Aurum™ Total RNA Mini Kit

Instruction Manual

Catalog # 732-6820

For technical support, call your local Bio-Rad office, or
in the US, call 1-800-4BIORAD (1-800-424-6723).

BIO-RAD

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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), real-time 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 or vacuum format using the Aurum vacuum manifold (catalog # 732-6470).

Section 2
Kit Components

The Aurum™ total RNA mini kit contains the following components:

Components*	**Quantity/Amount**
RNA binding columns	50
Capless wash tubes, 2 ml	50
Capped microcentrifuge tubes, 1.5 ml	50
Capped microcentrifuge tubes, 2.0 ml	100
DNase I (lyophilized)	1 vial
Lysis solution	50 ml
Low stringency wash solution (5x concentrate)	20 ml
High stringency wash solution	40 ml
Elution solution	20 ml
DNase dilution solution	20 ml

*There may be reagent remaining in some bottles.

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 reagents to be provided by the customer:

- Microcentrifuge (>12,000 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)
- Polyvinylpyrrolidone-40 (PVP), 2% (for plant isolation only)
- 95–100% ethanol
- Tris for DNase I reconstitution (catalog # 161-0716)

Additional equipment required for vacuum format:

- Aurum™ vacuum manifold with vacuum regulator and column adaptor plate (catalog # 732-6470), or other vacuum manifold with luer fittings
- Vacuum source (capability of –23 inHg required)

Section 5
Before 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 10^6 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 (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, 3 OD•ml of yeast would require 500 µl of an undiluted culture with an OD_{600} = 6.

** 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 column) of total RNA from various samples using the Aurum total RNA mini kit.

Starting Material	Avg. Yield (µg)*
Cultured cells (2 x 10^6)	
3T3	20–30
HeLa	20–40
Bacteria (2.4 x 10^9)	
E. coli	30–35
B. cereus	30–35
Yeast (3 x 10^7)	
S. cerevisiae	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)	
Arabidopsis	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 (80 ml) of 95–100% ethanol to the low stringency wash solution concentrate before initial use**
- Before using the lysis solution, add 500 µl 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 µl 10 mM Tris, pH 7.5 (not supplied) to the vial and pipetting 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 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 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 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 should 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 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 DEPC 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

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 straight forward 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 2 for a list of disruption and homogenization methods recommended for a particular starting material.

Table 2. 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 and 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 several times
- 18-gauge needle and syringe: pass the lysate through the needle several 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
Vacuum Manifold Setup and Use With the Column Adaptor Plate (CAP)

Guidelines for Vacuum Format

- The recommended operating range is –17 to –23 inHg. Do not exceed –25 inHg when performing this protocol. A vacuum regulator is strongly recommended to establish the appropriate negative pressure

Table 3. 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

About The 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 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.

Preparing the Aurum Vacuum Manifold

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

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

Vacuum Setup (Figure 1)

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.

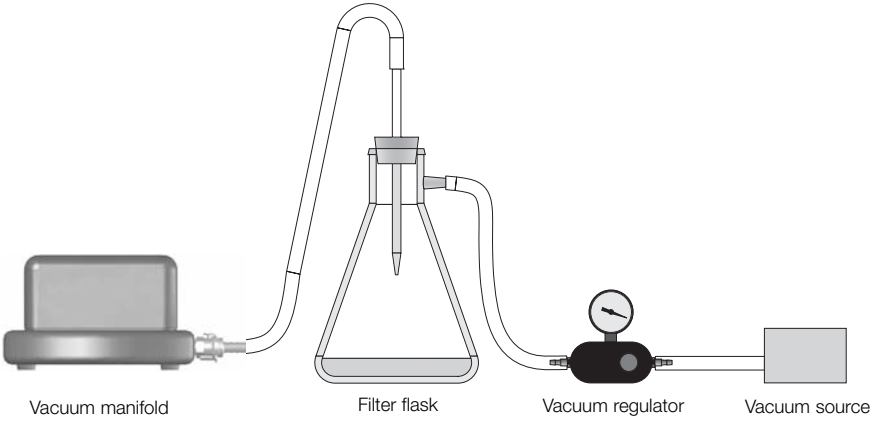

Fig. 1. Vacuum setup conditions.

Manifold Wash Setup (Figure 2)

1. Insert the CAP (luer ends up) into the depression in the vacuum manifold top. Ensure that the CAP rests evenly on the gasket.
2. Insert the luer ends of the desired columns into the available luer fittings, ensuring a tight fit.
3. Close the unused luer fittings 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.
4. The manifold is now ready for column processing according to the vacuum protocol of the appropriate column purification kit.
5. When ready to elute, proceed with the appropriate spin elution step as recommended by the protocol.
6. After finishing the elution, rinse the CAP and Aurum vacuum manifold with water and air dry or wipe with paper towels.

