
Aurum™ Total RNA Mini Kit and Mini Sample Pack

Instruction Manual

Catalog #732-6820

Catalog #732-6820S

For technical service, call your local Bio-Rad office, or
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Section 1

Introduction

The Aurum total RNA mini kit purifies total RNA samples rapidly, from mammalian cell cultures, bacteria, and yeast (*Saccharomyces cerevisiae*), as well as animal and plant tissue. Total RNA samples prepared using the Aurum total RNA mini kit are suitable for use in a variety of downstream applications, including reverse-transcription PCR (RT-PCR) and northern blots. A DNase I digest during the purification effectively removes genomic DNA contamination from the preparation, eliminating the need for separate DNase digests. All solutions and binding columns in the kit are RNase-free, ensuring the integrity of the isolated total RNA. The Aurum total RNA mini kit may be used in a spin format, or in a vacuum format using the Bio-Rad Aurum vacuum manifold.

Section 2

Kit Components

The Aurum total RNA mini kit and mini sample pack contain the following components:

	Mini Kit	Sample Pack
RNA binding columns	50	10
Capless wash tubes, 2 ml	50	10
Capped microcentrifuge tubes, 1.5 ml	50	10
Capped microcentrifuge tubes, 2.0 ml	100	20
DNase I (lyophilized)	1	1
Lysis solution	50 ml	10 ml
Low stringency wash solution (5x concentrate)	20 ml	4 ml
High stringency wash solution	40 ml	10 ml
Elution solution	10 ml	1 ml
DNase dilution solution	10 ml	1 ml

The solutions are specifically formulated for the Aurum total RNA mini kit. They are NOT interchangeable with solutions used in other kits or protocols.

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 non-frost-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:

- Microcentrifuge ($\geq 12,000 \times g$)
- Water bath (70°C)
- 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 base for DNase I reconstitution (catalog #161-0716)

Additional equipment required for vacuum format:

- Bio-Rad Aurum vacuum manifold (Figure 1) with vacuum regulator and column adaptor plate (catalog #732-6470), or other vacuum manifold with luer fittings

Note: Please read Section 7, Instrument Setup and Use of the Column Adaptor Plate, for proper vacuum setup conditions.

- Vacuum source (capability of -23 " Hg required)

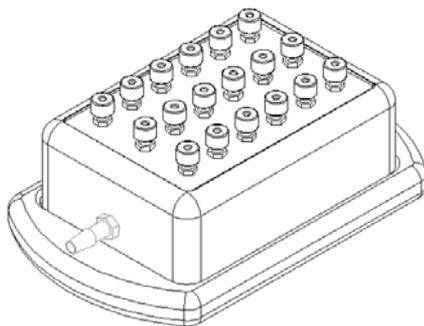


Fig. 1. Aurum vacuum manifold.

Section 5

Guidelines for Using the Aurum Total RNA Mini Kit

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

Starting Material Amounts

The Aurum total RNA mini kit is designed to process up to the amounts indicated below (per column):

- 2 x 10⁶ mammalian cultured cells
- 3 OD•ml* of gram-positive or gram-negative bacteria
3 OD•ml of bacteria roughly corresponds to a culture volume of 500–750 µl
- 3 OD•ml of yeast (*S. cerevisiae*)
3 OD•ml of yeast roughly corresponds to a culture volume of 600–1,000 µl
- 40 mg animal tissue
- 60 mg plant tissue

Processing larger amounts of starting material may lead to column 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 (OD₆₀₀), 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 OD₆₀₀ value of the undiluted bacterial or yeast culture. Depending upon the OD₆₀₀ 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:

$$(\text{OD}_{600} \text{ of undiluted culture})^{**} \times (\text{culture volume in ml}) = \# \text{ OD}\cdot\text{ml}$$

For example, 3 OD•ml of yeast would require 500 µl of an undiluted culture with an OD₆₀₀ = 6.

** 1 OD₆₀₀ is equivalent to approximately 8 x 10⁸ bacterial cells/ml, or 1 x 10⁷ yeast cells/ml.

Table 1. Yields (per column) of total RNA from various samples using the Aurum total RNA mini kit.

Starting Material	Avg. Yield (μg)*
Cultured cells (2×10^6)	
3T3	20–30
HeLa	20–40
Bacteria (2.4×10^9)	
<i>E. coli</i>	30–35
<i>B. cereus</i>	30–35
Yeast (3×10^7)	
<i>S. cerevisiae</i>	20–25
Animal tissue (up to 40 mg)	
Brain	15–20
Liver	8–12
Lung	8–12
Kidney	15–20
Spleen	15–25
Plant tissue (up to 60 mg)	
<i>Arabidopsis</i>	5–10
Maize	5–10
Potato	15–20
Spinach	10–15
Tomato	5–10
Tobacco	5–10

Starting material amounts in parentheses are the maximum amounts recommended for use with the total RNA mini kit.

*Yield figures are representative of a minimum of 20 mini column preps performed in both vacuum and spin formats.

Reagents Used With the Aurum Total RNA Mini Kit

- The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes of 95–100% ethanol to the low stringency wash solution concentrate before initial use.** This corresponds to 80 ml for the mini kit or 16 ml for the sample pack, respectively.
- Before using the lysis solution, add 500 μ l (or 100 μ l for the sample pack) of β -mercaptoethanol (catalog #161-0710) 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 μ l 10 mM Tris, pH 7.5 (not supplied, see note below) to the vial and pipetting up and down briefly to mix. Do not vortex. Store the reconstituted DNase I at -20°C in a non-frost-free freezer.

Note: 250 μ l of 10 mM Tris, pH 7.5 can be prepared by dissolving 303 mg RNase-free Tris base (catalog #161-0716) using the elution solution provided in the kit. Adjust the pH to 7.5, and make up to 250 μ l final volume.

