Technical Bulletin

MagneSil® Total RNA mini-Isolation System

INSTRUCTIONS FOR USE OF PRODUCT Z3351.

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Promega

MagneSil® Total RNA mini-Isolation System

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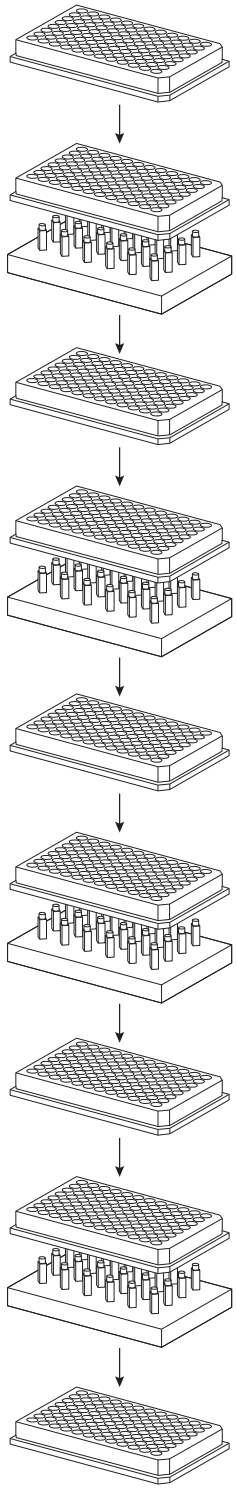
1. Description

The MagneSil® Total RNA mini-Isolation System[a] provides a high-throughput 96-well format for fast, simple preparation of intact, purified total RNA from small amounts of cell culture (≤1 × 10^5 tissue culture cells), tissue (≤2mg tissue lysate in 100µl), or freshly isolated whole blood (≤20µl). The protocol enables manual purification with the use of a multichannel pipettor and plate shaker or high-throughput automated purification on a variety of liquid-handling workstations. Isolation of total RNA in a 384-well format from cell culture (≤1 × 10^3 cells) and freshly isolated whole blood (≤5µl) may also be performed. Total RNA purification is achieved without the need for vacuum filtration, centrifugation or precipitation. The 96-well total RNA isolation procedure takes about 30 minutes to complete on a liquid-handling workstation.

The MagneSil® Total RNA mini-Isolation System uses the MagneSil® RNA Paramagnetic Particle technology. After sample lysis, nucleic acids are captured by MagneSil® RNA Paramagnetic Particles. The particles are washed and incubated with a DNase Solution to remove contaminating genomic DNA. The DNase is then inactivated, degraded genomic DNA is washed away, and the purified total RNA is eluted in Nuclease-Free Water (Figure 1). Sufficient reagents are supplied to isolate total RNA from four 96-well or 384-well plates. All components are guaranteed free of contaminating ribonucleases when used as directed and are thoroughly tested to ensure optimal performance.

The protocol provided in this technical bulletin provides instructions for automated or manual isolation of total RNA using the MagneSil® Total RNA mini-Isolation System. Specific instructions are provided for the Beckman Coulter Biomek® 2000 and Biomek® FX Laboratory Workstations. Information about obtaining automated methods for these workstations is available at: **www.promega.com/automethods/**

Total RNA purified using the MagneSil® Total RNA mini-Isolation System is suitable for a variety of molecular biology applications including end-point RT-PCR amplification and real-time RT-PCR.



1. Prepare sample plate containing cultured cells, 20l whole blood, or tissue lysate (maximum 2mg in 100µl RNA Lysis Buffer.)
2. Add RNA Lysis Buffer to each sample and mix.
3. Dispense MagneSil® RNA PMPs to processing plate.
4. Transfer processing plate to the MagnaBot® Magnetic Separation Device to capture MagneSil® PMPs to side of plate. Remove supernatant to waste. Remove Plate from MagnaBot® Device.
5. Transfer sample lysate to processing plate containing the MagneSil® RNA PMPs and mix.
6. Transfer plate to the MagnaBot® Magnetic Separation Device to capture MagneSil® PMPs to side of plate. Remove supernatant to waste.
7. Remove plate from the MagnaBot® Magnetic Separation Device.
8. Add 90% ethanol to each well and mix to wash MagneSil® RNA PMPs.
9. Transfer plate to the MagnaBot® Magnetic Separation Device to capture MagneSil® PMPs to side of plate. Remove supernatant to waste.
10. Remove plate from MagnaBot® Magnetic Separation Device.
11. Add DNase Solution and mix. Incubate at room temperature for 10 minutes.
12. Add DNase Stop Solution and mix.
13. Transfer plate to the MagnaBot® Magnetic Separation Device to capture MagneSil® PMPs to side of plate. Remove supernatant to waste.
14. Repeat Steps 8 and 9 twice more (for a total of two ethanol washes after DNase treatment step).
15. Add Nuclease-Free Water to MagneSil® RNA PMPs and mix.
16. Transfer plate to the MagnaBot® Magnetic Separation Device to capture MagneSil® PMPs to side of plate.
17. Remove eluted purified total RNA to new polypropylene 96-well plate. Store purified total RNA at –70C.

