Aurum™ Total RNA 96 Kit

Instruction Manual

Catalog #732-6800

BIO-RAD

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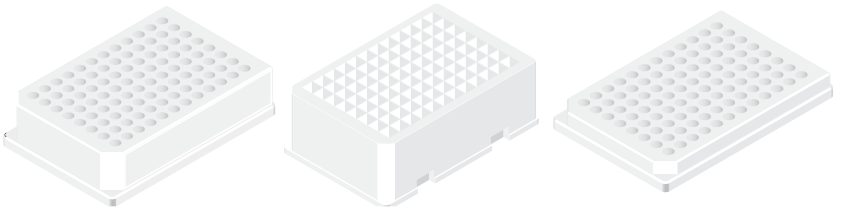
Section 1
Introduction

The Aurum™ Total RNA 96 kit rapidly purifies up to 192 total RNA samples from biological samples (e.g. mammalian cells, yeast, or bacteria) with minimal handling. Total RNA samples prepared using the Aurum™ Total RNA 96 kit are suitable for use in a variety of downstream applications, including reverse transcription-PCR (RT-PCR), quantitative real-time PCR, microarray analysis, and northern blots. A DNase I digestion during the purification effectively removes genomic DNA contamination from the preparation, eliminating the need for a separate DNase digestion. All solutions and binding plates in the kit are RNase-free, ensuring the integrity of the isolated total RNA. The Aurum™ Total RNA 96 kit is designed specifically for use with the Aurum™ Vacuum Manifold.

Section 2
Kit Components

The Aurum™ Total RNA 96 kit contains the following components:

Components	Quantity/Amount
Total RNA binding plates	2
Microtiter collection plates	2
Grow blocks	2
Sealing tape	2 pks of 4 pieces
DNase I (lyophilized)	2 vials
Lysis solution	85 ml
Low stringency wash solution (5x concentrate)	60 ml
High stringency wash solution	150 ml
Elution solution	20 ml
DNase dilution solution	20 ml



Total RNA binding plate | Grow block | Microtiter collection plate

Fig. 1. Selected components of the Aurum™ Total RNA 96 kit.

Section 3
Storage Conditions

All kit components (including lyophilized DNase I) should be stored at room temperature. Store reconstituted DNase I at –20°C in a nonfrost-free freezer, avoiding repeated freeze-thaw cycles. If precipitation is observed in any solution, warm the solution to 37°C to redissolve and allow the solution to return to room temperature before use.

Section 4
Necessary Supplies

Equipment and supplies to be provided by the customer:

- Plate centrifuge, capable of generating 1,500 x g
- β-mercaptoethanol, 14.2 M (catalog # 161-0710)
- Lyticase (for yeast RNA isolation only)
- Lysozyme (for bacterial RNA isolation only)
- Isopropanol (for bacterial RNA isolation only)
- 95–100% ethanol
- Tris for DNase I reconstitution (catalog # 161-0716)
- Multichannel pipettor (recommended)
- Reagent reservoirs (recommended)
- Additional sealing tape (catalog # MSB-1001) (optional)

Additional equipment required for vaccum format:

- Aurum™ Vacuum Manifold with regulator (catalog # 732-6470)
- Vacuum source (capability of –23 inHg or approximately –800 mbar required)

Section 5
Before Using the Aurum™ Total RNA 96 Kit

Please read the following guidelines before proceeding with the total RNA purification.

Starting Material Amounts

The Aurum™ Total RNA 96 kit is designed to process up to the amounts indicated below (per well):

- 1 x 10^6 mammalian cultured cells
- 1 OD/ml* of gram-positive or gram-negative bacteria
  1 OD/ml of bacteria roughly corresponds to a culture volume of 200–500 µl
- 2 OD/ml of yeast (*Saccharomyces cerevisiae*)
  2 OD/ml of yeast roughly corresponds to a culture volume of 300–600 µl

Average yields obtained from these starting amounts are shown in Table 1. Processing larger amounts of starting material may lead to well clogging and reduced RNA purity.

