Epigentek offers a series of genomic DNA isolation kits for your convenience.

DNA Storage: Isolated genomic DNA can be stored at 4°C or -20°C until use.

For the magnetic stand used for capturing **Binding Beads**, we recommend using Epigentek's EpiMag™ HT Magnetic Separator (Cat. No. Q10002), which has very strong magnetic power and is proven to quickly and efficiently achieve high, reproducible retention of magnetic bead-bound DNA in various 96-well plates, and specifically suitable for the Mag-96 well plate included in this kit.

Bisulfite DNA Conversion

1. For each well of a PCR plate or 0.2 ml PCR tube, add 30 µl of the **BisulFlash Conversion Mix** solution followed by adding 1-4 µl of your DNA sample (optimal 100 ng).
2. Tightly close the PCR well/tubes and place them in a thermal cycler with heated lid. Program and run the thermal cycler at 80°C for 45 min.

Converted DNA Clean-Up

1. Add 120 µl of **DNA Binding Solution** to each PCR well/tube followed by adding 5 µl of **Binding Beads**, mix by pipetting 8-10 times and then transfer the mixed samples from each PCR well/tube to the wells of the **Mag 96-well plate**. Incubate at RT for 10 min.

   ***Note**: The un-used plate wells can be covered with the **Adhesive Covering Film** include in the kit.*

2. Put the plate on an EpiMag™ HT Magnetic Separator or an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant *(Caution: Be careful not to disturb or discard the beads that contain DNA).*

3. Add 200 µl of *90% ethanol* solution to each well and resuspend the beads. Place the plate on the magnet stand for 2 minutes or until the solution is clear. Remove and discard supernatant.

4. Repeat Step 3 once for a total of 2 washes.

5. Prepare final desulphonation buffer by adding 25 µl of **Desulfonation Buffer** to every 1 ml of *90% ethanol*, and mix. Add 200 µl of the final desulphonation buffer to each well and resuspend beads. Incubate at room temperature for 15 min and then put the plate on the magnet stand for 2 minutes or until the solution is clear. Remove and discard the supernatant.

6. Add 200 µl of *90% ethanol* solution to each well and resuspend the beads. Place the plate on the magnet stand for 2 minutes or until the solution is clear. Remove and discard supernatant.

7. Repeat Step 6 once for a total of 2 washes. Make sure that the ethanol is completely removed after the final wash.

8. Air dry beads for 3-4 minutes at room temperature while the plate is on the magnetic stand to ensure all traces of ethanol are removed.

   ***Note**: Take care not to over dry the bead spot (an over dried bead spot appears cracked) as this will significantly decrease elution efficiency.*

Converted DNA Elution

1. Resuspend the beads in 20 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.

2. Capture the beads by placing the plate in the magnetic stand for 2 minutes or until the solution is completely clear.

3. Transfer 19-20 µl of supernatant to a new 0.2 ml PCR plate for immediate use or for storage at -20ºC after tightly capping the PCR plate.

WORKING WITH METHYLATION SPECIFIC qPCR

Working with MS-qPCR not only allows detection of gene-specific methylation, it also validates if the DNA is efficiently converted. For MS-qPCR, we recommend using the Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028) which has been optimized to decrease the overall methylation specific-qPCR amplification time and include primers specific for converted DNA. The master mix is provided at 2X

concentration for easier preparation of PCR reactions requiring only the addition of primers and templates. With this kit, the MS-qPCR can be finished in as short as 70 min.

Prepare the PCR Reactions

Component	Size (µl)	Final Concentration
Methylamp Master Mix (2X)	10 µl	1X
Forward Primer	1 µl	0.4-0.5 µM
Reverse Primer	1 µl	0.4-0.5 µM
DNA Template	1-2 µl	50 pg-0.1 µg
DNA/RNA-free H_2O	6-7 µl	
Total Volume	**20 µl**	

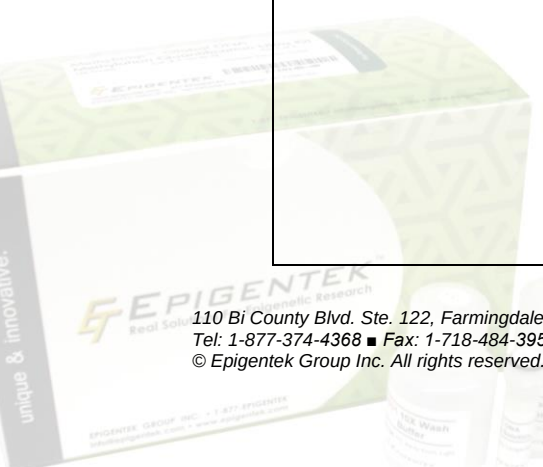
For the negative control, use DNA/RNA-free water instead of DNA template.

