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Supplementary Protocol

Purification of genomic DNA and Total RNA from cultured cells and tissue 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 cultured cells and tissue in one eluate. The purification procedures require use of the QIAamp RNA Blood Mini Kit in combination with Buffer AW1. Buffer AW1 (cat. no. 1067924) is not included in the QIAamp RNA Blood Mini Kit and 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.

Further information

- *QIAamp RNA Blood Mini Handbook*: **www.qiagen.com/1063021**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Equipment and reagents to be supplied by the user

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)
- Pipettes and sterile RNase-free pipette 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)

For cells protocol

- Equipment for sample disruption and homogenization depends on the method chosen; one or more of the following are required:
  - QIAGEN TissueRuptor® II (cat. no. 9002755) or equivalent rotor–stator homogenizer
  - QIAshredder (supplied)

For cells protocol using trypsin

- Phosphate-buffered saline (PBS)
- Trypsin
- Medium, which contains serum

For tissue protocol

- Equipment for sample disruption and homogenization (see "Lysis and homogenization" section in the *QIAamp RNA Blood Mini handbook*); depending on the method chosen, one or more of the following are required:
  - QIAGEN TissueRuptor® II (cat. no. 9002755) or equivalent rotor–stator homogenizer
  - QIAGEN TissueLyser II (cat. no. 85300)
  - Mortar and pestle
  - Liquid nitrogen

Starting amounts of samples

It is essential to use the correct amount of starting material in order to obtain optimal results. If the nucleic acid-binding capacity of the QIAamp spin column is exceeded, nucleic acid yields will not be consistent and less than the maximum possible nucleic acids may be recovered. If the starting material is incompletely lysed, the nucleic acid yield will be lower than expected even if the binding capacity of the QIAamp spin column is not exceeded.

Maximum amount of starting material

- Cells: 5×10^6
- Tissue: 15 mg

Important points before starting

- Use an appropriate starting amount of cells and tissue as described in the section "Starting amounts of samples".
- When using the QIAamp RNA Blood Mini Kits for the first time, please read "Important Notes" in the *QIAamp RNA Blood Mini Handbook*.
- When preparing RNA for the first time, please read "General Remarks for Handling RNA" in the *QIAamp RNA Blood Mini Handbook*.
- All steps in this protocol (including centrifugation) should be performed at room temperature (15–25°C). During the procedure, work quickly.

For cells protocol

- After harvesting cells, all centrifugation steps should be performed in a microcentrifuge at room temperature.
- Cell pellets can be stored at –90 to –65°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that cell pellets can be dislodged by flicking in step 2 of "Protocol: Purification of gDNA and Total RNA from cultured cells", page 5. Homogenized cell lysates (in Buffer RLT, step 2) can be stored at –90 to –65°C for several months. To process frozen lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 3 of "Protocol: Purification of gDNA and Total RNA from cultured cells", page 5.

For tissue protocol

- For best results, stabilize tissues immediately in RNAprotect® Tissue Reagent. Tissues can be stored in RNAprotect Tissue Tubes for up to 1 day at 37°C, 7 days at room temperature, 4 weeks at 2–8°C, or for archival storage at –30 to –15°C or –90 to –65°C. See the *RNAprotect Handbook* for more information about RNAprotect Tissue Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or RNAprotect-stabilized tissues can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen and immediately transfer to –90 to –65°C. Tissue can be stored for several months at –90 to –65°C. To process, do not allow tissue to thaw during weighing or handling before disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 1) can also be stored at –90 to –65°C for several months. To process frozen lysates, thaw samples at room temperature or at 37°C in a water bath until they are completely thawed and salts in the lysis buffer are dissolved. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA. Continue with step 2 of "Protocol: Purification of gDNA and Total RNA from tissue", page 4.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml of Buffer RLT. This solution is stable for 1 month at room temperature.
- 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.

