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Total RNA Purification 96-Well Kit — Product Insert

Product # 24300

Norgen's Total RNA Purification 96-Well Kit provides a rapid method for the high-throughput isolation and purification of total RNA from cultured animal cells, tissue samples, blood, plasma, serum, 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, RNase protection and primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on 96-well column chromatography using Norgen's 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 purification could be performed on either a vacuum manifold or using centrifugation. The process involves first lysing the cells or tissue of interest with the provided Buffer RL (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto the 96-Well Filter Plate. Norgen'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 flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Binding Capacity Per Well	50 µg
Maximum Loading Volume Per Well	500 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Animal Tissues Blood Plasma/Serum Bacteria Yeast Fungi Plant Tissues	 1 x 10^6 cells 10 mg 100 µL 150 µL 1 x 10^9 cells 1 x 10^8 cells 40 mg 40 mg
Time to Complete 96 Purifications	30 minutes
Average Yields* HeLa Cells (1 x 10^6 cells) *E. coli* (1 x 10^9cells)	 15 µg 50 µg

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

Advantages

- Fast and easy processing using either a vacuum manifold or centrifugation
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of sources
- RNA can be isolated and detected from as little as a single animal cell

Kit Components

Component	Product # 24300 (192 preps)
Buffer RL	2 x 40 mL
Wash Solution A	2 x 38 mL
Elution Solution A	2 x 20 mL
96-Well Filter Plate	2
Adhesive Tape	4
96-Well Collection Plate	2
96-Well Elution Plate	2
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

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. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

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.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Total RNA Purification 96-Well Kit:

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)
- 96 - 100% ethanol
- β-mercaptoethanol (optional)
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (single or 96-well format) for centrifugation. Two 96-Well Collection Plates are provided with the kit.

For Animal Cell Protocol

- PBS (RNase-free)

For Animal Tissue Protocol

- Liquid nitrogen
- Cell Disruption Tool such as mortar and pestle, rotor-stator homogenizer or bead mills

For Bacterial Protocol

- Lysozyme-containing TE Buffer:
    - For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer
    - For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

For Yeast Protocol

- Resuspension Buffer with Lyticase:
    - 50 mM Tris pH 7.5
    - 10 mM EDTA
    - 1 M Sorbital
    - 1 unit/μL Lyticase

For Fungi Protocol

- Liquid nitrogen
- Cell Disruption Tool such as mortar and pestle, rotor-stator homogenizer or bead mills

For Plant Protocol

- Liquid nitrogen
- Cell Disruption Tool such as mortar and pestle, rotor-stator homogenizer or bead mills

For Plasma/Serum Protocol

- MS2 RNA (0.8 µg/µl). (Roche, Cat. No. 10165948001)

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.

- The RNA area should be located away from microbiological work stations
- 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
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- 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
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

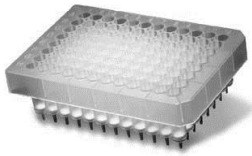
Flowchart

Procedure for Purifying Total RNA using Norgen's Total RNA Purification 96-Well Kit

Sample of cells or tissues

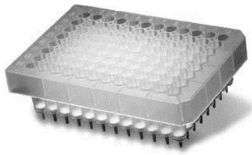
Lyse cells or tissues with Buffer RL

Add ethanol



Bind RNA

Wash three times with Wash Solution A



Elute RNA with Elution Solution A

Purified Total RNA

Procedures

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

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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.

Section 1. Preparation of Lysate From Various Cell Types

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 located in Appendix B*.**
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed, as indicated in the table below:

Sample Type	Lysate Preparation Page #
Cultured Cells	6
Animal Tissue	6
Blood	7
Plasma/Serum	14
Bacteria	7
Yeast	8
Fungi	9
Plant	9

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottles containing the concentrated **Wash Solution A**. This will give a final volume of 128 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 µL 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.
- It is important to work quickly during this procedure.

1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- The recommended input is 5 x 10^5 cells per well of the 96-Well Filter Plate. However, up to 1 x 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 µL 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 µL 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 µL 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 µL 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

Notes Prior to Use

- 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, particularly the Cell Lysate Preparation step.
- 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 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

1B. Cell Lysate Preparation from Animal Tissues

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 Filter Plate.
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.

Note: Thorough homogenization is required for optimal performance. For tissue inputs of ≤ 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 µL 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

Notes Prior to Use

- 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 µL of blood be used per well of the 96-Well Filter Plate 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.
- It is important to work quickly during this procedure.

