Quick-Start Protocol March 2016

# EpiTect® Plus DNA Bisulfite Kit – Protocol 1

MinElute® DNA spin columns, DNA Protect Buffer and Buffer BD from the EpiTect Plus DNA Bisulfite Kit (cat. no. 59124) should be stored at 2–8°C. All other buffers and Bisulfite Mix should be stored at room temperature (15–25°C) for up to 6 months if not otherwise stated on label. Dissolved Bisulfite Mix can be stored at –20°C for up to 4 weeks.

## Further information

- *EpiTect Plus Bisulfite Conversion Handbook*: **www.qiagen.com/HB-0388**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- Add 30 ml ethanol (96–100%) to Buffer BW and store at room temperature (15–25°C).
- 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.
- Carrier RNA (step 1) is not necessary if >100 ng DNA are used.

## Bisulfite conversion of DNA

1. Add 800 µl RNase-free water to each aliquot of Bisulfite Mix needed, and vortex until Bisulfite Mix is completely dissolved. This may take up to 5 min. Dissolving the Bisulfite Mix may require heating the solution to 60°C.
2. Set up the bisulfite reactions in 200 µl PCR tubes according to Table 1. Add each component in the order listed.
3. Close the PCR tubes and mix the bisulfite reactions thoroughly. DNA Protect Buffer should turn blue indicating sufficient mixing and correct pH.

4. Program the thermal cycler according to Table 2. Use a cycler with a heated lid. 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.
5. Place the PCR tubes in the thermal cycler and start the incubation. Converted DNA can be left in the thermal cycler overnight without loss of performance.
6. Proceed to Protocol 2 "Cleanup of converted DNA" included in the EpiTect Plus DNA Bisulfite Kit.

**Table 1. Bisulfite reaction setup**

| Component | High concentration samples (1ng – 2 µg) Volume per reaction (µl) | Low concentration samples (1 ng – 500 ng) Volume per reaction (µl) |
|---|---|---|
| DNA solution | Variable* (maximum 20 µl) | Variable† (maximum 40 µl) |
| RNase-free water | Variable* | Variable† |
| Bisulfite Mix | 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.

**Table 2. Bisulfite conversion thermal cycler conditions**

| Step | Time | 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.

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