User-Developed Protocol

REPLI-g® Cell WGA & WTA reaction of purified nucleic acid samples

This protocol is optimized for parallel whole genome amplification (WGA) and whole transcriptome amplification (WTA) using purified nucleic acid samples and the REPLI-g Cell WGA & WTA Kit (cat. nos. 150052 and 150054). Potential inhibitors present in the starting material may have negative effects on amplification. We recommend upstream nucleic acid purification by the QIAamp® Kits. Use intact nucleic acids for WGA and WTA reactions for highest sensitivity and reliability. For amplification of degraded nucleic acid, higher amounts of nucleic acids are necessary. The amount of nucleic acid necessary for parallel WGA and WTA increases with the fragmentation degree of nucleic acids**.**

This procedure has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please consult the Safety Information and Important Notes sections in the *REPLI-g Cell WGA & WTA Kit Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment and reagents

- Water bath, thermo cycler, or heating block
- Vortexer
- Microcentrifuge tubes
- Microcentrifuge
- Ice
- Pipets and pipet tips
- Nuclease-free water
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)

Note: This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important points before starting

- For best amplification, the template should be >10 kb.
- This protocol is optimized for use of nucleic acids from all vertebrate species (e.g., human, mouse, rat, sorted cells, tissue culture cells, or cells picked under the microscope). The protocol cannot be used for nucleic acids that are isolated from cells that have been fixed using formalin or other cross-linking agents (e.g., single cell samples obtained by laser microdissection from formalin-fixed, paraffin-embedded [FFPE] tissues).
- An amount of nucleic acids that corresponds to 25–1000 cells is optimal for WTA and WGA reactions using the REPLI-g Cell WGA & WTA Kit.
- Avoid DNA or RNA contamination of reagents by using separate laboratory equipment (e.g., pipettes, filter pipette tips, reaction vials, etc.). Set up the REPLI-g Cell WGA & WTA Kit reaction in a location free of nucleic acids.
- DNA yields of approximately 10 µg will be present in negative (no template) controls because DNA is generated during REPLI-g reaction by primer multimer formation, which generates high-molecular-weight DNA. This DNA will not affect the quality of the actual sample and will not give a positive result in downstream assays.

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Things to do before starting

- All buffers and reagents should be vortexed before use to ensure thorough mixing.

Procedure

1. **Place 15 µl nucleic acid solution (containing RNA and DNA) into a microcentrifuge tube. If using less than 15 µl of nucleic acid solution, add H_20 sc to bring the volume up to 15 µl.**

   Note: The mixture of RNA and DNA from the same sample material must contain both nucleic acids. Both nucleic acids must be presented as in ≥25 cells (~150 pg DNA, 250 pg RNA).

2. **Add 6 µl NA denaturation buffer. Mix carefully by flicking the tube, and centrifuge briefly.**

   Note: Ensure that no droplets stick to the wall of the tube above the meniscus.

3. **Incubate at 95°C for 3 min. Cool to 4°C.**

   Transfer a 10 µl aliquot to a fresh reaction tube and immediately perform WGA (see protocol "Amplification of Genomic DNA", *REPLI-g Cell WGA & WTA Handbook*).

4. **The tube should be kept on ice.**

5. **Transfer a second 10 µl aliquot to a second fresh reaction tube and immediately perform WTA (see protocol "Amplification of Total RNA", *REPLI-g Cell WGA & WTA Handbook*).**

   Important: Aliquots cannot be stored at this point. They must be processed immediately using the protocols "Amplification of Genomic DNA" and "Amplification of Total RNA" in the *REPLI-g Cell WGA & WTA Handbook*.

6. **The tube should be kept on ice.**

   Note: WGA with the protocol "Amplification of Genomic DNA" and WTA using the protocol "Amplification of Total RNA" in the *REPLI-g Cell WGA & WTA Handbook* should be processed in parallel. The protocols are identical from the preparation of ligation mix, through to the end of the procedures (step 3 of the protocol "Amplification of Genomic DNA" and step 4 of the protocol "Amplification of Total RNA" in *REPLI-g Cell WGA & WTA Handbook*).