- Bacterial total RNA isolations require the use of TE (10 mM Tris, 1 mM EDTA, pH 7.5), which is not supplied with the kit.
- Yeast total RNA isolations require the use 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 differing 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.

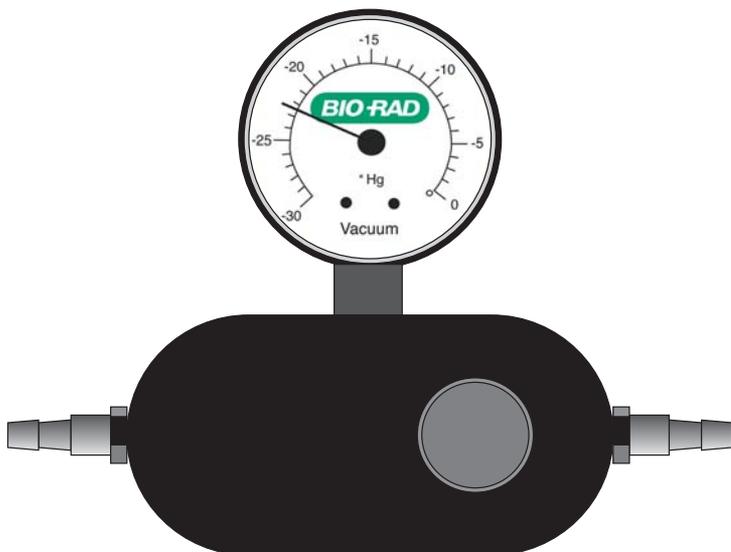


Fig. 2. Vacuum regulator with clear representation of the gauge.

Vacuum Guidelines (for Vacuum Format)

- The recommended operating range is -20 to -23 " Hg. Do not exceed -25 " Hg when performing this protocol. A vacuum regulator (Figure 2) is strongly recommended to establish the appropriate negative pressure.

Table 2. Pressure unit conversions.

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

Elution Guidelines

- Heat the elution solution to 70°C in a water bath prior to the elution step.
- Apply elution solution directly to the membranes at the base of each RNA binding column.

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 columns. Gloves should always be worn when handling RNA, and should be changed frequently. Care must be taken to proceed through the RNA isolation as quickly as possible.
- 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. Autoclave the solution to remove the DEPC.

Note: DEPC is destroyed by 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 down.

- 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 DEPC method described above for nonautoclavable plasticware, or by baking for 4 hr at 300°C.
- Working surfaces and micropipettors should be kept clean and wiped periodically.

Disruption and Homogenization

Disruption methods facilitate lysis of the starting material at the beginning of the RNA purification. Isolating RNA from nonadherent and adherent mammalian cultures and from unicellular organisms typically involves a straightforward disruption method such as repeated pipetting up and down. However, for animal and plant tissue, more vigorous disruption methods may be required in order to expose cells in the interior of the tissue sample to the lysis buffer. Grinding tissue with a mortar and pestle under liquid nitrogen greatly increases the cell surface area exposed to the lysis buffer while simultaneously inhibiting ribonucleases.

Following lysis, the lysate often becomes very viscous due to the release of genomic DNA into the solution. It is very important to reduce the viscosity of the lysate using a homogenization method, as a viscous, heterogeneous solution may cause the RNA binding column to clog. See Table 3 for a list of disruption and homogenization methods recommended for a particular starting material.

Table 3. Disruption and homogenization methods.

Starting Material	Disruption Method	Homogenization Method
Cultured mammalian cells	Lysis solution	Pipetting up and down 18-gauge needle and syringe
Bacteria	Lysozyme + lysis solution	Pipetting up and down 18-gauge needle and syringe
Yeast	Lyticase + lysis solution	Pipetting up and down 18-gauge needle and syringe
Animal tissue	Mortar and pestle + lysis solution	Rotor-stator homogenizer* Pipetting up and down 18-gauge needle and syringe
Plant tissue	Mortar and pestle + lysis solution	Rotor-stator homogenizer* Pipetting up and down 18-gauge needle and syringe

*Rotor-stator homogenizers are recommended for animal or plant tissue

- Mortar and pestle: Freeze the tissue with liquid nitrogen, then grind it into a fine powder under liquid nitrogen
- Pipetting up and down: Pass the lysate through a standard micropipettor tip at least 12 times
- 18-gauge needle and syringe: Pass the lysate through the needle at least 12 times
- Rotor-stator homogenizer: Immerse the tip of the homogenizer into the solution and homogenize for 30–60 sec

If column clogging occurs, switching to a more vigorous homogenization method may lower the incidence of column clogging.

Section 6

The Column Adaptor Plate (CAP)

The Aurum column adaptor plate (CAP) interfaces with the Aurum vacuum manifold to convert the manifold from a plate- to a column-processing system. The CAP has 18 luer fittings in a 6 x 3 array and comes supplied with 4 additional luer caps. Up to 18 Aurum miniprep columns can be accommodated on the CAP without the need for connectors or other manifold accessories. The CAP will also accommodate other columns with luer ends.

When vacuum is applied to the manifold, the CAP should self-seat, forming an airtight seal without the need to press down it. However, the application of gentle downward force may occasionally be required to facilitate seating.

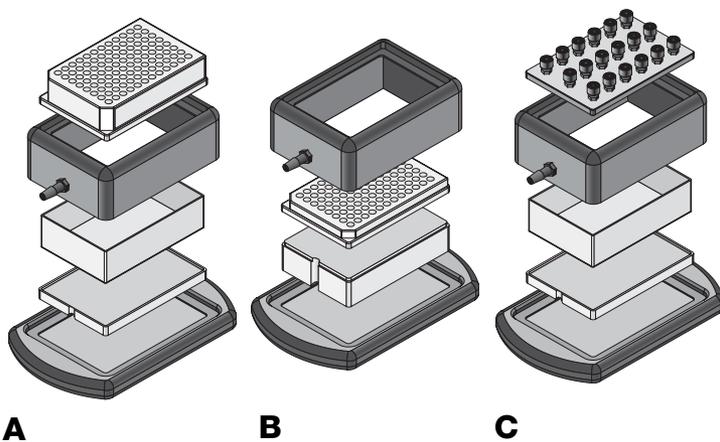


Fig. 3. Instrument setup for plate and column processing.
A, 96-well purification; B, 96-well elution; C, CAP purification.

