Quick-Start Protocol April 2016

EpiTect® FFPE Lysis Kit

The EpiTect FFPE Lysis Kit included in the EpiTect Fast FFPE Bisulfite Kits, EpiTect Fast 96 FFPE Bisulfite Kits and EpiTect Fast FFPE Bisulfite Kits can be stored at room temperature (15–25°C) for up to 6 months if not otherwise stated on label.

Further information

- *EpiTect Fast Bisulfite Conversion Handbook*: **www.qiagen.com/HB-1211**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Incubate Deparaffinization Solution and Lysis Buffer FTB at 30°C to dissolve any precipitates.
- Equilibrate samples and buffers to room temperature.
- Scrape any FFPE samples on slides into a 200 µl reaction tube or 8-well strip and proceed with step 1.
- Perform all centrifugation steps at room temperature (15–25°C).
- Do not place samples on ice after beginning the lysis procedure.

1. Place a FFPE slice (10 µm thick, with a surface area ≤100 mm^2) in a 200 µl reaction tube or 8-well strip (not provided) and add 150 µl Deparaffinization Solution.
2. Flick or vortex the tube until all paraffin is dissolved.
3. Add 20 µl distilled water, 15 µl Lysis Buffer FTB and 5 µl proteinase K. Vortex and briefly centrifuge the samples.

   Note: Deparaffinization Solution will form a layer above the Lysis Buffer FTB with the addition of proteinase K.
4. Program a thermal cycler according to Table 1 for lysis and decrosslinking.
5. Place the PCR tubes with the lysis reactions into the thermal cycler and start the lysis incubation. Proceed as soon as possible with bisulfite conversion.

Table 1. Lysis thermal cycler conditions

Step	Time	Temperature
Lysis	30 min*	56°C
Decrosslinking	60 min	95°C

* Ensure that the tissue is completely lysed; if not, add an additional lysis step (30 min at 56°C).

6. Make sure the Bisulfite Solution is completely dissolved. If necessary, heat the solution to 60°C and vortex until all precipitates are dissolved.

   Note: Do not place Bisulfite Solution on ice.

7. After the lysis incubation is completed, remove the Deparaffinization Solution (approximately 130 µl) from each reaction. Remove as much Deparaffinization Solution as possible without disturbing the lysed sample material to make sufficient space in the reaction tube for the bisulfite reaction components.

   Note: Small amounts of remaining Deparaffinization Solution have no effect on the bisulfite reaction.

8. For bisulfite reaction setup in 200 µl reaction tubes or 8-well strips, add the reagents listed in Table 2 directly to the lysis reactions, in the order listed. For bisulfite reaction setup in 96-well format, add the lysis reactions and reagents listed in Table 2, in the order listed, to an EpiTect 96 Conversion Plate. Use the provided EpiTect 96 DNA Protect Buffer Reservoir.

9. Proceed to step 3 of Part 1 of protocol "Bisulfite conversion of DNA" included in the EpiTect Fast DNA Bisulfite Kit or EpiTect Fast 96 DNA Bisulfite Kit.

Table 2. Bisulfite reaction setup

Component	Volume per reaction (µl)
Lysis reaction (step 7)	Approximately 40
Bisulfite Solution	85
DNA Protect Buffer	15
Total volume	**140**

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