Technical Information

Guide to Successful Bisulfite Conversion

Introduction

Bisulfite conversion is the most popular method for DNA methylation analysis and the most convenient and effective way to map DNA methylation to individual bases.

The method involves exposing DNA to sodium bisulfite, which causes unmethylated cytosines to be converted to uracil. Methylated cytosines (also called 5-methylcytosines or 5mC) are not affected. Therefore, bisulfite treatment gives rise to different DNA sequences for methylated and unmethylated DNA.

	Unmethylated DNA	Methylated DNA
Original sequences	N-C-G-N-C-G-N-C-G-N	N-C-G-N-C-G-N-C-G-N
Bisulfite converted	N-U-G-N-U-G-N-U-G-N	N-C-G-N-C-G-N-C-G-N

Starting material

Genomic and plasmid DNA can be used for bisulfite conversion. Genomic DNA should not undergo any previous restriction digestion step. Plasmid DNA should be linearized before starting, to prevent reannealing after the denaturation step, which can occur very quickly with unlinearized single-strand DNA.

Conversion reaction

The most critical step for the correct determination of a methylation pattern is the complete conversion of unmethylated cytosines. This is achieved by incubating the DNA in high concentrations of bisulfite salt at high temperature and low pH. These harsh conditions often lead to a high degree of DNA fragmentation and subsequent loss of DNA during purification, which is necessary to remove bisulfite salts and chemicals used in the conversion process that inhibit sequencing.

Figure 1. The DNA Protect Buffer for the EpiTect Fast Bisulfite kits. The buffer contains a pH indicator dye as a mixing control. It also enables confirmation of the correct pH for cytosine conversion.

Dealing with challenging reaction conditions

Common bisulfite procedures usually require high amounts of input DNA to compensate for the degradation during conversion and loss during purification. QIAGEN's EpiTect® Fast Bisulfite kits prevent DNA fragmentation during bisulfite conversion thanks to the unique DNA Protect Buffer, which is uniquely formulated to prevent the fragmentation usually associated with the harsh treatment conditions. It also ensures effective DNA denaturation to give the single-stranded DNA necessary for complete cytosine conversion. DNA Protect Buffer also contains a convenient pH indicator dye as a mixing control, allowing easy confirmation of the correct pH for cytosine conversion (Figure 1).

Checking the quality and quantity of bisulfite-converted DNA

Processing poor-quality samples through a multi-step workflow is a waste of money and time, so quality control is essential at every key step. The quantity of bisulfite-converted DNA can be determined using a spectrophotometer. A value of 40 µg/ml for A_{260}nm = 1.0 is needed, because the converted DNA resembles RNA. Check the quality by determining the A_{260}/A_{280} ratio of the converted DNA. It should be between 1.7 and 1.9.

Thanks to the DNA Protect Buffer, the EpiTect Fast Bisulfite kits consistently returns higher yields of DNA than methods that do not use DNA protection (Figure 2). The DNA is of appropriate quality for downstream applications.

Working with FFPE samples

The protection offered by this buffer is particularly important for FFPE tissue samples, where the starting DNA is already fragmented. The EpiTect Fast FFPE Bisulfite Kit contains a deparaffinization solution, lysis buffer and proteinase K to fully prepare the FFPE samples, releasing all the DNA so that it is accessible to the DNA Protect Buffer and Bisulfite Solution.