*Spectrophotometric determination of bacterial or yeast culture density is a REQUIREMENT for optimal total RNA isolation from these starting materials. To determine the density of a bacterial or yeast culture (OD_{600}), combine 50 µl of culture with 950 µl growth medium (20-fold dilution). Use the growth medium as a blank and take the spectrophotometric reading at λ = 600 nm. Multiply this figure by 20 to calculate the OD_{600} value of the undiluted bacterial or yeast culture. Depending upon the OD_{600} value, a specific volume of the culture will be selected to provide an optimum amount of bacteria or yeast for processing. To calculate the volume of culture required, use the following equation:

(OD_{600} of undiluted culture)** x (culture volume in ml) = # OD/ml

For example, 2 OD/ml of yeast would require 800 µl of an undiluted culture with an OD_{600} = 2.5.

**1 OD_{600} is equivalent to approximately 8 x 10^8 bacterial cells/ml, or 1 x 10^7 yeast cells/ml.

Table 1. Yield (per well) of total RNA from various samples using the Aurum™ Total RNA 96 kit.

Starting Material	Avg. Yield (µg)*
Cultured cells (1 x 10^6)	
3T3	7–10
HeLa	11–17
Bacterial (8 x 10^8)	
E. coli	5
B. cereus	5
Yeast (2 x 10^7)	
S. cerevisiae	9–11

Starting material amounts in parentheses are the maximum amounts recommended for use with the Aurum™ Total RNA 96 kit.

*Yield figures are representative of a minimum of four full plate experiments.

Reagents Used With the Aurum™ Total RNA 96 Kit

- The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes (240 ml) 95–100% ethanol to the low stringency wash solution concentrate before initial use.**
- Before using the RNA lysis solution, add 850 µl of β-mercaptoethanol to the solution, for a final concentration of 1%.
- The RNase-free DNase I is provided as a lyophilized powder. Reconstitute the DNase I by adding 250 µl 10 mM Tris, pH 7.5 (not supplied) to the vial. Pipet up and down briefly to mix. Do not vortex. Store the reconstituted DNase I at –20°C in a nonfrost-free freezer.
- Bacterial total RNA isolation with one RNA binding plate requires the use of 10 ml of TE (10 mM Tris, 1 mM EDTA, pH 7.5) for diluting the lysozyme. TE and lysozyme are not supplied with the kit.
- Yeast total RNA isolation with one RNA binding plate requires the use of 100 ml of lyticase dilution buffer (1 M sorbitol, 0.1 M EDTA, pH 7.4, 0.1% β-mercaptoethanol), which is not supplied with the kit.

- Vendors of lyticase, which is used to partially degrade the cell walls of yeast cells, may have different definitions of the enzyme's activity. As used in this instruction manual, 1 unit of lyticase produces a ΔA_{800} of 0.001/min at pH 7.5 at 25°C, using 3 ml of yeast suspension as a substrate in a 3 ml reaction volume.

Elution Guidelines

- Apply elution solution directly to the membrane stack at the base of each RNA binding plate.

Ribonucleases

- Although the components of this kit are provided free of contaminating ribonucleases, great care must be taken not to contaminate the solutions or the RNA binding plates. Gloves should always be worn when handling RNA and should be changed frequently. Proceed through the RNA isolation as quickly as possible with care.
- Solutions that are prepared by the user (e.g., TE) should be treated with diethyl pyrocarbonate (DEPC) to inactivate RNases. Add 1 ml DEPC per liter (final concentration 0.1%) of solution to be treated, mix thoroughly, and incubate the solution at 37°C for 1 hr, or at room temperature overnight. Autoclave the solution to remove the DEPC.

  Note: DEPC is destroyed by primary amines (e.g., Tris). If a solution containing a primary amine will be DEPC-treated, omit the amine in preparing the solution. Perform the DEPC treatment as described above, and add the amine to the autoclaved solution once the solution has cooled.
- Nondisposable, nonautoclavable plasticware should be rinsed with 0.1 M NaOH, 1 mM EDTA followed by several rinses with DEPC-treated water before use.
- Glassware and other autoclavable items may be treated using the method described above for nonautoclavable plasticware, or by baking for 4 hr at 300°C.
- Work surfaces and micropipettors should be kept clean and wiped periodically with an RNase removal reagent.

