

Vantage™ Total RNA Purification Kit**Overview and Intended Use**

OriGene's Vantage™ Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from cultured animal cells, tissue samples, blood, bacteria, yeast, fungi, plants and nasal or throat swabs. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension assays. The purified RNA can also be used in expression analysis studies and is compatible with microarray and Luminex™ profiling assays.

Principle of Method

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the cells or tissue of interest with the provided Lysis Solution. Ethanol is then added to the lysate, and the solution is loaded onto a RNA binding-column. The resin contained within the column binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flow through or retained on the top of the resin. The bound RNA is then washed with the provided Wash Buffer in order to remove any remaining impurities, and the purified total RNA is eluted with the RNA Elution Buffer. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including expression analysis.

Important Information**READ ENTIRE PROTOCOL BEFORE USE****ADDITIONAL PRECAUTIONS SHOULD BE TAKEN TO PREVENT THE DEGRADATION OF RNA.****Precautions to take when working with RNA**

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

1. The RNA area should be located away from microbiological work stations

2. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
3. There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
4. All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
Clean all surfaces with commercially available RNase decontamination solutions
5. When working with purified RNA samples, ensure that they remain on ice during downstream applications

Safety and Use Statement

This product contains hazardous reagents. It is the end-user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste organics, acids, bases, and radioactive materials must comply with all appropriate federal, state, and local regulations. If you have any questions concerning the hazards associated with this product, please contact OriGene at (888) 267-4436 or visit www.origene.com.

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

Components included with this kit:

Lysis Solution	35 ml
Wash Buffer	20 ml
RNA Elution Buffer	5 ml
RNA Binding Columns	50 columns
Wash Tubes	50 tubes
Recovery Tubes	50 tubes

Storage Conditions and Stability

Store all components at room temperature. All solutions should be kept tightly sealed and should remain stable for at least 2 years in their unopened containers.

Materials and Equipment Required but Not Supplied

The Vantage™ Total RNA Purification Kit isolates and purifies total RNA from a variety of different sample types that have different material and equipment requirements.

For All Protocols

Benchtop microcentrifuge (see Important Equipment Information below)

95 - 100% ethanol

β-mercaptoethanol (optional)

Table 1 - Sample Specific Materials and Equipment

Specific Sample Protocols	Materials and equipment required but not supplied
Cultured Cells	PBS (RNase-free)
Animal Tissue	Liquid nitrogen Mortar and pestle 70% ethanol
Bacteria	Lysozyme-containing TE Buffer: <ul style="list-style-type: none"> For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer
Fungi	Liquid nitrogen Mortar and pestle 70% ethanol
Yeast	Liquid nitrogen Mortar and pestle 70% ethanol
Plant	Liquid nitrogen Mortar and pestle 70% ethanol
Nasal or Throat Swabs	Sterile, single-use cotton swabs

