EPIGENTEK
Real Solutions For Epigenetic Research

www.epigentek.com

NEXT GENERATION DNA BISULFITE CONVERSION

INTRODUCTION

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.

Bisulfite modification of genomic DNA, followed by PCR amplification, cloning, and sequencing of individual PCR amplimers is a critical method to yield reliable and accurate information of DNA methylation states. By treating DNA with bisulfite, cytosine residues are converted to uracil while leaving 5-methylcytosine intact. For a complete cytosine conversion, single-stranded DNA is necessary.

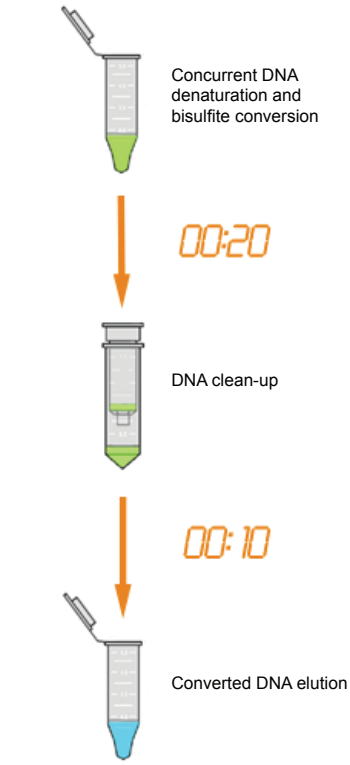
PERFECTION

To perfect DNA bisulfite treatment for better DNA methylation analysis, Epigentek developed a next generation DNA bisulfite conversion technology and incorporated this technology into its new product, the BisulFlash™ DNA Modification Kit.

The BisulFlash™ method enables the entire cytosine conversion reaction to be processed with 100% single stranded DNA, which leads to a significantly higher conversion efficiency and faster speeds.

Additionally, a unique protection reagent prevents over 90% of DNA loss, preserving important DNA recovery efficiency.

The BisulFlash™ DNA Modification Kit is suitable for MS-PCR, real time MS-PCR, methylation microarray, and pyrosequencing. Furthermore, based on its ability for a complete cytosine conversion, it is specifically suitable for next generation methylation sequencing and pyrosequencing.



▲ Schematic procedure of the BisulFlash™ DNA Modification Kit to obtain converted DNA.

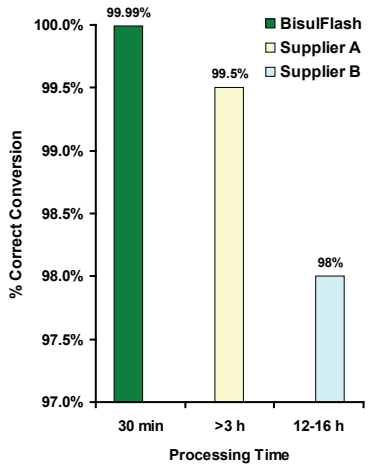
A NEXT GENERATION TOOL

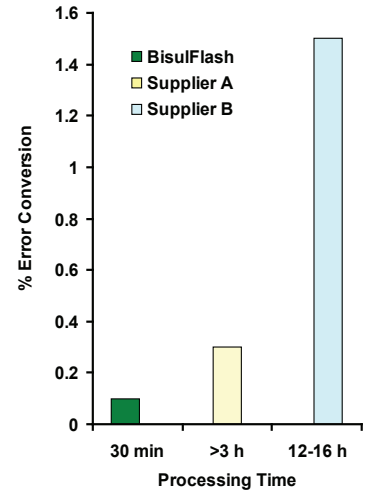
Current methods process bisulfite reaction under the condition that not all of the DNA are in single stranded form. But with the patent pending BisulFlash™ method, DNA denaturation status is sustained throughout the entire bisulfite DNA conversion process, which enables 100% of DNA to be modified in single stranded form. This breakthrough approach allows the reaction to be much faster with higher conversion efficiency and accuracy.

We continue to innovate with the development of the new BisulFlash™ kit by identifying four critical components of bisulfite conversion:

- *Speed:* Reduce the entire procedure to as short as 30 minutes without any reagent setup time.
- *Efficiency:* Completely convert unmethylated cytosine into uracil -- modified DNA > 99.99%.
- *DNA Protection:* Protect against DNA degradation of which more than 90% of DNA loss can be prevented, allowing for greater recovery.
- *Sensitivity:* Start with the lowest amount of input DNA for modification -- only 0.2 ng or just 50 cells.

The convenient DNA conversion mix solution and single temperature incubation along with the features mentioned above allow for true perfection in bisulfite conversion.





▲ Demonstration of high accuracy of DNA conversion achieved by BisulFlash™ DNA Modification Kit. 50 ng of genomic DNA methylated in all CpG sites by DNA methylase was treated with the BisulFlash™ DNA Modification Kit. Converted DNA was then amplified by real time qPCR using primers for multiple promoters containing numerous CpG sites and then directly sequenced. Correct conversion (C-U) and inappropriate or error conversion (mC-T) rates were culcalated as percentage of total cytosines or mCpGs. *Top Image:* correct conversion rate; *Bottom Image:* inappropriate or error conversion rate.

PRINCIPLE & PROCEDURE

As a next generation bisulfite conversion tool, the BisulFlash™ DNA Modification Kit contains all reagents required for an ultra-fast bisulfite conversion on a DNA sample. With the ready-to-use conversion mix solution, DNA denaturation status is sustained throughout the entire bisulfite DNA conversion proces. This proprietary solution allows the bisulfite reagents to rapidly convert all cytosine to uracil with negligible methylcytosine conversion. The unique DNA protection reagents contained in the mix can prevent the chemical and thermophilic degradation of DNA in the bisulfite treatment. The non-toxic DNA capture solution enables DNA to tightly bind to the column filter, thus DNA cleaning can be carried out on the column to effectively remove residual bisulfite and salts.

