QIAGEN Supplementary Protocol:

Isolation of total nucleic acids from tissue using the MagAttract® RNA Tissue Mini M48 Kit

This protocol is designed for the isolation of total nucleic acids (NA) from animal and human tissues using the MagAttract RNA Tissue 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 tissues. In brief, total nucleic acids are 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 lysate from animal or human soft tissue, 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 Tissue Mini M48 Kit is optimized for purification of nucleic acids from 1 to 10 mg soft tissue. If you use more than the maximum recommended amount of starting material, 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. Amount of Starting Material and Elution Volumes Used with the MagAttract RNA Tissue Mini M48 Kit and the Total Nucleic Acid Protocol

Sample	QIAsoft M Protocol	Amount of starting material	Elution volume
Soft tissue*	Total Nucleic Acid	1–10 mg†	50–200 µl

* For example, liver and kidney.

† Sample volume: 400 µl.

Yield of purified nucleic acids

Yields may 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 are shown in Table 2, below.

Table 2. Yields of Nucleic Acids Obtained from Tissue Using the MagAttract RNA Tissue Mini M48 Kit and the Total Nucleic Acid Protocol

Sample type	Amount of tissue	Yield (µg NA)
Pig liver*	1 mg	7.3
Pig liver*	5 mg	29.6

* 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 Tissue Mini M48 Kit (cat. no. 959236)
- 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 *MagAttract RNA M48 Handbook*).

† β-ME is 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 Tissue 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.
- For best results, stabilize animal tissues immediately in RNA*later*® RNA Stabilization Reagent. Tissues can be stored in RNA*later* TissueProtect Tubes for up to 1 day at 37°C, 7 days at 18–25°C, 4 weeks at 2–8°C, or for archival storage at –20°C or –80°C. See the *RNA*later *Handbook* for more information about RNA*later* RNA Stabilization Reagent and about stabilizing RNA in tissues.
- Fresh, frozen, or RNA*later* stabilized tissue can be used. To freeze tissue for long-term storage, flash-freeze in liquid nitrogen, and immediately transfer to –70°C. Tissue can be stored for several months at –70°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 4) can also 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. Continue with step 5.
- 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.
- 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. **Excise the tissue sample from the animal or remove it from storage. Remove RNA*later* stabilized tissues from the reagent using forceps.**
2. **Determine the amount of tissue. Do not use more than 10 mg. Proceed immediately with step 3.**

   Weighing tissue is the most accurate way to determine the amount.

   Note: For tissues of high cell density, such as spleen, do not use more than 5 mg.

3. **For RNA*later* stabilized tissues:**

   If the entire piece of RNA*later* stabilized tissue can be used for isolation of total nucleic acids, place it directly into a suitably sized vessel for disruption and homogenization, and proceed with step 4.

   If only a portion of the RNA*later* stabilized tissue is to be used, place the tissue on a clean surface for cutting, and cut it. Determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed with step 4.

   RNA in the RNA*later* treated tissue is still protected while the tissue is processed at 18–25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room. The remaining tissue can be placed into RNA*later* RNA Stabilization Reagent for further storage. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues:

   If the entire piece of tissue can be used for isolation of total nucleic acids, place it directly into a suitably sized vessel for disruption and homogenization, and proceed immediately with step 4.

   If only a portion of the tissue is to be used, determine the weight of the piece to be used, and place it into a suitably sized vessel for homogenization. Proceed immediately with step 4.

   RNA in tissues is not protected after harvesting until the sample is treated with RNA*later* RNA Stabilization Reagent, flash-frozen, or disrupted and homogenized in protocol step 4. Frozen animal tissue should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

   Note: The remaining fresh tissue can be placed into RNA*later* RNA Stabilization Reagent for stabilization (see *RNA*later *Handbook*). However, previously frozen tissue samples thaw too slowly in the reagent, thus preventing it from diffusing into the tissue quickly enough before the RNA begins to degrade.

4. **Disrupt tissue and homogenize lysate in Buffer RLT (do not use more than 10 mg tissue). Disruption and homogenization of tissue can be performed by 3 alternative methods (4a, 4b, or 4c).**

   See "Disruption and homogenization of starting material" in the *MagAttract RNA M48 Handbook* for a more detailed description of disruption and homogenization methods.

   After storage in RNA*later* RNA Stabilization Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization of tissue samples using standard methods is usually not a problem.

   Note: Incomplete homogenization will lead to significantly reduced yields. Homogenization with rotor–stator homogenizers generally results in higher total nucleic acid yields than with other homogenization methods.

4a. Rotor–stator homogenization:

Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a suitably sized vessel for the homogenizer. Add 400 µl Buffer RLT. Homogenize immediately using a conventional rotor–stator homogenizer until the sample is uniformly homogeneous (usually 20–40 s). Continue the protocol with step 5.

Rotor–stator homogenization simultaneously disrupts and homogenizes the sample.

4b. Mortar and pestle with QIAshredder homogenization:

Immediately place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.

Add 400 µl Buffer RLT. Pipet the lysate directly onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Continue the protocol with step 5.

Grinding the sample using a mortar and pestle will disrupt the sample, but it will not homogenize it. Homogenization is carried out by centrifugation through the QIAshredder Spin Column.

4c. TissueLyser homogenization:

Place the weighed (fresh, frozen, or RNA*later* stabilized) tissue in a 2 ml microcentrifuge tube (not supplied), add 400 µl Buffer RLT, and add one stainless steel bead (3–7 mm diameter). Homogenize on the TissueLyser for 2 min at 20 Hz. Rotate the TissueLyser rack, and homogenize for another 2 min at 20 Hz. Continue the protocol with step 5.

Note: The instructions in step 4c are only guidelines. They may need to be changed depending on the sample being processed and on the bead mill being used.

5. Centrifuge the tissue lysate for 3 min at maximum speed in a microcentrifuge. Carefully transfer the supernatant to a new microcentrifuge tube (not supplied) by pipetting. Use only this supernatant (lysate) in subsequent steps.

In some preparations, very small amounts of insoluble material will be present, making the pellet invisible.

6. 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.

7. Switch on the BioRobot M48.

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

8. Switch on the computer and monitor.

9. 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.

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

11. **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.

12. **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.**

13. **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.