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Figure 1. Overview of the MagneSil® Total RNA mini-Isolation System protocol.

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagneSil® Total RNA mini-Isolation System	4 plate	Z3351

Each system contains sufficient reagents for the isolation of total RNA from up to four 96- or 384-well plates. Includes:

- 100ml RNA Lysis Buffer (RLA), 100ml
- 20ml MagneSil® RNA Paramagnetic Particles (PMPs)
- 4 vials DNase I (lyophilized)
- 25ml Yellow Core Buffer
- 5ml $MnCl_2$, 0.09M
- 26.5ml DNase Stop Solution (concentrated)
- 150ml Nuclease-Free Water

Storage Conditions: Store the MagneSil® Total RNA mini-Isolation System at room temperature (22–25°C). Do not refrigerate or freeze the MagneSil® RNA PMPs.

Available Separately

The products listed below are required for isolation of RNA using the MagneSil® Total RNA mini-Isolation System but must be purchased separately.

96-well Purification

Product	Cat.#
MagnaBot® 96 Magnetic Separation Device	V8151
1/4 inch Foam Spacer	Z3301
Collection Plates (4 × 96-well U-bottom plates)	A9161

384-well Purification

Product	Cat.#
MagnaBot® 384 Magnetic Separation Device	V8241
384-well Plate, Flat	V5291

3. General Considerations

3.A. Direct Purification of RNA

The successful isolation of intact RNA requires four steps: 1) Effective disruption of cells or tissue; 2) Denaturation of nucleoprotein complexes; 3) Inactivation of endogenous ribonucleases (RNases); and 4) Removal of contaminating DNA and protein. The most important step is the immediate inactivation of endogenous RNases that are released from membrane-bound organelles upon cell disruption.

The MagneSil® Total RNA mini-Isolation System combines the disruptive and protective properties of guanidine thiocyanate (GTC) to lyse samples, denature nucleoprotein complexes and inactivate ribonucleases (1). Total RNA is captured from the sample lysate using MagneSil® RNA Paramagnetic Particles (PMPs). RNase-Free DNase is then added to digest contaminating genomic DNA. The bound total RNA is further purified from contaminating salts, proteins and cellular impurities by simple ethanol washes. Finally, total RNA is eluted by the addition of Nuclease-Free Water. The procedure yields an essentially pure fraction of total RNA in a single round of purification without organic extractions, precipitation or vacuum filtration. The procedure is easy to perform with small quantities of cultured cells or freshly isolated whole blood in 96- or 384-well formats, or tissue in a 96-well format.

3.B. Processing Capacity

The MagneSil® Total RNA mini-Isolation System is optimized for purifying total RNA from small sample sizes. A **maximum** of 1×10^5 cells, 2mg tissue lysate in 100µl RNA Lysis Buffer (RLA) or 20µl of whole blood (from a normal healthy adult) can be processed per well in 96-well plate format. A **maximum** of 1×10^3 cells or 5µl whole blood can be processed per well in 384-well plate format. Using sample sizes greater than those recommended will result in particle clumping, and yield and quality of the purified total RNA will be adversely affected.

3.C. Creating a Ribonuclease-Free Environment

Ribonucleases are very difficult to inactivate. Take care to avoid inadvertently introducing RNase activity into your RNA during or after the isolation procedure. This is especially important if the starting material has been difficult to obtain or is irreplaceable. The following notes may help you prevent accidental RNase contamination of your sample.

For all downstream applications, it is essential that you continue to protect your RNA samples from RNases by wearing gloves and using solutions and centrifuge tubes that are RNase-free. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with the system. Wear gloves at all times.

2. Whenever possible, sterile disposable plasticware should be used for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nondisposable glassware and plasticware before use to ensure that is RNase-free. Bake glassware at 200°C overnight and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA followed by RNAse-free water.
4. Treat solutions supplied by the user by adding diethyl pyrocarbonate (DEPC) to 0.1% and then incubating overnight at room temperature. Autoclave for 30 minutes to remove any trace of DEPC.

4. Before You Begin

Materials to be Supplied by the User

- 1X PBS for cell culture cells. This is used to wash cells gently before purification
- 90% ethanol
- 96-well U-bottom Collection Plates (Cat.# A9161)
- MagnaBot® Magnetic Separation Device [96-well (Cat.# V8151) or 384-well (Cat.# V8241)]
- 1/4 inch Foam Spacer (Cat.# Z3301) [The 1/4 inch Foam Spacer is required only for 96-well purifications and is placed on top of the MagnaBot® 96 Magnetic Separation Device.]
- Multichannel pipettor
- Plate shaker (manual purification)