Section 7

Instrument Setup and Use of the Column Adaptor Plate

Preparing the Aurum Manifold

Tubing provided in the Aurum vacuum manifold kit (catalog #732-6470) is 4' long and must be cut into appropriate pieces before processing.

Vacuum Setup (Figure 4A)

1. Cut tubing into 3 pieces of appropriate length.
2. Use one piece of tubing to connect the vacuum source to the **right** side of the vacuum regulator.
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 compatible) into the hole of the stopper.
5. Finally, use the last piece of tubing to connect the filter flask to the nozzle of the manifold.

Note: The Aurum vacuum regulator is strongly recommended to enable full control of the negative pressure with the manifold.

Vacuum Purification (Figures 4B and 4C)

6. Insert the CAP (luer ends up) into the depression in the vacuum manifold top. Ensure that the CAP rests evenly on the gasket.
7. Close the luer fittings that will not be used with the caps provided. Close caps by rotating clockwise until light resistance is encountered. Excessive tightening of a cap may cause the luer fitting to dislodge when the cap is removed.
8. Insert the luer ends of Aurum columns into the available luer fittings, ensuring a tight fit. The manifold is now ready for column processing according to the vacuum protocol.
9. After finishing all of the vacuum steps, rinse the CAP and the vacuum manifold with distilled water and air-dry or wipe with paper towels.

Elution

10. When ready to elute, proceed with the appropriate elution step as recommended by the protocol for your sample type.

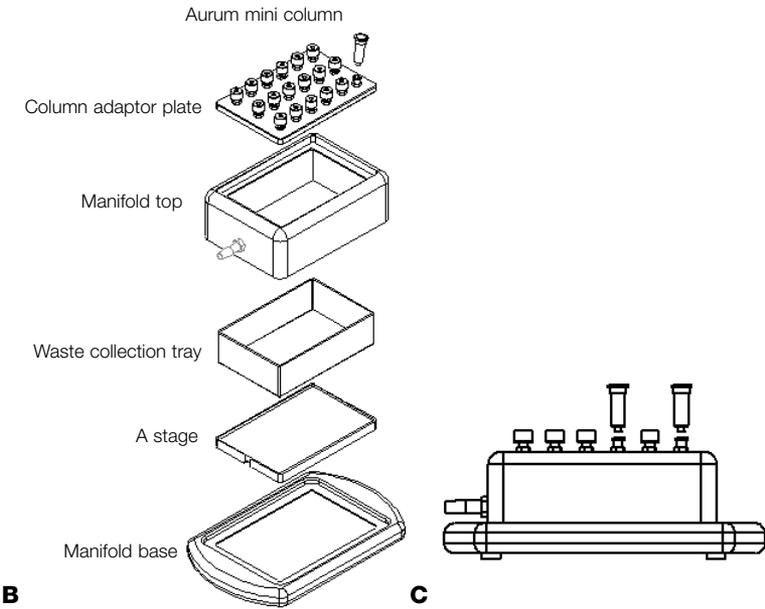
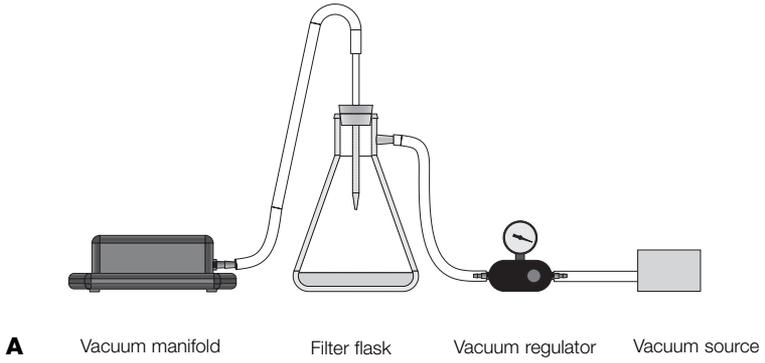


Fig. 4. Vacuum and manifold setup. A, vacuum setup; B, manifold and CAP setup; C, connection of mini columns to CAP.

Section 8

Vacuum Protocol for Cultured Cells, Bacteria, and Yeast

Important: Please read Section 5, “Guidelines for Using the Aurum Total RNA Mini Kit”, before proceeding.

This procedure requires the Bio-Rad Aurum vacuum manifold and column adaptor plate (catalog # 732-6470), or any vacuum manifold with luer fittings. For proper vacuum setup conditions, please read the previous section, Instrument Setup and Use of the Column Adaptor Plate. Vacuum filtration steps should be carried out at -20 to -23 ” Hg for optimum performance. Centrifugation steps can be performed on any commercially available microcentrifuge that can accommodate 1.5 ml and 2.0 ml tubes. All centrifugation steps are performed at maximum speed ($\approx 12,000 \times g$) and room temperature.

A 70°C water bath should be available to heat the elution solution prior to step 10.

Note: Except for the first few steps, the procedures for cultured cell lines, bacteria, and yeast share a common protocol. The vacuum protocols for animal and plant tissue follow in section 9.

Cultured Cell Lines

Follow steps A1–A3, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

A1. **For nonadherent cell cultures**, rinse the cells with PBS, and transfer up to 2×10^6 cells into a 2 ml capped microcentrifuge tube (provided). Centrifuge the tube at maximum speed for 2 min, decant the supernatant from the tube, and blot the tube with paper towels.

