Quick-Start Protocol April 2016

REPLI-g® Mitochondrial DNA Kit

The REPLI-g Mitochondrial DNA Kit (cat. no. 151023), including buffers and reagents, should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. When stored under these conditions and handled correctly, this product can be kept at least 6 months after shipping without showing any reduction in performance, if not otherwise stated on label. For longer storage, the kit should be stored at –65 to –90°C.

Further information

- *REPLI-g Mitochondrial DNA Handbook*: **www.qiagen.com/HB-1628**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for human mitochondrial genome amplification from purified genomic DNA. If used for amplification of mitochondrial genome from purified genomic DNA of another species, the REPLI-g Human mt Primer Mix should be substituted with an appropriate primer mix for that species. Template DNA should be suspended in TE buffer or water.
- QIAamp® and DNeasy® Kits enable purification of high-quality genomic DNA from a variety of sample types (including blood, tissue and body fluids). Genomic DNA purified with these kits is ideal for use in REPLI-g Mitochondrial DNA reactions. Alternative extraction methods may selectively enrich for nuclear DNA, reducing the amount of mitochondrial DNA.
- Degraded DNA is not suitable for use in this procedure.

- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 6). REPLI-g mt Reaction Buffer and REPLI-g Human mt Primer Mix should be thawed at room temperature (15–25°C).
- REPLI-g mt Reaction Buffer should be vortexed for at least 10 s before use to ensure thorough mixing.

Things to do before starting

- Set a water bath or heating block to 75°C for use in step 5.
- Set a water bath or heating block to 33°C for use in step 8.

1. Place 1 to 10 µl template DNA into a microcentrifuge tube

   The amount of template DNA should be >1 ng. Smaller amounts (≥0.1 ng) of starting material can be used if the DNA is of sufficient quality and a high proportion of the mitochondrial DNA has been purified.

2. Adjust the sample volume to 20 µl with RNAse-Free Water (supplied).

   The volume of water added depends on the volume of DNA template. The total volume of DNA template and water should be 20 µl.

3. Prepare a fresh amplification mix (see Table 1). Mix by vortexing and centrifuge briefly.

Table 1. Amplification mix

Component	Volume/reaction
REPLI-g mt Reaction Buffer	27 µl
REPLI-g Human mt Primer Mix	2 µl
Total volume	**29 µl**

4. Add 29 µl amplification mix to the DNA. Mix by vortexing and centrifuge briefly.
5. Incubate the sample for 5 min at 75°C. Allow sample to cool down to room temperature (15–25°C).
6. Thaw REPLI-g Midi DNA Polymerase on ice.
7. Add 1 µl REPLI-g Midi DNA Polymerase to the DNA (from step 5). Mix and centrifuge briefly.

8. Incubate the sample at 33°C for 8 h.
9. Inactivate REPLI-g Midi DNA Polymerase by heating the sample for 3 min at 65°C.
10. If not using directly, store the amplified DNA at 2–8°C for short-term storage or at –30 to –15°C for long-term storage.

    DNA amplified using the REPLI-g Mitochondrial DNA Kit should be treated as pure DNA with minimal freeze-thaw cycles. We therefore recommend storage of the DNA at a concentration of at least 100 ng/µl.

    Note: Optical density (OD) measurements overestimate REPLI-g amplified DNA. See Appendix B, page 14, for an accurate method of quantifying DNA amplified using the REPLI-g Mitochondrial DNA Kit.

    Note: In no-template controls, high-molecular-weight product can be generated by random extension of primer–dimers. This DNA will not affect the quality of actual samples or specific downstream genetic assays.
11. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing and quantitative PCR. For downstream applications, use the correct amount of REPLI-g amplified mt DNA in water or TE buffer according to the manufacturer's instructions.

    Note: If the amplified DNA will be used in next-generation sequencing, use up to 1 µg for shearing into a random library of fragments.

    Note: If the amplified DNA will be analyzed using PCR, dilute the DNA after inactivation 1:1000 in water or TE buffer. Use 2–3 µl diluted DNA for each PCR.

Scan QR code for handbook.

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