4.A. Preparation of Solutions

Solution	Preparation Steps	Notes
DNase I	Add the indicated volume of Nuclease-Free Water to the lyophilized DNase I	Gently mix by swirling. Do not vortex. One vial is sufficient for one 96- or 384-well plate. If processing less than a whole plate, we recommend dividing the rehydrated DNase into working aliquots using sterile, RNase-free microcentrifuge tubes. Store rehydrated DNase I at –20°C. Avoid multiple freeze-thaw cycles.
DNase Stop Solution	Add 40ml of 90% ethanol to the bottle of DNase Stop Solution	After adding ethanol, mark on the bottle that this step has been performed. The DNase Stop Solution is stable at 22–25°C when tightly capped.
DNase Solution	Combine the following in order: 5.2ml Yellow Core Buffer, 575µl $MnCl_2$ and 275µl DNase I	Prepare the DNase Solution fresh for each plate just before use. Mix by gentle pipetting. Do not vortex. DNase Solution **cannot** be stored.

Prepare DNase Solution **fresh** just before use.

4.B. Sample Preparation

Cell Culture Samples

Generally, a maximum of 1×10^5 cells per well can be processed. Exceeding the maximum cell number will reduce both yield and performance of purified total RNA.

1. Wash cells once with sterile 1X PBS.
2. Promega automated methods (i.e., the automated methods described below) begin with only tissue culture cells in the flat-bottom culture plate placed onto the deck of the automated liquid handler. Cell culture media or PBS should be gently removed before beginning the MagneSil® Total RNA purification process.

Tissue Homogenates

Completely disrupt the tissue of interest in the supplied RNA Lysis Buffer. The maximum amount of tissue that can be processed per purification well is 2mg in 100µl of RNA Lysis Buffer. If the volume of the tissue lysate is less than 100µl, add RNA Lysis Buffer to bring the volume to 100µl. If more RNA Lysis Buffer is required than is provided, SV RNA Lysis Buffer (Cat.# Z3051) may be used.

Whole Blood

Generally, a maximum of 20µl of whole blood can be processed per well. This is assuming that the whole blood sample is from a normal healthy adult whose white blood cell count will generally be between 4.5×10^6–1.1×10^7/ml (2). Efficient purification will be from a volume of sample that contains a maximum of 1×10^5 cells not to exceed a volume of 50µl. Processing samples that contain greater than 1×10^5 cells will reduce yield and affect performance of purified total RNA.

Use freshly isolated whole blood for the MagneSil® Total RNA mini-Isolation procedure.

5. Automated RNA Purification on the Biomek® 2000 Laboratory Workstation

The automated method for this procedure is available at:
www.promega.com/automethods
Instructions on importing Biomek® 2000 programs may be found at:
www.promega.com/automethods/beckman/biomek2000/default.asp

Materials to be Supplied by the User

- 96-well U-Bottom Collection Plate (Cat.# A9161)
- 96-well polypropylene U-Bottom plate (Greiner Cat.# 650201)
- Deep Well Plate, 2.2ml (Marsh Bio Products Cat.# AB0932)
- MagnaBot® 96 Magnetic Separation Device (Cat.# V8151)
- 1/4 inch Foam Spacer (Cat.# Z3301)
- 90% ethanol
- P250 barrier tips (Axygen Scientific Cat.# 22234-120)

5.A. Instrument Requirements

The following is a list of Beckman Coulter parts and corresponding catalog numbers that are required to use the MagneSil® Total RNA mini-Isolation System on the Biomek® 2000 Workstation.

Description	Quantity	Beckman Coulter Cat. #
Biomek® 2000 Workstation, 50/60 Hz, 100–120V	1	609000
Biomek® 2000 Controller NT	1	609875
IBM Monitor	1	974571
BioWorks™ Version 3.2 for Biomek® 2000	1	609983
Gripper Tool System for Biomek® 2000	1	609001
MP200 Pipetting Tool	1	609025
Tip Rack Holder	2	609121
Gray Labware Holders	5	609120
Reservoir Holder	1	372795
Quarter Vertical Reservoirs	2	372780
Quarter Single Reservoirs	2	372790
DPC Micromix® Shaker	1	380560
DPC Micromix® Shaker Integration Package	1	380561

5.B. Initial Deck Configuration for the Biomek® 2000

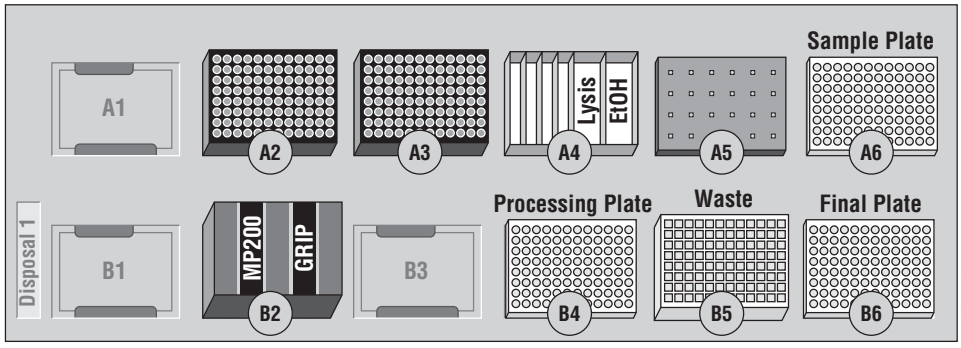
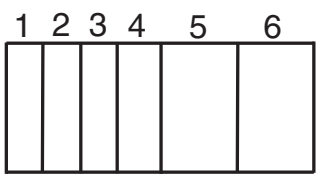


