Quick-Start Protocol February 2018

REPLI-g® Advanced DNA Single Cell Kit

The REPLI-g advanced DNA Single Cell Kit (cat. nos. 150363 and 150365) should be stored immediately upon receipt at –30 to –15°C for up to 6 months. For longer storage, the kit should be stored at –90 to –65°C. Reconstituted Buffer DLB can be stored for 6 months at –30 to –15°C. Buffers D1, N1 and D2 can be stored at –30 to –15°C for up to 3 months.

Further information

- *REPLI-g Advanced DNA Single Cell Handbook*: **www.qiagen.com/HB-2516**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Thaw REPLI-g sc DNA Polymerase on ice.
- Add 250 µl H_2O sc to Buffer DLB, mix well, and centrifuge briefly.
- Typical DNA yields are 20–30 µg per 50 µl reaction.

Table 1. Protocol selection according to starting material

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

Protocol 1: Amplification of genomic DNA from single cells

Notes before starting

- This protocol is optimized for whole genome amplification of eukaryotic single cell material. It cannot be used for cells that are treated with formalin or other cross-linking agents.
- Samples of 1–1000 intact eukaryotic cells are optimal.

Procedure

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

Table 2. Preparation of Buffer D2

Component	Volume*
DTT, 1M	3 µl
Reconstituted Buffer DLB	33 µl
Total volume	**36 µl**

* Volumes given are suitable for 12 reactions.

2. Place 4 µl cell material (supplied with REPLI-g Advanced Single Cell Storage buffer) into a 0.2 ml reaction tube.
3. Add 3 µl Buffer D2. Mix the tube by vortexing and centrifuge briefly.
4. Incubate at room temperature for 10 min.
5. Add 3 µl Stop Solution. Mix the tube by vortexing 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 advanced sc Reaction Buffer, briefly vortex and centrifuge before adding REPLI-g sc DNA Polymerase.

Table 3. Preparation of master mix

Component	Volume*
H_2O sc	9 µl
REPLI-g advanced 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 2 h.

   Note: If a thermal cycler is used with a heated lid for steps 8 and 9, the temperature of the lid should be set to 70°C.
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.

    Note: If amplified DNA has been stored at –20°C, we recommend heating the diluted DNA at 65°C for 3 min and then cooling on ice prior to PCR to ensure reproducible real-time PCR results.

    Note: Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing, array CGH, and quantitative PCR.

    Note: Typical DNA yields are approximately 20–30 µg per 50 µl reaction (depending on sample material) and often require dilution. Optical density (OD) measurements overestimate REPLI-g amplified DNA.

    Note: 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. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µl diluted DNA for each PCR reaction.

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) may be used.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.

Procedure

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

Table 4. Preparation of Buffer D1 and Buffer N1*

Component	Buffer D1†	Buffer N1†
Reconstituted Buffer DLB	7 µl	—
Stop Solution	—	18 µl
Nuclease-free water	57 µl	102 µl
Total volume	**64 µl**	**120 µl**

* Buffer D1 and Buffer N1 should not be stored longer than 3 months. † Volumes given are suitable for 24 reactions.

2. Place 2.5 µl template DNA into a 0.2 ml reaction 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. Follow steps 6–10 of Protocol 1, above.

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