

Total RNA Purification 96-Well Kit (Deep Well Format)

Product # 24350, 24380

Please note that a more detailed protocol is available online at www.norgenbiotech.com

Component	Product # 24350 (192 preps)	Product # 24380 (576 preps)
Buffer RL	2 x 40 mL	350 mL
Wash Solution A	2 x 38 mL	1 x 74 mL 1 x 148 mL
Elution Solution A	2 x 20 mL	60 mL
96-Well Isolation Plate (Deep Well)	2	6
Adhesive Tape	4	12
96-Well Collection Plate (Deep Well)	2	6
96-Well Elution Plate (Deep Well)	2	6
Product Insert	1	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. The **Buffer RL** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. 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.

Procedures

Notes Prior to Use:

- **The steps for preparing the lysate are different depending on the starting material (Step 1). However, the subsequent steps are the same in all cases (Steps 2 – 6), with the exception of the protocol for plasma/serum. A separate protocol for the isolation of total RNA from plasma/serum samples is available on our website.**
- Prepare a working concentration of the **Wash Solution A** by adding:
 - 90 mL of 96 - 100% ethanol (provided by the user) to each of the bottles containing 38 mL of concentrated **Wash Solution A**. This will give a final volume of 128 mL.
 - 175 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 74 mL concentrated **Wash Solution A**. This will give a final volume of 249 mL.
 - 350 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 148 mL concentrated **Wash Solution A**. This will give a final volume of 498 mL.

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- **Optional:** The use of β -mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (ex: pancreas), as well as for most plant tissues. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 μ L of β -mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required. β -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Buffer RL can be used as provided

Section 1. Preparation of Lysate From Various Cell Types

1A. Lysate Preparation from Cultured Animal Cells

- The recommended input is 5×10^5 cells per well of the 96-Well Isolation Plate (Deep Well). However, up to 1×10^6 cells may be processed for most cell lines. A hemocytometer can be used in conjunction with a microscope to count the number of cells.
- 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.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet (**Step 1A(ii) c**).

1A(i). Cell Lysate Preparation from Cells Growing in a Monolayer (96-Well Plate or other Multi-Well Plate)

- a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- b. Add 300 μ L of **Buffer RL** directly to each well of the multi-well culture plate.
- c. Lyse cells by gently tapping culture plate and swirling buffer around plate surface for two minutes.
- d. Add 120 μ L of 96 – 100% ethanol (provided by the user) to each well. Mix by pipetting up and down a few times.

1A (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

- a. 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.
- b. Aspirate supernatant carefully to ensure that the pellets are not dislodged.
- c. Add 300 μ L of **Buffer RL** to each well.
- d. Lyse cells by gently tapping culture plate and swirling buffer around plate surface for two minutes.
- e. Add 120 μ L of 96 - 100% ethanol (provided by the user) to each well. Mix by pipetting up and down a few times.

1B. Lysate Preparation from Animal Tissues

- 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.
 - The optimal amount of non-fibrous tissue be used per well of the 96-Well Isolation Plate (Deep Well) 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
- a. Excise the tissue sample from the animal.
 - b. 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 Isolation Plate (Deep Well).
 - c. Transfer the tissue samples to appropriate vessels for the desired disruption method.
 - d. Add 350 μ L of **Buffer RL** to each tissue sample.

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

- e. 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.

- f. Add 120 μ L of 96 - 100% ethanol (provided by the user) to each tissue sample. Mix by pipetting up and down a few times.

1C. Lysate Preparation from Blood

- This procedure is for the isolation of RNA from whole blood. **For the isolation of RNA from plasma or serum samples, please see Appendix B.**
 - 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.
 - It is recommended that no more than 100 μ L of blood be used per well of the 96-Well Isolation Plate (Deep Well) in order to prevent clogging of the plate.
 - We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.
- a. Transfer up to 100 μ L of non-coagulating blood to each well in an RNase-free 96-well microplate (not provided)
 - b. Add 200 μ L of **Buffer RL**. Lyse cells by gently tapping the 96-well microplate and swirling buffer around plate surface for two minutes
 - c. Add 120 μ L of 96 - 100% ethanol (provided by the user) to each well. Mix by pipetting up and down a few times.

