

AxyPrep Multisource Total RNA Maxiprep Kit

For the large-scale purification of total RNA from animal tissues, plant tissues, cultured cells, bacteria, yeast and filamentous fungi

Kit contents, storage and stability

Cat. No.	AP-MX-MS-RNA-2	AP-MX-MS-RNA-10	AP-MX-MS-RNA-25
Kit size	2 preps	10 preps	25 preps
Lysate Filtration column	2	10	25
Maxiprep RNA column	2	10	25
Buffer R-I	40 ml	180 ml	450 ml
Buffer R-II	15 ml	70 ml	160 ml
Buffer W1A concentrate	24 ml	120 ml	288 ml
Buffer W2 concentrate	24 ml	120 ml	2 x 150 ml
Buffer TE (nuclease-free)	6 ml	30 ml	80 ml
Protocol manual	1	1	1

All buffers are stable for a period of at least 12 months from the date of receipt when stored under ambient conditions. Please avoid exposure to direct sunlight or extremes in temperature.

Axygen Biosciences warrants the performance of this kit for a period of 12 months from the date of receipt when stored under the conditions specified.

Lysate Filtration column: Store at room temperature.

Maxiprep RNA column: Store at room temperature.

Buffer R-I: Cell lysis buffer. Store at room temperature.

Buffer R-II: Neutralization buffer. Store at room temperature.

Buffer W1A concentrate: Wash buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before use of the kit, add the amount of ethanol specified on the bottle label. Either 100% or 95% denatured ethanol can be used. Mix well and store at room temperature.

Buffer TE (nuclease-free): Eluent. Contains 10 mM Tris-Cl and 0.1 mM EDTA, pH 7.5. Store at room temperature.

Introduction

AxyPrep Multisource Total RNA Maxiprep Kit represents a new approach for total cellular RNA purification, which is designed to eliminate the problems associated with other column-type RNA kits, such as clogged columns and incomplete purification. Tissues and cells are first lysed by Cell Lysis Buffer R-I, which also inactivates any indigenous RNase activity. Proteins and genomic DNA are then precipitated by the addition of Neutralization Buffer R-II to the cell lysate. The Lysate Filtration column removes any remaining particulates and contains special media to remove residual traces of contaminating proteins. After addition of isopropanol to the supernatant, the total RNA is then bound to membranes within the Maxiprep RNA column for further washing and desalting. Highly purified, full-length total cellular RNA is then eluted in a small volume of TE (or DEPC-treated water) and is ready for use in any downstream application.* Each Maxiprep RNA column has sufficient capacity to bind up to 2 mg of total cellular RNA. Yields will vary, depending upon amount and type of starting material, lysis method and efficiency, etc.

**Some applications may require the use of the AxyPrep RNase-free DNase Digestion kit to remove residual amounts of genomic DNA.*

Caution

Buffer R-I and Buffer W1A contain chemical irritants. When working with these buffers, always wear protective clothing such as safety glasses, gloves and laboratory coat. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

Equipment and consumables required

- Centrifuge capable of 8,000xg
- Fixed angle rotor to accommodate 50 ml centrifuge tubes
- Nuclease-free 50 ml centrifuge tubes (capable of centrifugation at 8,000xg)
- Mortar and pestle
- Homogenizer (Dounce-type or motorized) *optional*
- Plastic syringe and 21-25-gauge syringe needle (see specific prep requirements)
- AxyVac Vacuum Manifold (#AP-VM) or other vacuum manifold
- Vacuum regulator
- Vacuum source (capable of -25-30 inches Hg)
- Liquid nitrogen
- 95-100% ethanol
- Isopropanol

Preparation before experiment

- 1). Before the use of the kit, add the amounts of 95-100% ethanol to the Buffer W1A concentrate and the Buffer W2 concentrate, specified on the bottle labels and mix well.
- 2). Use DEPC-treated materials whenever practical.

