EpiTect® Fast DNA Bisulfite Kit — Part 1

MinElute® DNA spin columns, DNA Protect Buffer, and Buffer BD from the EpiTect Fast DNA Bisulfite Kit (cat. nos. 59824 and 59826) should be stored at 2–8°C. All other buffers and Bisulfite Solution should be stored at room temperature (15–25°C) for up to 6 months.

For more information, please refer to the *EpiTect Fast Bisulfite Conversion Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- Add 30 ml ethanol (96–100%) to Buffer BW and store at room temperature.
- Add 27 ml ethanol (96–100%) to Buffer BD and store at 2–8°C.
- Add 310 µl RNase-free water to carrier RNA and store in aliquots at –20°C.
- Equilibrate samples and buffers to room temperature.
- Set up bisulfite reactions at room temperature.

Bisulfite conversion of DNA

1. Thaw DNA to be used in the bisulfite reactions. Make sure the Bisulfite Solution is completely dissolved. If necessary, heat the solution to 60°C and vortex until all precipitates are dissolved.
   Note: Do not place Bisulfite Solution on ice.
2. Set up the bisulfite reactions in 200 µl PCR tubes (not provided) according to Table 1. Add each component in the order listed.
3. Close the PCR tubes and mix the bisulfite reactions thoroughly. Keep the samples at room temperature.
   Note: DNA Protect Buffer should turn blue indicating sufficient mixing and correct pH for the bisulfite conversion reaction.

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Table 1. Bisulfite reaction setup

Component	High concentration samples (1ng – 2 µg) Volume per reaction (µl)	Low concentration samples (1–500 ng) Volume per reaction (µl)
DNA	Variable* (maximum 20 µl)	Variable† (maximum 40 µl)
RNase-free water	Variable*	Variable†
Bisulfite Solution	85	85
DNA Protect Buffer	35	15
Total volume	**140**	**140**

* The combined volume of DNA solution and RNase-free water must total 20 µl.
† The combined volume of DNA solution and RNase-free water must total 40 µl.

4. Program the thermal cycler according to Table 2. Use a cycler with a heated lid.
   Note: If using a thermal cycler that does not allow you to enter the reaction volume (140 µl), set the instrument to the largest volume setting available.

Table 2. Bisulfite conversion thermal cycler conditions

Step	Time	Temperature
Denaturation	5 min	95°C
Incubation	10 min‡	60°C
Denaturation	5 min	95°C
Incubation	10 min‡	60°C
Hold	Indefinite§	20°C

‡ In some cases it may be necessary to extend the 60°C cycle time up to 20 min to achieve complete bisulfite DNA conversion.
§ Converted DNA can be left in the thermal cycler overnight without any loss of performance.

5. Place the PCR tubes in the thermal cycler and start the incubation.

6. Proceed to Part 2 "Cleanup of converted DNA".