1D. Lysate Preparation from Bacteria

- 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.
- It is recommended that no more than 10^9 bacterial cells be used per well for 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.
- For RNA isolation, bacteria should be harvested in log-phase growth.
- 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 1Dc**).

- Pellet bacteria by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute for culture collected in 1.5 mL microfuge tubes or 3000 x g (~3,000 RPM) for 5 minutes for culture in a 96-well microplate.
- Carefully remove media by aspiration.
- Resuspend each bacterial pellet thoroughly in 75 μ L of the appropriate lysozyme-containing TE buffer (see Table 1). Incubate at room temperature for the time indicated in Table 1.
- Add 225 μ L of **Buffer RL** to each bacteria sample. Mix by pipetting up and down a few times.
- Add 120 μ L of 96 – 100% ethanol (provided by the user) to lysate. Mix by pipetting up and down a few times.

Table 1: Incubation Time for Different Bacterial Strains

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

1E. Lysate Preparation from Yeast

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 75 μ L of buffer is required for each preparation. The Resuspension Buffer should have the following composition: 50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbital, 0.1% β -mercaptoethanol and 1 unit/ μ L Lyticase. This solution should be prepared with sterile, RNase-free reagents, and kept on ice until needed. These reagents are to be provided by the user.
 - It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used per well of the 96-Well Isolation Plate (Deep Well)
 - For RNA isolation, yeast should be harvested in log-phase growth.
 - Yeast can be stored at -70°C for later use, or used directly in this procedure.
 - 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 1Ec**).
- Pellet yeast by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute for culture collected in 1.5 mL microfuge tubes or 3000 x g (~3,000 RPM) for 5 minutes for culture in a 96-well microplate.
 - Carefully remove media by aspiration.
 - Resuspend the yeast pellets thoroughly in 75 μ L of Lyticase-containing Resuspension Buffer. Incubate at 37°C for 10 minutes.
 - Add 225 μ L of **Buffer RL** to each yeast sample. Mix by pipetting up and down a few times.
 - Add 120 μ L of 96 – 100% ethanol (provided by the user) to the lysate. Mix by pipetting up and down a few times.

1F. Lysate Preparation from Fungi

- 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.
 - It is recommended that no more than 40 mg of fungi be used per well of the 96-Well Isolation Plate (Deep Well) to prevent clogging of the plate.
- Excise the tissue sample from the fungus.
 - Determine the amount of tissue by weighing. It is recommended that no more than 50 mg of tissue be used per well of the 96-Well Isolation Plate (Deep Well).
 - Transfer the tissue samples to appropriate vessels for the desired disruption method.
 - Add 350 μ L of **Buffer RL** to each tissue sample.
- Note:** Ensure that frozen tissues do not thaw during weighing or prior to the addition of Buffer RL. For maximum RNA recovery, homogenize frozen tissues to fine powder in liquid nitrogen prior to the addition of **Buffer RL**.
- Homogenize the tissues using the appropriate cell disruption tool.
 - Centrifuge the lysate at maximum speed for 2 minutes in an appropriate centrifuge. Transfer the supernatant to a new 96-well microplate.
 - Add 120 μ L of 96 - 100% ethanol (provided by the user) to each lysate sample. Mix by pipetting up and down a few times.

1G. Lysate Preparation from Plant

- The maximum recommended input of plant tissue is 40 mg or 5×10^6 plant cells per well of the 96-Well Isolation Plate (Deep Well).
- 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.
- It is important to work quickly during this procedure.

- a. Excise the tissue sample from the plant.
- b. Determine the amount of tissue by weighing. It is recommended that no more than 40 mg of tissue be used per well of the 96-Well Isolation Plate (Deep Well).
- c. Transfer the tissue samples to appropriate vessels for the desired disruption method.
- d. Add 350 μL of **Buffer RL** to each tissue sample.