Homogenization Methods

Depending upon the starting material and individual preference, different methods can be employed to achieve physical disruption of the source material, cell lysis and shearing of the genomic DNA. These methods include: mortar and pestle, manual Dounce-type homogenizer, motorized rotor-stator homogenizer, etc. The specific method selected to achieve physical disruption is left to the individual preference of the end user. Physical disruption and homogenization will either occur simultaneously (homogenizers) or successively (mortar and pestle), depending upon the method selected. Homogenization occurs in the presence of Buffer R-I. During homogenization, the individual cells are lysed, releasing their contents. Homogenization also inactivates nucleases and shears the genomic DNA, reducing the viscosity of the lysate (homogenate). Shearing the genomic DNA and reducing the lysate viscosity is important to achieving optimal RNA yield and purity.

The protocols provided utilize a mortar and pestle in conjunction with liquid nitrogen, followed by the use of a syringe needle to achieve complete homogenization. This disruption method is applicable to many different starting materials and obviates the requirement for special equipment. In the event that a homogenizer (either manual or motorized) is used, please follow the manufacturer's recommendations for achieving complete homogenization of the starting material. When using a homogenizer, freshly harvested tissue will generally have to be minced into small pieces in order to rapidly achieve complete homogenization and thoroughly nullify nuclease activity to preserve the integrity of the RNA.

IMPORTANT: The purification and handling of RNA requires particular attention to cleanliness to avoid contamination of work surfaces and laboratory equipments with nucleases. Please follow generally recommended practices for maintaining a nuclease-free work environment.

IMPORTANT: Grossly overloading the Maxiprep RNA columns with excessive RNA will often result in significantly diminished yields and purity. Please follow all guidelines for amounts of starting material.

I. Purification of Total RNA from Animal Tissues

Animal tissues can be efficiently disrupted by any one of the following methods:

- Mortar and pestle (tissue is freshly harvested and flash-frozen with liquid nitrogen)
- Dounce-type homogenizer (freshly harvested tissue, minced)
- Motorized homogenizer (freshly harvested tissue, minced)

In addition to physically disrupting the tissue, subsequent homogenization is important to shear the genomic DNA and achieve complete release of the cellular RNA. Throughout these protocols, homogenization is achieved by passing the lysate several times through a syringe needle. Shearing the genomic DNA reduces the viscosity of the lysate and results in higher yields and purity of the total cellular RNA. When using a manual or motorized homogenizer, the freshly harvested tissue should be quickly minced on ice to increase the efficiency of the homogenization process. Freshly harvested tissue can also be flash-frozen with liquid nitrogen and pulverized using a mortar and pestle before using a homogenizer.

Please use the following guidelines:

RNA-rich tissues (e.g., liver)	use up to 0.8 g
RNA-poor tissues (e.g., muscle)	use up to 1.5 g
When processing <0.4 g tissue	reduce R-I, R-II and isopropanol volumes by half
When processing >1.2 g tissue	increase R-I, R-II and isopropanol volumes proportionally

1. Select 0.25-1.5 g of freshly harvested animal tissue and immediately flash freeze by immersion in liquid nitrogen. Place a pestle into a mortar and freeze by adding liquid nitrogen to the mortar. Transfer the frozen tissue to the mortar and rapidly and vigorously grind to a finely pulverized powder. Depending upon the rapidity with which the tissue is pulverized, it may be necessary to add small amounts of liquid nitrogen intermittently so that the tissue remains frozen.

IMPORTANT: The tissue must remain frozen before and during grinding to prevent enzymatic degradation of the RNA.

2. Add 16 ml of Buffer R-I and continue to grind the tissue until the pulverized tissue and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a second nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided). Transfer the binding solution from Step 4 into the Maxiprep RNA column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube(not provided). Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE (nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Transfer the Maxiprep RNA column into a 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE (nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

Purification of Total RNA from Plant Tissues

Plant tissues can be efficiently disrupted by any one of the following methods:

- Mortar and pestle (tissue is freshly harvested and flash-frozen with liquid nitrogen)
- Dounce-type homogenizer (freshly harvested tissue, minced)
- Motorized homogenizer (freshly harvested tissue, minced)

In addition to physically disrupting the tissue, subsequent homogenization is important to shear the genomic DNA and achieve complete release of the cellular RNA. Throughout these protocols, homogenization is achieved by passing the lysate several times through a syringe needle or with a manual or motorized homogenizer. Shearing the genomic DNA reduces the viscosity of the lysate and results in higher yields and purity of the total cellular RNA. Due to the often fibrous nature of plant tissues, it is important to mince the starting material to increase the efficiency of manual or motorized homogenizers. Alternatively, freshly harvested plant tissues can be flash-frozen in liquid nitrogen and then pulverized with a mortar and pestle before homogenization.