Disruption and Homogenization

Proper disruption and homogenization of the starting materials are required to ensure complete lysis of the cells and to reduce the viscosity of the cell lysates. This procedure uses repeated pipetting up and down through micropipettor tips to aid in lysing the cells, as well as to prepare homogeneous lysates prior to loading onto the RNA binding plate. Make sure that cell lysates are thoroughly homogenized (i.e. the viscosity has been considerably reduced); failure to do so may result in well clogging and reduced RNA purity.

Section 6
Vacuum Manifold Setup and Use With 96-Well Plates

Guidelines for Vacuum Format

- The recommended operating range is –17 to –23 inHg (see Table 2 for pressure unit conversions). Do not exceed –25 inHg when performing this protocol. A vacuum regulator is strongly recommended to establish the appropriate negative pressure.

Table 2. Pressure unit conversions.

To convert from inches of mercury (inHg) to:	Multiply by:
millimeters of mercury or torr (mmHg, torr)	25.4
millibar (mbar)	33.85
atmospheres (atm)	0.03342
pounds per square inch (psi)	0.4912
kilopascals (kPa)	3.385

- The gasket used on the manifold allows the RNA binding plate to self-seat whenever vacuum is applied, without pressing down on the plate. However, under certain conditions, gentle downward force may be required to ensure that the plate forms a seal with the manifold.
- When applying a vacuum to the manifold, increase the negative pressure gradually by slowly closing the vacuum regulator over a 5–10 sec period. This will promote uniform movement of solutions through the wells of the binding plate, minimizing sample spraying and cross-contamination during elution.
- During purge steps, the negative pressure within the vacuum manifold may drop to below –15 inHg. This is normal and does not require corrective action.

Preparing the Aurum™ Vacuum Manifold

Tubing provided in the Aurum™ Vacuum Manifold kit is 4 ft long and must be cut into appropriate pieces before proceeding.

Prior to setup, you may ensure that the gauger pointer is adjusted to zero by removing the lens cover, followed by turning the adjustment pin located beneath the dial face.

Vacuum Setup (Figure 2)

1. Cut tubing into three pieces of appropriate length.
2. Use one piece of tubing to connect the **right** side of the vacuum regulator to the vacuum source.
3. Use another piece of tubing to connect the **left** side of the vacuum regulator to the sidearm of the filter flask.
4. Place a rubber stopper **with hole** into the mouth of the filter flask. Insert a serological pipette (or comparable) into the hole of the stopper.
5. Snap in the black sealed end of the quick connect fitting into the manifold base.
6. Finally, use the last piece of tubing to connect the filter flask to the quick connect fitting of the nozzle of the manifold.

   Note: Use of the Aurum™ vacuum regulator is strongly recommended to ensure full control of the negative pressure of the manifold.

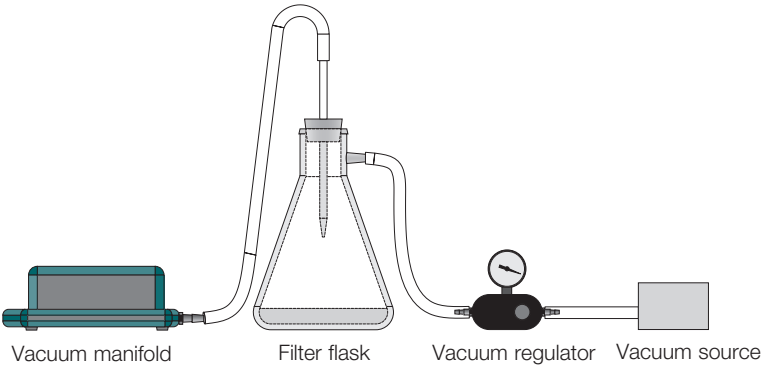


Fig. 2. Vacuum setup conditions.

Manifold Wash Setup (Figure 3A)

1. Place the manifold top on the base, ensuring complete and uniform contact between the manifold top and base.

2. Place the desired 96-well binding plate on the manifold top and apply the recommended vacuum pressure for your application.

Manifold Elution Setup (Figure 3B)

3. When eluting from the purification plate, place the stage on the manifold base, with the etched “This Side Up” facing up. Place a clean 96-well microtiter collection plate securely on top of the stage and replace the manifold top.

   Note: If desired, waste can be collected into an optional pipet tip box lid instead of draining directly into the vacuum trap.