1C. Cell Lysate Preparation from Blood

a. Transfer up to 100 µL of non-coagulating blood to each well in an RNase-free 96-well microplate (not provided)
b. Add 200 µL of **Buffer RL**. Lyse cells by gently tapping the 96-well microplate and swirling buffer around plate surface for two minutes
c. Add 120 µL 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

Notes Prior to Use

- 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 x 10^9 cells/mL has an OD_{600} 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)**.

1D. Cell Lysate Preparation from Bacteria

a. 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.
b. Carefully remove media by aspiration.
c. Resuspend each bacterial pellet thoroughly in 75 µL of the appropriate lysozyme-containing TE buffer (see Table 1). Incubate at room temperature for the time indicated in Table 1.
d. Add 225 µL of **Buffer RL** to each bacteria sample. Mix by pipetting up and down a few times.
e. Add 120 µL 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 Bufffer	Incubation Time
Gram-negative	1 mg/mL	5 min
Gram-positive	3 mg/mL	10 min

1E. Lysate Preparation from Yeast

Notes Prior to Use

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 75 µL 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/µL 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 Filter Plate
- 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)**.

1E. Cell Lysate Preparation

a. 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.
b. Carefully remove media by aspiration.

c. Resuspend the yeast pellets thoroughly in 75 µL of Lyticase-containing Resuspension Buffer. Incubate at 37°C for 10 minutes.
d. Add 225 µL of **Buffer RL** to each yeast sample. Mix by pipetting up and down a few times.
e. Add 120 µL 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

Notes Prior to Use

- 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 Filter Plate to prevent clogging of the plate.

1F. Cell Lysate Preparation from Fungi

a. Excise the tissue sample from the fungus.
b. 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 Filter Plate.
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) to each lysate sample. Mix by pipetting up and down a few times.

1G. Lysate Preparation from Plant

Notes Prior to Use

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

1G. Cell Lysate Preparation from Plant

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 Filter Plate.

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.

2. Binding RNA to 96-Well Filter Plate

a. Assemble the 96-Well Filter Plate and the vacuum manifold according to manufacturer's recommendations.

Note: The provided 96-Well Collection Plate 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 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.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. 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:

Norgen's Total RNA Purification 96-Well Kit 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 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 A 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.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a second time.
d. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a third time.
e. 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.
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. Complete the vacuum manifold assembly with the 96-Well Filter Plate.
b. Add 75 µL of **Elution Solution A** to each well of the plate.
c. Apply vacuum for 2 minutes.

5. Storage of RNA

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

a. Place the 96-Well Filter Plate on top of a provided 96-Well Collection Plate.
b. Apply up to 500 µL 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 *x 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

c. Discard the flowthrough. Reassemble the 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:

Norgen's Total RNA Purification 96-Well Kit 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 Filter Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~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 Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

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

4. RNA Elution

a. Stack the 96-Well Filter Plate on top of the 96-Well Elution Plate.
b. Add 75 µL of **Elution Solution A** to each well of the 96-Well Filter Plate.
c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 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 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 Filter Plate**" (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 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 µL of **Wash Solution A** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

4. Discard the flowthrough. Reassemble the 96-Well Filter Plate 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 Filter Plate.

   For Vacuum Manifold**:** Apply vacuum for 30 seconds.
   For Centrifugation**:** Centrifuge the assembly at maximum speed or 3,000 *x 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^{o}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).***
- It is important to work quickly during this procedure.
- 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 µg/µl 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 Filter Plate

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 x g (~2000 RPM) for the RNA Binding Step is recommended.**

Optional Step:

Norgen's 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. 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.***

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Wells of the plate have become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the wells of the plate show clogging below the recommended levels. See also "Clogged Wells in Plate" below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the wells of the plate.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure that the cell monolayer is washed with the appropriate amount of PBS in order to remove residual media from cells.
	Yeast: Lyticase was not added to the Resuspension Buffer	Ensure that the appropriate amount of Lyticase is added when making the Resuspension Buffer.
	Bacteria and Yeast: All traces of media not removed	Ensure that all media is removed prior to the addition of the Buffer RL through aspiration.
Clogged Wells in Plate	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications

Problem	Possible Cause	Solution and Explanation
Clogged Wells in Plate	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to isolation in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Buffer RL.
	Lysozyme or lyticase used may not be RNAse-free	Ensure that the lysozyme and lyticase being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the plate is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry vacuum or dry spin under the RNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000
Cytoplasmic & Nuclear RNA Purification Kit	21000
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

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 (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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

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