Please use the following guidelines:

For leaf tissue	routinely process 0.4-1.5 g
For fibrous tissue (stems, etc.)	routinely process 0.5-2.0 g
When processing <0.4 g leaf tissue	reduce R-I, R-II and isopropanol volumes by half
When processing >1.2 g leaf tissue	increase R-I, R-II and isopropanol volumes proportionally
When processing >1.5 g fibrous tissue	increase R-I, R-II and isopropanol volumes proportionally

1. Select 0.4-1.5 g of freshly harvested animal tissue and immediately flash freeze by immersion in liquid nitrogen. Place a pestle into a mortar and freeze by adding liquid nitrogen to the mortar. Transfer the frozen tissue to the mortar and rapidly and vigorously grind to a finely pulverized powder. Depending upon the rapidity with which the tissue is pulverized, it may be necessary to add small amounts of liquid nitrogen intermittently so that the tissue remains frozen.

IMPORTANT: The tissue must remain frozen before and during grinding to prevent enzymatic degradation of the RNA.

2. Add 16 ml of Buffer R-I and continue to grind the tissue until the pulverized tissue and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 18-23-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a

nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided). Transfer the binding solution from Step 4 into the Maxiprep RNA column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE (nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the

bottle label for future reference.

4. Transfer the Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE (nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

Purification of Total RNA from Cultured Cells

This protocol is designed for the isolation of total RNA from up to 1×10^9 mammalian cells grown in suspension, in monolayer or as a cell suspension isolated from animal tissues. If the number of cells is $\leq 1 \times 10^8$, reduce the volumes of R-I, R-II and isopropanol by half. All other buffer volumes should remain unchanged. If the number of cells is $> 5 \times 10^8$, the volumes of R-I, R-II and isopropanol should be scaled up proportionally. Mammalian cells are lysed without the use of homogenizers or mortar and pestle. Generally, resuspension in lysis buffer, followed by pipetting up and down several times is sufficient for complete lysis. Complete homogenization and DNA shearing is then achieved by passing the lysate several times through a syringe needle.

Table1. Describes the number of Hela cells growing in various culture vessels when cells are grown to confluence. It may be used as a guide for estimating the number of cells.

Table1. Estimated number of Hela cells grown in different vessels.

Vessels	Growth area (cm ²)	Cell number
Multi-well plate		
96-well	0.32-0.6	$4.0-5.0 \times 10^4$
48-well	1	1.3×10^5
24-well	2	2.5×10^5
12-well	4	5.0×10^5
6-well	9.5	1.2×10^6
Petri Dish		
35 mm	8	1.0×10^6
60 mm	21	2.5×10^6
100 mm	56	7.0×10^6
145-150 mm	145	2.0×10^7
Bottle		
40-50 ml	25	3.0×10^6
250-300 ml	75	1.0×10^7
650-750 ml	162-175	2.0×10^7
900 ml	225	3.0×10^7

1. Collect 1×10^8 - 5×10^8 cells in suspension and transfer into a 50 ml centrifuge tube (not provided). Centrifuge at 2,000xg for 5 minutes to pellet the cells. Discard the supernatant.
2. Add 16 ml of Buffer R-I and quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to

pellet DNA and protein.

4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a 50 ml centrifuge tube(not provided). Transfer the binding solution from Step 4 into the Maxiprep RNA column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of

Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Transfer the Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE (nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

Purification of Total RNA from Bacteria

This protocol is designed for the isolation of RNA from $1-6 \times 10^{10}$ bacterial cells. The following values may be used as a guide for estimating the number of bacterial cells. For an *E. coli* culture, an OD_{600} of $1 = 1 \times 10^9$ cells/ml. If the number of bacterial cells is $\leq 1 \times 10^{10}$, reduce the volumes of buffers R-I, R-II and isopropanol by half. All other buffer volumes remain unchanged. If the number of bacterial cells is $> 6 \times 10^{10}$, increase the volumes of R-I, R-II and isopropanol proportionally.