Figure 2. Initial deck layout for the MagneSil® Total RNA mini-Isolation System on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Empty
A2	Tip rack holder, P250 Barrier Tips
A3	Tip rack holder, P250 Barrier Tips
A4	Labware holder, reservoir holder, two quarter vertical reservoirs, two quarter single reservoirs (see below).
A5	Labware holder, MagnaBot® 96 Separation Device, 1/4 inch Foam Spacer on top of MagnaBot® Device
A6	Shaker Position, 96-well flat-bottom sample plate (e.g, Costar® cell culture plate).
B1	Empty
B2	Tool rack containing MP200 and Gripper tools.
B3	Labware holder
B4	Labware holder, 96-well U-bottom processing plate containing 30µl MagneSil® RNA PMPs per well.
B5	Labware holder, empty 2.2ml 96-well deep well plate
B6	Shaker Position, empty 96-well polypropylene U-bottom plate for eluted purified total RNA

Completely resuspend the MagneSil® RNA PMPs before dispensing.

Reagent Dispense Volumes for the Biomek® 2000: Prior to beginning run, the following system reagents need to be dispensed appropriately on the deck of the Biomek® 2000 workstation.



1. 6ml Nuclease-Free Water.
2. 12ml DNase Stop Solution (ethanol added)
3. Empty
4. 6ml DNase Solution (see Section 4.A)
5. 12ml RNA Lysis Buffer (leave empty when purifying RNA from tissue lysates)
6. 33ml 90% ethanol

5.C. Biomek® 2000 Specific Pre-Run Recommendations

Purification from Partial Plates on the Biomek® 2000

A "MagRNA" plate pattern has been defined in the Biomek® 2000 method for the MagneSil® Total RNA mini-Isolation System. This allows simple method editing to purify RNA from less than a full 96-well plate of sample material. The program refers to the defined "MagRNA" plate pattern for the number of samples to process in a purification run. The default setting is isolation from all 96-wells. If you need to process less than 96 samples, you can change the "MagRNA" plate pattern to accommodate this by following the instructions below:

Changing Plate Pattern

1. In the BioWorks™ Software, select "Patterns" from the "Edit" menu.
2. Select "MagRNA" to highlight the plate pattern
3. Click the "Edit" button.
4. Select "Allow Changes".
5. Click the "Clear" button to deselect all the wells.
6. Highlight the wells of the MagRNA plate pattern to the correct number of samples that you are running (default setting is 96 wells).
7. Select "OK".
8. Click the "Close" button in the "Edit Global Patterns" window.
9. Verify in the method that the number of wells highlighted corresponds to the changes just made to the "MagRNA" plate pattern.

6. Automated RNA Purification on the Biomek® FX Laboratory Workstation

Materials to Be Supplied By the User

96-well Purification

- Pyramid-bottom reservoir plates (Innovative Microplate Cat.# S30014)
- 96-well U-bottom Collection Plates (Cat.# A9161)
- Biomek® FX P250 barrier tips (Axygen Scientific Cat.# FXF-180-LRS)
- Deep Well Plate, 2.2ml (Marsh Bio Products Cat.# AB0932)
- 96-well U-bottom polypropylene plate, (Greiner Cat.# 650201) (for eluted purified total RNA)
- MagnaBot® 96 Separation Device (Cat.# V8151)
- 1/4 inch Foam Spacer (Cat.# Z3301)

384-well Purification

- Pyramid-bottom reservoir plates (Innovative Microplate Cat.# S30014)
- 96-well U-bottom Collection Plates (Cat.# A9161)
- Biomek® FX P250 barrier tips (Axygen Scientific Cat.# FXF-180-LRS)
- Deep Well Plate, 2.2ml (Marsh Bio Products Cat.# AB0932)
- MagnaBot® 384 Separation Device (Cat.# V8241)
- 384-well Plate, Flat (Cat.# V5291) (for eluted purified total RNA)

6.A. Instrument Requirements

The following is a list of Beckman Coulter parts that are required to use the MagneSil® Total RNA mini-Isolation System on the Biomek® FX Workstation.

