

### Overview and Intended Use

OriGene's Vantage™ Total RNA Purification 96-Well Kit provides a rapid method for the high-throughput isolation and purification of total RNA from cultured cells, tissues, blood, bacteria, yeast, fungi and plants. 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, and RNase protection and primer extension assays. The purified RNA can also be used in expression analysis studies and is compatible with the Vantage™ multiplex microRNA detection panels.

### Principle of Method

Purification is based on 96-well column chromatography using a proprietary resin as the separation matrix. The purification can be performed on either a vacuum manifold or by centrifugation. The process involves first lysing the cells or tissue of interest with Lysis Solution. Ethanol is then added to the lysate, and the solution is loaded onto the 96-Well Filter Plate. OriGene's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the resin in the wells, 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 Wash Solution in order to remove any remaining impurities, and the purified total RNA is eluted with RNA Elution Buffer.

Kit Specifications	
Binding Capacity Per Well	50 µg
Maximum Loading Volume Per Well	500 µL
Size of RNA Purified	All sizes, including RNA <200 nt
Time to Complete 96 Purifications	30 minutes

### 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
5. Clean all surfaces with commercially available RNase decontamination solutions
6. 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](http://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	2 x 35 mL
Wash Solution	2 x 25 mL
RNA Elution Buffer	2 x 15 mL
96-well Filter Plate	2 plates
Elution Plate with Lid	2 plates
Plate Sealers	4

## 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. See Table 1 for sample specific materials and equipment.

### For All Protocols

- For **Vacuum Format**:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads
- For **Centrifuge Format**:
  - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)
- 95 - 100% ethanol
- $\beta$ -mercaptoethanol (optional)
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (single or 96-well format) for centrifugation

**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
Bacteria	Lysozyme-containing TE Buffer: <ul style="list-style-type: none"> <li>For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer</li> <li>For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer</li> </ul>
Fungi	Liquid nitrogen Mortar and pestle
Yeast	Resuspension Buffer with Lyticase: <ul style="list-style-type: none"> <li>50 mM Tris pH 7.5</li> <li>10 mM EDTA</li> <li>1 M Sorbitol</li> <li>1 unit/<math>\mu</math>L Lyticase</li> </ul>
Plant	Liquid nitrogen Mortar and pestle

## Important Equipment Information

**For Vacuum Manifold:** All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

$$1 \text{ mbar} = 100 \text{ Pa} = 0.0394 \text{ in. Hg} = 0.75 \text{ mm Hg} = 0.0145 \text{ psi}$$

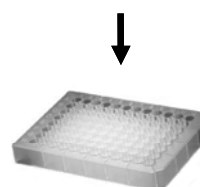
**For Centrifugation:** All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

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

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

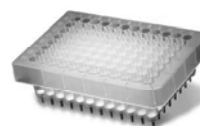
Sample of cells or tissues



Lyse cells or tissues with Lysis Solution



Add ethanol



Bind RNA



Wash three times with Wash Solution



Elute RNA with Elution Buffer



**Purified Total RNA**

### 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. Ensure that all solutions are at room temperature prior to use.
2. Prepare a working concentration of the **Wash Solution** by adding 90 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 120 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
3. The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
4. **Optional Step** - The use of  $\beta$ -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  $\mu$ L of  $\beta$ -mercaptoethanol (not provided) to each 1 mL of Lysis Solution required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the lysis solution can be used as provided.
5. It is important to work quickly during this procedure.

**Table 2**

Specific Sample Type	Maximum Amount of Starting Material
Cultured Cells	1 x 10 <sup>6</sup> cells
Animal Tissues	10 mg
Blood	100 $\mu$ L
Bacteria	1 x 10 <sup>9</sup> cells
Yeast	1 x 10 <sup>8</sup> cells
Fungi	40 mg
Plant Tissues	40 mg

### Preparation of Lysates from Cultured Cells

#### Usage Notes

##### 1A. Lysate Preparation from Cultured Animal Cells

1. The recommended input is 5 x 10<sup>5</sup> cells per well of the 96-Well Filter Plate. However, up to 1 x 10<sup>6</sup> cells may be processed for most cell lines. A hemocytometer can be used in conjunction with a microscope to count the number of 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 (96-Well Plate or other Multi-Well Plate)

1. Aspirate media and wash cell monolayer with an appropriate amount of PBS.
2. Aspirate PBS and add 300  $\mu$ L of **Lysis Solution** directly to culture plate.
3. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for two minutes.
4. Add 120  $\mu$ L of 95 – 100% ethanol (provided by the user) to each well. Mix by pipetting up and down a few times.