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

- e. Homogenize the tissues using the appropriate cell disruption tool.
- f. Centrifuge the lysate at maximum speed for 2 minutes in an appropriate centrifuge. Transfer the supernatant to a new 96-well microplate.
- g. Add 120 μL of 96 - 100% ethanol (provided by the user). Mix by pipetting up and down a few times.

Section 2. Total RNA Purification from All Types of Lysate

Note: 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 Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B

A. Total RNA Purification from All Types of Lysate Using Vacuum Manifold

Note: The remaining steps of the procedure for the purification of total RNA using a vacuum manifold are the same from this point forward for all the different types of lysate.

NOTE: The vacuum pressure must not exceed 350 mbar. Very high vacuum pressure may result in a low RNA yield.

2. Binding RNA to 96-Well Isolation Plate (Deep Well)

- a. Assemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold according to manufacturer's recommendations.

Note: The provided 96-Well Collection Plate (Deep Well) can be used as the collection/waste tray if desired.

- b. Apply up to 500 μL of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Isolation Plate (Deep Well). 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.

- c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) 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:

Norgen's Total RNA Purification 96-Well Kit (Deep Well Format) isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Plate 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.

3. RNA Wash

- a. Apply 400 μL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). 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 A has passed through into the collection/waste tray by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

- b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
- c. Reassemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a second time.
- d. Reassemble the 96-Well Isolation Plate (Deep Well) and vacuum manifold. Repeat steps **3a** and **3b** to wash column for a third time.
- e. Pat the bottom of the 96-Well Isolation Plate (Deep Well) dry. Reassemble the 96-Well Isolation Plate (Deep Well) and the vacuum manifold. Apply vacuum for an additional 15 minutes in order to completely dry the plate.
- f. Turn off vacuum and ventilate the manifold.

4. RNA Elution

- a. Replace the collection/waste tray in the vacuum manifold with the provided 96-Well Elution Plate (Deep Well). Complete the vacuum manifold assembly with the 96-Well Isolation Plate (Deep Well).
- b. Add 75 μL of **Elution Solution A** to each well of the plate.

- c. Apply vacuum for 5 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the 96-Well Elution Plate (Deep Well). The purified RNA samples may be stored at -20°C for a few days. It is recommended that samples be placed at -70°C for long term storage.

B. Total RNA Purification from All Types of Lysate Using Centrifugation

Note: The remaining steps of the procedure for the purification of total RNA using centrifugation are the same from this point forward for all the different types of lysate.

2. Binding RNA to 96-Well Isolation Plate (Deep Well)

- a. Place the 96-Well Isolation Plate on top of a provided 96-Well Collection Plate (Deep Well).
- b. Apply up to 500 μL of the lysate with the ethanol (from **Step 1**) into each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 $\times g$ ($\sim 3,000$ 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

- c. Discard the flowthrough. Reassemble the the 96-Well Isolation Plate (Deep Well) 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:

Norgen's Total RNA Purification 96-Well Kit (Deep Well Format) isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Plate 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

3. RNA Wash

- a. Apply 400 μL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 $\times g$ ($\sim 3,000$ RPM) for 2 minutes.

Note: Ensure the entire Wash Solution A has passed through into the bottom plate by inspecting the 96-Well Isolation Plate (Deep Well). If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

- b. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate.
- c. Repeat steps **3a** and **3b** to wash column for a second time.
- d. Repeat steps **3a** and **3b** to wash column for a third time.
- e. Pat the bottom of the 96-Well Isolation Plate (Deep Well) dry. Reassemble the 96-Well Isolation Plate (Deep Well) and the bottom plate. Centrifuge the assembly at maximum speed or 3,000 $\times g$ ($\sim 3,000$ RPM) for 15 minutes in order to completely dry the plate.

4. RNA Elution

- a. Stack the 96-Well Isolation Plate on top of the 96-Well Elution Plate (Deep Well).
- b. Add 75 μL of **Elution Solution A** to each well of the 96-Well Isolation Plate (Deep Well).
- c. Centrifuge the assembly at maximum speed or 3,000 $\times g$ ($\sim 3,000$ RPM) for 5 minutes.

