Aurum™ Total RNA Fatty and Fibrous Tissue Kit

Instruction Manual

Catalog # 732-6830

The Aurum Total RNA Fatty and Fibrous Tissue Kit is composed of:

Module 1 – 732-6870 (Aurum Total RNA Fatty and Fibrous Tissue Module)

Module 2 – 732-6880 (PureZOL™ RNA Isolation Reagent, 50 ml), packaged and shipped separately

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

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Section 1
Introduction

The Aurum™ total RNA fatty and fibrous tissue kit produces high yields of pure total RNA from samples that are difficult to disrupt. The kit is ideal for fatty or fibrous tissues, or samples that are rich in RNases. It also works well with most animal and plant tissues, cultured cells, yeast, and gram-negative and gram-positive bacteria. With this kit, greater than 100 µg of total RNA can be isolated from many sample types. Total RNA samples isolated using the Aurum total RNA fatty and fibrous tissue kit are suitable for use in a variety of downstream applications, including reverse transcription-PCR (RT-PCR), real-time PCR, *in vitro* translation, northern blots, and microarray analysis.

The Aurum total RNA fatty and fibrous kit uses a quick, easy-to-follow procedure for the purification of total RNA from difficult-to-disrupt samples. First, samples are disrupted and lysed using the PureZOL™ RNA isolation reagent. Subsequently, chloroform is added to the lysate, mixed, and centrifuged to achieve separation of the organic and aqueous phases. The aqueous phase, which contains the RNA, is carefully recovered and mixed with ethanol. The sample is then passed through a silica membrane packed in the Aurum RNA binding mini column, where nucleic acids get bound. Wash steps are performed to remove proteins and other cellular debris. An optional on-column DNase I digest is performed to remove any remaining genomic DNA. The RNA, which is eluted with the elution solution supplied in the kit, is now ready for downstream applications without further manipulation.

The membrane in the Aurum RNA binding mini column selectively binds to mRNA and larger rRNAs, while small RNA molecules less than 200 nucleotides, such as 5.8S rRNA, 5S rRNA, and tRNA (which together comprise 15–20% of total RNA), are removed. The on-column DNase I digest and subsequent wash steps that are performed during the purification effectively remove genomic DNA contamination as well as the residual DNase enzyme, eliminating the need for separate and lengthy DNase treatments and DNase removal protocols on the eluted RNA.

The Aurum total RNA fatty and fibrous tissue kit is designed to isolate total RNA from various amounts of tissue (5–100 mg) and cells (50–2.4 x 10^9 cells)*. The kit includes sufficient reagents and columns for 50 purifications. All solutions and RNA binding mini columns in the kit are RNase-free, ensuring the integrity of the isolated RNA. The Aurum total RNA fatty and fibrous tissue kit may be used in a spin format, or in a vacuum format using the Aurum vacuum manifold (catalog # 732-6470).

* The amount of starting material may be less for certain types of samples. For more detail, refer to Section 5.

Section 2
Kit Components

The Aurum™ total RNA fatty and fibrous tissue kit contains the following components:

Components*	Quantity/Amount
RNA binding mini columns	50
Capless wash tubes, 2.0 ml	50
Capped microcentrifuge tubes, 1.5 ml	50
Capped microcentrifuge tubes, 2.0 ml	100
DNase I (lyophilized)	1 vial
Low stringency wash solution (5x concentrate)	20 ml
High stringency wash solution	40 ml
Elution solution	20 ml
DNase dilution solution	20 ml
PureZOL™ RNA isolation reagent** (packaged and shipped separately)	50 ml

*There may be reagent remaining in the some bottles.

**PureZOL RNA isolation reagent contains poison (phenol) and an irritant (guanidine thiocyanate). The reagent causes burns and can be fatal if ingested. When working with PureZOL, use gloves and eye protection (lab glasses, shield, and safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read warning notice on bottle and MSDS.

Section 3
Storage Conditions

Store components of the kit at the recommended temperatures (see Table 1 below).

Table 1. Recommended storage temperature for the Aurum™ total RNA fatty and fibrous tissue kit components.

Kit Components	Storage Temperature
PureZOL™ RNA isolation reagent	Store at 4–25°C
Low stringency wash buffer	Room temperature (before and after the addition of ethanol)
High stringency wash buffer	Room temperature
Elution solution	Room temperature
DNase dilution solution	4°C; allow solution to equilibrate to room temperature before use
DNase I (lyophilized)	Room temperature in lyophilized form; once reconstituted in 250 µl of 10 mM Tris, pH 7.5, aliquot and store at –20°C in a nonfrost-free freezer. Do not freeze-thaw more than once after reconstitution
Aurum RNA binding mini columns and microcentrifuge tubes	Room temperature

Section 4
Materials and Equipment Required (Not Provided in the Kit)

- Microcentrifuge, capable of spinning at >12,000 x g at 4°C and room temperature
- 95–100% ethanol, ACS grade or better
- Tris for DNase I reconstitution (catalog #161-0716)
- Chloroform (without additives such as isoamyl alcohol) for phase separation; 0.2 ml of chloroform per 1 ml of PureZOL required

Supplies for Tissue Grinding, Disruption, and Homogenization

- Fresh tissue: tissue cutter
- Frozen tissue: liquid nitrogen, mortar and pestle
- Tissue homogenizer: rotor-stator homogenizers, or bead mill homogenizers recommended

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 Fatty and Fibrous Tissue Kit

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

*Maximum Starting Material Amounts**

The Aurum total RNA fatty and fibrous tissue kit is designed to process up to the amounts indicated below (per column):