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

1. Transfer cell suspension to the wells of an RNase-free 96-well microplate (not provided) and centrifuge at no more than 200 x g (~2,000 RPM) for 10 minutes to pellet cells.
2. Aspirate supernatant carefully to ensure that the pellets are not dislodged.
3. Add 300  $\mu$ L of Lysis Solution to each well.
4. Lyse cells by gently tapping culture plate and swirling buffer around plate surface for two minutes.
5. Add 120  $\mu$ L of 95 - 100% ethanol (provided by the user) to each well. Mix by pipetting up and down a few times.

## 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 homogenization.
3. The optimal amount of non-fibrous tissue be used per well of the 96-Well Filter Plate is up to 8 mg. However, for most tissues (except tissues with high cell number such as liver and spleen), up to 10 mg could be processed. For fibrous tissue such as heart, a maximum of 2 mg is recommended

### 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 10 mg of tissue be used for each well of the 96-Well Filter Plate.
3. Transfer the tissue samples to appropriate vessels for the desired disruption method.
4. Add 350 µL of **Lysis Solution** to each tissue sample.

**Note:** Ensure that frozen tissues do not thaw during weighing or prior to the addition of Lysis Solution. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Lysis Solution**.

5. Homogenize the tissues using the appropriate cell disruption tool.

**Note:** Thorough homogenization is required for optimal performance. For tissue inputs of  $\leq 8$  mg, it is not required to perform centrifugation to remove cell debris if the homogenization is complete. For tissue inputs larger than 8 mg, or if incomplete cell disruption is suspected, centrifuge the lysate at maximum speed for 2 minutes in an appropriate centrifuge. Transfer the supernatant to a new 96-well microplate.

6. Add 120 µL of 95 - 100% ethanol (provided by the user) to each tissue sample. Mix by pipetting up and down a few times.

## 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 per well in order to prevent clogging of plate.
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 96-well microplate (not provided).
2. Add 200 µL of **Lysis Solution** to the blood. Lyse cells by gently tapping the 96-well microplate and swirling buffer around plate surface for two minutes
3. Add 120 µL of 95 – 100% ethanol (provided by the user) to the each well. Mix by pipetting up and down a few times.

## 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. These reagents are to be provided by the user.
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  $1 \times 10^9$  cells/mL has an OD<sub>600</sub> of 1.0.
3. For RNA isolation, bacteria should be harvested in log-phase growth.
4. Bacterial pellets can be stored at  $-70^\circ\text{C}$  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 E3**).

### E Protocol for Preparing Cell Lysates from Bacteria

1. Pellet bacteria by centrifuging at  $14,000 \times g$  (~14,000 RPM) for 1 minute or  $3000 \times g$  (~3,000 RPM) for 5 minutes for culture in a 96-well microplate.
2. Carefully remove any remaining media by aspiration.
3. Resuspend the bacteria thoroughly in 75  $\mu\text{L}$  of the appropriate lysozyme-containing TE buffer (see Table 1) by vortexing. Incubate at room temperature for the time indicated in Table 3.
4. Add 225  $\mu\text{L}$  of **Lysis Solution** to each sample. Mix by pipetting up and down a few times.
5. Add 120  $\mu\text{L}$  of 95 – 100% ethanol (provided by the user) to lysate. Mix by pipetting up and down a few times.