For adherent cell cultures, rinse the growth vessel once with PBS and aspirate. Proceed with lysis if the expected number of cells in the vessel does not exceed 2×10^6 cells; otherwise, release the cells from the plate and transfer up to 2×10^6 cells into a 2 ml capped microcentrifuge tube (provided). Centrifuge the tube for 2 min. Decant the supernatant and blot the tube with paper towels.

A2. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube or growth vessel, and pipet up and down at least 12 times to lyse cells thoroughly.

A3. Add 350 μ l of 70% ethanol (not supplied) to each tube, 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–B4, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

- B1. Transfer up to the equivalent of 3 OD•ml bacterial culture into a 2 ml capped microcentrifuge tube (provided). Centrifuge at maximum speed for 1 min. Decant the supernatant, and blot the tube with paper towels.
- B2. Add 100 μ l of 500 μ g/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5) to each tube, and pipet up and down to resuspend the pellet thoroughly. Incubate at room temperature for 5 min.
- B3. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube, and pipet up and down at least 12 times to mix thoroughly.
- B4. Add 250 μ l of 70% isopropanol (not supplied) to each tube, 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–C6, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

- C1. Prepare 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. Transfer up to the equivalent of 3 OD•ml yeast culture into a 2 ml capped microcentrifuge tube (provided). Centrifuge at maximum speed for 1 min. Decant the supernatant, and blot the tube with paper towels.
- C3. Add 1 ml of 50 units/ml lyticase in lyticase dilution buffer equilibrated to 30°C, to each tube. Pipet up and down to resuspend the yeast pellet completely. Incubate for 10 min.
- C4. Centrifuge the tube at maximum speed for 5 min. Decant the supernatant, and gently blot the tube on paper towels.
- C5. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube, and pipet up and down at least 12 times to mix thoroughly.
- C6. Add 350 μ l of 70% ethanol (not supplied) to each tube, and pipet up and down to mix thoroughly. Make sure that no bilayer is visible, and that the viscosity is substantially reduced.

Cultured Cells, Bacteria, and Yeast (cont.)

1. At this time place the elution solution into a 70°C water bath to warm it for step 10.

Attach an Aurum total RNA binding column to a luer fitting of the column adaptor plate on the Aurum vacuum manifold or to a compatible vacuum manifold. Refer to Figures 4B and 4B for setup. The vacuum source should be turned off, and the vacuum regulator should be completely open.

2. Decant or pipet the homogenized lysate into the RNA binding column. Turn the vacuum on and adjust to -20 to -23" Hg by closing the vacuum regulator. Continue to apply vacuum until all the lysate has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
3. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes of 95–100% ethanol to the low stringency wash solution concentrate before initial use.** This corresponds to 80 ml for the mini kit or 16 ml for the sample pack, respectively.
4. Add 700 µl of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23" Hg. Continue to apply the vacuum until the low stringency wash solution has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
5. 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 provided) to the vial and pipetting up and down briefly to mix.

Note: 250 µl of 10 mM Tris, pH 7.5, can be prepared by dissolving 303 mg RNase-free Tris base (catalog #161-0716) using the elution solution provided in the kit. Adjust the pH to 7.5, and make up to 250 µl final volume.

6. For each column processed, mix 5 µl of reconstituted DNase I with 75 µl of DNase dilution solution in a 1.5 ml microcentrifuge tube (not provided). Scale up proportionally if processing more than one column. Add 80 µl of diluted DNase I to the membrane stack at the bottom of each column. Allow the digest to incubate at room temperature for 15 min. When the digest is complete, close the vacuum regulator dial until the gauge indicates -20 to -23" Hg. Continue to apply the vacuum until all of the DNase has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.

7. Add 700 μ l of high stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg. Continue to apply the vacuum until the high stringency wash solution has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
8. Add 700 μ l of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg.
9. Transfer the RNA binding column to a 2 ml capless tube (provided). Centrifuge for an additional 2 min to remove residual wash solution.

Note: The elution solution should be at 70°C before proceeding with the elution step.

10. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipet 80 μ l of the warmed elution solution onto the membrane stack at the bottom of the RNA binding column, and allow 1 min for the solution to saturate the membranes. Centrifuge for 2 min to elute the total RNA.

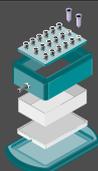
The eluted total RNA samples can be used immediately in RT-PCR reactions or in any other application. Alternatively, the total RNA can be stored at 4°C for later use.

Aurum™ Total RNA Mini Kit

Vacuum Format Protocol Overview*

Cultured cells		Bacterial cells	Yeast cells
<p>Adherent Rinse vessel with PBS, aspirate. Lyse in vessel if # of cells <math>2 \times 10^6</math>.</p>	<p>Nonadherent Rinse with PBS. Transfer up to 2×10^6 cells, centrifuge 2 min. Decant supernatant.</p>	<p>Transfer up to 2.4×10^9 cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant. Add 100 μl of 500 μg/ml lysozyme. Pipet up and down. Incubate at room temp. for 5 min.</p>	<p>Transfer up to 3×10^7 cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant. Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer. Pipet up and down. Incubate at room temp. for 10 min. Centrifuge at 5,000 rpm for 5 min. Discard supernatant.</p>
<p>Add 350 μl lysis solution. Pipet up and down 12x.</p>	<p>Add 350 μl lysis solution. Pipet up and down 12x.</p>	<p>Add 350 μl lysis solution. Pipet up and down 12x.</p>	<p>Add 350 μl lysis solution. Pipet up and down 12x.</p>
<p>Add 350 μl 70% EtOH. Pipet up and down.</p>	<p>Add 250 μl 70% isopropyl alcohol. Pipet up and down.</p>	<p>Add 250 μl 70% isopropyl alcohol. Pipet up and down.</p>	<p>Add 350 μl 70% EtOH. Pipet up and down.</p>

Continue with the following steps for all sample types:



Assemble manifold properly for isolation.

Transfer lysate to RNA binding column.
Apply vacuum.



Add 700 μ l low stringency wash.
Apply vacuum.



