Supplementary Protocol July 2019

Purification of genomic DNA and total RNA from human whole blood using the QIAamp RNA Blood Mini Kit and additional Buffer AW1; spin procedure

This protocol describes how to purify genomic DNA and total RNA from fresh, human whole blood in one eluate. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate or heparin can also be used. The purification procedure requires use of the QIAamp RNA Blood Mini Kit. Buffer AW1 must be purchased separately.

IMPORTANT: Please read the handbook supplied with the QIAamp RNA Blood Mini Kit carefully before beginning this procedure. The QIAamp RNA Blood Mini Kit is intended for molecular biology applications. The product is not intended for the diagnosis, prevention, or treatment of disease.

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.

- QIAamp RNA Blood Mini Kit (cat. no. 52304)
- Buffer AW1 (cat. no. 1067924)
- Tubes for erythrocyte lysis (1.5 to15 ml depending on sample size); the use of sterile, disposable, polypropylene tubes is recommended.
- Refrigerated (4°C) microcentrifuge (for blood samples ≤250 µl) or refrigerated laboratory centrifuge with rotor for 12 ml or 15 ml centrifuge tubes (for blood samples ≥250 µl).
- Pipets and sterile, RNase-free pipet tips
- Microcentrifuge with rotor for 2 ml tubes
- Ethanol (96-100%)
- 70% Ethanol in water
- 14.3 M β-Mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%) to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to dissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl of β-ME per 1 ml of Buffer RLT. Buffer RLT is stable for 1 month at room temperature (15–25°C) after addition of β-ME.

Procedure

1. **Mix 1 volume of human blood with 5 volumes of Buffer EL in an appropriately sized tube (not provided)**.

   For optimal results, the volume of the mixture (blood + Buffer EL) should not exceed ¾ of the volume of the tube to allow efficient mixing. For example, add 5 ml of Buffer EL to 1 ml of whole blood, and mix in a tube which has a total volume of >8 ml.

   Note: Use an appropriate amount of whole blood. Up to 1.5 ml of healthy blood (typically 4000–7000 leucocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used. (In this case, also adjust amount of Buffer RLT in step 6.)
2. **Incubate for 10 to 15 minutes on ice. Mix by vortexing briefly 2 times during incubation**.

   The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 minutes.
3. **Centrifuge at 400 x g for 10 minutes at 4°C, and completely remove and discard supernatant**.

   Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in the following wash step.
4. **Add Buffer EL to the cell pellet (use 2 volumes of Buffer EL per volume of whole blood used in step 1). Resuspend cells by vortexing briefly**.

   For example, add 2 ml of Buffer EL per 1 ml of whole blood used in step 1.
5. **Centrifuge at 400 x g for 10 minutes at 4°C, and completely remove and discard supernatant.**

   Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of nucleic acids to the QIAamp spin column, resulting in lower yield.
6. **Add Buffer RLT to pellet leukocytes according to the table below. Vortex or pipet to mix.**

   When not using healthy blood, refer to number of leukocytes to determine the volume of Buffer RLT required. Buffer RLT disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps.

Buffer RLT* (µl)	Healthy whole blood (ml)	No. of leukocytes
350	Up to 0.5	Up to 2 x 10^6
600	0.5 to 1.5	2 x 10^6 to 1 x 10^7

* Ensure β-ME is added to Buffer RLT (see "Things to do before starting").

7. **Pipet lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 minutes at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate**.

   To avoid aerosol formation, adjust pipet to >750 µl to ensure that the lysate can be added to the QIAshredder spin column in a single step.

   If too many cells have been used, after homogenization, the lysate will be too viscous to pipet. If this is the case, divide the sample into 2 aliquots and adjust the volumes of each aliquot to 600 µl with Buffer RLT. Continue with the procedure from step 7 using 2 QIAshredder and 2 QIAamp spin columns.

8. **Add 1 volume (350 µl or 600 µl) of 70% ethanol to the homogenized lysate and mix by pipetting. Do not centrifuge**.

   A precipitate may form after the addition of ethanol. This will not affect the QIAamp procedure.

9. **Carefully pipet sample, including any precipitate that may have formed, into a new QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 seconds at ≥8000 x g (>10,000 rpm). Maximum loading volume is 700 µl. If the volume of the sample exceeds 700 µl, successively load aliquots onto the QIAamp spin column and centrifuge as above**.

   Discard flow-through and collection tube.

10. **Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Apply 700 µl Buffer AW1 to the QIAamp spin column and centrifuge for 15 seconds at ≥8000 x g (>10,000 rpm)**.

    Discard flow-through and collection tube.

    Note: Buffer AW1 is not contained in the QIAamp RNA Blood Kit and must be purchased separately (see "Equipment and Reagents").

11. **Place QIAamp spin column in a new 2 ml collection tube (provided). Apply 500 µl Buffer RPE to the QIAamp spin column and centrifuge for 15 seconds at ≥8000 x g (≥10,000 rpm)**.

    Discard flow-through and collection tube.

    Note: Ensure ethanol is added to Buffer RPE (see "Things to do before starting").

12. **Carefully open the QIAamp spin column and add 500 µl of Buffer RPE. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes**.

13. **Recommended: Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute**.

    This helps to eliminate the chance of possible Buffer RPE carryover.

14. **Transfer QIAamp spin column into a 1.5 ml microcentrifuge tube (provided) and pipet 50 µl of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute. Repeat if >0.5 ml whole blood (or >2 x 10^6 leukocytes) has been processed**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor. The QIAamp RNA Blood Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.