4. After use, rinse the Aurum™ Vacuum Manifold with water and air dry or wipe with paper towels. Failure to rinse the vacuum manifold at the end of each use will cause the manifold to become cloudy and pitted.

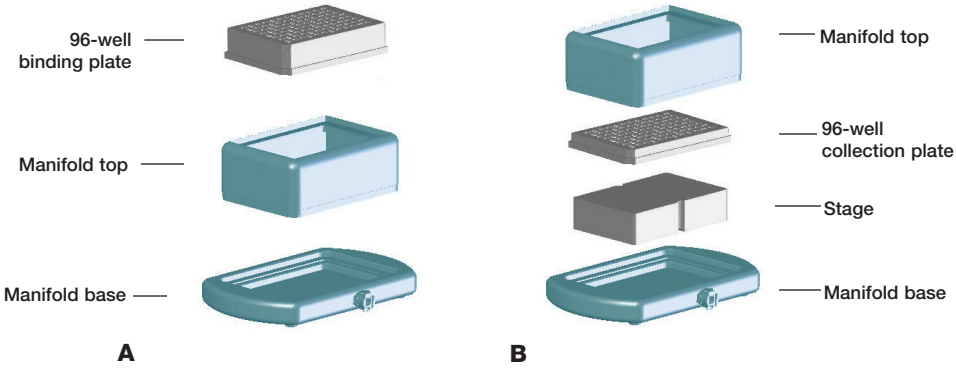


Fig. 3. Manifold setup for plate processing.
A) 96-well wash; B) 96-well elution.

Section 7
Vacuum Protocol

Please read Section 5, “Before Using the Aurum™ Total RNA 96 Kit” and Section 6, “Vacuum Manifold Setup and Use With 96-Well Plates” before proceeding.

Except for the first few steps that are specific for the starting sample types (A, for cultured cell lines; B, for bacteria; and C, for yeast), the remaining procedures within “All Starting Sample Types” share a common protocol.

Cultured Cell Lines

Follow steps A1–A3, then continue with step 1 of “All Starting Cell Types” on page 10.

A1. For nonadherent cell cultures, rinse the cells with PBS and transfer up to 1 x 10^6 cells into each well of a 96-well microplate (not provided). Centrifuge the plate at 300 x g for 5 min then aspirate the supernatant from each well.

For adherent cell cultures, rinse the wells (each containing up to 1 x 10^6 cells) of the growth vessel once with PBS and aspirate.

A2. Add 150 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each well and pipet up and down several times to lyse cells thoroughly.

A3. Add 150 µl of 70% ethanol (not supplied) to each well and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Bacteria

Follow steps B1–B3, then continue with step 1 of “All Starting Cell Types” on page 10. If starting with a grow block of bacterial culture (maximum 1 OD/ml/well), centrifuge the grow block at 1,500 x g for 10 min. Decant the supernatant, and blot the block with paper towels.

B1. Resuspend up to 8 x 10^8 bacterial cells per well (1 OD/ml/well), adding 100 µl of 500 µg/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5). Incubate at room temperature for 5 min.

B2. Add 350 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each sample and pipet up and down several times to mix thoroughly.

B3. Add 250 µl of 70% isopropanol (not supplied) to each sample and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Yeast

Follow steps C1–C5, then continue with step 1 of "All Starting Cell Types" on page 10. If starting with a grow block of yeast culture (maximum 2 OD/ml/well), centrifuge the grow block at 1,500 x g for 10 min. Decant the supernatant, and blot the block with paper towels.

C1. Prepare 100 ml lyticase dilution buffer:

1 M sorbitol
0.1 M EDTA, pH 7.4
0.1% (v/v) β-mercaptoethanol
Equilibrate the buffer at 30°C before use.

C2. Resuspend up to 2 x 10^7 yeast cells per well (2 OD/ml/well), adding 1 ml of 50 units/ml lyticase in lyticase dilution buffer equilibrated to 30°C to each well. Incubate for 10 min.

C3. Centrifuge at 1,500 x g for 5 min. Decant the supernatant.

C4. Add 350 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each sample and pipet up and down several times to mix thoroughly.