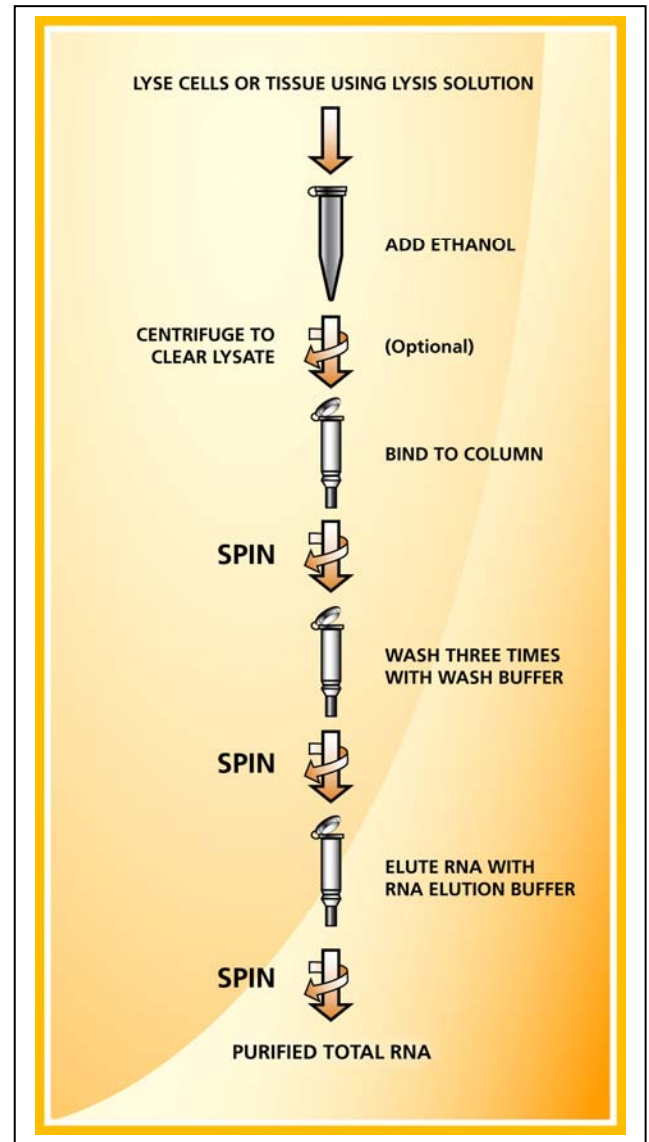
Important Equipment Information

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Procedure Workflow



Step 1 Preparation of Lysates

IMPORTANT: Vantage™ Total RNA Purification Kit isolates and purifies total RNA from a variety of different sample types that have different lysate preparation requirements (Step 1). All subsequent purification steps are the same in all cases (Step 2). Please ensure that the correct procedure for preparing the lysate from your starting material is followed.

Usage Notes

1. All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
2. A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
3. Ensure that all solutions are at room temperature prior to use.
4. Prepare a working concentration of the **Wash Buffer** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Buffer**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
5. **Optional Step** - The use of β -mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNase content (e.g. pancreas), as well as for most plant tissues and nasal and throat swabs. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μ L of β -mercaptoethanol (not provided) to each 1 mL of Lysis Solution required. β -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the lysis solution can be used as provided.
6. It is important to work quickly during this procedure.

Table 2

Specific Sample Type	Maximum Amount of Starting Material
Cultured Cells	3 x 10 ⁶ cells
Animal Tissues	25 mg
Blood	100 μ L
Bacteria	1 x 10 ⁹ cells
Yeast	1 x 10 ⁸ cells
Fungi	50 mg
Plant Tissues	50 mg

Preparation of Lysates from Cultured Cells

Usage Notes

1. The maximum recommended input of cells is 3 x 10⁶. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10⁶ cells.
2. Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing. Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
3. Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Lysis Solution directly to the frozen cell pellet (**Step B2**).

A. Protocol for Preparing Lysates from Cells Growing in a Monolayer

1. Aspirate media and wash cell monolayer with an appropriate amount of PBS.
2. Aspirate PBS and add 350 μ L of **Lysis Solution** directly to culture plate.
3. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
4. Transfer lysate to an RNase-free microcentrifuge tube and add 200 μ L of 95 – 100% ethanol (not provided) to the lysate. Mix by vortexing for 10 seconds.

Note: For input amounts greater than 10⁶ cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

5. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation**.

B. Protocol for Preparing Lysates from Cells Growing in Suspension and Lifted Cells

1. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 x g (~2,000 RPM) for 10 minutes to pellet cells. Carefully decant the supernatant. A few μ L of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
2. Add 350 μ L of **Lysis Solution** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
3. Add 200 μ L of 95 - 100% ethanol (not provided) to the lysate. Mix by vortexing for 10 seconds.

Note: For input amounts greater than 10⁶ cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

4. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation.**

Preparation of Lysates from Animal Tissues Usage Notes

1. RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible.
2. Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
3. It is recommended that no more than 25 mg of tissue be used, in order to prevent clogging of the column.

C. Protocol for Preparing Cell Lysates from Animal Tissues

1. Excise the tissue sample from the animal.
2. Determine the amount of tissue by weighing. It is recommended that no more than 25 mg of tissue be used for the protocol.
3. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
4. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
5. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.
6. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
7. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
8. Add an equal volume of 70% ethanol (not provided) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
9. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation.**

Preparation of Lysates from Blood Usage Notes

1. Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
2. It is recommended that no more than 100 µL of blood be used in order to prevent clogging of the column.
3. We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.
4. It is important to work quickly during this procedure.

D. Protocol for Preparing Cell Lysates from Blood

1. Transfer up to 100 µL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
2. Add 350 µL of **Lysis Solution** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
3. Add 200 µL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
4. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation.**

Preparation of Lysates from Nasal or Throat Swabs Usage Notes

1. Body fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with the samples.
2. It is important to work quickly during this procedure.

