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# Total RNA Purification 96-Well Kit (Magnetic Bead System)
## Product Insert

**Product #75500**

Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) offers a fast, reliable and high throughput method for isolating and purifying total RNA from a wide range of sample types, including cultured animal cells, tissue samples, blood, plasma, serum, bacteria, yeast, fungi, plants, and viruses. The kit efficiently purifies RNA of all sizes, from large mRNA and ribosomal RNA to microRNA (miRNA) and small interfering RNA (siRNA). The purification process selectively isolates RNA from other cellular components, such as proteins, without requiring phenol or chloroform. The resulting RNA is of exceptional integrity and is suitable for various downstream applications, including real-time PCR, reverse transcription PCR, next-generation sequencing (NGS), Northern blotting, RNase protection assays, primer extension, and expression array analyses.

### Norgen's Purification Technology

Purification relies on magnetic beads that selectively bind RNA under optimized conditions. The process begins with lysing cells or tissues using Buffer RL. Isopropanol is added to the lysate, enabling RNA to bind to the magnetic beads based on ionic concentrations. The binding chemistry ensures only RNA attaches to the beads, while contaminating proteins are removed in the supernatant. The bound RNA is then washed with Wash Solution A to eliminate residual impurities, followed by elution with Elution Solution A. The resulting RNA is highly pure and suitable for various downstream applications.

### Kit Components

| Component | Product # (192 samples) |
|---|---|
| Buffer RL | 4 x 40 mL<br>1 x 30 mL |
| Magnetic Beads C | 8.5 mL |
| Wash Solution A | 3 x 38 mL |
| Elution Solution A | 1 x 30 mL |
| 96-Well Plate | 2 |
| 96-Well Elution Plate | 2 |
| Adhesive Tape | 2 |
| Product Insert | 1 |

### Advantages

- Fast, reproducible and easy processing of samples.
- Isolates total RNA including microRNAs.
- Isolates RNA from a variety of microbial samples (Bacteria, Viruses, Yeast).
- Isolates RNA from cells and a variety of tissues (Lung, Heart, Spleen).
- Tested on a variety of bodily fluids (Blood, Plasma, Saliva, Swabs).
- Magnetic Bead protocol can easily be adapted on a variety of automation systems (IsoPure™, KingFisher, Hamilton Star/Vantage, and Tecan)
- Purified RNA is suitable for a variety of downstream applications, including qPCR or NGS sequencing.

**Specifications**

| Kit Specifications | |
|---|---|
| Number of Preps | 192 |
| Size of Purified RNA | All sizes including Small RNA (<200 nt) |
| Maximum Amount of Starting Material:<br>Animal Cells<br>Animal Tissues<br>Blood<br>Plasma/Serum<br>Bacteria<br>Yeast<br>Fungi<br>Plant Tissues<br>Virus | <br>3 x $10^6$ cells<br>10 mg (for most tissues*)<br>100 µL<br>200 µL<br>1 x $10^9$ cells<br>1 x $10^8$ cells<br>50 mg<br>50 mg or ≤5x$10^6$ plant cells<br>100 µL viral suspension |
| Average Yield * | 4 – 20 µg |
| Average Purity (OD260/280) | 1.8 – 2.0 |
| Time to Complete 96 Purifications (automated) | 15 minutes (hands-on time) |
| Time to Complete 96 Purifications (manual) | 60 minutes (hands-on time) |

* Average RNA yield will vary depending on the sample type

**Storage Conditions and Product Stability**

All solutions should be kept tightly sealed and stored at room temperature. These kits are 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. For more information, please consult the appropriate Safety Data Sheets (SDS). 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**

*For All Protocols*

- Magnetic Bead 96-Well Separation Plate (For manual isolation)
- IsoPure™ 96 or IsoPure™ Mini Purification System (For automated isolation)
- Micropipettors
- Microcentrifuge tube
- β-mercaptoethanol (optional)
- Isopropanol