- 1 x 10^7 cultured mammalian cells grown in suspension
- One 10^2 cm plate mammalian cultured cells grown in monolayer
- 2.4 x 10^9 of gram-positive or gram-negative bacteria (equivalent to 3 OD•ml of bacteria)
- 3.0 x 10^7 of yeast (equivalent to 3 OD•ml of yeast)
- 100 mg of animal tissue (a 4 mm cube of most animal tissue weighs approximately 70–85 mg)
- 100 mg of plant tissue
- 50 mg filamentous fungi

Warning: Processing larger amounts of starting material may lead to column clogging and reduced RNA purity. It is crucial that the appropriate amount of starting material be used. For samples that are known to be rich in RNA, it is highly recommended that less than the maximum amount of starting material be used so that the binding capacity of the column is not exceeded. In addition, complete disruption and homogenization of the starting material is critical to prevent column clogging and reduced RNA yields.

Minimum Starting Material Amounts

- 50 cultured mammalian cells
- 5 mg of animal tissue
- 5 mg plant tissue

* 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 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.

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

Table 2. Yield (per column) of total RNA from various samples using the Aurum total RNA fatty and fibrous tissue kit.

Starting Material (Amount Used)*	Average Yield (µg)**
Cultured cells (1 x 10^7)	
293H	145
Bacteria (2.4 x 10^9 or 3 OD•ml)	
E. coli	30
Yeast (3 x 10^7 or 3 OD•ml)	
S. cerevisiae	64
Fatty animal tissue (100 mg)	
Brain	96
Breast	58
Adipose	14
Fibrous animal tissue (100 mg)	
Heart	85
Cartilage	54
Skin	63
Plant tissue (100 mg)	
Potato	91
Arabidopsis	5
Filamentous fungi (50 mg)	
Aspergillus niger	12

* Starting material amounts in parentheses are the maximum amounts recommended for this kit. Note: The elution volume should be decreased to 30 µl if only a small amount of starting material (<500,000 cells or <10 mg of animal or plant tissue) is used.

** 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 Fatty and Fibrous Tissue Kit

PureZOL™ RNA Isolation Reagent for Sample Lysis

Use 1 ml of PureZOL for up to:

- 100 mg of tissue
- 1 x 10^7 cultured cells grown in suspension
- One 10 cm^2 plate of cultured cells grown in monolayer
- 2.4 x 10^9 of gram-positive or gram-negative bacteria (equivalent to 3 OD•ml of bacteria)
- 3 x 10^7 of yeast (equivalent to 3 OD•ml of yeast)

Low Stringency Wash Solution

- 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

DNase I

- 10 mM Tris, pH 7.5 prepared in DEPC-treated water (not supplied) is required to reconstitute the RNase-free DNase I that is provided as a lyophilized powder
- Reconstitute the lyophilized DNase I by adding 250 µl of 10 mM Tris, pH 7.5 to the vial and mix by briefly pipetting up and down. Do not vortex
- Aliquot and store the reconstituted DNase I at –20°C in a nonfrost-free freezer. Avoid freeze-thaw cycles

  Note: 5 µl of the DNase I stock is needed per column or prep. When the DNase is ready to be used, it must be mixed with 75 µl of the DNase dilution solution (provided in the kit) per column. Once diluted with DNase dilution solution, use the DNase immediately and do not refreeze for later use

Elution Guidelines

- Apply elution solution directly to the membrane stack at the base of each RNA binding mini column

Preparation of DEPC-Treated Water

- Autoclaving of laboratory solutions and buffers used for RNA preparation does not guarantee the complete inactivation of RNases, which can maintain residual activity causing RNA samples to be degraded. For this reason, solutions and buffers should be treated with diethyl pyrocarbonate

(DEPC) to inactivate RNases. DEPC is an efficient, strong, and nonspecific RNase inhibitor that is usually used at a concentration of 0.1%

- To prepare a 0.1% (v/v) solution of DEPC-treated water, add 1.0 ml of liquid DEPC per 1 L of water. Incubate the solution at 37°C for 1 hr while mixing thoroughly. Autoclave the treated water to remove the DEPC
- **Warning:** DEPC is suspected to be a carcinogen and should be handled with care. Always use gloves and open under a fume hood

Preparation of 10 mM Tris, pH 7.5, for DNase I Reconstitution

- To prepare 50 ml of 10 mM Tris, pH 7.5, add 60.6 mg of Tris (catalog # 161-0716) to 45 ml of DEPC-treated water. Mix until Tris is completely dissolved. Adjust the pH of the solution by dropwise addition of 6N HCl. Once the pH is adjusted to 7.5, add more DEPC-treated water to make a final volume of 50 ml

  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

Preparation of 70% Ethanol

- To prepare 100 ml of 70% ethanol, add 70 ml of 95–100% ethanol to 30 ml of DEPC-treated water. Mix well before use.

Maintaining an RNase-Free Environment

- 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. Proceed through the RNA isolation as quickly as possible with care
- Solutions that are prepared by the user should be treated with DEPC to inactivate RNases as described above.

- 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 micropipets should be kept clean and wiped periodically

Sample Disruption and Homogenization

Successful isolation of total RNA is dependent on the efficient disruption and homogenization of cells and tissues. Cell and tissue disruption is the physical breakdown of cell walls and plasma membranes, usually done using mechanical or enzymatic techniques. Efficient disruption facilitates the lysis of the starting material and release of all the RNA contained in the sample, ensuring a high yield of RNA. Incomplete disruption results in column clogging and significantly reduced RNA yields. After disruption, proper mixing of the lysate is necessary to produce a homogenous solution for efficient passage of the lysate through the Aurum RNA binding mini column and for RNA to properly bind to the silica membrane.