Dilute 5 μ l reconstituted* DNase I with 75 μ l DNase I dilution solution.

Add 80 μ l diluted DNase I.
Incubate 15 min at room temp. Apply vacuum.



Add 700 μ l high stringency wash.
Apply vacuum.



Add 700 μ l low stringency wash.
Apply vacuum. Spin-purge 2 min into a capless 2 ml tube.



Place RNA binding column into a 1.5 ml capped tube.

Add 80 μ l 70PC elution solution onto membrane stack.

Incubate 1 min. Centrifuge 2 min to elute.
* Refer to manual for detailed protocol.



Aurum Total RNA Mini Kit: Cat. #732-6820

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Section 9

Vacuum Protocol for Animal and Plant Tissue

1. Cut the tissue into small pieces (<5 mm long), and grind it into a fine powder with a mortar and pestle containing liquid nitrogen. Make sure that the tissue does not thaw, by periodically adding liquid nitrogen to the mortar.
2. Transfer up to 20 mg hard animal tissue, up to 40 mg soft animal tissue, or up to 60 mg plant tissue into an RNase-free 2.0 ml capped microcentrifuge tube (provided). Let the tissue thaw before adding the lysis solution, or precipitation may occur.

Note: Plant tissues require the Aurum lysis solution to be supplemented with 2% polyvinylpyrrolidone-40 (PVP). For each column processed, add 14 μ l PVP to every 700 μ l of lysis solution before proceeding to step 3.

3. Add 700 μ l of lysis solution to the tube, and disrupt the sample by pipetting up and down, by passing the sample twelve times through an 18-gauge needle and syringe, or by using a rotor-stator homogenizer for 30–60 sec.
4. Centrifuge the lysate for 3 min, and transfer the supernatant into a new 2.0 ml capped microcentrifuge tube (provided).
5. Add 700 μ l of 70% ethanol for plant tissue or 60% ethanol for animal tissue to the supernatant, and mix thoroughly by pipetting up and down, or by using a rotor-stator homogenizer. Make sure that no bilayer is visible.
6. At this time place the elution solution into a 70°C water bath to warm it for step 15.

Attach an Aurum total RNA binding column to a luer fitting of the column adaptor plate on the Aurum vacuum manifold or to a compatible vacuum manifold. The vacuum source should be turned off, and the vacuum regulator should be completely open. Refer to Figures 4B and 4C for setup.

7. Decant or pipet the homogenized lysate into the RNA binding column. Turn the vacuum on and adjust to -20 to -23 " Hg by closing the vacuum regulator. Continue to apply vacuum until all the lysate has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.

8. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes of 95–100% ethanol to the low stringency wash solution concentrate before initial use.** This corresponds to 80 ml for the mini kit or 16 ml for the sample pack, respectively.
9. Add 700 μ l of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg. Continue to apply vacuum until the low stringency wash solution has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
10. The RNase-free DNase I is provided as a lyophilized powder. Reconstitute the DNase I by adding 250 μ l 10 mM Tris, pH 7.5 (not provided) to the vial and pipetting up and down briefly to mix.

Note: 250 μ l of 10 mM Tris, pH 7.5, can be prepared by dissolving 303 mg RNase-free Tris base (catalog #161-0716) using the elution solution provided in the kit. Adjust the pH to 7.5, and make up to 250 μ l final volume.

11. For each column processed, mix 5 μ l of reconstituted DNase I with 75 μ l of DNase dilution solution in a 1.5 ml microcentrifuge tube (not provided). Scale up proportionally if processing more than one column. Add 80 μ l of diluted DNase I to the membrane stack at the bottom of each column. Allow the digest to incubate at room temperature (25 min for animal tissue, 15 min for plant tissue). When the digest is complete, close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg. Continue to apply vacuum until all of the DNase has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
12. Add 700 μ l of high stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg. Continue to apply vacuum until the high stringency wash solution has passed through the column. Open the vacuum regulator until the gauge indicates 0" Hg.
13. Add 700 μ l of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates -20 to -23 " Hg.
14. Transfer the RNA binding column to a 2 ml capless tube (provided). Centrifuge for 2 min to remove residual wash solution.

Note: The elution solution should be at 70°C before proceeding with the elution step.

15. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipette 80 μ l of the warmed elution solution onto the

membrane stack at the bottom of the RNA binding column, and allow 1 min for the solution to saturate the membranes. Centrifuge for 2 min to elute the total RNA.

The eluted total RNA samples can be used immediately in RT-PCR reactions or any other application. Alternatively, the total RNA can be stored at 4°C for later use.

Aurum™ Total RNA Mini Kit

Vacuum Format Protocol Overview*

Animal tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 20 mg (hard tissue) or
40 mg (soft tissue) to a capped 2 ml tube.



Plant tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 60 mg to
a capped 2 ml tube.



Continue with the following steps for all sample types:

Add 700 µl lysis solution.

Disrupt vigorously with rotor-stator for 30–60 sec.



Centrifuge lysate at maximum speed for 3 min.

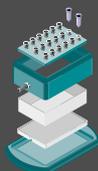
Transfer supernatant to a new 2 ml capped tube.



Add 700 µl EtOH (use 60% for animal tissue, 70% for plant tissue) to supernatant.

Homogenize with rotor-stator 30 sec.

700 µl
60% EtOH
or
70% EtOH



Assemble manifold properly for isolation.

Transfer lysate.

Apply vacuum.



Add 700 µl low stringency wash.

Apply vacuum.



Dilute 5 µl reconstituted* DNase I with 75 µl DNase I dilution solution.

Add 80 µl diluted DNase I.

Incubate at room temp. 25 min for animal tissue, 15 min for plant tissue. Apply vacuum.



Add 700 µl high stringency wash.

Apply vacuum.



Add 700 µl low stringency wash.

Apply vacuum. Spin-purge 2 min into 2 ml capless tube.