- Temperature adjustable (50ºC) incubator
- Nuclease-free water
- DNase I (optional)
- Phosphate Buffered Saline (PBS) – (for Cultured Animal Cells protocol)
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer - (for Animal Tissue protocol)
- Syringe with a 22G needle - (for Animal Tissue protocol)
- Orbital Shaker

*For Animal Cell Protocol*

- PBS (RNase-free)

*For Animal Tissue Protocol*

- Liquid nitrogen
- Mortar and pestle

*For Nasal or Throat Swabs*

- Sterile, single-use cotton swabs

*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/L Lyticase

*For Fungi Protocol*

- Liquid nitrogen
- Mortar and pestle

*For Plant Protocol*

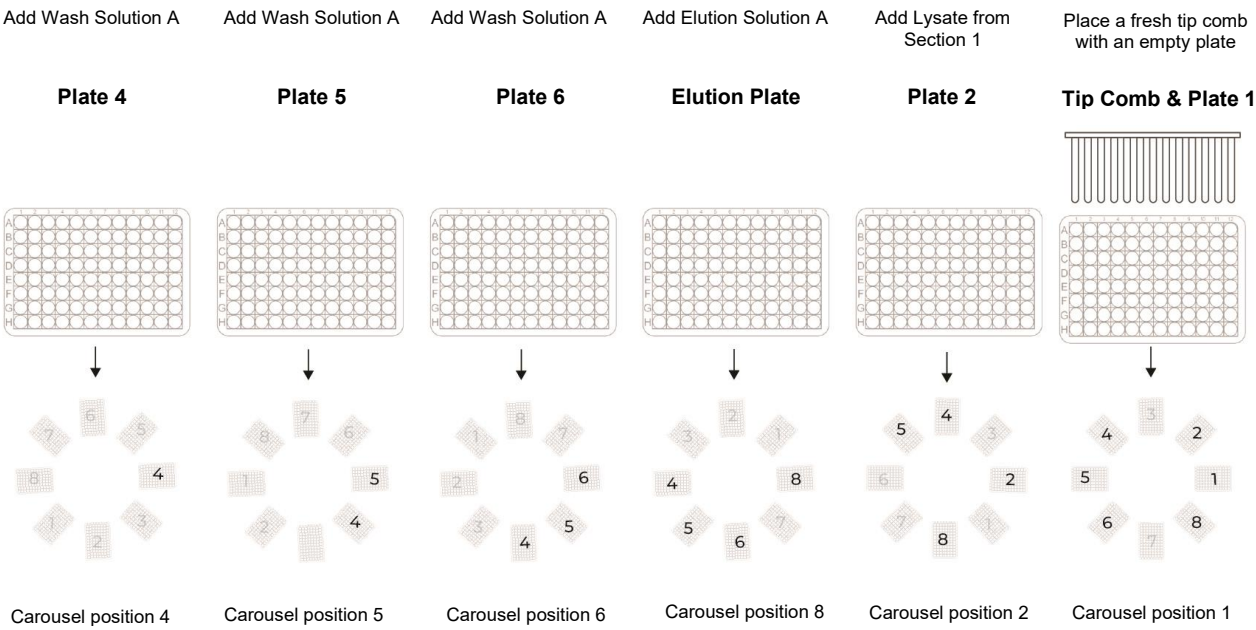
- Liquid nitrogen
- Mortar and pestle

*For Plasma/Serum Protocol*

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

## Flow Chart

Procedure for using Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) –IsoPure™ 96




## Run Instrument

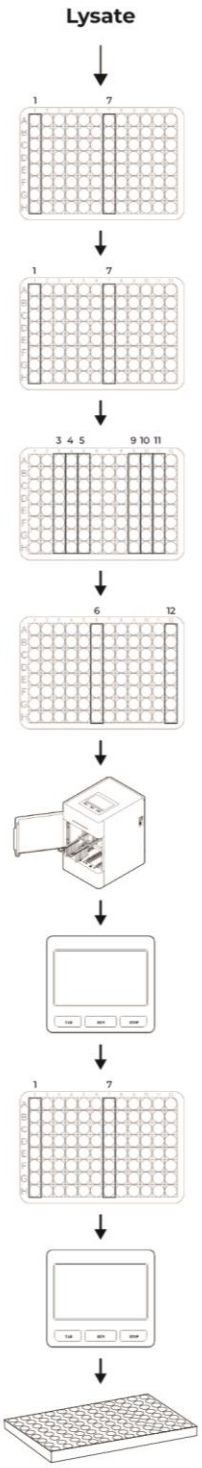
Add Isopropanol & bead mix to Plate 2 when prompted

Press "OK" after placing plate 2 back at position 2

Run Complete. Take Elution plate and store RNA.

