Quick-Start Protocol

REPLI-g® Mini Kits

REPLI-g Mini Kits (cat. nos. 150023 and 150025) should be stored at –30 to –15°C for up to 6 months if not otherwise stated on label. For longer storage, the kits should be stored at –70°C. Reconstituted Buffer DLB can be stored for 6 months at –20°C if not otherwise stated on label. Buffers D1, N1 and D2 should not be stored longer than 3 months if not otherwise stated on label.

Further information

- REPLI-g Mini/Midi Handbook: www.qiagen.com/HB-0469
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Amplification of purified genomic DNA

Notes before starting

- This protocol is optimized for whole genome amplification from >10 ng of purified genomic DNA template. The template DNA should be suspended in TE. Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For best results, template DNA should be >2 kb in length with some fragments >10 kb.
- Typical DNA yields from a REPLI-g Mini Kit reaction are approximately 10 μg per 50 μl reaction.
- Add 500 μl nuclease-free water to Buffer DLB, mix well and centrifuge briefly.
- Thaw REPLI-g Mini DNA Polymerase on ice.
- ▲ denotes instructions for 2.5 μl template genomic DNA.
- ● denotes instructions for 5 μl template genomic DNA.
1. Prepare sufficient Buffer D1 and Buffer N1 for the total number of amplification reactions (see Table 1).

**Table 1. Preparation of Buffer D1 and Buffer N1**

<table>
<thead>
<tr>
<th>Component</th>
<th>Buffer D1*</th>
<th>Buffer N1*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted Buffer DLB</td>
<td>9 µl</td>
<td>–</td>
</tr>
<tr>
<td>Stop solution</td>
<td>–</td>
<td>12 µl</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>32 µl</td>
<td>68 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>41 µl</strong></td>
<td><strong>80 µl</strong></td>
</tr>
</tbody>
</table>

* Volumes given are suitable for up to ▲ 15 or ● 7 reactions.

2. Place ▲ 2.5 µl or ● 5 µl template DNA into a microcentrifuge tube.

3. Add ▲ 2.5 µl or ● 5 µl Buffer D1. Mix by vortexing and centrifuge briefly.

4. Incubate the samples at room temperature (15–25°C) for 3 min.

5. Add ▲ 5 µl or ● 10 µl Buffer N1. Mix by vortexing and centrifuge briefly.

6. Prepare a master mix on ice according to Table 2. Add components in the order listed. Mix and centrifuge briefly.

**Table 2. Preparation of Master Mix for genomic DNA**

<table>
<thead>
<tr>
<th>Component</th>
<th>▲ 2.5 µl gDNA</th>
<th>● 5 µl gDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free water</td>
<td>10 µl</td>
<td>–</td>
</tr>
<tr>
<td>REPLIg Mini Reaction Buffer</td>
<td>29 µl</td>
<td>29 µl</td>
</tr>
<tr>
<td>REPLIg Mini DNA Polymerase</td>
<td>1 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td><strong>Total volume per reaction</strong></td>
<td><strong>40 µl</strong></td>
<td><strong>30 µl</strong></td>
</tr>
</tbody>
</table>

7. Add ▲ 40 µl or ● 30 µl master mix to ▲ 10 µl or ● 20 µl denatured DNA, from step 5.

8. Incubate at 30°C for 10–16 h.

9. Inactivate REPLIg Mini DNA Polymerase by heating samples for 3 min at 65°C.

10. If performing PCR analysis, dilute amplified DNA 1:20 with TE, and use 3 µl of diluted DNA for each PCR.
Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A of the REPLI-g Mini/Midi Handbook for an accurate method of quantifying REPLI-g amplified DNA.

11. Store amplified DNA at 4°C for short-term storage, or −20°C for long-term storage. We recommend storage at a concentration of at least 100 ng/µl.

Amplification of genomic DNA from blood or cells

Notes before starting

- Add 500 µl nuclease-free water to Buffer DLB, mix well and centrifuge briefly.
- Thaw REPLI-g Mini DNA Polymerase on ice.
- The protocol is optimized for 0.5 µl whole blood or cell material.
- Cell concentration should be >600 cells/µl.
- High concentrations of heparin in blood can inhibit the REPLI-g reaction.
- Additional protocols for the amplification of DNA from dried blood cards, buccal cells, tissue, serum, plasma and laser-microdissected cells are available from QIAGEN Technical Services or online at www.qiagen.com/literature.

1. Prepare sufficient Buffer D2 for the total number of amplification reactions (see Table 3).

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted Buffer DLB</td>
<td>55 µl</td>
</tr>
<tr>
<td>Dithiothreitol (DTT), 1 M</td>
<td>5 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>60 µl</td>
</tr>
</tbody>
</table>

* Volumes given are suitable for up to 15 reactions.

2. Mix 2.5 µl PBS with 0.5 µl cell material or blood in a microcentrifuge tube.
3. Add 3.5 µl Buffer D2. Mix by vortexing and centrifuge briefly.
4. Incubate the samples on ice for 10 min.
5. Add 3.5 µl Stop Solution. Mix by vortexing and centrifuge briefly.
6. Prepare a master mix on ice according to Table 4. Add components in the order listed. Mix and centrifuge briefly.

Table 4. Preparation of Master Mix for blood or cells

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleasefree water</td>
<td>10 µl</td>
</tr>
<tr>
<td>REPLIg Mini Reaction Buffer</td>
<td>29 µl</td>
</tr>
<tr>
<td>REPLIg Mini DNA Polymerase</td>
<td>1 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>40 µl</strong></td>
</tr>
</tbody>
</table>

7. Add 40 µl master mix to 10 µl denatured DNA from blood or cells from step 5.

8. Incubate at 30°C for 10–16 h.

9. Inactivate REPLIg Mini DNA Polymerase by heating samples for 3 min at 65°C.

10. If performing PCR analysis, dilute amplified DNA 1:20 with TE and use 3 µl of diluted DNA for each PCR.

   Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A of the REPLIg Mini/Midi Handbook for an accurate method of quantifying REPLIg amplified DNA.

11. Store amplified DNA at 4°C for short-term storage or −20°C for long-term storage.

   We recommend storage at a concentration of at least 100 ng/µl.

Scan QR code for handbook.

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