Quick-Start Protocol April 2016

REPLI-g® WTA Single Cell Kit

The REPLI-g WTA Single Cell Kit (cat. nos. 150063 and 150065) 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 WTA Single Cell Handbook*: **www.qiagen.com/HB-1686**
- 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 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, 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: Amplification of total RNA or 3'-enriched mRNA

1. Place 7 µl cell material (suspended in PBS) into a microcentrifuge tube.

   If using <7 µl of cell material, add H_20 sc to bring the volume up to 7 µl.

2. Add 4 µl Lysis Buffer. Mix by vortexing and centrifuge briefly.

   Note: Ensure that 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. Add 2 µl gDNA Wipeout Buffer, WTA. Mix by vortexing and centrifuge briefly.

5. Incubate at 42°C for 10 min.

6. Prepare Quantiscript RT mix (Table 1). Add 7 µl Quantiscript RT mix to the lysed cell sample. Mix by vortexing and centrifuge briefly.

   Note: Quantiscript RT mix must be prepared fresh.

   IMPORTANT: Omit Random Primer from the Quantiscript RT mix if the WTA amplification product should be enriched for mRNA poly A+ sequences.

Table 1. Preparing Quantiscript RT mix*

Component	Volume/reaction
RT/Polymerase Buffer	4 µl
Random Primer†	1 µl
Oligo dT Primer	1 µl
Quantiscript RT Enzyme Mix	1 µl
Total volume‡	**7 µl**

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

† Omit Random Primer from the Quantiscript RT mix if the WTA amplification product should be enriched for mRNA (poly A+ only). The final reaction volume will be 6 µl.

‡ Mix by vortexing and centrifuge briefly.

7. Incubate at 42°C for 60 min. Stop the reaction by incubating at 95°C for 3 min, then cool on ice.

8. Prepare the ligation mix (Table 2). Add 10 µl ligation mix to the Quantiscript RT reaction mix reaction from step 7. 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.

9. Incubate at 24°C for 30 min. Stop the reaction by incubating at 95°C for 5 min.
10. Prepare the REPLI-g SensiPhi amplification mix (Table 3). Add 30 µl REPLI-g SensiPhi amplification mix to the ligation reaction from step 9. 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.

11. Incubate at 30°C for 2 h.
12. Stop the reaction by incubating at 65°C for 5 min.

    Note: Concentration of amplified cDNA typically ranges between 150–350 ng/µl.
13. 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.
14. Amplified cDNA behaves like purified genomic DNA and has an approximate length of 2000 bp up to 70,000 bp. It is highly suited for use in a variety of downstream applications, including next-generation sequencing and quantitative PCR. See Table 3 for

further handling of amplified cDNA. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix C of the *REPLI-g WTA Single Cell 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 3. 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 Kits
Microarray	Purify amplified cDNA and use the appropriate amount	n.a.
Sanger sequencing, Pyrosequencing®	PCR has to be performed from the region of interest prior to sequencing. See advice for PCR.	PyroMark® products

Protocol 2: Amplification of purified RNA

1. Place 8 µl purified RNA into a microcentrifuge tube. If using less than 8 µl of purified RNA, add H_20 sc to bring the volume up to 8 µl.

   Note: Purified total RNA can be total RNA, rRNA-depleted RNA or mRNA-enriched RNA (poly A+ RNA). Use a starting amount of >10 pg – 100 ng RNA.
2. Add 3 µl NA Denaturation Buffer. Mix by vortexing and centrifuge briefly.
3. Incubate at 95°C for 3 min.
4. Continue with step 4 of Protocol 1, page 2.

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