Quick-Start Protocol April 2016

REPLI-g® Cell WGA & WTA Kit

The REPLI-g Cell WGA & WTA Kit (cat. nos. 150052 and 150054) should be stored immediately upon receipt at –30°C to —15°C for up to 6 months if not otherwise stated on label. For longer storage, the kit should be stored at –65°C to –90°C.

Further information

- *REPLI-g Cell WGA & WTA Handbook*: **www.qiagen.com/HB-1687**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- If performing NGS or microarray analysis, use Protocol 1 with oligo dT only or Protocol 2 with poly A+ RNA or rRNA-depleted-RNA to omit rRNA from the amplification.
- If performing PCR/real-time PCR, the amplified cDNA **must be** diluted 1:100.* Add 2–3 µl diluted cDNA to a 20 µl reaction volume.
- Purification of amplified cDNA is only necessary for labeling of cDNA for microarrays, for example. All other downstream applications such as NGS or PCR do not interfere with the remaining nucleotides.
- The high-molecular-weight DNA generated by random extension of primers (primer-multimer formation) in no-template controls (NTC) does not contain genetic information and will not affect the quality of downstream applications.
- Thaw Quantiscript® RT Enzyme mix, WGA Ready Enzyme, Ligase Mix, REPLI-g SensiPhi DNA Polymerase and gDNA Wipeout Buffer, WTA on ice. All other components can be thawed at room temperature (15–25°C).
- Vortex all buffers and reagents before use to ensure thorough mixing.

* To avoid artifacts due to overloading effects.

Protocol 1: Sample lysis

1. Place 13 µl cell material (suspended in PBS) into a microcentrifuge tube. If using less than 13 µl of cell material, add H_20 sc to bring the volume up to 13 µl.

2. Add 8 µl Lysis Buffer. Mix by vortexing and centrifuge briefly.

   Note: Ensure that the cell material does not stick to the wall of the tube above the buffer line.
3. Incubate at 24°C for 5 min, followed by 95°C for 3 min. Cool to 4°C.
4. Transfer a 10 µl aliquot to a fresh reaction tube and immediately perform whole genome amplification (WGA) of genomic DNA (Protocol 2, page 2). The tube should be kept on ice.
5. Transfer a second 10 µl aliquot to a second fresh reaction tube and immediately perform whole transcriptome amplification (WTA) of RNA (Protocol 3, page 4). The tube should be kept on ice.

Protocol 2: Whole genome amplification (WGA) reaction

1. Prepare the WGA ready mix (Table 1).
2. Add 10 µl WGA ready mix to the first aliquot of the lysed cell sample (from Protocol 1, step 4). Mix by vortexing and centrifuge briefly. **Note**: The WGA ready mix must be prepared fresh.

Table 1. Preparing WGA ready mix*

Component	Volume/reaction
RT/Polymerase Buffer	4 µl
gDNA Wipeout Buffer, WTA†	2 µl
H_2O sc	1 µl
Random Primer	1 µl
Oligo dT Primer	1 µl
WGA Ready Enzyme	1 µl
Total volume‡	**10 µl**

* Scale up accordingly if performing several reactions at one time. † Necessary for the reaction; do not remove. ‡ Mix by vortexing and centrifuge briefly.

3. Incubate at 42°C for 60 min. Stop the reaction by incubating at 95°C for 3 min, then cool on ice. **Important**: The following protocol steps are identical to those used in Protocol 3 (WTA), and can be processed in parallel. If processing both reactions in parallel, make sure to set up the correct amount of ligation mix and REPLI-g SensiPhi amplification mix for both reactions.
4. Prepare the ligation mix (Table 2). Add 10 µl ligation mix to the WGA ready reaction from step 3 (or to the Quantiscript RT reaction from Protocol 3, step 4).

Mix by vortexing and centrifuge briefly. **IMPORTANT**: When preparing the ligation mix, add the components in the order shown in Table 2. **Note**: The ligation mix must be prepared fresh.

Table 2. Preparing ligation mix*

Component	Volume/reaction
Ligase Buffer	8 µl
Ligase Mix	2 µl
Total volume†	**10 µl**

* Scale up accordingly if performing several reactions at one time. † Mix by vortexing and centrifuge briefly.

5. Incubate at 24°C for 30 min. Stop the reaction by incubating at 95°C for 5 min.
6. Prepare REPLI-g SensiPhi amplification mix (Table 3). Add 30 µl REPLI-g SensiPhi amplification mix to the ligation reaction from step 5. Mix by vortexing and centrifuge briefly. **Note**: REPLI-g SensiPhi amplification mix must be prepared fresh.

Table 3. Preparing REPLI-g SensiPhi amplification mix*

Component	Volume/reaction
REPLI-g sc Reaction Buffer	29 µl
REPLI-g SensiPhi DNA Polymerase	1 µl
Total volume†	**30 µl**

* Scale up accordingly if performing several reactions at one time. † Mix by vortexing and centrifuge briefly.

7. Incubate at 30°C for 2 h.
8. Stop the reaction by incubating at 65°C for 5 min.
9. If not being used directly, store the amplified cDNA at –15°C to –30°C until required for downstream applications. We recommend storage of the amplified cDNA at a concentration of at least 100 ng/µl.
10. Amplified cDNA is highly suited for use in a variety of downstream applications, including next-generation sequencing. See Table 4 for further handling of amplified cDNA. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix C of the *REPLI-g Cell WGA & WTA Handbook* for PicoGreen® measurements. Alternatively, purify the reaction with ethanol using the protocol "Purification of DNA amplified using REPLI-g Kits". Following purification, determine the amount of cDNA by standard OD measurement.

Table 4. Advice for downstream applications

Downstream applications*	Use of amplified cDNA	QIAGEN® products
Real-time PCR, PCR	Dilute amplified cDNA 1:100 and use 2–3 µl for real-time PCR	QuantiTect®, QuantiFast®, QuantiNova™ Kits
NGS	Use 3–10 µl for shearing, purify sheared cDNA and start DNA library preparation	GeneRead™ Library Prep Kit

* If performing arrays, refer to "Notes before starting" (page 1).

Protocol 3: Whole transcriptome amplification (WTA) reaction

1. Add 2 µl gDNA Wipeout Buffer to the second aliquot of the lysed cell sample (from Protocol 1, step 5). Mix by vortexing and centrifuge briefly.
2. Incubate at 42°C for 10 min.
3. Prepare Quantiscript RT mix (Table 5). Add 8 µl Quantiscript RT mix to the sample. Mix by vortexing and centrifuge briefly. **Note**: Quantiscript RT mix must be prepared fresh. **IMPORTANT**: If performing NGS, see "Notes before starting" (page 1).

Table 5. Preparing Quantiscript RT mix*

Component	Volume/reaction
RT/Polymerase Buffer	4 µl
H_20 sc	1 µl
Random Primer†	1 µl
Oligo dT Primer	1 µl
Quantiscript RT Enzyme Mix	1 µl
Total volume‡	**8 µl**

* Scale up accordingly if performing several reactions at one time. † Omit Random Primer from the RT mix if the WTA amplification product should be enriched for mRNA (poly A+ RNA only). ‡ Mix by vortexing and centrifuge briefly.

4. Incubate at 42°C for 60 min. Stop the reaction by incubating at 95°C for 3 min, then cool on ice. Continue with step 4 of Protocol 2, page 2.

Scan QR code for handbook.

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