Place RNA binding column into a 1.5 ml capped tube.

Add 80 µl 70PC elution solution onto



* Refer to manual for detailed protocol.

Section 10

Spin Protocol for Cultured Cells, Bacteria, and Yeast

Important: Please read Section 5, “Guidelines for Using the Aurum Total RNA Mini Kit” before proceeding.

The Aurum total RNA mini kit and sample pack can be used with any commercially available microcentrifuge that can accommodate 1.5 ml and 2.0 ml tubes. All centrifugation steps are performed at maximum speed ($\approx 12,000 \times g$) and room temperature.

A 70°C water bath should be available to heat the elution solution prior to step 10.

Note: Except for the first few steps, the procedures for cultured cell lines, bacteria, and yeast share a common protocol. The spin protocols for animal and plant tissue follow in section 11.

Cultured Cell Lines

Follow steps A1–A3, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

A1. **For nonadherent cell cultures**, rinse the cells with PBS, and transfer up to 2×10^6 cells into a 2 ml capped microcentrifuge tube (provided). Centrifuge the tube at maximum speed for 2 min, decant the supernatant from the tube, and blot the tube with paper towels.

For adherent cell cultures, rinse the growth vessel once with PBS and aspirate. Proceed with lysis if the expected number of cells in the vessel does not exceed 2×10^6 cells; otherwise, release the cells from the plate and transfer up to 2×10^6 cells into a 2 ml capped microcentrifuge tube (provided). Centrifuge the tube for 2 min. Decant the supernatant and blot the tube with paper towels.

A2. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube or growth vessel, and pipet up and down at least 12 times to lyse cells thoroughly.

A3. Add 350 μ l of 70% ethanol (not supplied) to each tube, 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–B4, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

- B1. Transfer up to the equivalent of 3 OD•ml bacterial culture into a 2 ml capped microcentrifuge tube (provided). Centrifuge at maximum speed for 1 min. Decant the supernatant, and blot the tube with paper towels.
- B2. Add 100 μ l of 500 μ g/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5) to each tube, and pipet up and down to resuspend the pellet thoroughly. Incubate at room temperature for 5 min.
- B3. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube, and pipet up and down at least 12 times to mix thoroughly.
- B4. Add 250 μ l of 70% isopropanol (not supplied) to each tube, 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–C6, then continue with step 1 of “Cultured Cells, Bacteria, and Yeast (cont.)”

- C1. Prepare 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. Transfer up to the equivalent of 3 OD•ml yeast culture into a 2 ml capped microcentrifuge tube (provided). Centrifuge at maximum speed for 1 min. Decant the supernatant, and blot the tube with paper towels.
- C3. Add 1 ml of 50 units/ml lyticase in lyticase dilution buffer equilibrated to 30°C, to each tube. Pipet up and down to resuspend the yeast pellet completely. Incubate for 10 min.
- C4. Centrifuge the tube at maximum speed for 5 min. Decant the supernatant and gently blot the tube on paper towels.
- C5. Add 350 μ l of lysis solution (already supplemented with 1% β -mercaptoethanol) to each tube, and pipet up and down at least 12 times to mix thoroughly.
- C6. Add 350 μ l of 70% ethanol (not supplied) to each tube, and pipet up and down to mix thoroughly. Make sure that no bilayer is visible, and that the viscosity is substantially reduced.

Cultured Cells, Bacteria, and Yeast (cont.)

1. At this time place the elution solution into a 70°C water bath to warm it for step 10. Insert an RNA binding column into a 2 ml capless wash tube (provided).

2. Decant or pipet the homogenized lysate into the RNA binding column. Centrifuge for 30 sec. Remove the RNA binding column from the wash tube, discard the filtrate from the wash tube, and replace the column into the same wash tube.
3. The low stringency wash solution is provided as a 5x concentrate. Add **4 volumes of 95–100% ethanol to the low stringency wash solution concentrate before initial use.** This corresponds to 80 ml for the mini kit or 16 ml for the sample pack, respectively.
4. Add 700 μ l of low stringency wash solution to the RNA binding column. Centrifuge for 30 sec. Discard the low stringency wash solution from the wash tube, and replace the column into the same wash tube.
5. The RNase-free DNase I is provided as a lyophilized powder. Reconstitute the DNase I by adding 250 μ l 10 mM Tris, pH 7.5 (not provided) to the vial and pipetting up and down briefly to mix.

Note: 250 μ l of 10 mM Tris, pH 7.5, can be prepared by dissolving 303 mg RNase-free Tris base (catalog #161-0716) using the elution solution provided in the kit. Adjust the pH to 7.5, and make up to 250 μ l final volume.

6. For each column processed, mix 5 μ l of reconstituted DNase I with 75 μ l of DNase dilution solution in a 1.5 ml microcentrifuge tube (not provided). Scale up proportionally if processing more than one column. Add 80 μ l of diluted DNase I to the membrane stack at the bottom of each column. Allow the digest to incubate at room temperature for 15 min. When the digest is complete, centrifuge the columns for 30 sec. Discard the digest buffer from the wash tube, and replace the column into the same wash tube.
7. Add 700 μ l of high stringency wash solution to the RNA binding column. Centrifuge for 30 sec. Discard the high stringency wash solution from the wash tube, and replace the column into the same wash tube.
8. Add 700 μ l of low stringency wash solution to the RNA binding column. Centrifuge for 1 min. Discard the low stringency wash solution from the wash tube, and replace the column into the same wash tube.
9. Centrifuge for an additional 2 min to remove residual wash solution.

Note: The elution solution should be 70°C before proceeding with the elution step.

10. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipette 80 μ l of the warmed elution solution onto the membrane stack at the bottom of the RNA binding column, and allow

1 min for the solution to saturate the membranes. Centrifuge for 2 min to elute the total RNA.

The eluted total RNA samples can be used immediately in RT-PCR reactions or in any other application. Alternatively, the total RNA can be stored at 4°C for later use.

