Quick-Start Protocol March 2016

EpiTect® Plus DNA Bisulfite Kit – Protocol 2

See *Quick-Start Protocol: EpiTect Plus DNA Bisulfite Kit – Protocol 1* for instructions about kit storage and reagent preparation.

Further information

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

Cleanup of converted DNA

1. For starting material <100 ng DNA, add dissolved carrier RNA to Buffer BL. Prepare only the amount needed for the number of reactions (Table 1).
2. Upon completion of the bisulfite conversion (Protocol 1), briefly centrifuge the PCR tubes. Transfer the reactions to clean 1.5 ml microcentrifuge tubes.
3. Add 310 µl Buffer BL (with 10 µg/ml carrier RNA for <100 ng DNA; step 1) to each sample. Mix by vortexing and then centrifuge briefly.
4. Add 250 µl ethanol (96–100%) to each sample. Mix by pulse vortexing for 15 s and then centrifuge briefly to remove drops from inside the lid.
5. Place MinElute® DNA spin columns and collection tubes in a rack. Transfer the entire contents of each tube (step 4) to a corresponding spin column.
6. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
7. Add 500 µl Buffer BW to each spin column. Centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
8. Add 500 µl Buffer BD to each spin column, close the spin column lids, and incubate for 15 min at room temperature (15–25°C).

   Important: Minimize exposure of Buffer BD to air to prevent acidification.

9. Centrifuge the spin columns at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
10. Add 500 µl Buffer BW to each spin column. Centrifuge at maximum speed for 1 min. Discard the flow-through and place the spin columns back into the collection tubes.
11. Repeat step 10.
12. Add 250 µl ethanol (96–100%) to each spin column and centrifuge at maximum speed for 1 min.
13. Place the spin columns into new 2 ml collection tubes and centrifuge at maximum speed for 1 min to remove any residual liquid.

    Optional: Incubate the spin columns on a heating block at 60°C for 5 min to evaporate the liquid.
14. Place the spin columns into clean 1.5 ml microcentrifuge tubes (not provided). Add 15 µl Buffer EB directly onto the center of each spin-column membrane and close the lids gently.

    Note: As little as 10 µl Buffer EB can be used for elution.
15. Incubate the spin columns at room temperature for 1 min.
16. Centrifuge for 1 min at 15,000 x *g* (12,000 rpm) to elute the DNA.

    Note: Store purified DNA at 2–8°C for up to 24 h. For longer storage, we recommend storage at –20°C.

Table 1. Carrier RNA and Buffer BL volumes

Number of samples	1	4	8	16	24	48
Volume of Buffer BL*	350 µl	1.4 ml	2.8 ml	5.6 ml	8.4 ml	16.8 ml
Volume of carrier RNA solution*	3.5 µl	14 µl	28 µl	56 µl	84 µl	168 µl

* Volumes give10 µg/ml carrier RNA in Buffer BL with a 10% surplus for pipetting inaccuracies.

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