Quick-Start Protocol April 2016

EpiTect® 96 Bisulfite Kit – Part 2

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

- Unless otherwise stated, centrifugation is performed at 5800 x *g*.

Table 2. Bisulfite conversion thermal cycler conditions

Step	Time	Optimized temperature
Denaturation	5 min	95°C
Incubation	25 min	60°C
Denaturation	5 min	95°C
Incubation	85 min (1 h 25 min)	60°C
Denaturation	5 min	95°C
Incubation	175 min	(2 h 55 min) 60°C
Hold	Indefinite*	20°C

* Converted DNA can be left in the thermal cycler overnight without any loss of performance.

4. Perform the bisulfite DNA conversion using a thermal cycler. Program the thermal cycler according to Table 2.
5. Place the EpiTect Conversion Plate containing the bisulfite reactions into the thermal cycler. Start the thermal cycling incubation. **IMPORTANT**: Only thermal cyclers with heated lids are suitable for this procedure. Samples can be stored at –20°C for up to 24 h. Before further processing, precipitates must be dissolved by heating to 60°C and vortexing.

Cleanup of bisulfite converted DNA

6. Briefly centrifuge the plate containing the bisulfite reactions at 650 x *g*.
7. Place an EpiTect 96 Plate on top of an S-Block. Mark for later identification.
8. Dispense 560 µl freshly prepared Buffer BL containing 10 µg/ml carrier RNA into the required wells of the EpiTect 96 Plate. Proceed within 5 min.
9. Transfer the complete bisulfite reactions from step 5 to the EpiTect 96 Plate and mix with the Buffer BL by pipetting up and down 4 times.
10. Seal the EpiTect 96 Plate with an AirPore Tape Sheet (provided). Load the S-Block and EpiTect 96 Plate into the centrifuge plate holder, and then place the holder into the rotor bucket. Centrifuge for 1 min.
11. Remove the tape. Carefully add 500 µl Buffer BW to each sample and seal with a new tape. Centrifuge for 1 min. Carefully empty the S-Block.
12. Remove the tape. Carefully add 250 µl Buffer BD to each sample and seal the plate with a new tape. Incubate for 15 min at room temperature (15–25°C). Avoid transferring precipitates to the plate. Centrifuge for 1 min.
13. Remove the tape. Carefully add 500 µl Buffer BW to each sample and seal the plate with a new tape. Centrifuge for 1 min. Carefully empty the S-Block.
14. Remove the tape. Carefully add 500 µl Buffer BW to each sample and seal the plate with a new tape. Centrifuge for 1 min.
15. Remove the tape. Carefully add 250 µl ethanol (96–100%) to each sample and seal the plate with a new tape. Centrifuge for 1 min.
16. Dispose of the S-Block appropriately. Remove the tape and place the EpiTect 96 Plate on top of an EpiTect Elution Plate (provided). Centrifuge for 15 min.
17. Place the EpiTect 96 Plate on top of a new EpiTect Elution Plate (provided).
18. Dispense 70 µl Buffer EB directly onto the center of the EpiTect membrane.
19. Centrifuge for 1 min. Seal the elution plate for storage using a tape sheet.

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