Aurum™ Total RNA Mini Kit

Spin Format Protocol Overview*

Cultured cells		Bacterial cells	Yeast cells
<p>Adherent Rinse vessel with PBS, aspirate. Lyse in vessel if # of cells <2 x 10⁶.</p>	<p>Nonadherent Rinse with PBS. Transfer up to 2 x 10⁶ cells, centrifuge 2 min. Decant supernatant.</p>	<p>Transfer up to 2.4 x 10⁹ cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant. Add 100 µl of 500 µg/ml lysozyme. Pipet up and down. Incubate at room temp. for 5 min.</p>	<p>Transfer up to 3 x 10⁷ cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant. Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer. Pipet up and down. Incubate at room temp. for 10 min. Centrifuge at 5,000 rpm for 5 min. Discard supernatant.</p>
<p>Add 350 µl lysis solution. Pipet up and down 12x.</p> 		<p>Add 350 µl lysis solution. Pipet up and down 12x.</p> 	<p>Add 350 µl lysis solution. Pipet up and down 12x.</p> 
<p>Add 350 µl 70% EtOH. Pipet up and down.</p> 		<p>Add 250 µl 70% isopropyl alcohol. Pipet up and down.</p> 	<p>Add 350 µl 70% EtOH. Pipet up and down.</p> 

Continue with the following steps for all sample types:

Insert RNA binding column into a 2 ml capless tube.

Transfer lysate.

Centrifuge 30 sec. Discard filtrate.

Add 700 µl low stringency wash.

Centrifuge 30 sec. Discard filtrate.

Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.

Add 80 µl diluted DNase I.

Incubate 15 min at room temp. Centrifuge 30 sec. Discard filtrate.

Add 700 µl high stringency wash.

Centrifuge 30 sec. Discard filtrate.

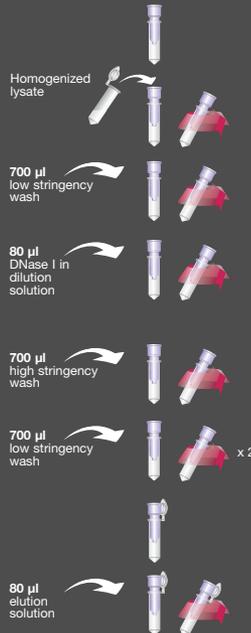
Add 700 µl low stringency wash.

Centrifuge 1 min. Discard filtrate.
Centrifuge additional 2 min.

Place RNA binding column into a 1.5 ml capped tube.

Add 80 µl 70°C elution solution onto membrane stack.

Incubate 1 min. Centrifuge 2 min to elute.
* Refer to manual for detailed protocol.



Aurum Total RNA Mini Kit: Cat. #732-6820

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Section 11

Spin Protocol for Animal and Plant Tissue

1. Cut the tissue into small pieces (<5 mm long), and grind it into a fine powder with a mortar and pestle containing liquid nitrogen. Make sure that the tissue does not thaw, by periodically adding liquid nitrogen to the mortar.
2. Transfer up to 20 mg hard animal tissue, up to 40 mg soft animal tissue, or up to 60 mg plant tissue into an RNase-free 2.0 ml capped microcentrifuge tube (provided). Let the tissue thaw before adding the lysis solution, or precipitation may occur.

Note: Plant tissues require the Aurum lysis solution to be supplemented with 2% w/v polyvinylpyrrolidone-40 (PVP).

3. Add 700 μ l of lysis solution to the tube, and disrupt the sample by pipetting up and down, by passing the sample ten times through an 18-gauge needle and syringe, or by using a rotor-stator homogenizer for 30–60 sec.
4. Centrifuge the lysate for 3 min, and transfer the supernatant into a new 2.0 ml capped microcentrifuge tube (provided).
5. Add 700 μ l of 70% ethanol for plant tissue and 60% ethanol for animal tissue to the supernatant, and mix thoroughly by pipetting up and down, or by using a rotor-stator homogenizer. Make sure that no bilayer is visible.
6. At this time place the elution solution into a 70°C water bath to warm it for step 15. Insert an RNA binding column into a 2 ml capless wash tube (provided).
7. Decant or pipet up to 700 μ l of the homogenized lysate into the RNA binding column. Centrifuge for 60 sec. Remove the RNA binding column from the wash tube, discard the filtrate from the wash tube, and replace the column into the same wash tube. Repeat for the remainder of the homogenized lysate.
8. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes of 95–100% ethanol to the low stringency wash solution concentrate before initial use.** This corresponds to 80 ml for the mini kit or 16 ml for the sample pack, respectively.
9. Add 700 μ l of low stringency wash solution to the RNA binding column. Centrifuge for 30 sec. Discard the low stringency wash solution from the wash tube, and replace the column into the same wash tube.

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

Note: 250 μ l of 10 mM Tris, pH 7.5, can be prepared by dissolving 303 mg RNase-free Tris base (catalog #161-0716) using the elution solution provided in the kit. Adjust the pH to 7.5, and make up to 250 μ l final volume.

11. For each column processed, mix 5 μ l of reconstituted DNase I with 75 μ l of DNase dilution solution in a 1.5 ml microcentrifuge tube (not provided). Scale up proportionally if processing more than one column. Add 80 μ l of diluted DNase I to the membrane stack at the bottom of each column. Allow the digest to incubate at room temperature for (25 min animal tissue, 15 min plant tissue). When the digest is complete, centrifuge the columns for 30 sec. Discard the digest buffer from the wash tube, and replace the column into the same wash tube.
12. Add 700 μ l of high stringency wash solution to the RNA binding column. Centrifuge for 30 sec. Discard the high stringency wash solution from the wash tube, and replace the column into the same wash tube.
13. Add 700 μ l of low stringency wash solution to the RNA binding column. Centrifuge for 30 sec. Discard the low stringency wash solution from the wash tube, and replace the column into the same wash tube.
14. Centrifuge for an additional 1 min to remove residual wash solution.

