ORIGENE
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Vantage™ microRNA Purification Kit

Store at Room Temperature

Catalog No. NP100028 (11520-025) 25 Reactions

Overview and Intended Use

OriGene's *Van*tage™ microRNA Purification Kit provides a rapid method for the isolation and purification of Low Molecular Weight (LMW) RNA molecules (< 200 nt) from cultured animal cells, small tissue samples, bacterial cells, plants and blood. These LMW RNAs include regulatory RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA), as well as tRNA and 5S rRNA. Small RNA molecules are often studied due to their ability to regulate gene expression. miRNAs and siRNAs are typically 20-25 nucleotides long, and regulate gene expression by binding to mRNA molecules and affecting their stability or translation. The LMW RNA molecules isolated using OriGene's *Van*tage™ microRNA Purification Kit can be used in various downstream applications relating to gene regulation and functional analysis, including RT-PCR, Northern blotting and microarray and Luminex™ profiling assays.

Principle of Method

Purification is based on spin column chromatography using a proprietary resin as the separation matrix. The resin binds RNA in a manner that depends on ionic concentrations. The small RNA molecules are preferentially purified from other cellular components such as ribosomal RNA without the use of phenol or chloroform. The process involves the use of two different spin columns: the Large RNA Removal Column and the microRNA Enrichment Column. Briefly, the cells or tissues of interest are lysed using the provided Lysis Buffer. Ethanol is then added to the sample and if any precipitates are present the sample is centrifuged. The cleared lysate is then applied to the Large RNA Removal Column, and the larger RNA molecules will bind to the resin in the spin column while the smaller RNA species will pass through into the flow through. Ethanol is then added to the flow through, and the sample is applied to the microRNA Enrichment Column. The LMW RNA molecules will then bind to the resin, and any impurities are removed through a series of washes with the provided Wash Buffer. The LMW RNA molecules are then eluted using the RNA Elution Buffer, and are ready for use in various applications.

Important Information

READ ENTIRE PROTOCOL BEFORE USE

ADDITONAL 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.

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.

Components included with this kit:

Lysis Solution	35 ml
Wash Buffer	20 ml
RNA Elution Buffer	5 ml
Large RNA Removal Columns	25 columns
microRNA Enrichment Columns	25 columns
Wash Tubes	50 tubes
Recovery Tubes	50 tubes

Materials and equipment required but not supplied:
*Van*tage™ microRNA Purification Kit isolates and purifies LMW RNAs 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)
RNase-free microcentrifuge tubes

Table 1 - Sample Specific Materials and Equipment

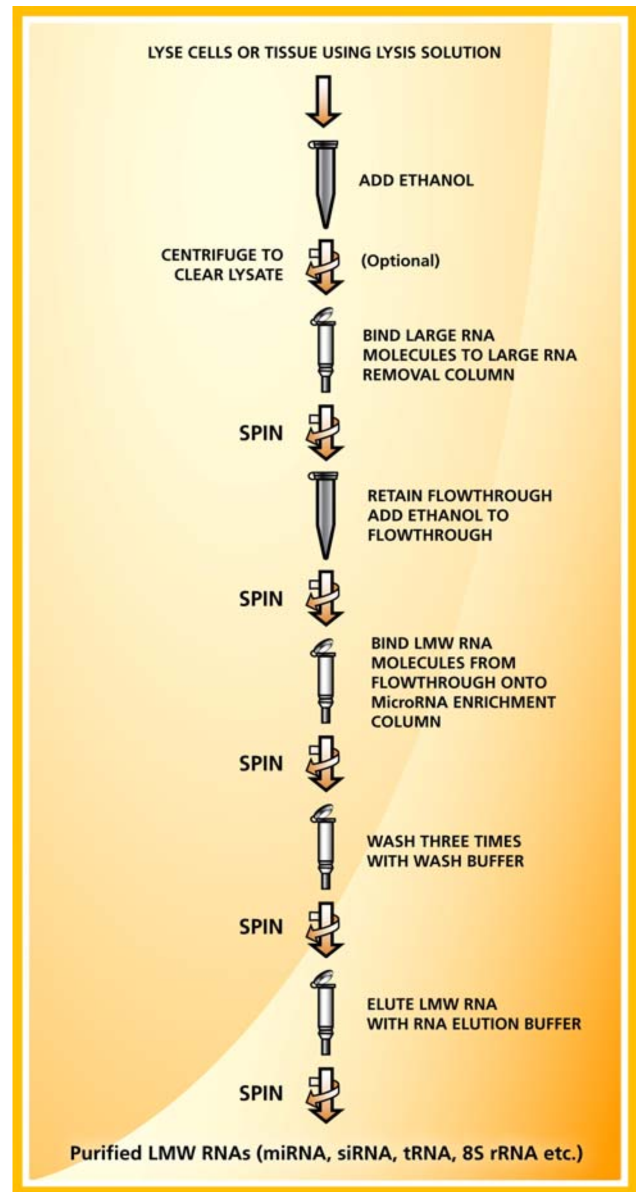
Specific Sample Protocols	Materials and equipment required but not supplied
Cultured Cells	PBS (RNase-free)
Animal Tissue	Liquid nitrogen Mortar and pestle 25 gauge needle and syringe
Bacteria	Lysozyme-containing TE Buffer: a. For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer b. For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer
Plant	Liquid nitrogen Mortar and pestle 70% ethanol

Important Equipment Information
All centrifugation steps are carried out in a benchtop microcentrifuge at room temp. Various speeds are required for different steps; check your microcentrifuge specifications to ensure that it is capable of the proper speeds. 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: *Van*tage™ microRNA Purification Kit isolates and purifies LMW RNAs 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. There are 2 different spin columns provided with this kit; the Large RNA Removal Column and the microRNA Enrichment Column. Ensure that the correct column is used for each step of the procedure.
5. 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.
6. Prepare an appropriate amount of Lysis Solution by adding 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
7. It is important to work quickly during this procedure.

