QIAGEN Supplementary Protocol:

Isolation of total nucleic acids from animal and human cells using the EZ1 RNA Cell Mini Kit

This protocol is designed for the isolation of total nucleic acids (NA) from animal and human cells using the EZ1 RNA Cell Mini Kit in combination with the BioRobot® EZ1 workstation.

Introduction

The BioRobot EZ1 RNA system allows fully automated purification of RNA from animal and human cells. In brief, total nucleic acids are first isolated from sample lysates, then DNA is removed by DNase digestion; this is followed by washing and elution of pure RNA. **In this supplementary protocol, however, the DNase treatment step is omitted, to allow purification of high-quality total nucleic acids (DNA and RNA) in high yields.**

The high-quality nucleic acids obtained using the BioRobot EZ1 System are well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot EZ1 performs all steps of the purification procedure.

The procedure given below describes the preparation of animal or human cells from suspension or monolayer cultures, and the subsequent procedure for setting up the BioRobot EZ1and starting a run.

IMPORTANT: Please read the *EZ1 RNA Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Starting material

The EZ1 RNA Cell Mini Kit is optimized for purification of nucleic acids from 10 to 1 x 10^6 animal or human cultured cells, and from 10 to 2 x 10^6 human white blood cells. If you use more than these amounts, you may not achieve further increases in nucleic acid yields. The starting and elution volumes to use in this procedure are given in Table 1.

Table 1. Amounts of Starting Material and Elution Volumes Used with the EZ1 RNA Cell Mini Kit and the Total Nucleic Acid Protocol

Sample	EZ1 RNA Card Protocol	Amount of starting material	Elution volume
Cultured cells	Total Nucleic Acid	10 – 1 x 10^6 cells*	50–200 µl
White blood cells	Total Nucleic Acid	10 – 2 x 10^6 cells*	50–200 µl

* Sample volume: 300 µl.

Yield of purified nucleic acids

Using this procedure, the typical yield of nucleic acids is 5–25 µg per 1 x 10^6 cultured cells, dependent upon cell type and growth conditions. Yields may also vary depending on the elution volume used. Elution in smaller volumes increases the final concentration of nucleic acids in the eluate, but slightly reduces the overall yield. We recommend using an elution volume appropriate for the intended downstream application. Typical yields of total nucleic acids obtained from cell cultures are shown in Table 2, below.

Table 2. Yields of Nucleic Acids Obtained from Cells Using the EZ1 RNA Cell Mini Kit with the Total Nucleic Acid Protocol

Sample type	Number of cells	Yield (µg NA)
Diluted human HL60 cells*	1 x 10^5	1
Diluted human HL60 cells*	5 x 10^5	4
Diluted human HL60 cells*	1 x 10^6	8.5

* Total nucleic acids were eluted in 200 µl RNase-free water.

Equipment and reagents to be supplied by user

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

- EZ1 RNA Cell Mini Kit, cat. no. 958034
- EZ1 RNA Card, cat. no. 9015590
- BioRobot EZ1, cat. no. 9000705, and disposables (see the *EZ1 RNA Handbook*)
- 14.3 M β-mercaptoethanol (β-ME)† (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipet tips
- Disposable gloves
- Equipment for disruption and homogenization (see "Disrupting and homogenizing starting material" in the *EZ1 RNA Handbook*)

For suppliers of bead-mill homogenizers and rotor–stator homogenizers, see "Equipment and Reagents to Be Supplied by User" in the *EZ1 RNA Handbook*.

† β-ME may be optionally added to Buffer RLT before use (see "Things to do before starting", below).

Important points before starting

- If using the EZ1 RNA Cell Mini Kit for the first time, read "Important Notes" in the kit handbook.
- If working with RNA for the first time, read "Appendix A" in the kit handbook.
- If working with blood cells, read "Appendix D" in the kit handbook.
- Cell pellets can be stored at –70°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. Homogenized cell lysates (in Buffer RLT, step 3) can be stored at –70°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. If any insoluble material is visible, centrifuge for 5 minutes at 3000–5000 x *g*. Transfer supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.
- Buffer RLT and the reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information" in the kit handbook. Take appropriate safety measures and wear gloves when handling.
- All steps of the protocol should be performed at room temperature (15–25°C). During the procedure, work quickly.
- RNase-free DNase and Buffer RDD are not required in this procedure.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature.
- β-Mercaptoethanol (β-ME) may be optionally added to Buffer RLT before use to increase RNA yields. We do not recommend using β-ME unless RNA yields from previous purification procedures were low and the troubleshooting guidelines (in the kit handbook) have already been followed. If using β-ME, add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of β-ME.

Procedure

1. **Harvest cells according to step 1a (for cells grown in suspension) or 1b (for cells grown in a monolayer).**

1a. **Cells grown in suspension (do not use more than 1 x 10^6 cells):**

Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell culture medium will inhibit lysis and dilute the lysate, which may reduce the yield by affecting the conditions for binding of nucleic acids to the magnetic particles.