Description	Quantity	Ordering Information
Minimum: Biomek® FX Software Version 2.1	1	Contact Beckman Coulter
96-channel POD	1	Contact Beckman Coulter
Orbital Shaker	1	Contact Beckman Coulter
Minimum number of labware positions accessible by 1 POD		
96-well purification	12	Contact Beckman Coulter
384-well purification	14	Contact Beckman Coulter

6.B. Initial Deck Configuration for the Biomek® FX

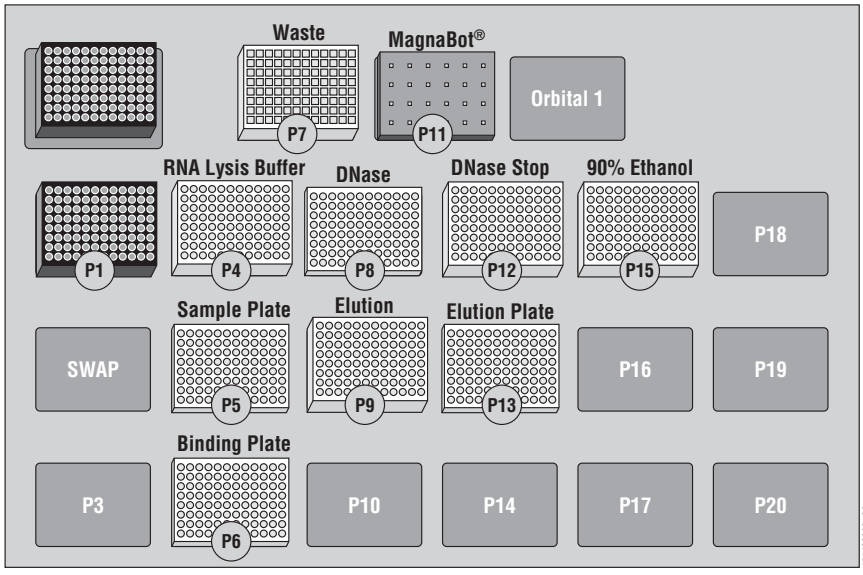


Figure 3. Biomek® FX initial deck configuration—96-well purification. This is an example of a MagneSil® Total RNA mini-Isolation System deck layout on a Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Equipment
Tip Loader	Biomek® P250 Barrier Tips
P1	Biomek® P250 Barrier Tips
P2	Swap position
P3	Empty
P4	Pyramid-bottom reservoir plate containing 12ml of RNA Lysis Buffer
P5	96-well, flat-bottom culture plate containing sample
P6	96-well, U-bottom plate (Binding Plate) containing 30µl of MagneSil® RNA Paramagnetic Particles per well
P7	Empty 2.2ml deep-well, 96-well plate (used for waste)
P8	96-well, U-bottom plate containing 55µl of prepared DNase Solution per well
P9	Pyramid-bottom reservoir plate containing 25ml of Nuclease-Free Water (Elution)
P10	Empty
P11	MagnaBot® 96 Magnetic Separation Device with 1/4 inch Foam Spacer
P12	Pyramid-bottom reservoir plate containing 12ml of DNase Stop Solution (ethanol added)
P13	96-well polypropylene U-bottom Collection Plate for purified total RNA (Elution Plate)
P14	Empty
P15	Pyramid-bottom reservoir plate containing 50ml of 90% ethanol

Completely resuspend the MagneSil® RNA PMPs before dispensing.