1. Collect $1-6 \times 10^{10}$ bacteria. Centrifuge at 8,000xg for 10 minutes to pellet the bacteria. Decant or pipette off as much of the supernatant as possible. Resuspend the bacterial pellet in 200 μ l of PBS by vortexing. Transfer the sample to a mortar, completely frozen in liquid nitrogen. The bacterial suspension should freeze on contact. Grind rapidly and vigorously to form a finely pulverized powder.

Note: If using a microfuge, simply centrifuge the bacteria for 2 minutes at top speed to pellet.

2. Add 16 ml of Buffer R-I and continue to grind the sample until the pulverized sample and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided). Transfer the binding solution from Step 4 into the Maxiprep RNA column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Transfer the Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

Purification of Total RNA from Yeast

This protocol is designed for the isolation of total cellular RNA from 2×10^8 - 3×10^9 yeast cells. The following values may be used as a guide to estimating the number of yeast cells. For yeast cultures, an OD_{600} of $1 = 3 \times 10^7$ cells/ml. If the number of yeast cells is $\leq 5 \times 10^8$, reduce the volumes buffers R-I, R-II and isopropanol by half. All other buffer volumes remain unchanged. If the number of yeast cells is $> 1 \times 10^9$, the volumes of R-I, R-II and isopropanol should be increased proportionally.

There are two different methods which can be used to achieve the disruption and lysis of yeast. The **mechanical disruption method** (a. below) employs a mortar and pestle to grind yeast to form a fine powder, followed by homogenization with a syringe needle. The **enzymatic lysis method** (b. below) requires digestion of the cell walls with lyticase to convert the yeast to spheroplasts.

a. Mechanical Disruption

1. Collect $0.5-1 \times 10^9$ yeast cells. Centrifuge at 8,000xg for 10 minutes to pellet the yeast. Decant or pipette off as much of the supernatant as possible. Resuspend the yeast pellet in 200 μ l of PBS by vortexing. Transfer to a mortar, completely frozen with liquid nitrogen. The yeast suspension should freeze upon contact. Grind rapidly and vigorously to form a finely pulverized powder.
2. Add 16 ml of Buffer R-I and continue to grind the sample until the pulverized sample and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

b. Enzymatic Disruption

Prepare Buffer YE:

- a) 1 M sorbitol
- b) 0.1 M EDTA, pH 7.5

Just before use, add the following to Buffer YE:

- 0.1% β -mercaptoethanol
- 50 U lyticase per 1×10^7 cells

1. Collect $0.5-1 \times 10^9$ yeast cells. Centrifuge at 8,000xg for 10 minutes to pellet the yeast. Decant or pipette off as much of the supernatant as possible.
2. Resuspend the yeast pellet in 10 ml freshly prepared Buffer YE containing lyticase. Use 50 unit of lyticase for each 1×10^7 yeast cells. Incubate for 20-30 minutes at 30°C with occasional gentle inversion to generate spheroplasts.

IMPORTANT: Spheroplasts must be handled gently in the next step. Do not shake or agitate.

3. Pellet the spheroplasts by centrifuging for 5 minutes at 3,000xg. Carefully remove and discard the supernatant.
4. Add 16 ml of Buffer R-I and continue to grind the sample until the pellet and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

5. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
6. Transfer the supernatant from Step 5 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided). Transfer the binding solution from Step 4 (for mechanical disruption) or Step 6 (for enzymatic disruption) into the Maxiprep RNA column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml

centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Transfer the Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minute at room temperature. Centrifuge at 8,000xg for 5 minutes.

Purification of Total RNA from Filamentous Fungi

When processing <0.4 g of starting material, please

reduce the volumes of buffers R-I, R-II and isopropanol by half. All other buffer volumes remain unchanged. When processing >1.5 g, increase the volumes of R-I, R-II and isopropanol proportionally.