Isolation of RNA from nonadherent and adherent mammalian cultures typically involves a straightforward disruption method such as repeated pipetting up and down or passing through an 18-gauge needle and syringe. For animal and plant tissues, more vigorous disruption methods may be required to increase the cell surface area exposed to the PureZOL RNA isolation reagent while simultaneously inhibiting RNases. Tissue disruption can be performed by first grinding with a mortar and pestle under liquid nitrogen and then using either a rotor-stator homogenizer or a bead mill homogenizer (see manufacturer instructions for more detail). If a homogenizer is not available, passing the tissue sample through an 18-gauge needle and syringe may also work. However, RNA yields will not be as high as when using a homogenizer.

Bacteria and yeast cells have thick cells walls that are difficult to break. Repeated pipetting up and down or passing the sample through an 18-gauge needle and syringe may not be sufficient for lysing bacteria and yeast cells. More vigorous physical disruption methods, such as using a rotor-stator homogenizer or a bead mill homogenizer (see manufacturer instructions for more detail), may be required in order to lyse the bacterial and yeast cells.

Table 3. Disruption and homogenization methods.

Starting Material	Disruption Method	Homogenization Method
Animal tissue	Grind tissue with a mortar and pestle under liquid nitrogen, use a rotor-stator homogenizer, bead mill homogenizer, or 18-gauge needle and syringe	Pipetting up and down, rotor-stator homogenizer, bead mill homogenizer, or 18-gauge needle and syringe
Plant tissue	Grind tissue with a mortar and pestle under liquid nitrogen, use a rotor-stator homogenizer, bead mill homogenizer, or 18-gauge needle and syringe	Pipetting up and down, rotor-stator homogenizer, bead mill homogenizer, or 18-gauge needle and syringe
Cultured cells	Pipet up and down, or use 18-gauge needle and syringe	Pipetting up and down, or 18-gauge needle and syringe
Bacteria	Rotor-stator homogenizer, bead mill homogenizer, pipet up and down, or 18-gauge needle and syringe	Pipetting up and down, rotor-stator homogenizer, or bead mill homogenizer
Yeast	Rotor-stator homogenizer, bead mill homogenizer, or 18-gauge needle and syringe	Pipetting up and down, rotor-stator homogenizer, or bead mill homogenizer

- Mortar and pestle: freeze the tissue with liquid nitrogen, then grind it into a fine powder under liquid nitrogen
- Pipet up and down: pass the lysate through a standard micropipet 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
- For bead mill homogenizers, follow manufacturer's instructions

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

Guidelines for Vacuum Format

- The recommended operating range is –17 to –20 inHg. Do not exceed –25 inHg when performing this protocol. A vacuum regulator is strongly recommended to establish the appropriate negative pressure.

Table 4. 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 Column Adaptor Plate (CAP)

The Aurum™ CAP interfaces with the Aurum vacuum manifold to convert the manifold from a plate-processing 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 RNA binding mini 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 it down. 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 to 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.

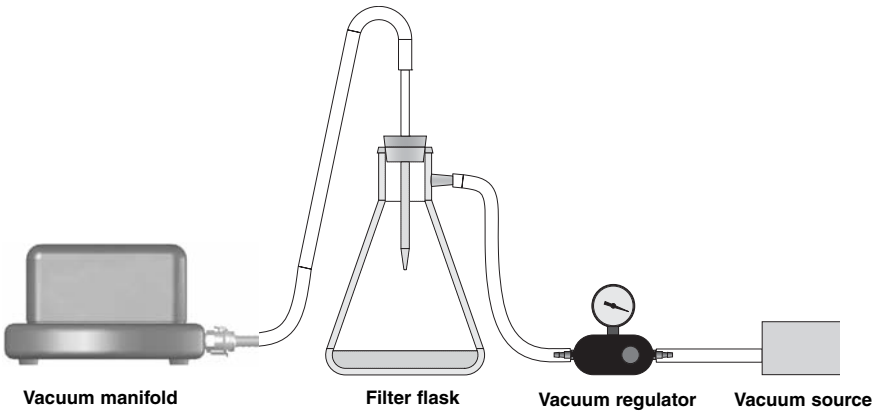

Fig. 1. Vacuum setup conditions.

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.

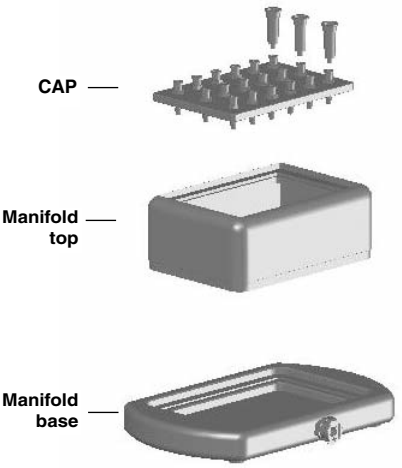

Fig. 2. Manifold setup for column processing.

Section 7
Vacuum Protocol

All steps are carried out at room temperature unless otherwise indicated. Vacuum filtration steps should be carried out at –17 to –23 inHg for optimum performance.

Important: If using the kit for the first time, please read Section 5, "Before Using the Aurum™ Total RNA Fatty and Fibrous Tissue Kit," and Section 6, "Vaccum Manifold Setup and Use With the Column Adaptor Plate" before proceeding.