E. Protocol for Preparing Cell Lysates from Nasal or Throat Swabs

1. Add 600 µL of **Lysis Solution** to an RNase-free microcentrifuge tube (not provided).
2. Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.
3. Using sterile technique, cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the **Lysis Solution**. Close the tube. Vortex gently and incubate for 5 minutes at room temperature.
4. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube (not provided). Note the volume of the lysate.
5. Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
6. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation.**

Preparation of Lysates from Bacteria

Usage Notes

1. Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 1. This solution should be prepared with sterile, RNase-free TE Buffer, and kept on ice until needed.
2. It is recommended that no more than 10^9 bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an *E. coli* culture containing 1×10^9 cells/mL has an OD₆₀₀ of 1.0.
3. For RNA isolation, bacteria should be harvested in log-phase growth.
4. Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure. Frozen bacterial pellets should not be thawed prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet (**Step F3**).

F Protocol for Preparing Cell Lysates from Bacteria

1. Pellet bacteria by centrifuging at $14,000 \times g$ (~14,000 RPM) for 1 minute.
2. Decant supernatant, and carefully remove any remaining media by aspiration. Resuspend the bacteria thoroughly in 100 μL of the appropriate lysozyme-containing TE buffer (see Table 1) by vortexing. Incubate at room temperature for the time indicated in Table 3.
3. Add 300 μL of **Lysis Solution** and vortex vigorously for at least 10 seconds.
4. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
5. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation**.

Table 3

Incubation Time for Different Bacterial Strains

Bacteria Type	Lysozyme Concentration in TE Buffer	Incubation Time
Gram-	1 mg/mL	5 min
Gram-	3 mg/mL	10 min

Preparation of Lysates from Yeast

Usage Notes

1. Prepare the appropriate amount of Lyticase-containing Resuspension Buffer (not provided), considering that 100 μL of buffer is required for each preparation.
2. The Resuspension Buffer should have the following composition:
 - 50 mM Tris, pH 7.5
 - 10 mM EDTA
 - 1M Sorbitol
 - 0.1% β -mercaptoethanol
 - 1 unit/ μL Lyticase.

This solution should be prepared with sterile, RNase-free reagents, and kept on ice until needed. The Resuspension Buffer is not provided with this kit and should be prepared fresh by the user.

3. It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used for this procedure.
4. For RNA isolation, yeast should be harvested in log-phase growth.
5. Yeast can be stored at -70°C for later use, or used directly in this procedure.
6. Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet (**Step G4**).

G. Protocol for Preparing Cell Lysates from Yeast

1. Pellet yeast by centrifuging at $14,000 \times g$ (~14,000 RPM) for 1 minute.
2. Decant supernatant, and carefully remove any remaining media by aspiration.
3. Resuspend the yeast thoroughly in 100 μL of Lyticase-containing Resuspension Buffer (see above) by vortexing. Incubate at 37°C for 10 minutes.
4. Add 300 μL of **Lysis Solution** and vortex vigorously for at least 10 seconds.
5. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
6. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation**.

Preparation of Lysates from Fungi

Usage Notes

1. Fresh or frozen fungi may be used for this procedure. Fungal tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Fungi may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
2. It is recommended that no more than 50 mg of fungi be used for this procedure in order to prevent clogging of the column.

H. Protocol for Preparing Cell Lysates from Fungi

1. Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
2. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle.
Note: At this stage the ground fungus may be stored at -70°C, such that the RNA purification can be performed at a later time.
3. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
4. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
5. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
6. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
7. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation**.

Preparation of Lysates from Plant

Usage Notes

1. The maximum recommended input of plant tissue is 50 mg or 5×10^6 plant cells.
2. Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
3. It is important to work quickly during this procedure.

I. Protocol for Preparing Cell Lysates from Plant

1. Transfer ≤ 50 mg of plant tissue or 5×10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.
Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.
2. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
3. Add 600 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.
4. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
5. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
6. Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.
7. If any visible precipitates are present, spin the lysate for 1 minute in a benchtop microcentrifuge to pellet any debris. Otherwise, proceed directly to **Step 2 without centrifugation**.

Step 2. Total RNA Purification from All Sample Type Lysates

IMPORTANT: *Vantage*[™] Total RNA Purification Kit isolates and purifies total RNA from a variety of different sample types that have different lysate preparation requirements (Step 1). All subsequent purification steps are the same in all cases (Step 2).