1. Select 0.4-1.5 g of freshly harvested animal tissue and immediately flash freeze by immersion in liquid nitrogen. Place a pestle into a mortar and freeze by adding liquid nitrogen to the mortar. Transfer the frozen tissue to the mortar and rapidly and vigorously grind to a finely pulverized powder. Depending upon the rapidity with which the tissue is pulverized, it may be necessary to add small amounts of liquid nitrogen intermittently so that the tissue remains frozen.

IMPORTANT: The tissue must remain frozen before and during grinding to prevent enzymatic degradation of the RNA.

2. Add 16 ml of Buffer R-I and continue to grind the tissue until the pulverized tissue and buffer are completely mixed. Quickly homogenize the sample by passing it 8-10x through a 50 ml syringe fitted with a 21-25-gauge needle. Be careful to minimize foaming. Transfer the homogenate to a nuclease-free 50 ml centrifuge tube (not provided).

Note: Thorough homogenization of the lysate is essential for high yield and purity of the RNA. The fully homogenized lysate should flow easily dropwise through the syringe needle. A viscous, stringy lysate indicates incomplete shearing of the genomic DNA and further homogenization is required.

3. Add 6 ml of Buffer R-II and vortex for 15-30 seconds. Centrifuge at 8,000xg for 10 minutes at 4°C to pellet DNA and protein.
4. Transfer the supernatant from Step 3 into the Lysate Filtration column. Carefully insert the plunger into the Lysate Filtration column and push with a slow, steady motion. Discharge the filtrate into a nuclease-free 50 ml centrifuge tube (not provided). Add 10 ml of isopropanol to the 50 ml centrifuge tube and mix by vortexing.

Note: The supernatant should be carefully removed without disturbing the pellet. The transfer of pellet material may result in column clogging and contamination of the RNA with DNA and protein.

Follow either Protocol A (centrifuge) or Protocol B (vacuum), below.

A. Using Centrifugation

1. Place a Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided). Transfer the binding solution from Step 4 into the spin/vac column. Centrifuge at 8,000xg for 5 minutes at room temperature or 4°C.
2. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 15 ml of Buffer W1A to the Maxiprep RNA column and centrifuge at 8,000xg for 5 minutes.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Discard the filtrate and place the Maxiprep RNA column back into the same 50 ml centrifuge tube. Add 20 ml of Buffer W2 and centrifuge at 8,000xg for 5 minutes. Discard the filtrate from the 50 ml centrifuge tube and repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Discard the filtrate from the 50 ml centrifuge tube. Place the Maxiprep RNA column back into the 50 ml centrifuge tube. Centrifuge at 8,000xg for 5 minutes to remove residual wash solution.
5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

B. Using Vacuum

1. Attach the vacuum manifold base to a vacuum source. Firmly position the Maxiprep RNA column(s) into the complementary fittings on the manifold top. Transfer the binding solution from Step 4 to the Maxiprep RNA column. Turn on the vacuum source and adjust to -25 inches Hg. Continue to apply the vacuum until no solution remains in the Maxiprep RNA column.

Note: -25 inches Hg is equivalent to -850-1,000 mbar or -12-15 psi.

2. Add 15 ml of Buffer W1A and draw all of the solution through the Maxiprep RNA column.

Note: Make sure that ethanol has been added into Buffer W1A concentrate. Make a notation on the bottle label for future reference.

3. Add 20 ml of Buffer W2 along the wall of Maxiprep RNA column to wash off residual Buffer W1A and draw all of the solution through the column. Repeat this wash step with a 10 ml aliquot of Buffer W2.

Note: Make sure that ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

4. Transfer the Maxiprep RNA column into a nuclease-free 50 ml centrifuge tube (not provided) and centrifuge at 8,000xg for 5 minutes to remove residual wash solution.

5. Transfer the Maxiprep RNA column into a clean nuclease-free 50 ml centrifuge tube (not provided). To elute the total RNA, add 2 ml of Buffer TE(nuclease-free) to the center of the membrane. Let it stand for 5 minutes at room temperature. Centrifuge at 8,000xg for 5 minutes.

