Quick-Start Protocol April 2016

EpiTect® 96 Bisulfite Kit – Part 1

Upon arrival of the EpiTect 96 Bisulfite Kit (cat. no. 59110), the DNA Protect Buffer and the Buffer BD should be stored at 2–8°C. All other kit components can be stored at room temperature (15–25°C) and are stable for at least 6 months if not otherwise stated on label.

Further information

- *EpiTect 96 Bisulfite Handbook*: **www.qiagen.com/HB-0244**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for sodium bisulfite conversion of unmethylated cytosines in DNA using a centrifuge. DNA (1 ng – 2 µg) can be processed in a volume of up to 20 µl. For other protocols, refer to the handbook.
- Perform all centrifugation steps at room temperature (15–25°C).
- Add 120 ml ethanol (96–100%) to Buffer BW, and store at room temperature. Invert the bottle several times before starting the procedure.
- Add 27 ml ethanol (96–100%) to Buffer BD, and store at 2–8°C. Invert the bottle several times before starting the procedure. Avoid transferring precipitates to the EpiTect 96 Plate.
- Add 1350 µl RNase-free water to the lyophilized carrier RNA (1350 µg) to obtain a solution of 1 µg/µl. Aliquots can be stored at –20°C for up to 1 year.
- Add 600 µl of the dissolved carrier RNA to one bottle of Buffer BL to obtain a final concentration of 10 µg/ml. Carrier RNA is not necessary if >100 ng DNA is used. Dissolve precipitates in Buffer BL by heating (maximum 70°C).
- Equilibrate samples and buffers to room temperature.
- **Optional**: set a thermomixer, heating block or heated orbital incubator to 60°C for use in step 1.

Bisulfite DNA conversion

1. Thaw DNA to be used in the bisulfite reactions. Add 9 ml RNase-free water to the Bisulfite Mix and vortex until the Bisulfite Mix is completely dissolved. This can take up to 5 min.
2. Prepare the bisulfite reactions in the provided EpiTect Conversion Plate according to Table 1. Add each component in the order listed.

Table 1. Bisulfite reaction components

Component	Volume per reaction, µl
DNA solution (1 ng – 2 µg)	Variable* (maximum 20 µl)
RNase-free water	Variable*
Bisulfite Mix (dissolved), see step 1	85
DNA Protect Buffer	35
Total volume	140 µl

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

3. Seal the EpiTect Conversion Plate using EpiTect Cover Foil (provided) and mix the bisulfite reactions by vortexing thoroughly. Centrifuge the plate briefly at 650 x *g* (approximately 2000 rpm) to collect the reactions in the bottom of the wells. Store the plate at room temperature (15–25°C).

   Note: DNA Protect Buffer should turn from green to blue after addition to DNA–Bisulfite Mix, indicating sufficient mixing and correct pH for DNA bisulfite conversion reaction. Proceed with Part 2.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.