Quick-Start Protocol April 2016

REPLI-g® FFPE Kit

The REPLI-g FFPE Kit (cat. nos. 150243 and 150245) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer, with the exception of Proteinase K and FFPE Lysis Solution, which should be stored at room temperature (15–25°C). The kit can be stored for 6 months (up to 1 year for Proteinase K and FFPE Lysis Solution) if not otherwise stated on label.

Further information

- *REPLI-g FFPE Handbook*: **www.qiagen.com/HB-0842**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Tissue sections should be between 10 and 40 µm. One tissue section is sufficient per REPLI-g reaction. Depending on the size of the tissue within the section, a fraction of a tissue section may be sufficient. The tissue portion used should exceed 1 cm in diameter. For smaller tissue portions, use thicker sections or two or more sections.
- If paraffin-embedded tissue is limited or the tissue section contains tissue <1 cm in diameter, the section should be trimmed to reduce the paraffin content. A smaller, sufficient volume of FFPE Lysis Solution (1x) (<100 µl) should be added in step 3 to ensure complete coverage of the tissue section.
- Preferably, the starting material for DNA amplification should be freshly cut sections of FFPE tissue. If the sample surface has been exposed to air, discard the first 2–3 sections.
- All frozen enzymes (Ligation Enzyme, FFPE Enzyme and REPLI-g Midi DNA Polymerase) should be thawed on ice just before reaction setup. All other components can be thawed at room temperature.

- We recommend using the procedure detailed in the *REPLI-g FFPE Handbook* Appendix C to determine the quality of the DNA in FFPE tissue section samples and to estimate the success rate of the REPLI-g FFPE whole genome amplification reactions with the tested samples. After this assessment, the DNA in the lysed tissue sections can be directly amplified using the REPLI-g FFPE Kit without prior purification.
- DNA yields are dependent on the reaction conditions and the quality of the template DNA. The 2 amplification times indicated in step 15 – 2 h (standard reaction) and 8 h (high-yield reaction) – provide typical DNA yields of ≤10 µg and ≤40 µg per 50 µl reaction, respectively.

1. Cut one tissue section from a paraffin-embedded tissue block and transfer the tissue section into a microcentrifuge tube.
2. Prepare 1x FFPE Lysis Solution according to Table 1. Mix and centrifuge briefly.

Table 1. Preparation of FFPE Lysis Solution (1x)

Component	Volume/reaction
FFPE Lysis Solution(10x)	10 µl
Nuclease-Free Water	90 µl
Total volume	**100 µl**

3. Add 100 µl of FFPE Lysis Solution (1x) to the tissue section from step 1. Mix and centrifuge briefly.
4. Incubate the sample at 95°C for 10 min to melt the paraffin.
5. Cool the sample to room temperature.

   Note: At this stage, a thin layer of paraffin with tissue forms on the surface of the liquid portion of the sample.
6. Add 2 µl of Proteinase K. Mix and centrifuge briefly.
7. Incubate for 60 min at 60°C followed by 10 min at 95°C.
8. Transfer 10 µl of the lysed tissue section into a new microcentrifuge tube.

   IMPORTANT: Avoid carryover of paraffin to the microcentrifuge tube.

9. Prepare FFPE master mix on ice according to Table 2. Vortex and centrifuge briefly.

   IMPORTANT: Add the FFPE master mix components in the order listed in Table 2. The master mix should be kept on ice and used immediately after preparation.

Table 2. Preparation of FFPE master mix

Component	Volume/reaction
FFPE Buffer	8 µl
Ligation Enzyme	1 µl
FFPE Enzyme	1 µl
Total volume	**10 µl**

10. Add 10 µl FFPE master mix to 10 µl DNA from the lysed tissue from step 8. Mix and centrifuge briefly.

11. Incubate the samples at 24°C for 30 min.

    Note: DNA fragments prepared from tissue sections are now ligated randomly to form DNA of high molecular weight.

12. Stop the reaction by incubating at 95°C for 5 min and cool to 4°C using a thermal cycler or ice.

13. Prepare the REPLI-g master mix according to Table 3.

    IMPORTANT: Add the master mix components in the order listed in Table 3. The master mix should be kept on ice and used immediately upon addition of the REPLI-g Midi DNA Polymerase.

Table 3. Preparation of REPLI-g master mix

Component	Volume/reaction
REPLI-g Midi Reaction Buffer	29 µl
REPLI-g Midi Polymerase	1 µl
Total volume	**30 µl**

14. Add 30 µl of the REPLI-g master mix to the denatured DNA prepared in step 12. Mix and centrifuge briefly.

15. Incubate at 30°C for either 2 h (standard reaction) or 8 h (high-yield reaction).

16. Stop the reaction by incubating at 95°C for 10 min.

    Note: During the amplification reaction the solution becomes turbid, which indicates the presence of high DNA concentrations. If performing PicoGreen® quantification of REPLI-g amplified DNA, please see Appendix E of the *REPLI-g FFPE Handbook*.

17. Store the amplified DNA at –20°C until required for downstream applications.

    Note: If performing PCR analysis, see guidelines in Appendix A of the *REPLI-g FFPE Handbook*. The amplified DNA should be treated as genomic DNA (and the number of freeze–thaw cycles should be minimized). As long-term storage of nucleic acids at a low concentration may result in acid hydrolysis, we recommend a minimum storage concentration of 50 ng/µl for the amplified DNA.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.