Quick-Start Protocol February 2016

EpiTect® Fast 96 DNA Bisulfite Kit – Part 2

See *Quick-Start Protocol: EpiTect Fast 96 DNA Bisulfite Kit* – Part 1 for instructions about kit storage and reagent preparation.

Further information

- *EpiTect Fast 96 DNA Bisulfite Kit Handbook*: **www.qiagen.com/HB-1212**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Equilibrate samples and buffers to room temperature.
- Carrier RNA is not necessary if >100 ng DNA is used.
- Symbols: ●centrifuge processing; ▲vacuum processing.

Cleanup of converted DNA

1. Upon completion of the bisulfite conversion (refer to Part 1), briefly centrifuge the EpiTect 96 Conversion Plate at 650 x *g*.
2. Place an EpiTect 96 Plate ● on top of an S-Block, and mark the plate for later identification, or ▲ securely onto a prepared vacuum manifold (e.g., QIAvac 96), and seal unused wells with Tape Pads.
3. Add 310 µl freshly prepared Buffer BL to the required wells of the EpiTect 96 Plate (add dissolved carrier RNA to Buffer BL if samples contain <100 ng DNA starting material).

   Note: Proceed with the cleanup protocol within 5 min of applying Buffer BL.
4. Transfer the complete reactions from step 1 to the EpiTect 96 Plate. Mix by pipetting up and down 4 times. Precipitates in the bisulfite reactions will not affect the performance or yield. Add 250 µl ethanol (96–100%) to each sample and mix with the Buffer BL-bisulfite reaction solution by pipetting up and down 4 times.
5. Process samples. ● Seal the EpiTect 96 Plate with an AirPore

Tape Sheet. Centrifuge the S-Block and EpiTect 96 Plate at 5800 x *g* for 1 min at room temperature. Carefully empty the S-Block. Remove the AirPore Tape Sheet. ▲ Apply vacuum. Switch off vacuum after 1 min (or until all liquid passes through the membrane).

6. Add 500 µl buffer BW to each sample and process as described in step 5.
7. Add 250 µl Buffer BD to each sample. Cover and incubate for 15 min at room temperature (15–25°C). Avoid transferring precipitates to the plate. Repeat step 5.

   IMPORTANT: The bottle containing Buffer BD should be closed immediately after use to avoid acidification from carbon dioxide in the air.
8. Add 500 µl Buffer BW to each sample and repeat step 5.
9. Repeat step 7 to wash samples again.
10. Add 250 µl ethanol (96–100%) to each sample and repeat step 5.
11. Remove residual ethanol: ● Place the EpiTect 96 Plate on top of an EpiTect Elution Plate. Centrifuge at 5800 x *g* for 15 min. **Optional**: Centrifuge at 40°C to ensure evaporation of residual ethanol. ▲ Apply maximum vacuum for 10 min. Switch off the vacuum source and slowly ventilate. Lift the QIAvac 96 Top Plate from the base (not the EpiTect 96 Plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the EpiTect 96 Plate with clean absorbent paper.
12. Prepare Elution Plate: ● Place the EpiTect 96 Plate on top of a new EpiTect Elution Plate (provided), or ▲ remove the waste tray and insert the vacuum manifold adapter for elution plates. Place Elution Plate directly on the adapter and top plate back on the base.
13. Add 70 µl Buffer EB to each sample using a multichannel pipet. ● Centrifuge at 5800 x *g* for 1 min. ▲ Add 10 µl Top Elute Fluid to each sample. Switch on the vacuum source for 1 min. Switch off the vacuum source and slowly ventilate.

    IMPORTANT: For complete elution of bound DNA, ensure that the elution buffer is dispensed directly onto the center of the membrane.
14. Seal the elution plate for storage using Tape Pads. Store purified DNA at 2–8°C for up to 24 h. For longer storage, we recommend storage at –20°C.

    Note: The average eluate volume is 40–50 µl from 70 µl elution buffer.

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