C5. Add 350 µl of 70% ethanol (not supplied) to each sample and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

All Starting Cell Types

1. Set up the Aurum™ Vacuum Manifold and one RNA binding plate according to "Manifold Wash Setup" instructions on page 7.

2. With the vacuum turned off and the regulator open, transfer the lysate from each sample to a well of the RNA binding plate.

3. Turn the vacuum on and gradually increase the negative pressure between –17 to –23 inHg over a 5–10 sec period by slowly closing the vacuum regulator. Once all wells have emptied, open the vacuum regulator fully. Check that the regulator gauge reads approximately 0 inHg.

   Note: Gradual application of negative pressure is required to ensure uniform flow of lysates through all 96 wells of the RNA binding plate.

4. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes (240 ml) 95–100% ethanol to the low stringency wash solution concentrate before initial use**.

5. Add 700 µl of low stringency wash solution to each well of the RNA binding plate. Gradually increase the negative pressure between –17 to –23 inHg over a 5–10 sec period by slowly closing the vacuum regulator. After all wells have emptied, open the vacuum regulator until the gauge reads approximately 0 inHg.

6. The RNase-free DNase I is provided as a lyophilized powder. Reconstitute the DNase I by adding 250 µl 10 mM Tris, pH 7.5 (not supplied) to the vial. Pipet up and down briefly to mix.

7. For each well (of a 96-well plate) processed, mix 2.5 µl of reconstituted DNase I with 77.5 µl of DNase dilution solution. For one 96-well plate, mix the entire contents of one vial of reconstituted DNase I with 7.75 ml DNase dilution solution in a 15 ml sterile conical tube. Scale proportionally if processing more or less than one full plate at a time. Add 80 µl diluted DNase I to the membrane at the bottom of each well of the RNA binding plate. Cover the plate with sealing tape and allow the digestion to incubate at room temperature for 10 min.

8. Add 700 µl of high stringency wash solution to each well of the RNA binding plate. Gradually increase the negative pressure between –17 to –23 inHg over a 5–10 sec period by slowly closing the vacuum regulator. After all wells have emptied, open the vacuum regulator until the gauge reads approximately 0 inHg.

9. Add 700 µl of low stringency wash solution to each well of the RNA binding plate. Gradually increase the negative pressure between –17 to –23 inHg over a 5–10 sec period by slowly closing the vacuum regulator. After all wells have emptied, continue to apply vacuum for an additional 4 min to purge the wells of residual wash solution. When completed, open the vacuum regulator until the gauge reads approximately 0 inHg.

10. Set up the Aurum™ Vacuum Manifold for elution according to “Manifold Elution Setup” on page 8.

11. Pipet 80 µl (or 40 µl)† of the elution solution onto the membrane stack at the base of each well of the RNA binding plate and allow 1 min for the solution to saturate the membranes. Gradually increase the negative pressure between –17 to –23 inHg over a 5–10 sec period by slowly closing the vacuum regulator. Continue to apply vacuum for 5 min. Open the vacuum regulator and turn off the vacuum source.

    †Note: Pipet 40 µl when isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells).

Note: Gradual application of negative pressure is required to prevent sample spraying and cross-contamination.

The eluted total RNA samples in the sample collection plate can be used immediately in downstream applications. Alternatively, the sample collection plate can be sealed with sealing tape and stored at –20°C or –80°C for later use.

Section 8
Spin Protocol

Please read Section 5, "Before Using the Aurum™ Total RNA 96 Kit" before proceeding.

Except for the first few steps that are specific for the starting sample types (A, for cultured cell lines; B, for bacteria; and C, for yeast), the remaining procedures within "All Starting Cell Types" share a common protocol.

Cultured Cell Lines

Follow steps A1–A3, then continue with step 1 of "All Starting Cell Types" on page 13.

A1. For nonadherent cell cultures, rinse the cells with PBS and transfer up to 1 x 10^6 cells into each well of a 96-well microplate (not provided). Centrifuge the plate at 300 x g for 5 min then aspirate the supernatant from each well.

For adherent cell cultures, rinse the wells (each containing up to 1 x 10^6 cells) of the growth vessel once with PBS and aspirate.

A2. Add 150 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each well and pipet up and down several times to lyse cells thoroughly.