1b. **Cells grown in a monolayer (do not use more than 1 x 10^6 cells):**

Cells grown in a monolayer in cell culture vessels can either be lysed directly in the culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet before lysis. Cells grown in a monolayer in cell culture flasks should always be trypsinized.

To lyse cells directly in culture dish:

Determine the number of cells. Completely aspirate the cell culture medium, and continue immediately with step 2 of the procedure.

Note: Incomplete removal of the cell culture medium will inhibit lysis and dilute the lysate, which may reduce the yield by affecting the conditions for binding of nucleic acids to the magnetic particles.

To trypsinize cells:

Determine the number of cells. Aspirate the medium, and wash cells with PBS. Aspirate the PBS and add 0.10–0.25% trypsin in PBS to trypsinize the cells. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and pellet by centrifugation at 300 x *g* for 5 min. Completely aspirate the supernatant, and continue with step 2 of the procedure.

Note: Incomplete removal of the cell culture medium will inhibit lysis and dilute the lysate, which may reduce the yield by affecting the conditions for binding of nucleic acids to the magnetic particles.

2. **Disrupt cells by addition of Buffer RLT. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 300 µl Buffer RLT. Vortex or pipet to mix, and proceed to step 3.**

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced yields.

For direct lysis of cells grown in a monolayer, add 300 µl Buffer RLT to the cell culture dish. Collect cell lysate with a rubber policeman. Pipet lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

3. Homogenize the sample according to step 3a, 3b, 3c, or 3d.

One of four methods may be used to homogenize the sample. After homogenization, proceed with step 4. See "Disruption and homogenization of starting material", in the kit handbook, for a more detailed description of homogenization methods.

If $\leq 1 \times 10^5$ cells are processed, the cells can be homogenized by vortexing for 1 min.

Note: Incomplete homogenization can affect binding of nucleic acids to the magnetic particles and lead to significantly reduced yields. Homogenization with rotor–stator or QIAshredder™ homogenizers generally results in higher nucleic acid yields than with a syringe and needle.

3a. Pipet the lysate directly onto a QIAshredder spin column (not supplied; see kit handbook for ordering information) placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed.

3b. Homogenize cells for 30 s using a rotor–stator homogenizer.

3c. Pass the lysate at least 5 times through a 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe.

3d. Transfer the lysate to a 2 ml microcentrifuge tube, and add one stainless steel bead (5 mm diameter). Homogenize the lysate on the TissueLyser for 2 min at 20 Hz. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant using a pipet.

Note: The instructions in step 3d are only guidelines. They may need to be changed depending on the cell sample being processed and on the bead mill being used.

4. Transfer the homogenized lysates to the 2 ml sample tubes supplied with the kit.

5. Insert the EZ1 RNA Card completely into the EZ1 Card slot of the BioRobot EZ1.

6. Switch on the BioRobot EZ1.

7. Press "START" to display the "Protocols" menu.

8. Press "2" to select purification of total nucleic acids.

9. Press "1" to select purification of total nucleic acids from cells.

10. Press any key to proceed through the text displayed in the LCD.

11. Press "1" to select an elution volume of 50 µl, press "2" to select an elution volume of 100 µl, or press "3" to select an elution volume of 200 µl.

12. Press any key to proceed through the text displayed in the LCD.

The text summarizes the following steps, which describe the loading of the worktable.

13. Open the workstation door.

14. Invert 1–6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.

15. **Load the reagent cartridges into the cartridge rack.*†**

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

    If there are fewer than 6 reagent cartridges, you can load them in any order on the rack. However, when loading the other labware in steps 16–18 ensure that they also follow the same order.

16. **Load 1–6 opened elution tubes into the elution tube rack.†**
17. **Load 1–6 tip holders containing filter-tips into the front 2 rows of the tip rack.†**
18. **Load 1–6 opened sample tubes containing lysate (from step 4) into the back row of the tip rack.†**
19. **Close the workstation door.**
20. **Press "START" to start the protocol.**
21. **When the protocol ends, the LCD displays "Protocol finished". Open the workstation door.**
22. **Remove the elution tubes containing the purified total nucleic acids. Discard the sample-preparation waste.**
23. **To run another protocol, press "ESC", prepare samples as described in steps 1–4, and follow the procedure from step 8 onward. Otherwise, press "STOP" twice to return to the first screen of the LCD, close the workstation door, and switch off the BioRobot EZ1.**
24. **Clean the BioRobot EZ1.**

    Follow the maintenance instructions in the *BioRobot EZ1 User Manual*.

Troubleshooting

For troubleshooting, please consult the Troubleshooting Guide in the kit handbook.

* See Figure 2B in the kit handbook.

† See Figure 3 in the kit handbook.

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.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.