EpiTect[®] LyseAll Lysis Kit

The EpiTect LyseAll Lysis Kit included in the EpiTect Fast LyseAll Bisulfite Kits, EpiTect Fast 96 LyseAll Bisulfite Kits, and EpiTect Plus LyseAll Bisulfite Kits can be stored at room temperature (15–25°C) for up to 6 months.

For more information, please refer to the EpiTect Fast Bisulfite Conversion Handbook or EpiTect Fast 96 Bisulfite Conversion Handbook, which can be found at <u>www.qiagen.com/handbooks</u>.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at <u>www.qiagen.com/contact</u>.

Notes before starting

- Equilibrate samples and buffers to room temperature.
- Perform all centrifugation steps at room temperature (15–25°C).
- Do not place samples on ice after beginning lysis procedure.

Lysis of whole blood samples

- 1. Dilute blood samples (maximum $20 \,\mu$ l) 1:20 with Buffer EL.
- 2. Incubate at room temperature (15–25°C) for 10–15 min, inverting the tubes several times during incubation.
- 3. Centrifuge at maximum speed for 5 min. Discard supernatant and add an additional 125 μ l Buffer EL.
- 4. Centrifuge at maximum speed for 1 min. Discard supernatant.
- 5. Resuspend cells in 10 μ l PBS and transfer into a 200 μ l reaction tube or into 8–well strips (not provided).
- Add 10 μl distilled water, 15 μl Lysis Buffer FTB, and 5 μl proteinase K. Vortex and centrifuge briefly. Proceed to step 12.

Lysis of cultured cells

7. Harvest cells according to your current protocol.



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- 8. Resuspend cells in 10 μ l PBS in a 200 μ l reaction tube or in 8-well strips (not provided). Do not use more than 1 x 10⁵ cells per 10 μ l PBS.
- Add 10 μl distilled water, 15 μl Lysis Buffer FTB, and 5 μl proteinase K. Vortex and centrifuge briefly. Proceed to step 12.

Lysis of tissue samples

- 10. Place the tissue sample ($\leq 100 \ \mu$ g) in a 200 μ l reaction tube or 8-well strip and add 20 μ l distilled water.
- 11. Add 15 μ l Lysis Buffer FTB and 5 μ l proteinase K. Vortex and centrifuge briefly.

Bisulfite conversion

- 12. Incubate samples for 30 min at 56°C.
- 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.
- 14a. For bisulfite reaction setup in $200 \,\mu$ l reaction tubes or 8-well strips, add the reagents listed in Table 1 directly to the lysis reactions, in the order listed.
- 14b. For bisulfite reaction setup in 96-well format, add the lysis reactions and reagents listed in Table 1, in the order listed, to an EpiTect 96 Conversion Plate. Use the provided EpiTect 96 DNA Protect Buffer Reservoir.
- 15. 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.

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

Table 1. Bisulfite reaction setup

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