Note: The elution solution should be at 70°C before proceeding with the elution step.

15. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipette 80 μ l of the warmed elution solution onto the membrane stack at the bottom of the RNA binding column, and allow 1 min for the solution to saturate the membranes. Centrifuge for 2 min to elute the total RNA.

The eluted total RNA samples can be used immediately in RT-PCR reactions or any other application. Alternatively, the total RNA can be stored at 4°C for later use.

Aurum™ Total RNA Mini Kit

Spin Format Protocol Overview*

Animal tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 20 mg (hard tissue) or
40 mg (soft tissue) to a capped 2 ml tube.



Plant tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 60 mg to
a capped 2 ml tube.



Continue with the following steps for all sample types:

Add 700 μ l lysis solution.

Disrupt vigorously with rotor-stator for 30–60 sec.

700 μ l
lysis solution

Rotor-stator

Centrifuge lysate at maximum speed 3 min.

Transfer supernatant to a new 2 ml capped tube.

New tube

Add 700 μ l EtOH (60% EtOH for animal tissue, 70% EtOH for plant tissue) to supernatant.

Homogenize with rotor-stator 30 sec.

700 μ l
60% EtOH
or
70% EtOH

Insert RNA binding column into a 2 ml capless tube.

Transfer lysate, centrifuge 60 sec.

Discard filtrate. Repeat if necessary.

Add 700 μ l low stringency wash.

Centrifuge 30 sec. Discard filtrate.

700 μ l
low stringency
wash

Dilute 5 μ l reconstituted* DNase I with 75 μ l DNase dilution solution.

Add 80 μ l diluted DNase I.
Incubate at room temp. 25 min for animal tissue,
15 min for plant tissue. Centrifuge column 30 sec.
Discard filtrate.

80 μ l
DNase I in
dilution
solution

Add 700 μ l high stringency wash.

Centrifuge 30 sec. Discard filtrate.

700 μ l
high stringency
wash

Add 700 μ l low stringency wash.

Centrifuge 30 sec. Discard filtrate.
Centrifuge additional 1 min.

700 μ l
low stringency
wash

x 2

Place RNA binding column into a 1.5 ml capped tube.

Add 80 μ l 70PC elution solution onto membrane stack.

Incubate 1 min. Centrifuge 2 min to elute.

80 μ l
elution
solution

Section 12

Troubleshooting Guide

Problem	Possible Cause	Recommended Solution
Genomic DNA contamination	Incomplete DNase I digest	Increase DNase I digest time
	Inactive DNase I	Store reconstituted DNase I in a non-frost-free freezer. Avoid freeze-thaw cycles. Aliquot reconstituted DNase I for single use only
	Incorrect preparation of DNase dilution	Use only the DNase dilution solution provided in the kit to dilute the DNase
RNA degradation	Excessive amount of starting material	Reduce amount of starting material used
	RNase contamination of user-made solutions and/or plasticware	DEPC-treat all hand-made solutions. Decontaminate all work surfaces. See Section 5 for more details
Clogging of RNA binding column	Endogenous RNases	Work quickly through the steps prior to the addition of lysis solution
	Excessive amount of starting material	Reduce amount of starting material used
	Poor disruption and/or homogenization	Increase intensity/duration of disruption and/or homogenization Switch to more intense disruption and/or homogenization method
	Incomplete digest with lysozyme or lyticase	Increase duration of lysozyme or lyticase digest. Use fresh enzyme

Problem	Possible Cause	Recommended Solution
Low eluate volume (<60 µl)	Insufficient centrifugation time during elution	Add 1–3 min to the centrifugation time during elution
	Column clogging	See problem “Clogging of RNA binding column”
High eluate volume (>80 µl)	Low stringency wash carryover in eluate	Add 1–3 min to the centrifugation time after the final wash step
Low RNA yield	Low amount of starting material	Increase starting material amount up to the maximum indicated for the specific starting material type
	Excessive amount of starting material	Reduce amount of starting material used
	Poor disruption and/or homogenization	Increase intensity/duration of disruption and/or homogenization
		Switch to more intense disruption and/or homogenization method
	Incorrect use of wash solutions	Add the appropriate volume of 95–100% ethanol to the wash solutions before initial use
	Incorrect preparation of DNase dilution	Use only the DNase dilution solution provided in the kit to dilute the DNase
	Low sample eluate volume	See problem “Low eluate volumes (<60 µl)”

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 wash solutions before initial use
	RNA is degraded	See problem “RNA degradation”
	Ethanol contamination in prep (eluate volumes >80 µl)	Add 1–3 min to the centrifugation time after the final wash step

Section 13

Ordering Information

Catalog #	Description
732-6820	Aurum Total RNA Mini Kit, 50 preps, includes 50 RNA binding columns, 50 capless collection tubes, (2.0 ml), 2 x 50 capped sample tubes, (2.0 ml), 50 capped sample tubes (1.5 ml), reagents, protocol overview, instruction manual
732-6820S	Aurum Total RNA Mini Sample Pack, 10 preps, includes 10 RNA binding columns, 10 capless collection tubes, (2.0 ml), 2 x 10 capped sample tubes, (2.0 ml), 10 capped sample tubes (1.5 ml), reagents, instruction manual
732-6800	Aurum Total RNA 96 Kit, 2 x 96 well preps, includes 2 grow blocks, 2 growth membranes, 2 RNA binding plates, 2 microtiter collection plates, reagents, protocol overview, instruction manual
732-6470	Aurum Vacuum Manifold, includes column adaptor plate, 4 replacement luer caps, A stage and B stage, waste collection tray, vacuum regulator and gauge, tubing, protocol overview, instruction manual

Bio-Rad Laboratories, Inc.

2000 Alfred Nobel Dr.

Hercules, CA 94547 USA

(510) 741-1000

1-800-424-6723