RAPID RESULTS

Only 30 minutes are required for the entire BisulFlash™ procedure -- from inputting your sample DNA to obtaining pure bisulfite modified DNA. The BisulFlash™ kit provides everything required for a successful bisulfite conversion and DNA cleanup in the shortest time with the fewest steps possible.

PERFECT DNA CONVERSION

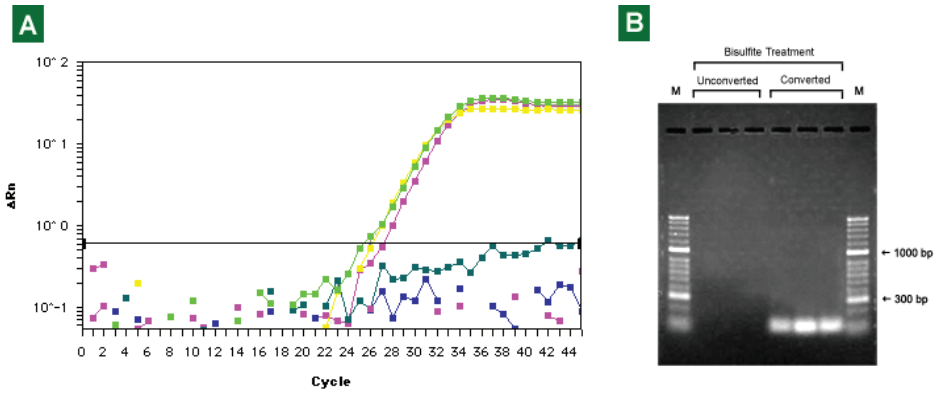
Each reaction with the BisulFlash™ kit can use 0.2 ng – 1 µg of DNA. For optimal conversion, the DNA amount is 20-200 ng. The novel procedure and proprietary ready-to-use DNA conversion mix solution allow DNA denaturation to remain during the entire bisulfite conversion process, resulting in 100% single-stranded DNA in cytosine conversion processing. This enables all cytosines to be converted to uracil (>99.999%), while 5-methylcytosine remains the same.

POWERFUL DNA PROTECTION

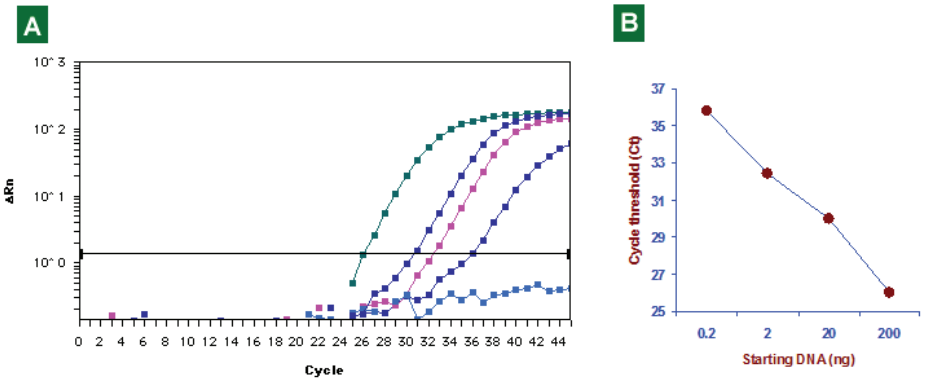
DNA protection reagents included in the DNA conversion mix solution prevents 90% of DNA from chemical and thermophilic degradation in the bisulfite treatment.

	BISULFLASH	SUPPLIER 1	SUPPLIER 2	SUPPLIER 3
Processing Time	30 min	>3 hours	>6 hours	12-16 hours
Correct Conversion	99.99%	99.5%	99.4%	98%
Error Conversion	<0.1%	>0.3%	N/A	>1.5%
Correct/Error Conv. Ratio	~1000	~330	N/A	~65
DNA Degradation	Very low	Medium	Low	High
Min. Starting DNA	0.2 ng	0.5 ng	1 ng	1 ng
Convenience	Very High	High	Medium	Low

▲ **Comparative Overview of Commercial Kits.** Data was obtained through actual use of the kit, customer feedback, or information provided by the supplier's datasheet or website.



▲ **Complete Cytosine Conversion.** 200 ng of genomic DNA isolated from 3 cancer cell lines was treated with the BisulFlash™ DNA Modification Kit. Next, the unconverted and converted DNA in each treated sample were determined using unconverted DNA-specific and converted DNA-specific primers (β-actin, 110 bps), respectively. *Image A:* real time PCR with Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028); *Image B:* end-point PCR. The BisulFlash™ kit treated DNA was completely converted and no unconverted DNA in the treated samples was determined after 45 cycles.



▲ **Effective DNA Protection.** Fully methylated human genomic DNA at various amounts (0.2 ng-200 ng) were converted using the BisulFlash™ DNA Modification kit. 1 µl of 20 µl eluate was used for real time qPCR and a pair of primers was used to amplify converted DNA. As little as 0.2 ng DNA is sufficient for bisulfite conversion using the BisulFlash™ DNA Modification Kit. *Image A:* real time PCR with Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028); *Image B:* starting DNA amount-CT value curve.

CATALOG INFORMATION

Catalog #	Description	Size	Price
	BisulFlash™ DNA Modification Kit	50 reactions	

EPIGENTEK
Real Solutions For Epigenetic Research

a product of Epigentek Group Inc., USA