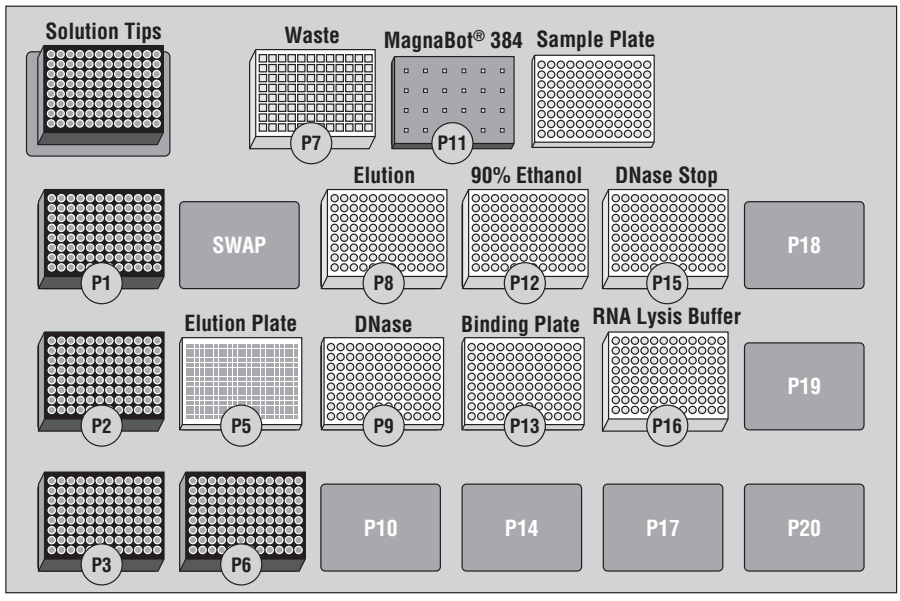


Figure 4. Biomek® FX initial deck configuration—384-well purification. This is an example of a MagneSil® Total RNA mini-Isolation System deck layout on a Biomek® FX. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Equipment
Tip Loader	Biomek® P250 Barrier Tips
P1	Biomek® P250 Barrier Tips
P2	Biomek® P250 Barrier Tips
P3	Biomek® P250 Barrier Tips
P4	Swap position (leave empty)
P5	384-well plate for purified total RNA
P6	Biomek® P250 Barrier Tips
P7	Empty 2.2ml deep-well, 96-well plate (used for waste)
P8	Pyramid-bottom reservoir plate containing 25ml of Nuclease-Free Water
P9	96-well, U-bottom plate containing 55µl of prepared DNase Solution per well
P10	Empty
P11	MagnaBot® 384 Magnetic Separation Device
P12	Pyramid-bottom reservoir plate containing 60ml of 90% ethanol
Orbital	384-well, flat-bottom cell culture plate containing sample
P13	96-well, U-bottom plate (Binding Plate) containing 50µl of MagneSil® RNA Paramagnetic Particles per well
P14	Empty
P15	Pyramid-bottom reservoir plate containing 15ml of DNase Stop Solution (ethanol added)
P16	Pyramid-bottom reservoir plate containing 20ml of RNA Lysis Buffer

Note: Use a 96-channel head for the 384-well method.

Completely resuspend the MagneSil® RNA PMPs before dispensing.

7. Description of MagneSil® Total RNA mini-Isolation Protocol

This overview describes the general liquid handling and purification steps required for RNA isolation from samples in a 96-well plate format using the MagneSil® Total RNA mini-Isolation System. This protocol can be performed manually or adapted to a variety of automated liquid handling robots. Section 7.C provides information on RNA isolation in a 384-well format. For additional information about adaptation to liquid handling robots other than those already discussed, see Section 8.

In the protocol described below, a plate shaker (such as the DPC Micromix® 5 Shaker) is used for mixing steps. For all steps where shaking is indicated, shake the plate vigorously but not so vigorously to cause splashing/spilling, because this will cause cross-contamination of samples. Recommended shaker settings for the DPC Micromix® 5 Shaker are provided, but these may need to be optimized because settings often differ slightly between shakers.

Alternatively, mixing may be performed manually using a multichannel pipettor. Manual mixing will slow the purification process considerably. Ensure that the MagneSil® RNA PMPs are thoroughly resuspended during the purification procedure.

7.A. Sample Preparation

Before beginning the purification procedure, prepare the starting materials as follows:

Cultured Cells: Wash cultured cells gently once with 1X PBS. If using adherent cells, be careful not to wash the cells off the bottom of the plate. Remove all 1X PBS to waste. Purification begins with cell culture plate containing cells alone.

Tissue Lysate: Homogenize the tissue in RNA Lysis Buffer. Place up to 2mg of tissue lysate in a total volume of 100µl of Lysis Buffer per well of a 96-well plate.

Whole Blood: Place up to 20µl of whole blood into each well of a 96-well sample plate.

7.B. Protocol

1. Thoroughly resuspend the MagneSil® RNA Paramagnetic Particles (PMPs) in the reagent bottle. Dispense 30µl of particles to each well of 96-well U-bottom plate.

   ! Completely resuspend the MagneSil® RNA PMPs before dispensing.

2. **Remove Storage Buffer.** Move the 96-well processing plate onto the MagnaBot® Device and pause for 1 minute to capture the MagneSil® RNA PMPs to the side of the wells. Carefully remove supernatant to waste.

3. **Sample Lysis.** Add 100µl RNA Lysis Buffer to each sample and mix either by tip mixing or by vigorous shaking (DPC settings: form 47, amplitude 7) for 1 minute.

   Note: Addition of RNA Lysis Buffer is not required for purification from tissue lysates, as these are homogenized in RNA Lysis Buffer.

4. **Capture of Nucleic Acids.** Transfer the sample in RNA Lysis Buffer to each well of the 96-well U-bottom processing plate containing the MagneSil® RNA PMPs. Mix vigorously on plate shaker (DPC settings: form 47, amplitude 7) for 2 minutes.

5. Move the 96-well processing plate onto the MagnaBot® Device and pause for 1 minute to capture MagneSil®-bound nucleic acids to the sides of the wells. Remove and discard the supernatant, taking care to avoid disturbing the captured MagneSil® RNA PMPs.

6. **Wash.** Add 100µl of 90% ethanol to each well. Move the processing plate from the MagnaBot® Device to the shaker and shake (DPC settings: form 42, amplitude 5) vigorously for 1 minute to resuspend and wash the MagneSil® RNA PMPs.

   Note: For larger sample sizes (1×10^5 cells or 2mg of tissue lysate), some particle clumping may occur at Step 6. This should disperse after DNase treatment and subsequent washes.