This procedure requires the Aurum vacuum manifold and column adaptor plate (catalog # 732-6470), or any vacuum manifold with luer fittings. If the necessary vacuum manifold is not available, follow the spin protocol in Section 8.

Centrifugation steps can be performed on any commercially available microcentrifuge that can accommodate 1.5 ml and 2.0 ml microcentrifuge tubes and can spin at $\geq$ 12,000 x g. It is recommended that sterile, disposable polypropylene tubes be used throughout the procedure.

Procedure

1. **Measure the amount of starting material**. Note that the Aurum total RNA fatty and fibrous tissue kit is designed to process up to the amounts indicated below (per column):

   - 1 x 10^7 cultured mammalian cells grown in suspension
   - One 10^2 cm plate mammalian cultured cells grown in monolayer
   - 2.4 x 10^9 of gram-positive or gram-negative bacteria
   - 3.0 x 10^7 of yeast
   - 100 mg of animal tissue (a 4 mm cube of most animal tissue weighs 70–85 mg)
   - 100 mg of plant tissue
   - 50 mg filamentous fungi

Warning: Processing larger amounts of starting material may lead to column clogging and reduced RNA purity. It is crucial that the appropriate amount of starting material be used. For samples that are known to be rich in RNA, it is highly recommended that less than the maximum amount of starting material be used so that the binding capacity of the column is not exceeded. In addition, complete disruption and homogenization of the starting material is critical to prevent column clogging and reduced RNA yields.

2. **Disrupt and homogenize the sample**. Below are recommended procedures for disruption and homogenization.

   Note: Incomplete disruption will clog the column in subsequent steps and result in reduced yields of total RNA.

   Fresh Tissue

   Fresh tissues can be processed in PureZOL™ immediately after dissection. Alternatively, freshly dissected tissue can be immediately frozen in liquid nitrogen and processed using instructions for frozen tissue. Transfer up to 100 mg of freshly dissected tissue into a 2.0 ml microcentrifuge tube and add 1 ml of PureZOL. Disrupt the sample for 30–60 sec using a rotor-stator homogenizer or a bead mill homogenizer (refer to manufacturer instructions for more details). Although not as effective, passing the tissue sample through an 18-gauge needle and syringe can be used for sample disruption if a homogenizer is not available. Pass the sample through the needle and syringe until no more solid tissue is left in the lysate. The sample volume should not exceed 10% of the volume of PureZOL used for disruption. Proceed to step 3.

   Frozen Tissue

   Grind up the frozen tissues to fine powder with a mortar and pestle under liquid nitrogen. Avoid thawing the sample by periodically adding liquid nitrogen to the mortar. Weigh up to 100 mg of tissue and transfer the sample into a 2.0 ml microcentrifuge tube. Add 1 ml of PureZOL and disrupt for 30–60 sec using a rotor-stator homogenizer or a bead mill homogenizer (refer to manufacturer's instructions for more details). Although not as effective, passing the tissue sample through an 18-gauge needle and syringe can be used for sample disruption if a homogenizer is not available. Pass the sample through the needle and syringe until no more solid tissue is left in the lysate. The sample volume should not exceed 10% of the volume of PureZOL used for disruption. Proceed to step 3.

Cells Grown in a Monolayer

Cells grown in a monolayer should be lysed with PureZOL directly in the culture dish. Aspirate the culture medium and immediately add 1 ml of PureZOL to a 10 cm^2 dish. Pass the lysate through a pipet several times. The amount of PureZOL added is dependent on the area of culture dish (1 ml per 10 cm^2) and not on cell number. Insufficient volumes of PureZOL may result in DNA contamination. Proceed to step 3.

Note: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

Suspension Cells (Mammalian, Plant, Bacterial, or Yeast)

Pellet the cells by centrifuging at 3,000–5,000 x g for 2 min. Immediately lyse by adding 1 ml of PureZOL to 1 x 10^7 cultured mammalian and plant cells, 2.4 x 10^9 of gram-positive or gram-negative bacteria, or 3.0 x 10^7 of yeast (equivalent to 3 OD•ml of yeast). Pass the lysate through a pipet or an 18-gauge needle and syringe several times. To improve the efficiency of the cell lysis process, a rotor-stator homogenizer or a bead mill homogenizer is recommended to disrupt the cell walls of yeast and bacteria. Bacteria and yeast lysate can also be heated to 55°C for 10 min prior to adding chloroform to increase lysis effectivity of PureZOL. Proceed to step 3.

Note: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

3. **Once the sample has been disrupted in PureZOL, incubate the lysate at room temperature for 5 min to allow the complete dissociation of nucleoprotein complexes**.

   Note: Following the disruption step, the sample can be stored at –70°C for at least one month. To process frozen lysates, samples should be thawed at room temperature. If necessary, heat samples to 37°C in a water bath for 5–10 min to completely dissolve salts. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA.

   It is recommended that lysate from tissues that are rich in fat, polysaccharides, proteins, and extracellular material be centrifuged at 12,000 x g for 10 min at 4°C following the 5 min incubation at room temperature. This step removes any solid insoluble debris that was left after the disruption step. Transfer the supernatant into a new 2.0 ml microcentrifuge tube without aspirating the pellet, then proceed to step 4. For lipid-rich samples, avoid transferring the excess fat that collects as a top layer. Carryover of the solid debris can cause column clogging and affect RNA sample purity.

4. **Add 0.2 ml of chloroform to the lysate, then cover and shake vigorously for 15 sec. Do not vortex!**

5. **Incubate for 5 min at room temperature while periodically mixing the sample**.

6. **Centrifuge at 12,000 x g for 15 min at 4°C**.