**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 75  $\mu\text{L}$  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%  $\beta$ -mercaptoethanol
  - 1 unit/  $\mu\text{L}$  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 per well of the 96-Well Filter Plate.
4. For RNA isolation, yeast should be harvested in log-phase growth.
5. Yeast can be stored at  $-70^\circ\text{C}$  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 F4**).

### F. Protocol for Preparing Cell Lysates from Yeast

1. Pellet yeast by centrifuging at  $14,000 \times g$  (~14,000 RPM) for 1 minute for culture collected in 1.5 mL microfuge tubes or  $3000 \times g$  (~3,000 RPM) for 5 minutes for culture in a 96-well microplate.
2. Carefully remove any remaining media by aspiration.
3. Resuspend the yeast thoroughly in 75  $\mu\text{L}$  of Lyticase-containing Resuspension Buffer (see above). Incubate at  $37^\circ\text{C}$  for 10 minutes.
4. Add 225  $\mu\text{L}$  of **Lysis Solution** to each yeast sample. Mix by pipetting up and down a few times.
5. Add 120  $\mu\text{L}$  of 95 – 100% ethanol (provided by the user) to the lysate. Mix by pipetting up and down a few times.

## 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 40 mg of fungi be used per well of the 96-Well Filter Plate to prevent clogging of the plate.

### G. 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 tissue be used per well of the 96-Well Filter Plate.
2. Transfer the tissue samples to appropriate vessels for the desired disruption method.
4. Add 350 µL of **Lysis Solution** to each tissue sample.  
**Note:** Ensure that frozen tissues do not thaw during weighing or prior to the addition of Lysis Solution. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Lysis Solution**.
5. Homogenize the tissues using the appropriate cell disruption tool.
6. Centrifuge the lysate at maximum speed for 2 minutes in an appropriate centrifuge. Transfer the supernatant to a new 96-well microplate.
7. Add 120 µL of 95 - 100% ethanol (provided by the user) to each lysate sample. Mix by pipetting up and down a few times.

## Preparation of Lysates from Plant

### Usage Notes

1. The maximum recommended input of plant tissue is 40 mg or  $5 \times 10^6$  plant cells per well of the 96-Well Filter Plate.
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.

### H. Protocol for Preparing Cell Lysates from Plant

1. Excise the tissue sample from the plant.
2. Determine the amount of tissue by weighing.  
**Note:** It is recommended that no more than 40 mg of tissue be used per well of the 96-Well Filter Plate.
3. Transfer the tissue samples to appropriate vessels for the desired disruption method.
4. Add 350 µL of **Lysis Solution** to the tissue sample.  
**Note:** Ensure that frozen tissues do not thaw during weighing or prior to the addition of Lysis Solution. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Lysis Solution**.
5. Homogenize the tissues using the appropriate cell disruption tool.
6. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free 96-well microplate.
7. Add 120 µL of 95 - 100% ethanol (provided by the user). Mix by pipetting up and down a few times.

## Step 2. Total RNA Purification from All Sample Type Lysates

**IMPORTANT:** *Vantage*<sup>™</sup> 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.**

The purification of total RNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Step 2(i). For purification using centrifugation, please follow the procedure outlined in Step 2(ii).

### Step 2(i). Total RNA Purification from All Types of Lysate Using Vacuum Manifold

#### A. Binding RNA to 96-Well Filter Plate

1. Assemble the 96-Well Filter Plate and the vacuum manifold according to manufacturer's recommendations.
2. Apply up to 500 µL of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

**Note:** Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

3. Turn off vacuum and ventilate the manifold. Discard the flow through. Reassemble the 96-Well Filter Plate and the vacuum manifold.

**Note:** Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

#### Optional Step:

*Vantage*<sup>™</sup> 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 B for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

#### B. Plate Wash

1. Apply 400 µL of **Wash Solution** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

**Note:** Ensure the entire **Wash Solution** has passed through into the collection/waste tray by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

2. Turn off vacuum and ventilate the manifold. Discard the flow through.
3. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **B1** and **B2** to wash column for a second time.
4. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **B1** and **B2** to wash column for a third time.
5. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.
6. Turn off vacuum and ventilate the manifold.

