QIAGEN Supplementary Protocol:

Isolation of total nucleic acids from cells using the MagAttract® RNA Cell Mini M48 Kit

This protocol is designed for the isolation of total nucleic acids (NA) from animal and human cells using the MagAttract RNA Cell Mini M48 Kit in combination with the BioRobot® M48 workstation.

Introduction

The MagAttract RNA M48 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 MagAttract technology are well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M48 performs all steps of the purification procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 48 samples, in multiples of 6, are processed in a single run.

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 M48 and starting a run.

IMPORTANT: Please read the *MagAttract RNA M48 Handbook*, paying careful attention to the Safety Information and Important Notes sections, before beginning this procedure.

Starting material

The MagAttract RNA Cell Mini M48 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 MagAttract RNA Cell Mini M48 Kit and the Total Nucleic Acid Protocol

Sample	QIAsoft M 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: 400 µl.

Yield of purified nucleic acids

Using this procedure, the typical yield of nucleic acids is 8–25 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 MagAttract RNA Cell Mini M48 Kit with the Total Nucleic Acid Protocol

Sample type	Number of cells	Yield (µg NA)
Diluted human HL60 cells*	1 x 10^5	1.1
Diluted human HL60 cells *	5 x 10^5	4.1
Diluted human HL60 cells *	1 x 10^6	8.3

* 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.

- MagAttract RNA Cell Mini M48 Kit (cat. no. 958236)
- App. Package, M48, Gene Expression (cat. no. 9016149)
- BioRobot M48 workstation (cat. no. 9000708) and disposables (see the *MagAttract RNA M48 Handbook*)
- 14.3 M β-mercaptoethanol (β-ME)* (commercially available solutions are usually 14.3 M)
- Sterile, RNase-free pipet tips
- Ethanol (96–100%)†
- Disposable gloves
- Equipment for disruption and homogenization (see "Disruption and homogenization of starting material" in the *MagAttract RNA M48 Handbook*)

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

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

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important points before starting

- If using the MagAttract RNA Cell Mini M48 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 Buffer MW 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

- Before starting the procedure, ensure that MagAttract Suspension E is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses.
- 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.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

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 400 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 400 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 1.5 ml or 2 ml sample tubes that are compatible with the sample rack of the BioRobot M48.

We recommend use of 1.5 ml sample tubes.

5. Switch on the BioRobot M48.

The power switch is on the left side of the instrument.

6. Switch on the computer and monitor.

7. Launch the QIAsoft M Operating System.

Upon startup, the computer displays the QIAsoft M startup window. Click "Start" to continue. If the QIAsoft M startup window does not appear, either double-click the QIAsoft M icon on the desktop or click the Microsoft® Windows® "Start" menu and select QIAsoft M Operating System →QIAsoft M V2.0 for BioRobot M48.

8. Select the protocol group "Gene Expression" from the drop-down menu by clicking on the dark green arrow; select "Total NA" and then "NA Cell".

9. Click the "Select" button to choose the elution tube type. Select the number of samples and the sample and elution volumes in the corresponding dialog fields. Click "Next".

The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected; these steps include the option of entering names for your samples. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.

10. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are automated. The software displays a table of results when the protocol is finished.**
11. **Retrieve the elution tubes containing the purified nucleic acids from the cooling block. The eluate is ready to use, or can be stored at –20°C or –70°C for longer periods.**

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to a clean tube (see Appendix B in the kit handbook).

Troubleshooting

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