Protocol: Purification of gDNA and Total RNA from tissue

1. Disrupt tissue and homogenize the lysate according to step 1a, 1b, or 1c in the ***QIAamp RNA Mini Blood Handbook***.
   Important: Do not use more than 15 mg tissue as input amount.
   Note: Some tissues, including heart, skeletal muscle, and fiber-rich tissues, are difficult to homogenize. To facilitate complete homogenization and to avoid reduced yields, perform the following procedure:
   Place the weighed tissue in a suitable-sized vessel for a rotor–stator homogenizer. Add 210 µl of Buffer RLT and 140 µl of RNase-free water. Immediately disrupt and homogenize the tissue using the TissueRuptor II or equivalent rotor–stator homogenizer until it is uniformly homogenous (usually 20-40 s). Add 20 µl QIAGEN Proteinase K solution and mix thoroughly by pipetting. Incubate at room temperature for 15 min. Centrifuge the lysate for 3 min at maximum speed. Transfer 350 µl of the supernatant to a new 2 ml collection tube (not provided). Proceed to step 2.
2. Add 1 volume (350 µl) of 70% ethanol to the cleared lysate and mix well by pipetting. Do not centrifuge.
   If some lysate was lost during homogenization, reduce volume of ethanol accordingly. A precipitate may form after the addition of ethanol but, this will not affect the QIAamp procedure.
3. Carefully pipet 700 µl of the sample, including any precipitate that may have formed, into a QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge at full speed for 1 min. Discard the flow-through and the collection tube.
4. Transfer the QIAamp spin column to a new 2 ml collection tube (provided). Pipet 700 µl Buffer AW1 (must be purchased separately) onto the QIAamp spin column and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash.
   Discard the flow-through and the collection tube.
5. Transfer QIAamp spin column to a new 2 ml collection tube (provided). Pipet 500 µl of Buffer RPE onto the QIAamp spin column and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash. Discard the flow-through.
   Note: Ensure ethanol is added to Buffer RPE before use (see "Things to do before starting").
6. Carefully open the QIAamp spin column and add 500 µl Buffer RPE. Close the cap and centrifuge at full speed (20,000 x *g*, 14,000 rpm) for 3 min.
   Note: Some centrifuge rotors may distort slightly upon deceleration, resulting in flow-through, containing Buffer RPE, and contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.
   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 min.
   This step helps to eliminate the chance of possible Buffer RPE carryover.

7. Transfer the QIAamp spin column into a 1.5 ml microcentrifuge tube (provided) and pipet 30–50 µl of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at 8000 x *g* (10,000 rpm) to elute.

Protocol: Purification of gDNA and Total RNA from cultured cells

1. Harvest cells according to step 1a or 1b in the *QIAamp RNA Mini Blood Handbook*.

   Important: Do not use more than 5 x 10^6 cells as input amount.

2. Disrupt cells by adding Buffer RLT. Proceed to step 3.

   Note: Ensure β-ME is added to Buffer RLT before use (see "Things to do before starting"). **For pelleted cells, loosen cell pellet by flicking the tube and add Buffer RLT (according to Table 1 below). Vortex or pipet to mix. No cell clumps should be visible before proceeding to step 3.**

Table 1 Volume of Buffer RLT for lysing pelleted cells

Number of pelleted cells	Volume of Buffer RLT (µl)
Up to 5 x 10^6	350

For monolayer cells, add Buffer RLT (according to Table 2 below) to monolayer cells. Collect cell lysate with a rubber cell scraper. Vortex or pipet to mix. No cell clumps should be visible before proceeding to step 3.

Table 2. Volume of Buffer RLT for direct cell lysis

Dish diameter (cm)*	Volume of Buffer RLT (µl)
<6	350
6-10	600

* Regardless of the cell number, use the buffer volumes indicated to completely cover the surface of the dish.

3. Homogenize the sample using one of the methods below:

   Note: Cultured cells can be homogenized using a rotor–stator homogenizer, such as the QIAGEN TissueRuptor II or a QIAshredder spin column. Rotor–stator homogenizers simultaneously disrupt and homogenize the cells, whereas QIAshredder spin columns only homogenize the cells. Thus, use of a rotor–stator homogenizer is the preferred method.

   3a. Homogenization using the QIAGEN TissueRuptor II or equivalent rotor-stator homogenizer:
   Homogenize the cells using the TissueRuptor II or equivalent rotor-stator homogenizer until it is uniformly homogenous (usually 20-40 s). Centrifuge the lysate for 3 min at maximum speed and use only the supernatant. Proceed to step 4.

   3b. Homogenization using a QIAshredder homogenizer:
   Pipet the lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 min at maximum speed to homogenize. Discard the QIAshredder spin column and save the homogenized lysate. Proceed to step 4.

   If too many cells have been used after homogenization, the lysate will be too viscous to pipet. If this is the case, please refer to the "Troubleshooting Guide" in the *QIAamp RNA Blood Mini Handbook*.

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

   If some lysate was lost during homogenization, adjust the volume of ethanol accordingly. Precipitate may form after the addition of ethanol, but this will not affect the QIAamp procedure.

5. Carefully pipet the sample, including any precipitate which may have formed, into a new QIAamp spin column in a 2 ml collection tube (provided) without moistening the rim. Centrifuge for 15 s 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 the flow-through and the collection tube.

6. Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Pipet 700 µl Buffer AW1 (must be purchased separately) into the QIAamp spin column and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash.

   Discard the flow-through and the collection tube.

7. Transfer the QIAamp spin column into a new 2 ml collection tube (provided). Pipet 500 µl Buffer RPE into the spin column and centrifuge for 15 s at 8000 x *g* (10,000 rpm) to wash.

   Discard the flow-through.

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

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

   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 min.

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

9. Transfer the QIAamp spin column into a 1.5 ml collection tube (provided) and pipet 30–50 µl of RNase-free water (provided) directly onto the QIAamp membrane. Centrifuge for 1 min at 8000 x *g* (10,000 rpm) to elute.

   Discard flow-through and collection tube.

Document Revision History

Date	Changes
12/2019	Initial release

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