Overview

Add sample and 16 ml of Buffer R-I

Lysis

Add 6 ml of Buffer R-II
Centrifuge at 8,000xg for 10 minutes

Neutralization

Filter the supernatant with Lysate Filtration column

Particulate & protein removal

Add 10 ml of isopropanol

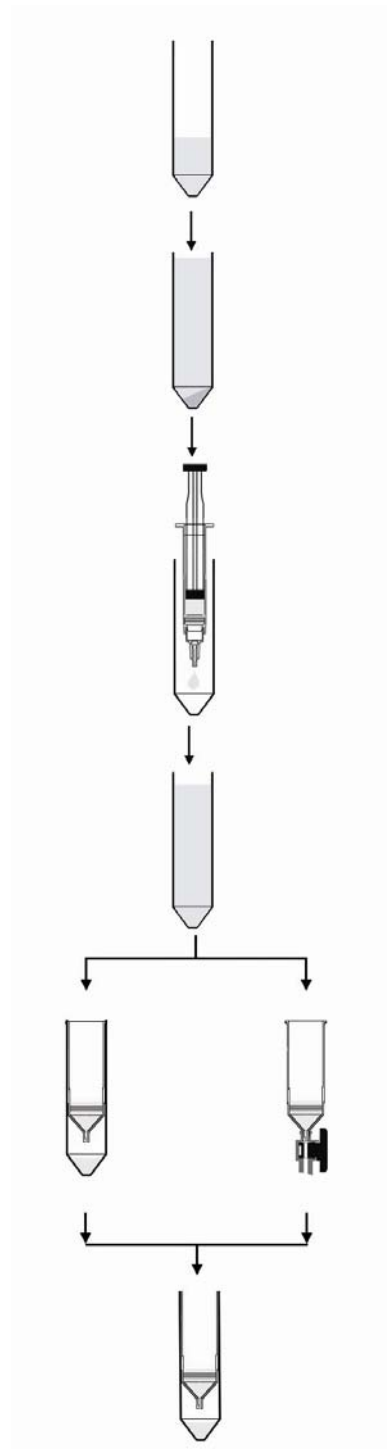
Binding to Maxiprep RNA column

Add 15 ml of Buffer W1A
Add 20 ml of Buffer W2
Add 10 ml of Buffer W2

Washing

Add 2 ml of Buffer TE
(nuclease-free)

Elution



Troubleshooting

1. Little or no RNA eluted

- Too much starting material. Overloading significantly reduces yield. Reduce the amount of starting material.
- Incomplete removal of supernatant after pelleting of cultured cells. Ensure complete removal of the supernatant after harvesting cells.
- Buffer temperatures too low. All buffers must be equilibrated to room temperature before starting the procedure.

2. Low A₂₆₀/A₂₈₀ value

- Inefficient cell lysis due to insufficient mixing of the samples with Buffer R-I.
- Buffer W1A or Buffer W2 prepared incorrectly with the correct amount of ethanol.
- Inadvertent transfer of pellet material to spin/vac column after Buffer R-II addition and centrifugation.

3. RNA degraded

- Inappropriate handling of starting material. Ensure that cells have been properly handled and that the RNA extraction has been performed without interruptions, especially during the initial steps involving cell lysis.
- RNase contamination: check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase-free, RNase can be introduced during use. Be certain not to introduce any RNase during the procedure or later handling.

For technical inquiries about AxyPrep Kits, please contact Axygen Biosciences at

support.axyprepkits@axygenbio.com

Warranty/Disclaimer

Axygen Biosciences warrants that this kit will perform as indicated for the specified application for a period of up to 12 months from the date of receipt when stored in the specified manner indicated and used according to the instructions provided. In using this product, the customer agrees that Axygen Biosciences shall not be held liable for any direct or indirect damages, including, but not limited to, personal injury, property damage or lost profits (or other economic loss) resulting from the use or inability to use this product. In the event that this product fails to perform in the specified manner, remedial measures on the part of Axygen Biosciences shall be limited to the replacement of this product and will be implemented at the discretion of Axygen Biosciences.