A3. Add 150 µl of 70% ethanol (not supplied) to each well and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Bacteria

Follow steps B1–B3, then continue with step 1 of "All Starting Cell Types" on page 13. If starting with a grow block of bacterial culture (maximum 1 OD/ml/well), centrifuge the grow block at 1,500 x g for 10 min. Decant the supernatant, and blot the block with paper towels.

B1. Resuspend up to 8 x 10^8 bacterial cells per well (1 OD/ml/well), adding 100 µl of 500 µg/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5). Incubate at room temperature for 5 min.

B2. Add 350 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each sample and pipet up and down several times to mix thoroughly.

B3. Add 250 µl of 70% isopropanol (not supplied) to each sample and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Yeast

Follow steps C1–C5, then continue with step 1 of "All Starting Cell Types" on page 13. If starting with a grow block of yeast culture (maximum 2 OD/ml/well), centrifuge the grow block at 1,500 x g for 10 min. Decant the supernatant, and blot the block with paper towels.

C1. Prepare 100 ml lyticase dilution buffer:

1 M sorbitol
0.1 M EDTA, pH 7.4
0.1% (v/v) β-mercaptoethanol
Equilibrate the buffer at 30°C before use.

C2. Resuspend up to 2 x 10^7 yeast cells per well (2 OD/ml/well), adding 1 ml of 50 units/ml lyticase in lyticase dilution buffer equilibrated to 30°C to each well. Incubate for 10 min.

C3. Centrifuge at 1,500 x g for 5 min. Decant the supernatant.

C4. Add 350 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each sample and pipet up and down several times to mix thoroughly.

C5. Add 350 µl of 70% ethanol (not supplied) to each sample and pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

All Starting Cell Types

1. Place an RNA binding plate on top of a waste tray or a pipet tip box lid.

2. Transfer the lysate from each well of the 96-well plate into a corresponding well of the RNA binding plate.

3. Centrifuge for 2 min. Discard the filtrate from the waste tray and replace the RNA binding plate on top of the same waste tray.

4. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes (240 ml) 95–100% ethanol to the low stringency wash solution concentrate before initial use**.

5. Add 700 µl of low stringency wash solution to each well of the RNA binding plate. Centrifuge for 2 min at 1,500 x g. Discard the low stringency wash solution from the waste tray and replace the RNA binding plate on top of the same waste tray.

6. The RNase-free DNase I is provided as a lyophilized powder. Reconstitute the DNase I by adding 250 µl 10 mM Tris, pH 7.5 (not supplied, see note below) to the vial. Pipet up and down briefly to mix.

7. For each well (of a 96-well plate) processed, mix 2.5 µl of reconstituted DNase I with 77.5 µl of DNase dilution solution. For one 96-well plate, mix the entire contents of one vial of reconstituted DNase I with 7.75 ml DNase dilution solution in a 15 ml sterile conical tube. Scale proportionally if processing more or less than one full plate at a time. Add 80 µl diluted DNase I to the membrane at the bottom of each well of the RNA binding plate. Cover the plate with sealing tape and allow the digestion to incubate at room temperature for 10 min.

8. Add 700 µl of high stringency wash solution to each well of the RNA binding plate. Centrifuge for 2 min at 1,500 x g. Discard the high stringency wash solution from the same waste tray and replace the RNA binding plate on top of the tray.

9. Add 700 µl of low stringency wash solution to each well of the RNA binding plate. Centrifuge for 2 min at 1,500 x g. Discard the low stringency wash solution from the waste tray and replace the RNA binding plate on top of the same tray. Centrifuge for an additional 2 min at 1,500 x g.

10. Place the RNA binding plate on top of the provided collection plate.

11. Pipette 80 µl (or 40 µl)† of the elution solution onto the membrane stack at the base of each well of the RNA binding plate and allow 1 min for the solution to saturate the membranes. Centrifuge for 2 min at 1,500 x g.

    †Note: Pipet 40 µl when isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells).

    The eluted total RNA samples in the sample collection plate can be used immediately in downstream applications. Alternatively, the sample collection plate can be sealed with sealing tape and stored at –20°C or –80°C for later use.