5. Storage of RNA

Use the provided adhesive tape to seal the 96-Well Elution Plate. 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

Norgen's Total RNA Purification 96-Well Kit (Deep Well Format) 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 Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 10 μL of **DNase I** and 65 μL of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/ μL RNase-free DNase I solution according to the manufacturer's instructions. A 75 μL aliquot is required for each column to be treated.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding RNA to 96-Well Isolation Plate (Deep Well)**" (Steps 1 and 2 of all protocols).
3. **For Vacuum Manifold:** Apply 400 μL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). 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 μL of **Wash Solution A** to each well of the 96-Well Isolation Plate (Deep Well). Centrifuge the assembly at maximum speed or 3,000 $\times g$ (~3,000 RPM) for 2 minutes.

4. Discard the flowthrough. Reassemble the 96-Well Isolation Plate (Deep Well) with the vacuum manifold or the bottom plate.
5. Apply 75 μL of the RNase-free DNase I solution prepared in Step 1 to each well of the 96-Well Isolation Plate (Deep Well).

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. After the centrifugation or vacuum in Step 5, pipette the flowthrough that is present in the collection plate back onto the top of the column.

Note: Ensure Step 6 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species

7. Incubate the assembly at 25 - 30°C for 15 minutes.
8. Without any further centrifugation, proceed directly to “RNA Wash” Section 2A, Step 3b for **Vacuum Manifold** procedure or Section 2B, Step 3c for **Centrifugation** procedure.

Appendix B

Protocol for Total RNA Purification from Plasma or Serum

Notes Prior to Use

- Plasma or serum 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 plasma or serum.
- We recommend the use of this kit to isolate RNA from plasma or serum prepared by standard protocol from non-coagulating fresh blood using EDTA or sodium citrate as the anti-coagulant.
- It is recommended that no more than 150 μL of plasma or serum be used in order to prevent clogging of the column.
- Avoid multiple freeze-thaw cycle of the plasma or serum sample. Aliquot to the appropriate volume for usage prior to freezing.
- **Substitute the provided Wash Solution A with 96 – 100 % Ethanol (User provided).**
- The yield of RNA from plasma and serum is highly variable. In general, the expected yield could vary from 1 to 100 ng per 100 μL plasma or serum used. In addition, the expected A260:A280 ratio as well as the A260:A230 ratio will be lower (<1.80) than the normal acceptable range from other cells or tissues. Nonetheless, these isolated RNA could still be used effectively in different downstream applications such as RT-qPCR or microarrays.

1. Cell Lysate Preparation from Plasma/Serum

- a. Transfer up to 150 μL of plasma or serum to an RNase-free microcentrifuge tube or an RNase-free Deep-Well 96-well microplate (not provided).
- b. Add 250 μL of Buffer RL to every 100 μL of plasma or serum. Mix by vortexing for 10 seconds.
- c. **(Optional):** Add 0.7 μL of 0.8 $\mu\text{g}/\mu\text{l}$ MS2 RNA per sample.

Note: The addition of MS2 RNA could increase the consistency of RNA isolation

- d. Add 350 μL of 96 – 100% ethanol (provided by the user) to every 350 μL of the lysate (equivalent to every 100 μL plasma or serum used). Mix by agitation or by pipetting up and down a few times. Proceed to Step 2 below.

2. Purification of RNA using 96-Well Isolation Plate (Deep Well)

Proceed to Section 2 for Total RNA Purification using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B. **For both protocols, use 96 – 100 % Ethanol (provided by the user) instead of the provided Wash Solution A for the Wash Step.**

**** NOTE:** For higher recovery of small RNA species using the centrifugation protocol, a lower centrifugation speed of ~1500 $\times g$ (~2000 RPM) for the RNA Binding Step is recommended.

Optional Step:

Norgen's Total RNA Purification 96-Well Kit (Deep Well Format) 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. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol. **Use 96 – 100 % Ethanol (provided by the user) instead of the provided Wash Solution A for the Wash Step.**

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website or through email at techsupport@norgenbiotech.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotech.com/patents