Table 2

Specific Sample Type	Maximum Amount of Starting Material
Cultured Cells	3×10^6 cells
Animal Tissues	25 mg
Blood	100 μL
Bacteria	1×10^9 cells
Plant Tissues	50 mg

Preparation of Lysates from Cultured Cells
Usage Notes
1. The maximum recommended input of cells is 3×10^6. 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 10^6 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 300 μL 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 40 μL of 95 – 100% ethanol (provided by the end-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.**

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.
2. Carefully decant the supernatant. A few μL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
3. Add 300 μL 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.
4. Add 40 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.**

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 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 400 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 a volume of 95-100% ethanol (provided by the user) that is equivalent to 15% of the lysate volume (15 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 L 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 L of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
2. Add 250 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 40 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 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 x 10^9 cells/mL has an OD_{600} 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.
5. 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 *x 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 200 µL of **Lysis Solution** and vortex vigorously for at least 10 seconds.
4. Add 50 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 Bufffer	Incubation Time
Gram-negative	1 mg/mL	5 min
Gram-positive	3 mg/mL	10 min

Preparation of Lysates from Plant
Usage Notes
1. The maximum recommended input of plant tissue is 50 mg or 5 x 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.

F. Protocol for Preparing Cell Lysates from Plant
1. Transfer ≤50 mg of plant tissue or 5 x 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. Add 400 µL of **Lysis Solution** to the tissue sample and continue to grind until the sample has been homogenized.
3. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
4. 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.
5. Add a volume of 95-100% ethanol (provided by the user) that is equivalent to 15% of the lysate volume (15 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.**

Step 2. LMW RNA Purification from All Sample Type Lysates
IMPORTANT: The remaining steps of the procedure for the purification of LMW RNA are the same from this point forward for all the different types of Lysate prepared in Step 1.

A. Removal of Large RNAs
1. Assemble a **Large RNA Removal Column** with one of the provided **Wash Tubes**.
2. Apply the clarified lysate with the ethanol (from **Step 1**) onto the column and centrifuge for 1 minute. **Retain the flow through, which contains the LMW RNA species.**
If LMW RNA-depleted RNA is to be isolated, retain the column and proceed to the Optional "**Large RNA Purification Protocol" (Appendix A)**. Otherwise, discard the column.
3. Transfer the flow through to an RNase-free microcentrifuge tube (not provided).
IMPORTANT NOTE: The flow through contains the LMW RNA species, thus ensure that this fraction is not discarded.

B. Capture of LMW RNA to microRNA Enrichment Column
1. **For cultured cells, bacteria or blood**: Add 450 µL of 95-100% ethanol (provided by the user) to the flow through collected in **Step 2A3** above. Mix by vortexing for 10 seconds.
2. **For animal tissues or plant:** Based on the lysate volume determined in **Steps 1C7** or **1F4**, add 1.5 volumes of 95 – 100% ethanol (provided by the user) to the flow through collected in **Step 2A3** above (150 µL of ethanol is added to every 100 µL of flow through)**.** Mix by vortexing for 10 seconds.
3. Assemble a **microRNA Enrichment Column** with one of the provided **Wash Tubes**.
4. Apply half of the lysate mix with ethanol onto the **microRNA Enrichment Column** and centrifuge for 1 minute.
5. Discard the flow through and reassemble the **microRNA Enrichment Column** with the **Wash Tubes**.
6. Repeat steps **B4** and **B5** with the remaining lysate mix to complete the capture of the LMW RNAs.

C. Column Wash
1. Apply 400 µL of **Wash Buffer** to the **microRNA Enrichment 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 **microRNA Enrichment Column** with its **Wash Tube**.
3. Repeat column wash steps C**1** and C**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 **Wash Tube** with the flow through.

D. Elution of LMW RNA Species
1. Place the **microRNA Enrichment Column** into a fresh 1.7 mL **Recovery Tube** (provided).
2. Add 50 µL of **RNA Elution Buffer** to the column.
Note: For higher concentrations of LMW RNAs, a lower elution volume may be used. A minimum volume of 20 L is recommended
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 recovery of the LMW RNA species, it is recommended that a second elution be performed into a separate microcentrifuge tube by repeating D**2** and **D3**.

E. RNA Analysis
To determine the quality and quantity of the eluted LMW RNAs 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 RNA recovered in µgs using the formula:

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

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

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

F. 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.

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Appendix A

Protocol for Optional Purification of Large RNAs depleted of LMW RNA species

A. Column Wash

1. Reassemble the **Large RNA Removal Column** with the **Wash Tube** used in Step **2A**
2. Apply 400 µL of **Wash Buffer** to the **Large RNA Removal Column** and centrifuge for 1 minute.

Note: Ensure that the entire **Wash Buffer** has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

3. Discard the flow through and reassemble the **Large RNA Removal Column** with the **Wash Tube**.
4. Repeat steps A**2** and **A3** to wash the **Large RNA Removal Column** a second time.
5. Wash column a third time by adding another 400 µL of **Wash Buffer** and centrifuging for 2 minutes.
6. Ensure that the column is dry. Spin for an additional minute, if necessary.
7. Discard the **Wash Tube** with the flow through.

B. Elution of Large RNAs

1. Place the **Large RNA Removal Column** into a fresh 1.7 mL **Recovery Tube** provided with the kit.
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 volume 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 (Repeat **Steps B2 and B3**).

C. RNA Analysis

To determine the quality and quantity of the eluted LMW RNAs 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 µgs using the formula:

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

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

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

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.

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.

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