7. Move the 96-well processing plate onto the MagnaBot® Device and pause for 1 minute to capture MagneSil®-bound nucleic acids to the sides of the wells. Remove and discard the supernatant.

8. Ensure that all of the ethanol wash has been removed from the wells. Incubate the plate for 1 minute on the MagnaBot® Device to allow residual ethanol to evaporate.

9. **DNase Treatment.** Add 50µl of prepared DNase Solution to each well of the processing plate. Move the plate from the MagnaBot® Device to the shaker and shake to resuspend the MagneSil® RNA PMPs. Shake (DPC settings: form 47, amplitude 6) for 10–15 minutes to allow the DNase Solution to digest contaminating genomic DNA.

10. **DNase Inactivation.** Add 100µl DNase Stop Solution to each well of the processing plate and shake (DPC settings: form 42, amplitude 4) for 2 minutes to resuspend the MagneSil® RNA PMPs.

11. Move the processing plate onto the MagnaBot® Device and pause for 1 minute to capture the MagneSil®-bound nucleic acids to the sides of the wells. Remove and discard the supernatant.

12. **Wash.** Add 100µl of 90% ethanol to each well. Move the processing plate from the MagnaBot® Device to the shaker and shake vigorously (DPC settings: form 42, amplitude 5) for 1 minute to resuspend and wash the MagneSil® RNA PMPs.

13. Move the processing plate onto the MagnaBot® Device and pause for 1 minute to capture MagneSil®-bound total RNA to the sides of the wells. Remove and discard the supernatant.

14. Repeat Steps 12 and 13 for a total of two 90% ethanol washes.

15. After complete removal of the last 90% ethanol wash, pause for 2 minutes with the 96-well processing plate on the MagnaBot® Device to allow the MagneSil® RNA PMPs to dry.

16. **Elution.** Add 50µl of Nuclease-Free Water to each well of the 96-well processing plate.

    Note: Elution volumes as low as 25µl can be used to increase the concentration of the eluted total RNA.

17. Move the processing plate from the MagnaBot® Device to the shaker. Shake (DPC settings: form 47, amplitude 6) vigorously for 2 minutes to resuspend the MagneSil® RNA PMPs and elute the purified total RNA.

18. Move the 96-well processing plate onto the MagnaBot® Device and pause for 1 minute to capture the MagneSil® RNA PMPs. Remove the supernatant containing purified total RNA to a new 96-well polypropylene plate. Store the purified total RNA at –70°C.

    Note: Addition of 0.5µl of RNasin® Plus RNase Inhibitor (Cat.# N2611) to samples can help protect eluted total RNA from post-purification degradation. During elution, 0.5µl of RNasin® Plus RNase Inhibitor can be added per 50µl of Nuclease-Free Water.

7.C. Use of MagneSil® Total RNA mini-Isolation System in a 384-Well Format

A 96-well purification procedure has been described. 384-well purification is achieved by scaling down the 96-well purification procedure. Below is a table of relative volumes of reagents for each purification format. The purification limits for 384-well purification are: ≤1 × 10^3 cells or ≤5µl of whole blood. We do not recommend 384-well purification of tissue lysates.

Reagent	Volume/Well 96-well Purification	Volume/Well 384-well Purification
RNA Lysis Buffer	100µl	50µl
MagneSil® RNA PMPs	30µl	10µl
90% Ethanol Washes	100µl	50µl
DNase Solution (prepared)	50µl	12.5µl
DNase Stop Solution	100µl	25µl
Nuclease-Free Water for elution	50µl	15µl

The recommended volume for the MagneSil® Total RNA mini-Isolation System is 50µl for 96-well purification. This elution volume can be decreased to 25µl to increase the concentration of purified total RNA without a significant drop in total yield. Elution volumes less than 25µl will result in concomitant decrease in RNA yield. We do not recommend decreasing the elution volume for 384-well purifications.

8. General Guidelines for Adaptation to Alternative Robotic Platforms

The use of aerosol-resistant tips is recommended for the MagneSil® Total RNA mini-Isolation System to decrease the chance of contaminating samples with RNases. If your robotic platform uses fixed tips, be sure that the tips are washed thoroughly between pipetting steps. Also, if system liquid is used to perform pipetting steps, be sure to limit the exposure of samples to system liquid, a potential source of RNase contamination, during all pipetting steps by increasing the volume of leading air gaps that are used for pipetting.

Do not exceed 1×10^5 cultured cells, 2mg of tissue lysate, or 20µl of whole blood per well. Purification from tissue lysates can present particular problems. Do not exceed 2mg of tissue per sample well, and ensure that the sample volume is 100µl. If sample volume is less than 100µl, add Lysis Buffer to bring the volume to 100µl.

Complete resuspension of MagneSil® RNA Paramagnetic Particles (PMPs) is necessary for efficient purification of total RNA. MagneSil® RNA PMPs need to be equivalently dispensed into the sample processing plate and thoroughly resuspended during wash steps. Failure to resuspend MagneSil® RNA PMPs could result in variable yields from well-to-well, genomic DNA contamination, low yields or low purity of the purified RNA.