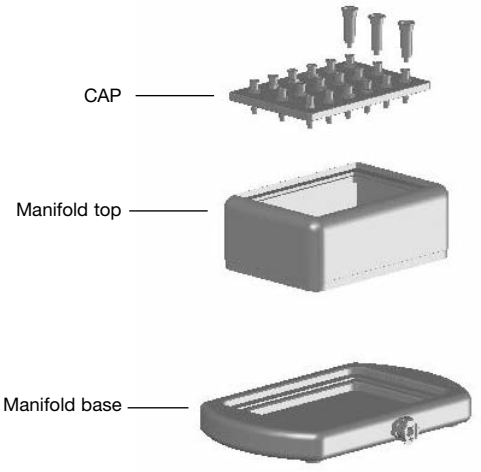

Fig. 2. Manifold setup for column processing.

Section 7
Vacuum Protocol

Important: Please read Section 5, “Before Using the Aurum™ Total RNA Mini Kit” and Section 6 "Vacuum Manifold Setup and Use With the Column Adaptor Plate (CAP)," before proceeding.

This procedure requires the Aurum vacuum manifold and column adaptor plate (catalog # 732-6470), or any vacuum manifold with luer fittings. Vacuum filtration steps should be carried out at –17 to –23 inHg for optimum performance. Centrifugation steps can be performed on any commercially available microcentrifuge that can accommodate 1.5 and 2.0 ml tubes. All centrifugation steps are performed at maximum speed (>12,000 x g) and room temperature.

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

Cultured Cell Lines

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

A1. **For nonadherent cell cultures**, rinse the cells with PBS, and transfer up to 2 x 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 x 10^6 cells; otherwise, release the cells from the plate and transfer up to 2 x 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 µl of lysis solution (already supplemented with 1% β-mercapto-ethanol) to each tube or growth vessel. Pipet up and down several times to lyse cells thoroughly.

A3. Add 350 µl of 70% ethanol (not supplied) to each tube. 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 "All Starting Sample Types" on page 13.

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 µl of 500 µg/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5) to each tube. Pipet up and down to resuspend the pellet thoroughly. Incubate at room temperature for 5 min.

B3. Add 350 µl of lysis solution (already supplemented with 1% β-mercapto-ethanol) to each tube. Pipet up and down several times to mix thoroughly.

B4. Add 250 µl of 70% isopropanol (not supplied) to each tube. 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 "All Starting Sample Types" on page 13.

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 5,000 rpm for 5 min. Decant the supernatant and gently blot the tube on paper towels.

C5. Add 350 µl of lysis solution (already supplemented with 1% β-mercapto-ethanol) to each tube. Pipet up and down several times to mix thoroughly.

C6. Add 350 µl of 70% ethanol (not supplied) to each tube. Pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Animal and Plant Tissue

Follow steps D1–D5, then continue with step 1 of "All Starting Sample Types" on page 13.

D1. 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.

D2. 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).

Note: Plant tissue requires the Aurum lysis solution to be supplemented with 2% (w/v) polyvinylpyrrolidone-40 (PVP). For each column processed, add 14 µl PVP to every 700 µl of lysis solution before proceeding to step 3.

D3. Add 700 µl of lysis solution to the tube. Resuspend the sample by pipetting up and down. Use a rotor-stator homogenizer for 30–60 sec or other equivalent method to disrupt the sample.

D4. Centrifuge the lysate for 3 min and transfer the supernatant into a new 2.0 ml capped microcentrifuge tube (provided).

D5. Add 700 µl of 70% ethanol for plant tissue or 60% ethanol for animal tissue to the supernatant. Mix thoroughly by pipetting up and down or by using a rotor-stator homogenizer. Make sure that no bilayer is visible.

All Starting Sample Types:

1. 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 Figure 2 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 –17 to –23 inHg 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 inHg.

3. The low stringency wash solution is provided as a 5x concentrate. **Add 4 volumes (80 ml) of 95–100% ethanol to the low stringency wash solution concentrate before initial use.**

4. Add 700 µl of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates –17 to –23 inHg. 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 inHg.

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.

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 (25 min for animal tissue).

7. Add 700 µl of high stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates –17 to –23 inHg. 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 inHg.

8. Add 700 µl of low stringency wash solution to the RNA binding column and close the vacuum regulator dial until the gauge indicates –17 to –23 inHg.

9. Transfer the RNA binding column to a 2 ml capless tube (provided). Centrifuge for an additional 2 min to remove residual wash solution.

10. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipet 80 µl (or 40 µl)† of the 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.

    †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 can be used immediately in downstream applications. Alternatively, the total RNA can be aliquoted stored at –20°C or at –80°C for later use.

Section 8
Spin Protocol

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

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

Note: Except for the first few steps that are specific for the starting sample types (A. for cultured cell lines, B. for bacteria, C. for yeast D. for animal and plant tissue), 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 Sample Types" on page 17.

A1. **For nonadherent cell cultures**, rinse the cells with PBS and transfer up to 2 x 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 x 10^6 cells; otherwise, release the cells from the plate and transfer up to 2 x 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 µl of lysis solution (already supplemented with 1% β-mercapto-ethanol) to each tube or growth vessel. Pipet up and down several times to lyse cells thoroughly.

A3. Add 350 µl of 70% ethanol (not supplied) to each tube. 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 “All Starting Sample Types” on page 17.

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 µl of 500 µg/ml lysozyme in TE (10 mM Tris, 1 mM EDTA, pH 7.5) to each tube. Pipet up and down to resuspend the pellet thoroughly. Incubate at room temperature for 5 min.

B3. Add 350 µl of lysis solution (already supplemented with 1% β-mercapto-ethanol) to each tube. Pipet up and down several times to mix thoroughly.

B4. Add 250 µl of 70% isopropanol (not supplied) to each tube. 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 "All Starting Sample Types" on page 17.

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 5,000 rpm for 5 min. Decant the supernatant and gently blot the tube on paper towels.

C5. Add 350 µl of lysis solution (already supplemented with 1% β-mercaptoethanol) to each tube. Pipet up and down several times to mix thoroughly.

C6. Add 350 µl of 70% ethanol (not supplied) to each tube. Pipet up and down to mix thoroughly. Make sure that no bilayer is visible and that the viscosity is substantially reduced.

Animal and Plant Tissue

Follow steps D1–D5, then continue with step 1 of "All Starting Sample Types" on page 17.

D1. 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.

D2. 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 tissue requires the Aurum lysis solution to be supplemented with 2% (w/v) polyvinylpyrrolidone-40 (PVP). For each column processed, add 14 µl PVP to every 700 µl of lysis solution before proceeding to step 3.

D3. Add 700 µl of lysis solution to the tube. Disrupt the sample by pipetting up and down. Use a rotor-stator homogenizer for 30–60 sec or other equivalent method to disrupt the sample.

D4. Centrifuge the lysate for 3 min and transfer the supernatant into a new 2.0 ml capped microcentrifuge tube (provided).

D5. Add 700 µl of 70% ethanol for plant tissue or 60% ethanol for animal tissue to the supernatant. Mix thoroughly by pipetting up and down or by using a rotor-stator homogenizer. Make sure that no bilayer is visible.

All Starting Sample Types

1. 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 (for cultured, bacterial, or yeast cells) or 60 sec (for animal or plant tissue). 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 (80 ml) of 95–100% ethanol to the low stringency wash solution concentrate before initial use.**
4. Add 700 µl 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 µl 10 mM Tris, pH 7.5 (not provided) to the vial and pipetting up and down briefly to mix.

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 (25 min for animal tissue).

7. Add 700 µl 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 in the same wash tube.

8. Add 700 µl of low stringency wash solution to the RNA binding column. Centrifuge for 1 min (for cultured, bacterial, or yeast cells) or 30 sec (for animal or plant tissue). Discard the low stringency wash solution from the wash tube and replace the column in the same wash tube.

9. Centrifuge for an additional 2 min to remove residual wash solution.

10. Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided). Pipette 80 µl (or 40 µl)† of the 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.

    †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 can be used immediately in downstream applications. Alternatively, the total RNA can be stored at –20°C or at –80°C for later use.

Section 9
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 nonfrost-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
	Excessive amount of starting material	Reduce amount of starting material used
RNA degradation	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
	Endogenous RNases	Work quickly through the steps prior to the addition of lysis solution
Clogging of RNA binding column	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)”
	Inefficient elution	Preheat the elution solution to 70°C in water bath prior to the elution step

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 10
Ordering Information

Catalog #	Description
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™ Isolation Reagent)
732-6800	Aurum Total RNA 96 Kit
732-6470	Aurum Vacuum Manifold

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Dr.
Hercules, CA 94547 USA
(510) 741-1000

1-800-424-6723

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD*
Australia *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11*
Brazil *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808*
Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0*
Greece *30 210 777 4396* ***Hong Kong*** *852 2789 3300*
Hungary *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
Mexico *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99*
Portugal *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723*
Spain *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

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