User-Developed Protocol

Whole transcriptome amplification of purified RNA samples using the REPLI-g® Cell WGA & WTA kit

This protocol is optimized for whole transcriptome amplification (WTA) purified RNA 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 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 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 to be supplied by user

- Water bath, thermo cycler, or heating block
- Vortexer
- Microcentrifuge tubes
- Microcentrifuge
- Ice
- Pipettes and pipette 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

- This protocol is optimized for use of RNA 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 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 RNA that corresponds to 25–1000 cells is optimal for WTA 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.

Things to do before starting

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

Procedure

1. **Place 7 µl purified RNA (containing >75 pg RNA) into a microcentrifuge tube. If using less than 7 µl of isolated RNA, add H_20 sc to bring the volume up to 7 µl.**
2. **Add 3 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.**

   Important: Aliquots cannot be stored at this point. They must be processed immediately using the protocol “Amplification of Total RNA”, *REPLI-g Cell WGA & WTA Kit Handbook*.
4. **Immediately perform WTA (see protocol “Amplification of Total RNA”, *REPLI-g WGA & WTA Handbook*).**