   Following centrifugation, the mixture will separate into three phases: an upper, colorless aqueous phase, a white interphase, and a lower, red organic phase. RNA will be exclusively in the aqueous phase, while DNA and proteins remain in the interphase and organic phase. The volume of the aqueous phase should be approximately 600 µl, or 60% of the volume of PureZOL used in the initial disruption.

If removal of contaminating DNA is a requirement, prepare the DNase I enzyme by following steps a-b below while centrifuging the samples for phase separation:

   a. DNase I is provided as a lyophilized powder. If the DNase has already been reconstituted, skip to step b. Otherwise, reconstitute the DNase I by adding 250 µl of 10 mM Tris, pH 7.5 (not provided) to the vial and mix by pipetting up and down briefly. Do not vortex! See Section 4, Materials and Equipment Required (Not Provided in the Kit), on how to prepare 10 mM Tris, pH 7.5.

   b. For each column to be processed, mix 5 µl of reconstituted DNase I with 75 µl of DNase dilution solution in a 1.5 ml microcentrifuge tube. Scale up proportionally if processing more than one column. Once diluted with DNase dilution solution, do not refreeze for later use.

7. **Without disturbing the interphase, immediately transfer the aqueous phase to a 2.0 ml microcentrifuge tube**.

   Note: It is crucial that **none** of the interphase or organic phase is transferred with the aqueous phase. It is recommended that some of the aqueous phase be left behind to avoid the risk of contaminating the RNA with contaminants such as phenol, which can interfere with downstream applications.

8. **Add an equal volume (approximately 600 µl) of 70% ethanol (not provided) to the tube and mix thoroughly by pipetting up and down**.

9. **Attach an Aurum total RNA binding mini 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.

10. **Pipet 700 µl of the RNA sample into the RNA binding mini column**. Turn the vacuum on and adjust to –17 to –23 inHg by closing the vacuum regulator. Continue to apply vacuum until all of the RNA sample passes through the column. Open the vacuum regulator until the gauge indicates 0 inHg.

11. **Repeat step 11 for the remainder of the sample**.

    The Aurum total RNA fatty and fibrous tissue kit supplies RNase-free DNase I to be used to treat samples for complete removal of contaminating genomic DNA. If removal of genomic DNA is not a requirement, proceed directly to step 14. Otherwise, perform on-column DNase I digest by proceeding to step 12.

12. **Add 700 µl of low stringency wash solution (*already supplemented with ethanol*) 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 passes through the column. Open the vacuum regulator until the gauge indicates 0 inHg.

13. **Remove any contaminating genomic DNA from the RNA sample**.

    a. Add 80 µl of the diluted DNase I to each column processed, making sure to add the DNase to the center of the membrane stack at the bottom of each column.

    b. Allow the DNase digest to incubate at room temperature for 15 min.

14. **Add 700 µl of high stringency wash solution to the RNA binding mini 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 passes through the column. Open the vacuum regulator until the gauge indicates 0 inHg.

15. **Add 700 µl of low stringency wash solution (*already supplemented with ethanol*) 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.

16. **Transfer the RNA binding mini column to a 2.0 ml capless tube (provided). Centrifuge for 2 min at >12,000 x g to remove the residual wash solution**.

17. **Transfer the RNA binding column to a 1.5 ml microcentrifuge tube (provided)**.

18. **Pipet 40 µl (or 30 µl)† of the elution solution onto the center of the membrane at the bottom of the RNA binding column.**

    † **Note**: When isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells), perform a **single** elution with **30 µl** of the elution solution. **Do not perform step 21**.

19. **Incubate 1 min for complete soaking and saturation of the membrane**.

20. **Centrifuge for 2 min at >12,000 x g to elute the total RNA**.

21. **Repeat steps 18 and 19 using 40 µl of the elution solution if the starting amounts of starting material is more than 10 mg of tissue or 500,000 cells**.

Note: The eluted total RNA samples can be used immediately in downstream applications. Alternatively, the RNA sample can be aliquoted and stored at –20°C or –70°C for later use.

Section 8
Spin Protocol

Important: If using the kit for the first time, please read Section 6, "Before Using the Aurum™ Total RNA Fatty and Fibrous Tissue Kit," before proceeding.

Centrifugation steps can be performed on any commercially available microcentrifuge that can accommodate 1.5 and 2.0 ml microcentrifuge tubes and can spin at ≥12,000 x g. It is recommended that sterile, disposable polypropylene tubes be used throughout the procedure.

Protocol

1. **Measure the amount of starting material**. Note that the Aurum total RNA fatty and fibrous tissue kit is designed to process up to the amounts indicated below (per column):

   - 1 x 10^7 cultured mammalian cells grown in suspension
   - One 10^2 cm plate mammalian cultured cells grown in monolayer
   - 2.4 x 10^9 gram-positive or gram-negative bacteria
   - 3.0 x 10^7 yeast cells
   - 100 mg of animal tissue (a 4 mm cube of most animal tissue weighs 70–85 mg)
   - 100 mg of plant tissue
   - 50 mg filamentous fungi

Warning: Processing larger amounts of starting material may lead to column clogging and reduced RNA purity. It is crucial that the appropriate amount of starting material be used. For samples that are known to be rich in RNA, it is highly recommended that less than the maximum amount of starting material be used so that the binding capacity of the column is not exceeded. In addition, complete disruption and homogenization of the starting material is critical to prevent column clogging and reduced RNA yields.

2. **Disrupt and homogenize the sample**. Below are recommended procedures for disruption and homogenization.