Addition of 0.5µl of RNasin® Plus RNase Inhibitor (Cat.# N2611) to samples can help protect eluted total RNA from post-purification degradation. During elution, 0.5µl of RNasin® Plus RNase Inhibitor can be added per 50µl of Nuclease-Free Water.

For RT-PCR analysis, RNA volumes exceeding 10% of the final reaction volume are not recommended.

9. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low RNA yield	Initial sample mass too large. The MagneSil® Total RNA mini-Isolation System is optimized for purification from ≤1 × 10^5 cells, ≤2mg tissue lysate in 100µl, or ≤20µl whole blood. Exceeding these limits will significantly reduce both yield and concentration of the purified RNA and will cause excessive clumping of MagneSil® RNA Paramagnetic Particles, making handling of the particles difficult.
	Use of sample lysate that has been stored at –20°C or –70°C. Lysate samples that have been frozen may have a decreased amount of total RNA. For optimal performance, purify the total RNA as soon as the lysate is prepared.
	Sample lysates have undergone multiple freeze-thaw cycles. Samples that have been frozen and thawed repeatedly will have some RNA degradation. Use fresh samples whenever possible.
	Tissue culture cells low in total RNA. The yield of total RNA may vary depending on the sample type. If total RNA yields are low, increase the amount of starting material processed.
	RNA Lysis Buffer not added to tissue lysates. Make sure that RNA Lysis Buffer is added to all sample lysates.
	Steps not followed correctly, or the wrong reagents were used. Use the MagneSil® Total RNA mini-Isolation System reagents in the order specified. This ensures that the RNA remains bound to the MagneSil® RNA PMPs during the purification process.
	Ethanol not added to DNase Stop Solution. Ensure that ethanol is added to the DNase Stop Solution. See Section IV.A.
	Incorrect concentration of ethanol wash. Ethanol concentrations lower than 90% will result in reduced yields. Make 90% ethanol wash solution fresh before each purification.

Symptoms	Causes and Comments
Low RNA yield (continued)	Failure to resuspend MagneSil® RNA PMPs in reagent bottle. Thoroughly resuspend MagneSil® RNA PMPs in the reagent bottle before dispensing to the sample processing plate to ensure even distribution of particles.
	Inaccurate dispensing of MagneSil® RNA PMPs. The volumes of MagneSil® RNA PMPs used in the protocol are optimized for yield and purity of RNA. Use of 25% greater than or less than the recommended amount will result in decreased yield (e.g., purification from 1×10^5 cultured cells requires 30µl of MagneSil® RNA PMPs; no less than 22.5µl and no more than 37.5µl of particles should be used per isolation.)
DNA contamination	DNase Solution not prepared correctly. For each plate purification 5.2ml Yellow Core Buffer, 575µl $MnCl_2$ and 275µl of DNase I are mixed to make a DNase Solution.
	Insufficient incubation with DNase Solution. Incubate DNase Solution for at least 10 minutes. DNase incubation time can be lengthened up to 20 minutes.
	DNase Solution stored or frozen before use. Make DNase Solution fresh before each use. It cannot be prepared and then stored.
MagneSil® RNA PMPs clump	Too much sample material used. MagneSil® RNA mini-Isolation System input limitations are $\leq 1 \times 10^5$ cells, ≤2mg tissue lysate in 100µl volume and ≤20µl whole blood
	Lysate too concentrated. If the lysate is too viscous, dilute with RNA Lysis Buffer until it becomes easier to pipette.
	Insufficient mixing. Vigorously mix during wash and incubation steps to resuspend the MagneSil® RNA PMPs.
RNA degradation	RNase introduced during purification/handling. Use RNase-free plastic- or glassware during the purification process. Use filter tips during all pipetting steps. Wear gloves at all times. RNases introduced after elution will degrade RNA.

10. References

1. Chirgwin, J.M. *et al*. (1979) Isolation of biologically active ribonucleic acid from sources enriched with ribonucleases. *Biochemistry* **18**, 5294–9.
2. Henry, J.B. (2001) *Clinical Diagnosis and Management by Laboratory Methods*, 20th Edition, W.B. Saunders Company, Chapter 24, p. 509.

11. Related Products

Product	Size	Cat. #
RNasin® Plus RNase Inhibitor	2,500 units	N2611
	10,000 units	N2615
SV 96 Total RNA Isolation System	1 × 96	Z3500
	5 × 96	Z3505
SV RNA Lysis Buffer	50ml	Z3051
Reverse Transcription System	100 reactions	A3500
AMV Reverse Transcriptase	300 units	M5101
	1,000 units	M5108
ImProm-II™ Reverse Transcription System	100 reactions	A3800
ImProm-II™ Reverse Transcriptase	100 reactions	A3802
	500 reactions	A3803
M-MLV Reverse Transcriptase	10,000 units	M1701
	50,000 units	M1705

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.

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