QIAGEN Supplementary Protocol

Whole genome amplification from residual cells in plasma/serum samples using the REPLI-g® Single Cell Kit

This protocol is optimized for amplification of DNA associated with residual cells and cell debris within plasma/serum samples using the REPLI-g Single Cell Kit (cat. nos. 150343 and 150345). Note that the whole genome may not be amplified with complete genome coverage, depending on the number and quality of residual cells in the plasma/serum samples. Cells should be intact (i.e., not apoptotic) to ensure optimal results.

IMPORTANT: Please refer to the *REPLI-g Single Cell Handbook* for general information on the handling and storage of kit components.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Water bath, heating block, or thermocycler
- Vortexer
- Ice
- 1.5 ml microcentrifuge tube
- Microcentrifuge

Important points before starting

- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Single Cell Kit reaction in a location free of DNA.
- DNA yields of approximately 40 µg will be present in negative (no template) controls because DNA is generated during the REPLI-g Single Cell reaction by random extension of primer dimers, generating high-molecular-weight product. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

- The correct centrifugation speed (rpm) for your centrifuge can be calculated using the known g-force value with the following formula:

$$rpm = 299\sqrt{\frac{RCF}{r}}$$

RCF = Relative Centrifugal Force (g-force)

r = radius of rotor (in cm)

Things to do before starting

- Prepare Buffer DLB by adding 500 µl H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.
  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 13). All other components can be thawed at room temperature (15–25°C).
- All buffers and reagents should be vortexed before use to ensure thorough mixing.

Procedure

1. **Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (see Table 1). Vortex Buffer D2 thoroughly and centrifuge briefly.**

   Note: The total volumes of Buffer D2 given in Table 1 are suitable for 10 reactions. Buffer D2 should not be stored longer than 3 months.

Table 1. Preparation of Buffer D2

Component	Volume*
DTT, 1 M	9 µl
Buffer DLB (reconstituted)†	99 µl
Total volume	**108 µl**

* Volumes given are sufficient for 10 reactions.

† Reconstitution of Buffer DLB is described in "Things to do before starting".

2. **Thaw the plasma sample.**
3. **Centrifuge for 10 min at 300 x *g* to eliminate denatured protein.**

   Refer to the formula in "Important points before starting" to calculate the rpm value for your centrifuge.

   Note: Avoid resuspension of the precipitated protein pellet.

4. **Pipet 1 ml of supernatant into a new 1.5 ml microcentrifuge tube.**
5. **Centrifuge for 10 min at 6000 x *g* to pellet residual cells.**

   Refer to the formula in "Important points before starting" to calculate the rpm value for your centrifuge.
6. **Carefully aspirate 700 µl of supernatant and discard.**

   Note: Avoid re-suspension of cells collected at the bottom of the tubes.
7. **Tap on the microcentrifuge tube while the tube is standing open-side down on clean, absorbent material to remove all residual plasma from the cell pellet.**

   Important: Take care not to lose the cell pellet during this step.
8. **Centrifuge briefly and resuspend by vortexing.**

   Note: Do not vortex for longer than 3 sec.
9. **Add 10 µl Buffer D2 to the cell pellet. Centrifuge briefly and mix by vortexing.**
10. **Incubate on ice for 10 min.**
11. **Add 10 µl Stop Solution. Centrifuge briefly and mix by vortexing.**
12. **Transfer 10 µl of lysed plasma cells into a new microcentrifuge tube.**

    Note: Do not store lysed plasma cells.
13. **Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly.**

    The REPLI-g sc Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
14. **Prepare a master mix on ice according to Table 2. Mix and centrifuge briefly.**

    Important: Add the master mix components in the order listed in Table 2. After addition of water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge the mixture before addition of REPLI-g sc DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of the REPLI-g sc DNA Polymerase.

Table 2. Preparation of master mix[*†]

Component	Volume/reaction
H_2O sc	9 µl
REPLI-g sc Reaction Buffer	29 µl
REPLI-g sc DNA Polymerase	2 µl
Total volume	**40 µl**

* Volumes provided are sufficient for one 50 µl reaction. To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%.

† After the addition of H_2O sc and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge.

15. **Add 40 µl of master mix to the lysed plasma cells (from step 12).**

16. **Incubate at 30°C for 8 h.**

    A thermocycler can be used for programming the incubation and reaction inactivation temperatures (see step 17).

17. **Inactivate REPLI-g sc DNA Polymerase at 65°C for 3 min.**

18. **If not being used directly, store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**

    DNA amplified using the REPLI-g Single Cell Kit should be treated as genomic DNA with minimal freeze-thaw cycles. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

19. **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 40 µg per 50 µl reaction and need to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix B of the *REPLI-g Single Cell Handbook* for an accurate method of quantifying REPLI-g amplified DNA.

20. **Use the correct amount of REPLI-g amplified DNA diluted in water or TE buffer according to the manufacturer's instructions. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µl of diluted DNA for each PCR reaction.**

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Australia ■ 1-800-243-800
Austria ■ 0800/281010
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