Section 9
Troubleshooting Guide

Problem	Possible Cause	Recommended Solution
Difficulty achieving –17 to –23 inHg negative pressure	Purge step in protocol	If sealing of wells with sealing tape restores negative pressure, no corrective action is required; however, do not leave sealed
	Open vacuum regulator	Turn control knob fully clockwise to close regulator
	Plate not seating on vacuum manifold	Press down gently on RNA binding plate to seat
	Manifold top not seated properly on base	Ensure that manifold top is seated properly on base
	Residue on gasket	Rinse manifold and gasket with distilled water and wipe dry
	Gasket detached from manifold	Press down to reestablish uniform adhesive contact or replace gasket
	Worn manifold gasket	Replace gasket
Cross-contamination between adjacent wells	Abrupt application of negative pressure during elution	Increase negative pressure gradually over 5–10 sec using the vacuum regulator
	Residual wash buffer on drip directors	Blot RNA binding plate drip directors with paper towels; ensure that the plate underside is dry

Problem	Possible Cause	Recommended Solution
Low or highly variable eluate volumes among wells	Elution solution applied to RNA binding plate well walls	Apply elution solution directly to membranes at base of each well
	RNA binding plate not seated properly	Set plate properly and press down gently to seat
	Abrupt application of negative pressure during elution	Increase negative pressure gradually over 5–10 sec using the vacuum regulator
	Residual wash buffer on drip directors	Blot RNA binding plate drip directors with paper towels; ensure that the plate underside is dry
Genomic DNA contamination	Incomplete DNase I digestion	Increase DNase I digestion time
	Inactive DNase I	Store reconstituted DNase I in a nonfrost-free freezer; avoid freeze-thaw cycles; aliquot reconstituted DNase I for single use only
	Excessive amount of starting material per well	Reduce volume of culture used
	Incorrect preparation of DNase dilution	Use only the DNase dilution solution provided in the kit to dilute the DNase
RNA degradation	RNase contamination of user-made solutions and/or plasticware	DEPC-treat all handmade solutions; decontaminate all work surfaces; see Section 5 for more details
	Endogenous RNases	Work quickly through the steps prior to the addition of lysis solution

Problem	Possible Cause	Recommended Solution
Clogging of RNA binding plate	Excessive amount of starting material per well	Reduce volume of culture used
	Incomplete mixing of lysis and alcohol solutions	Mix RNA lysis solution and alcohol thoroughly by pipetting up and down
	Incomplete homogenization of cell lysate	Pipette lysate up and down further to reduce lysate viscosity
	Incomplete digestion with lysozyme or lyticase	Increase duration of lysozyme or lyticase digestion; use fresh enzyme
Low RNA yield	Low amount of starting material	Increase starting material amount up to the maximum indicated for the specific starting material type
	Incorrect use of wash solutions	Add the appropriate volume of 95–100% ethanol to the low stringency wash solution before initial use
	Incorrect preparation of DNase dilution	Use only the DNase dilution solution provided in the kit to dilute the DNase I
	Low sample eluate volume	See problem “Low or highly variable eluate volumes among wells”
	Inefficient elution	Preheat the elution solution to 70°C in water bath prior to the elution step
	Incomplete lysis	Pipet lysate up and down further to facilitate lysis

Problem	Possible Cause	Recommended Solution
Total RNA prep performs poorly in downstream applications	Incorrect use of wash solutions	Add the appropriate volume of 95–100% ethanol to the low stringency wash solution before initial use
	RNA is degraded	See problem “RNA degradation”
	Ethanol contamination in prep (eluate volumes >60 µl)	Add 1–3 min to the purge time after the final wash step
		Blot RNA binding plate drip directors with paper towels; ensure that the plate underside is dry
	Inefficient drying	Dry for a full 4 min and ensure no droplets of wash buffer remain on the walls or the bottom of the plate

Section 10
Ordering Information

Catalog #	Description
732-6800	**Aurum™ Total RNA 96 Kit**
732-6470	**Aurum™ Vacuum Manifold**
732-6820	**Aurum™ Total RNA Mini Kit**
732-6830	**Aurum™ Total RNA Fatty and Fibrous Tissue Kit**
732-6870	**Aurum™ Total RNA Fatty and Fibrous Tissue Module (without PureZOL™ RNA isolation reagent)**

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Italy *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
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