THUS THE REMAINING STEPS OF THE PROCEDURE FOR THE PURIFICATION OF TOTAL RNA ARE THE SAME FROM THIS POINT FORWARD FOR ALL THE DIFFERENT TYPES OF LYSATE.

A. Binding RNA to Column

1. Assemble an **RNA Binding Column** with one of the provided **Wash Tubes**.
2. Apply up to 600 μL of the clarified lysate with the ethanol (from **Step 1**) onto the column and centrifuge for 1 minute.
3. Discard the flow through. Reassemble the **RNA Binding Column** with its **Wash Tube**.
4. Depending on your lysate volume, repeat **2** and **3** as necessary.

Optional Step:

Vantage[™] Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional “**On-Column DNA Removal Protocol**” is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

B. Column Wash

1. Apply 400 μL of **Wash Buffer** to the **RNA Binding Column** and centrifuge for 1 minute.
Note: Ensure the entire **Wash Buffer** has passed through into the **Wash Tube** by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
2. Discard the flow through and reassemble the **RNA Binding Column** with its **Wash Tube**.
3. Repeat column wash steps **1** and **2** to wash column a second time.
4. Wash column a third time by adding another 400 μL of **Wash Buffer** and centrifuging for 2 minutes.
5. Ensure that the column is dry. Spin for an additional minute, if necessary.
6. Discard the collection tube with the flow through.

C. RNA Elution

1. Place the **RNA Binding Column** into a fresh 1.7 mL **Recovery Tube** (provided).
2. Add 50 μL of **RNA Elution Buffer** to the column.
3. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 1 minute at 14,000 x g (~14,000 RPM) Note the volume eluted from the column. If the entire 50 μL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube by repeating **2** and **3**.

D. RNA Analysis

To determine the quality and quantity of the eluted RNA refer to the following protocol:

1. Dilute an aliquot of the eluted RNA in nuclease free 10mM Tris-HCl, pH 7.5. Mix well. Transfer to a cuvette or to a UV transparent plate.
2. Using 10 mM Tris-HCl as blank determine the A_{260} and A_{280} of the diluted RNA solution.
3. Calculate the amount of Total RNA recovered in μg using the formula:

$$\frac{A_{260} \times 40 \mu\text{g}}{(1 A_{260} \times 1 \text{ mL}) \times \text{dilution factor} \times \text{sample volume (mL)}}$$

4. Determine the A_{260}/A_{280} ratio. The value should be 1.8-2.4.

Table 4

Average Yields*	Amounts of RNA (μg)
HeLa Cells (1×10^6 cells)	15 μg
<i>E. coli</i> (1×10^9 cells)	50 μg

* average yields will vary depending upon a number of factors including species, growth conditions used and developmental stage.

Alternatively, an Agilent Bioanalyzer can be used to determine the quality and quantity of eluted RNA following the manufacturer’s recommended protocol.

E. Storage

The purified RNA sample may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

Appendix A

Protocol for Optional On-Column DNA Removal

Vantage™ Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that an RNase-free DNase I be used.

1. Prepare a working stock of 0.25 Kunitz unit/ μ L RNase-free DNase I solution according to the manufacturer's instructions. A 100 μ L aliquot is required for each column to be treated. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 8.0, 10 mM MgCl₂ and 3 mM CaCl₂, made RNase-free) to give a final concentration of 0.25 Kunitz unit/ μ L.
2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding RNA to Column**" (Step 1 through Step 2A)
3. Apply 400 μ L of **Wash Buffer** to the column and centrifuge for 2 minute. Discard the flow through. Reassemble the **RNA Binding Column** with its **Wash Tube**.
4. Apply 100 μ L of the RNase-free DNase I solution prepared in Step 1 to the column. Centrifuge for 30 seconds at 200 x g (~2000 RPM). Alternatively, centrifuge for a 5 second pulse at 14, 000 x g (~14 000 RPM) if only a single speed centrifuge is available. Approximately half of the DNase I solution will pass through the column.
5. Incubate the column assembly at 25 - 30°C for 15 minutes.
6. Without any further centrifugation, proceed directly to "**Column Wash**" (Step 2B).

Technical Support

For further technical assistance please contact us at (888) 267-4436 or by email at techsupport@origene.com. Technical support and troubleshooting guides for these products can also be found on our website at www.origene.com.

Related Products

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