   Note: Incomplete disruption will clog the column in subsequent steps and result in reduced yields of total RNA.

   #### *Fresh Tissue*

   Fresh tissue can be processed in PureZOL™ immediately after dissection. Alternatively, freshly dissected tissue can be immediately frozen rapidly in liquid nitrogen and processed using instructions for frozen tissue. Transfer

up to 100 mg of freshly dissected tissue into a 2.0 ml microcentrifuge tube and add 1 ml of PureZOL. Disrupt the sample for 30–60 sec using a rotor-stator homogenizer or a bead mill homogenizer (refer to manufacturer instructions for more details). Although not as effective, passing the tissue sample through an 18-gauge needle and syringe can be used for sample disruption if a homogenizer is not available. Pass the sample through the needle and syringe until no more solid tissue is left in the lysate. The sample volume should not exceed 10% of the volume of PureZOL used for disruption. Proceed to step 3.

Frozen Tissue

Grind up the frozen tissue to fine powder with a mortar and pestle under liquid nitrogen. Avoid thawing the sample by periodically adding liquid nitrogen to the mortar. Weigh up to 100 mg of tissue and transfer the sample into a 2.0 ml microcentrifuge tube. Add 1 ml of PureZOL and disrupt for 30–60 sec using a rotor-stator homogenizer or a bead mill homogenizer (refer to manufacturer instructions for more details). Although not as effective, passing the tissue sample through an 18-gauge needle and syringe can be used for sample disruption if a homogenizer is not available. Pass the sample through the needle and syringe until no more solid tissue is left in the lysate. The sample volume should not exceed 10% of the volume of PureZOL used for disruption. Proceed to step 3.

Cells Grown in a Monolayer

Cells grown in a monolayer should be lysed with PureZOL directly in the culture dish. Aspirate the culture medium and immediately add 1 ml of PureZOL to a 10 cm^2 dish. Pass the lysate through a pipet several times. The amount of PureZOL added is dependent on the area of culture dish (1 ml per 10 cm^2) and not on cell number. Insufficient volumes of PureZOL may result in DNA contamination. Proceed to step 3.

Note: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

Suspension Cells (Mammalian, Plant, Bacterial, or Yeast)

Pellet the cells by centrifuging at 3,000–5,000 x g for 2 min. Immediately lyse by adding 1 ml of PureZOL to 1 x 10^7 cultured mammalian and plant cells, 2.4 x 10^9 of gram-positive or gram-negative bacteria, or 3.0 x 10^7 of yeast (equivalent to 3 OD•ml of yeast). Pass the lysate through a pipet or an 18-gauge needle and syringe several times. To improve the efficiency of the cells lysis process, a rotor-stator homogenizer or a bead mill homogenizer is recommended to disrupt the cell walls of yeast and bacteria. Bacteria and yeast lysate can also be heated to 55°C for 10 min prior to adding chloroform to increase lysis effectivity of PureZOL. Proceed to step 3.

Note: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

3. **Once the sample has been disrupted in PureZOL, incubate the lysate at room temperature for 5 min to allow the complete dissociation of nucleoprotein complexes**.

   Note: Following the disruption step, the sample can be stored at –70°C for at least one month. To process frozen lysates, samples should be thawed at room temperature. If necessary, heat samples to 37°C in a water bath for 5–10 min to completely dissolve salts. Avoid extended treatment at 37°C, which can cause chemical degradation of the RNA.

   It is recommended that lysate from tissues that are rich in fat, polysaccharides, proteins, and extracellular material be centrifuged at 12,000 x g for 10 min at 4°C following the 5 min incubation at room temperature. This step removes any solid insoluble debris that was left after the disruption step. Transfer the supernatant into a new 2.0 ml microcentrifuge tube without aspirating the pellet, then proceed to step 4. For lipid-rich samples, avoid transferring the excess fat that collects as a top layer. Carryover of the solid debris can cause column clogging and affect RNA sample purity.

4. **Add 0.2 ml of chloroform to the lysate, then cover and shake vigorously for 15 sec. Do not vortex!**

5. **Incubate for 5 min at room temperature while periodically mixing the sample**.

6. **Centrifuge at 12,000 x g for 15 min at 4°C**.

   Following centrifugation, the mixture will separate into three phases: an upper, colorless aqueous phase, a white interphase, and a lower, red organic phase. RNA will be exclusively in the aqueous phase, while DNA and proteins remain in the interphase and organic phase. The volume of the aqueous phase should be approximately 600 µl, or 60% of the volume of PureZOL used in the initial disruption.

If removal of contaminating DNA is a requirement, prepare DNase I enzyme by following steps a–b below while centrifuging the samples for phase separation:

   a. DNase I is provided as a lyophilized powder. If the DNase has already been reconstituted, skip to step b. Otherwise, reconstitute the DNase I by adding 250 µl of 10 mM Tris, pH 7.5 (not provided) to the vial and mix by pipetting up and down briefly. Do not vortex! See Section 4, Materials and Equipment Required (Not Provided in the Kit), on how to prepare 10 mM Tris, pH 7.5.

b. For each column to be processed, mix 5 µl of reconstituted DNase I with 75 µl of DNase dilution solution in a 1.5 ml microcentrifuge tube. Scale up proportionally if processing more than one column. Once diluted with DNase dilution solution, do not refreeze for later use.

7. **Without disturbing the interphase, immediately transfer aqueous phase to a 2.0 ml microcentrifuge tube**.

   Note: It is crucial that **none** of the interphase or organic phase is transferred with the aqueous phase. It is recommended that some of the aqueous phase be left behind to avoid the risk of contaminating the RNA with contaminants such as phenol, which can interfere with downstream applications.