Program the PCR Reactions

Cycle Step	Temp	Time	Cycle
Activation	95°C	7 min	1
Cycling	95°C 55°C 72°C	10 sec 10 sec 8 sec	40-45
Final Extension	72°C	1 min	1

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
DNA is Poorly Modified	Poor DNA quality (DNA is severely degraded).	Check if the sample DNA 260/280 ratio is between 1.6-1.9 and if DNA is degraded by running gel.
	Too little DNA or too much DNA (i.e., <100 pg or >1 µg).	Increase or decrease input DNA to within the correct range, or to the optimal amount of 100 ng.
	Temperature or thermal cycling condition is incorrect.	Check for appropriate temperature or thermal cycling conditions.
	BisulFlash Conversion Mix solution was contaminated by other chemicals or affected by long-term exposure to air.	Check if **BisulFlash Conversion Mix** solution has any color change (deep yellow or brown) or insoluble precipitates. If so, use/order new **BisulFlash Conversion Mix** solution.
	Kit is not stored or handled properly.	Store all components of the kit at room temperature except **Binding Beads**. Tightly cap the **BisulFlash Conversion Mix** solution after each opening or use.



Eluate Contains Little or No DNA	Poor input DNA quality (degraded).	Check if DNA is degraded by running gel.
	DNA Binding Solution is not sufficiently added into the sample.	Ensure that **DNA Binding Solution** is added in Step 1 of **Converted DNA Clean-Up**.
	Concentration of ethanol solution used for DNA clean-up is not correct.	Use *90% ethanol* for DNA clean-up.
	DNA is not completely bound to the beads at the DNA binding step or beads are overdried at the DNA elution step.	Make sure the incubation time is sufficient (10 min) at Step 1 of **Converted DNA Clean-Up** and/or the beads are not over dried at Step 8 of **Converted DNA Clean-Up.**
Poor Results in Downstream Methylation-Specific PCR	Little or no PCR product even in positive control.	Ensure that all PCR components were added and that a suitable PCR program is used (PCR cycles should be >40).
		PCR primers and probes were not appropriate or were incorrectly designed. Ensure the primer and probes are suitable for MS-PCR.
		Ensure the amount of template DNA used for PCR was sufficient.
	Insufficient DNA clean-up.	Ensure that 25 µl of **Desulfonation Buffer** is added into every 1 ml of *90% ethanol* at Step 5 of **Converted DNA Clean-Up**.
	Beads were not sufficiently re-suspended at the wash and desulfonation steps.	Ensure that the beads have been completely re-suspended during the wash and desulfonation steps.
	Significant non-specific PCR products.	Failed bisulfite conversion. Ensure that all steps of the modification and clean-up protocol were followed and that the input DNA amount is within the recommended range.
		Primers and probes are not specific for converted DNA and target genes. Check the primer and probe design.

RELATED PRODUCTS

DNA Sample Preparation

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1007	FitAmp™ Gel DNA Isolation Kit

P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

DNA Bisulfite Modification

P-1001	Methylamp™ DNA Modification Kit
P-1016	Methylamp™ Whole Cell Bisulfite Modification Kit
P-1026	BisulFlash™ DNA Modification Kit

DNA Methylation Analysis

P-1005	TuMinute™ PCR Clean-Up Kit
P-1011	Methylamp™ Universal Methylated DNA Kit
P-1019	Methylamp™ Universal Methylated DNA Preparation Kit
P-1028	Methylamp™ MS-qPCR Fast Kit

Magnetic Devices

Q10002	EpiMag™ HT (96-Well) Magnetic Separator

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