Quick-Start Protocol April 2016

REPLI-g® Single Cell Kit

The REPLI-g Single Cell Kit (cat. nos. 150343 and 150345) should be stored immediately upon receipt at –30 to –15°C for up to 6 months if not otherwise stated on label. For longer storage, the kit should be stored at –70°C. Reconstituted Buffer DLB can be stored for 6 months at –20°C. Reconstituted Buffers D1, N1 and D2 can be stored at –20°C for up to 3 months.

Further information

- *REPLI-g Single Cell Handbook*: **www.qiagen.com/HB-1306**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Thaw REPLI-g sc DNA Polymerase on ice.
- Add 500 µl H_2O sc to Buffer DLB, mix well and centrifuge briefly.
- Typical DNA yields are 40 µg per 50 µl reaction.
- Supplementary protocols for use with the REPLI-g Single Cell Kit for different starting material such as dried blood cards, buccal cells, tissue, serum, plasma and laser-microdissected cells are available online at **www.qiagen.com/literature**, from the REPLI-g Single Cell Kit product page on the QIAGEN website under the "Resources" tab or from QIAGEN Technical Services.

Table 1. Protocol selection according to starting material

Starting material	Protocol
Single cells, 2–1000 cells	Amplification of genomic DNA from single cells (page 2)
Purified genomic DNA (1–10 ng)	Amplification of purified genomic DNA (page 4)

Protocol 1: Amplification of genomic DNA from single cells

Notes before starting

- This protocol is optimized for whole genome amplification of single cell material. It cannot be used for cells that are treated with formalin or other cross-linking agents. Refer to "Notes before starting" on page 1 for information on other starting materials.
- Samples of 1–1000 intact cells (e.g., human or bacterial) are optimal.

1. Prepare sufficient Buffer D2 for the total number of reactions (Table 2).

Table 2. Preparation of Buffer D2*

Starting material	Volume†
DTT, 1M	3 µl
Reconstituted Buffer DLB	33 µl
Total volume	**36 µl**

* Buffer D2 should not be stored longer than 3 months.

† Volumes given are suitable for 12 reactions.

2. Place 4 µl cell material (supplied with PBS sc) into a microcentrifuge tube.
3. Add 3 µl Buffer D2. Mix by flicking the tube and centrifuge briefly.
4. Incubate at 65°C for 10 min.
5. Add 3 µl Stop Solution. Mix by flicking the tube and centrifuge briefly. Store on ice.
6. Prepare a master mix on ice according Table 3. Mix and centrifuge briefly.

   IMPORTANT: Add the master mix components in the order listed in Table 3. After the addition of water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge the mixture before the addition of REPLI-g sc DNA Polymerase.

Table 3. Preparation of master mix

Starting material	Volume*
H_2O sc	9 µl
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total volume	**40 µl**

* Scale up accordingly if performing several reactions at one time.

7. For each amplification reaction, add 40 µl master mix to 10 µl denatured DNA (from step 5).
8. Incubate at 30°C for 8 h.
9. Inactivate REPLI-g sc DNA Polymerase by heating the sample for 3 min at 65°C.
10. If not being used directly, store amplified DNA at 4°C for short-term storage or –20°C for long-term storage. We recommend a storage concentration of at least 100 ng/µl.
11. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing, array CGH and quantitative PCR.

    Typical DNA yields are approximately 40 µg per 50 µl reaction and often need to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix A of the *REPLI-g Single Cell Handbook* for more information.
12. Use the correct amount of REPLI-g amplified DNA diluted in water or TE buffer according to the manufacturer's recommendations for the downstream application you are using. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µl of diluted DNA for each PCR reaction. If bacterial cells were used, dilute the amplified DNA at least 1:10,000.

Protocol 2: Amplification of purified genomic DNA

Notes before starting

- This protocol is optimized for whole genome amplification of 10 ng purified genomic DNA. The template DNA should be suspended in TE buffer. If the DNA is of sufficient quality, smaller amounts (1–10 ng or 10–100 pg when amplifying bacterial DNA) may be used.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.

1. Prepare sufficient Buffer D1 and Buffer N1 for the total number of amplification reactions (Table 4).

Table 4. Preparation Buffer D1 and Buffer N1*

Component	Buffer D1†	Buffer N1†
Reconstituted Buffer DLB	7 µl	–
Stop Solution	–	9 µl
Nuclease-free water	25 µl	51 µl
Total volume	**32 µl**	**60 µl**

* Buffer D1 and Buffer N1 should not be stored longer than 3 months.
† Volumes given are suitable for 12 reactions.

2. Place 2.5 µl template DNA into a microcentrifuge tube.
3. Add 2.5 µl Buffer D1 to the DNA. Mix by vortexing and centrifuge briefly.
4. Incubate the samples at room temperature (15–25°C) for 3 min.
5. Add 5.0 µl Buffer N1. Mix by vortexing and centrifuge briefly.
6. Proceed as in steps 6–12 of Protocol 1.

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