8. **Add an equal volume (approximately 600 ml) of 70% ethanol (not provided) to the tube and mix thoroughly by pipetting up and down**.

9. **Insert an RNA binding column into a 2.0 ml capless wash tube (provided)**.

   For steps 10–21, all centrifugation steps are performed at room temperature.

10. **Pipet up to 700 µl of the RNA sample into the RNA binding mini column. Centrifuge for 60 sec at >12,000 x g**. 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.

11. **Repeat step 10 for the remainder of the sample**.

    The Aurum total RNA fatty and fibrous tissue kit supplies RNase-free DNase I to be used to treat samples for complete removal of contaminating genomic DNA. If removal of genomic DNA is not a requirement, proceed directly to step 14. Otherwise, perform on-column DNase I digest by proceeding to step 12.

12. **Add 700 µl of low stringency wash solution (*already supplemented with ethanol*) to the RNA binding column. Centrifuge for 30 sec at >12,000 x g**. Discard the low stringency wash solution from the wash tube and replace the column into the same wash tube.

13. **Remove any contaminating genomic DNA from the RNA sample**.

    a. Add 80 µl of the diluted DNase I to each column processed, making sure to add the DNase to the center of the membrane stack at the bottom of each column.

    b. Allow the DNase digest to incubate at room temperature for 15 min.

14. **Add 700 µl of high stringency wash solution to the RNA binding column. Centrifuge for 30 sec at ≥12,000 x g**. Discard the high stringency wash solution from the wash tube and place the column back into the same wash tube.

15. **Add 700 µl of low stringency wash solution (*already supplemented with ethanol*) to the RNA binding column**. Centrifuge for 1 min at ≥12,000 x g. Discard the filtrate from the wash tube and place the column back into the same wash tube.

16. **Centrifuge for an additional 2 min at ≥12,000 x g to remove residual wash solution**.

17. **Transfer the RNA binding column to a 1.5 ml capped microcentrifuge tube (provided)**.

18. **Pipet 40 µl (or 30 µl)† of the elution solution onto the center of the membrane at the bottom of the RNA binding column.**

    † **Note**: When isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells), perform a **single** elution with **30 µl** of the elution solution. **Do not perform step 21**.

19. **Incubate 1 min for complete soaking and saturation of the membrane**.

20. **Centrifuge for 2 min at >12,000 x g to elute the total RNA**.

21. **Repeat steps 18 and 19 using 40 µl of the elution solution if the starting amounts of starting material is more than 10 mg of tissue or 500,000 cells**.

Note: The eluted total RNA samples can be used immediately in downstream applications. Alternatively, the RNA sample can be aliquoted and stored at –20°C or –70°C for later use.

Section 9
Troubleshooting Guide

Problems that may be encountered during RNA purification:

Problem	Possible Cause	Recommended Solution
Incomplete separation of phases after centrifugation	Lysate was not mixed properly after adding chloroform (see step 4 in protocol)	Once chloroform is added, mix tubes vigorously by shaking for 15 sec. Do not vortex! Let the lysate incubate for 5 min at room temperature, mix again before centrifuging
	Lysate was not centrifuged at the right temperature	Make sure that centrifugation step is performed at 4°C following the addition of chloroform in order to achieve complete separation of the phases
	Incorrect amount of chloroform was added	For every 1 ml of PureZOL™ used, add 0.2 ml of chloroform
RNA binding mini column is clogging	Incomplete disruption of starting material	Increase the duration or intensity of sample disruption. Make sure to use the 1 ml of PureZOL for each prep
	Excessive amount of starting material	Do not exceed the maximum starting amount limit for the kit (see Section 5). If clogging persists when using the maximum starting amount, reduce the amount of material used

Problem	Possible Cause	Recommended Solution
RNA binding mini column is clogging *(continued)*	Starting material is high in fat, proteins, polysaccharides, or extracellular material, causing RNA eluate impurities	After the sample disruption step, centrifuge the lysate at 12,000 x g for 10 min at 4°C to pellet any debris present. Transfer the supernatant into a new 2.0 ml micro-centrifuge tube, leaving behind the pellet. Avoid transferring the excess fat that collects as a top layer in lipid-rich samples. Perform this step before adding the chloroform
	Not enough vacuum pressure was applied for the filtrate to pass through the columns	Make sure vacuum filtration steps are carried out at –17 to –23 inHg for optimum performance. Alternatively, transfer the RNA binding columns to a 2.0 ml capless tube and centrifuge for 60 sec at ≥12,000 x g
Problems that may be encountered after RNA is eluted from the column:		
Low RNA yield	Excessive amount of starting material	Do not exceed the maximum starting amount limit for the kit (see Section 5). If clogging occurs when using the maximum starting amount, reduce the amount of material used
	Inefficient elution	Preheat the elution solution to 70°C in water bath prior to the elution step

Problem	Possible Cause	Recommended Solution
Low RNA yield *(continued)*	Low amount of starting material	Do not use less than the recommended minimum starting amount (see Section 5). When processing small amounts of starting material (<500,000 cells or <10 mg of tissue), perform a single elution with 30 µl of elution solution
	Incomplete disruption of starting material that causes cells not to be lysed, and thus fail to release RNA into the lysate to be recovered	Increase the duration or intensity of sample disruption. Make sure that 1 ml of PureZOL is used per prep
	Incorrect use of wash solutions. (Incorrect ethanol concentration in the low stringency wash can cause accidental elution of the RNA from the membrane)	Add the required amount of ethanol to the low stringency wash buffer before use
	Incorrect DNase I dilution solution used, causing accidental elution of the RNA from the membrane	Use only the DNase dilution solution included in the kit to dilute the DNase I
	Elution solution was not pipetted directly onto the center of the membrane	Avoid pipetting the elution solution onto the side of the column or on top of the ring that holds the membrane stack in place
	Elution contamination of the eluate	Prior to eluting the RNA, make sure to perform the purge spin step (see step 16 in spin protocol) to remove residual ethanol in the wash solution