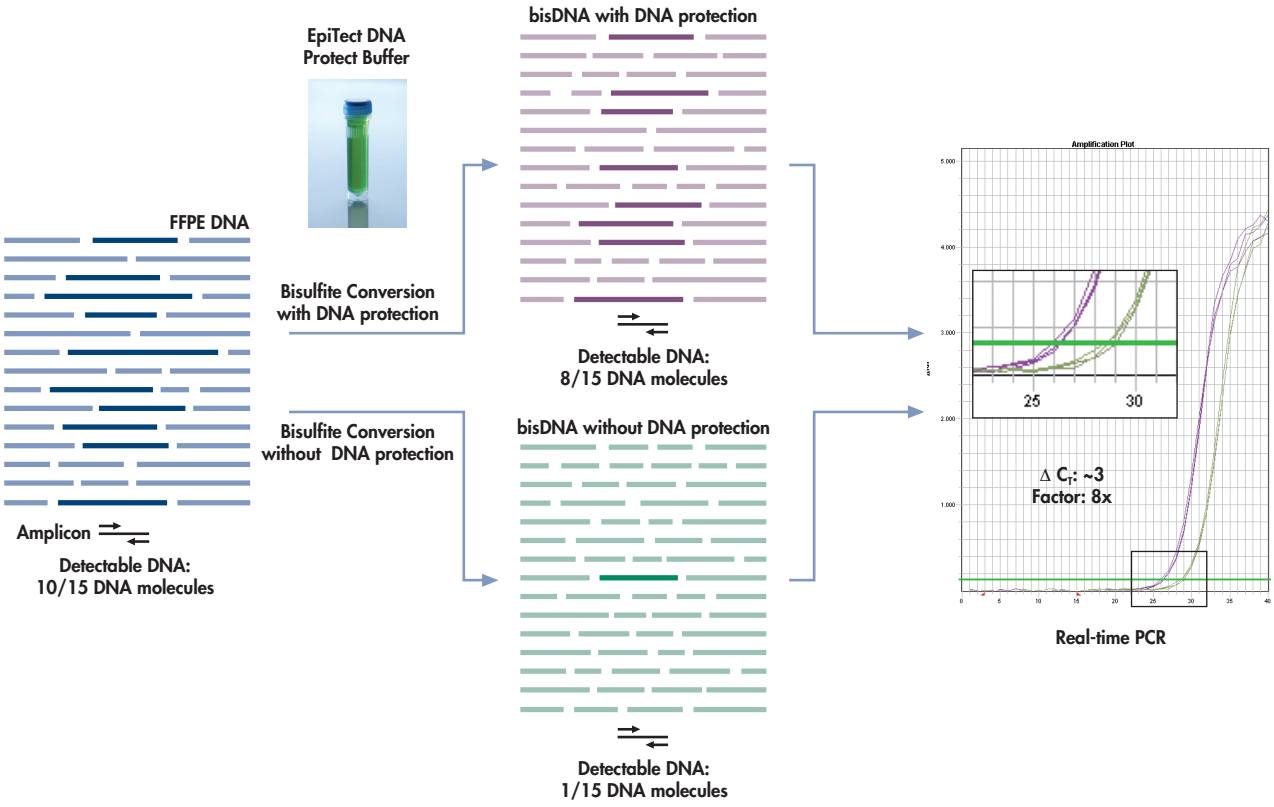

Figure 2. The DNA Protect Buffer in the EpiTect Fast Bisulfite kits ensures appropriate DNA recovery. Degradation of DNA during the bisulfite conversion reaction results in low yields of the target sequence for downstream applications. The DNA Protect Buffer minimizes DNA damage during conversion. Along with minimal loss of DNA during sample lysis and thorough cleanup of the resulting converted DNA, this innovative DNA protect technology maximizes recovery of the converted DNA.

Storage of bisulfite-converted DNA

Purified bisulfite-converted DNA can be stored at 2–8°C for up to 24 h. When storing purified DNA for longer than 24 h, storage at –20°C is recommended.

Conclusion

Successful bisulfite conversion requires appropriately treated starting material and the correct incubation conditions. It is essential to use an appropriate DNA protective buffer, such as the DNA Protect Buffer in the EpiTect Fast Bisulfite kits. This prevents excessive DNA degradation and loss. Appropriate quality control and storage ensures that the converted DNA can be used for a range of sensitive downstream applications.

Ordering Information

Kit name	Contents	Cat. no.
EpiTect Fast Bisulfite Kit (10)	Trial kit for 10 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute® DNA Spin Columns, Carrier RNA and Buffers	59802
EpiTect Fast DNA Bisulfite Kit (50)	For 50 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59824
EpiTect Fast DNA Bisulfite Kit (200)	For 200 preps: Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59826
EpiTect Fast FFPE Bisulfite Kit (50)	For 50 preps: Deparaffinization Solution, Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59844
EpiTect Fast LyseAll Bisulfite Kit (50)	For 50 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59864
EpiTect Fast LyseAll Bisulfite Kit (200)	For 200 preps: Lysis Buffer, Proteinase K, Bisulfite Solution, DNA Protect Buffer, MinElute DNA Spin Columns, Carrier RNA and Buffers	59866
EpiTect Fast 96 Bisulfite Kit	2x EpiTect 96-well Plates, Bisulfite Solution, DNA Protect Buffer, Carrier RNA and Buffers	59720
EpiTect Fast 96 FFPE Bisulfite Kit	Deparaffinization Solution, Lysis Buffer, Proteinase K, 2x EpiTect 96-well Plates, Bisulfite Solution, DNA Protect Buffer, Carrier RNA and Buffers	59740

Discover the reliable EpiTect Fast Bisulfite kits at **www.qiagen.com/Bisulfite-Conversion**.