#### C. RNA Elution

1. Remove the lid from the provided Elution Plate. Replace the collection/waste tray in the vacuum manifold with the Elution Plate. Complete the vacuum manifold assembly with the 96-Well Filter Plate.
2. Add 75 µL of **Elution Buffer** to each well of the plate.
3. Apply vacuum for 2 minutes.

#### D. 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.

## Step 2(ii). Total RNA Purification from All Types of Lysate Using Centrifugation

### A. Binding RNA to 96-Well Filter Plate

1. Place the 96-Well Filter Plate on top of an RNase-Free 96-well bottom plate (single well or 96-well, not provided)

**Note:** Whether a deep or shallow bottom plate is needed depends on the brand of the centrifuge or the type of rotor used. The user should ensure that the 96-Well Filter Plate/bottom plate stack fits into the rotor without interfering with the centrifugation process. The bottom plate chosen should be able to handle 500  $\mu\text{L}$  of liquid per well for the 96-well format or 50 mL total for the single well format.

2. Apply up to 500  $\mu\text{L}$  of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 2 minutes.

**Note:** Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

3. Discard the flow through. Reassemble the 96-Well Filter Plate and the bottom plate.

**Note:** Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

### Optional Step:

*Vantage*<sup>™</sup> 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 B for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

### B. Plate Wash

1. Apply 400  $\mu\text{L}$  of **Wash Solution** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 2 minutes.

**Note:** Ensure the entire wash solution has passed through into the bottom plate by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

2. Discard the flow through. Reassemble the 96-Well Filter Plate and the bottom plate.

3. Repeat steps **B1** and **B2** to wash column for a second time.

4. Repeat steps **B1** and **B2** to wash column for a third time.

5. Pat the bottom of the 96-Well Filter Plate dry.

Reassemble the 96-Well Filter Plate and the bottom plate. Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 5 minutes in order to completely dry the plate.

### C. RNA Elution

1. Remove the lid from the provided Elution Plate. Stack the 96-Well Filter Plate on top of the Elution Plate.

b. Add 75  $\mu\text{L}$  of **Elution Buffer** to each well of the 96-Well Filter Plate.

c. Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 2 minutes.

### D. Storage

The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  for long term storage.

## Appendix A 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  $\mu\text{gs}$  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 ( $\mu\text{g}$ )
HeLa Cells ( $1 \times 10^6$ cells)	15 $\mu\text{g}$
<i>E. coli</i> ( $1 \times 10^9$ cells)	50 $\mu\text{g}$

\* 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.

## Appendix B 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/  $\mu\text{L}$  RNase-free DNase I solution according to the manufacturer's instructions. A 75  $\mu\text{L}$  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  $\text{MgCl}_2$  and 3 mM  $\text{CaCl}_2$ , made RNase-free) to give a final concentration of 0.25 Kunitz unit/  $\mu\text{L}$ .
2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding RNA to 96-Well Filter Plate**" (Steps 1 and 2A of all protocols)
3. **For Vacuum Manifold:** Apply 400  $\mu\text{L}$  of **Wash Solution** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or a pad (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.  
**For Centrifugation:** Apply 400  $\mu\text{L}$  of **Wash Solution** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 2 minutes.
4. Discard the flow through. Reassemble the 96-Well Filter Plate with the vacuum manifold or the bottom plate.
5. Apply 75  $\mu\text{L}$  of the RNase-free DNase I solution prepared in Step 1 to each well of the 96-Well Filter Plate.  
**For Vacuum Manifold:** Apply vacuum for 30 seconds.  
**For Centrifugation:** Centrifuge the assembly at maximum speed or 3,000  $\times g$  (~3,000 RPM) for 30 seconds.
6. Incubate the assembly at 25 – 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to "**RNA Wash**" Step 2(i); B2 for **Vacuum Manifold** procedure or Step 2(ii); B2 for **Centrifugation** procedure.

## Technical Support

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

## Related Products

To see our full line of **Vantage™** microRNA analysis products visit our website at [www.origene.com](http://www.origene.com).