Problem	Possible Cause	Recommended Solution
Genomic DNA contamination	On-column DNase I digest was not performed	Perform the on-column DNase I digest (see step 14 in protocol)
	Incomplete DNase I digest	Increase the digest time for starting materials that are known to contain a high level of genomic DNA
	DNase I is inactive	Reconstitute the lyophilized DNase I with 10 mM Tris, pH 7.5. Aliquot and store the reconstituted DNase I enzyme in a nonfrost-free freezer. Avoid freeze-thaw cycles by aliquoting the enzyme for single use only
	Some of the white interphase (after phase separation) was transferred with the aqueous phase	Leave some of the aqueous phase solution behind to avoid transferring the white interphase with the aqueous phase (see step 7 in the protocol)
RNA is degraded	RNase contamination of solutions provided in the kit	Make sure to use RNase-free plasticware. See Section 5, Maintaining an RNase-free Environment, for detailed instructions
	RNase contamination of solutions supplied by the user	Treat all user-made solutions with DEPC before use (see Section 6 for instructions)
	RNase contamination of plasticware and work station	See Section 6, Maintaining an RNase-free Environment, for detailed instructions

Problem	Possible Cause	Recommended Solution
RNA is degraded *(continued)*	Frozen tissue samples were allowed to thaw or sit at room temperature	Add PureZOL directly to frozen samples before they thaw. Do not let starting materials sit at room temperature
	Cells grown in either monolayer or suspension were washed prior to lysis with PureZOL	Cells grown in monolayer: aspirate the growth medium and then add PureZOL directly to the plate. No trypsinization is necessary
		Cells grown in suspension: pellet the cells and aspirate growth medium, then add PureZOL directly to the pellet
	Starting tissue sample was not immediately frozen, or had gone through several freeze-thaw cycles before RNA purification was performed	Make sure that starting material is immediately processed following dissection. Alternatively the starting material must be immediately frozen after dissection. Once frozen, do not subject starting material to freeze-thaw cycles
Low RNA A_{260}/A_{280} ratio	Lysate was not incubated at room temperature for 5 min after the disruption step (see step 3 in protocol)	Make sure to incubate the lysate after the disruption step for 5 min at room temperature to allow complete dissociation of nucleoprotein complexes
	Ethanol contamination of the eluate	Prior to eluting the RNA, make sure to perform the purge spin step (see step 16 in spin protocol) to remove residual ethanol in the wash solution

Problem	Possible Cause	Recommended Solution
Low RNA A_{260}/A_{280} ratio *(continued)*	Some of the white interphase and the organic phase (containing proteins) were transferred with the aqueous phase during aspiration into a new tube	Leave some of the aqueous phase solution behind to avoid transferring the white interphase with the aqueous phase (see step 7 in the protocol)
	Starting material is high in fat, proteins, polysaccharides, or extracellular material, causing RNA eluate to be impure	After the sample disruption step, centrifuge the lysate at 12,000 x g for 10 min at 4°C to pellet any debris present. Transfer the supernatant into a new 2.0 ml micro-centrifuge tube, leaving behind the pellet. Avoid transferring the excess fat that collects as a top layer in lipid-rich samples. Perform this step before adding the chloroform
	Wash solutions and elution solution were contaminated with proteins and other contaminants	Make sure that the pipets that are being used for RNA preparation are not used for protein and DNA applications
	The solution used to dilute the RNA for spectrophotometric reading has a low pH (less than pH 6.5)	$A_{260}/_{280}$ may vary based on the pH of the solution used to dilute RNA samples. To get more accurate and consistent $A_{260}/_{280}$ values, dilute your RNA samples with a solution that has a pH within the 6.5–8.5 range

Problem	Possible Cause	Recommended Solution
Prepared total RNA performs poorly in downstream applications	RNA is degraded	See troubleshooting section "RNA is degraded"
	Ethanol contamination of the eluate	Make sure to perform the purge spin step (see step 16) to remove residual wash solution prior to eluting the RNA
	RNA is contaminated	See troubleshooting section "Low RNA $A_{260}/_{280}$ ratio"

Section 10
Ordering Information

Catalog #	Description
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)**
732-6470	**Aurum Vacuum Manifold**
732-6890	**PureZOL RNA Isolation Reagent**, 100 ml
732-6880	**PureZOL RNA Isolation Reagent**, 50 ml

Related Products

Catalog #	Description
732-6820	**Aurum Total RNA Mini Kit**
732-6800	**Aurum Total RNA 96 Kit**
732-6828	**DNase I,** RNase-Free, 1 vial
732-6826	**Aurum RNA Binding Mini Columns**, 50
732-6802	**Aurum Total RNA Lysis Solution**, 85 ml
732-6804	**Aurum Total RNA Wash Solution**, Low Stringency, 60 ml
732-6803	**Aurum Total RNA Wash Solution**, High Stringency, 150 ml
732-6805	**Aurum DNase Dilution Solution**, 20 ml
732-6801	**Aurum Total RNA Elution Solution**, 20 ml

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