## Flow Chart

Procedure for using Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) –IsoPure™ Mini




Add Lysate

Add Isopropanol and Magnetic Beads C

Add Wash Solution A

Add Elution Solution A

Install fresh Tip Comb

Run Program "TOTRNA16"

Add Isopropanol and Bead Mix

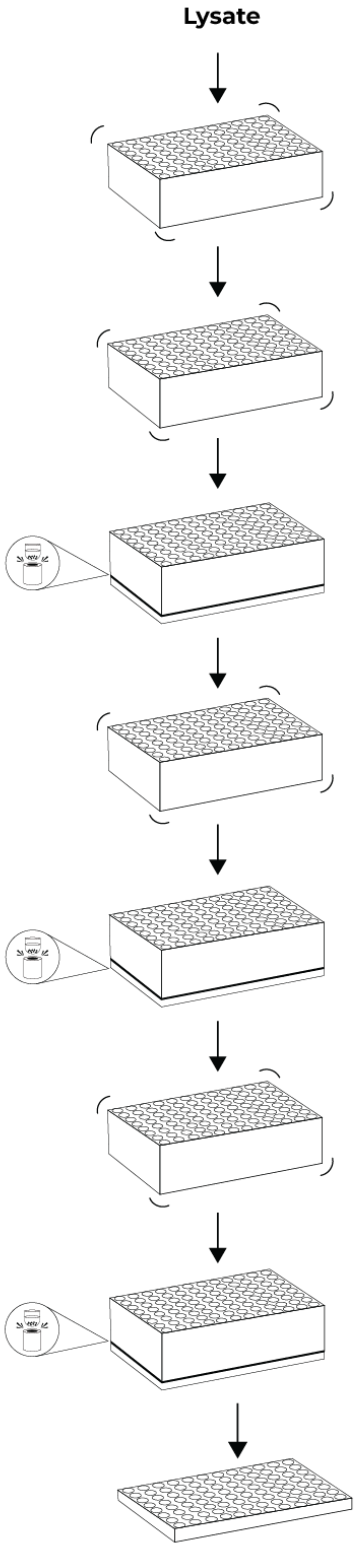
Press "OK"

Transfer RNA to fresh Plate

**Flow Chart**

## Flow Chart

Procedure for Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) - Manual Method




Add Lysate and mix.

Add Isopropanol and Magnetic Bead C and mix.

Place Plate on magnetic rack.
Discard supernatant.

Add Wash Solution A and mix.

Place Plate on magnetic rack.
Discard supernatant.
Repeat the Wash A step, 2 more times.

Add Elution Buffer A. Incubate at 50°C for 2 minutes.

Place Plate on magnetic rack.

Transfer RNA to a fresh Plate.

# Procedures

### Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware are 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 workstations.
- 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.

***Notes Prior to Use:***

- The procedure provides information and steps to follow for extraction of Total RNA using an Automated as well as a Manual method.
- In each method, the steps for preparing the lysate are different depending on the starting material **(Section 1)**. However, the subsequent steps from all lysate types for automation as well as manual extraction are mentioned in **Section 2**.
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Wash Solution A** by adding:
  a) 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.
- The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- **Always** vortex the **RNA Magnetic Bead C** before use.
- ***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 and nasal and throat swabs. 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.
- Preheat the incubator(s) according to the temperatures required (50ºC). For automation procedures, the automation platform that is being used may have a heat block to provide appropriate temperatures for incubation and thus an incubator might not be required.

## Total RNA Isolation using Automation and Manual Method

This section outlines step-by-step instructions for extracting Total RNA from various sample types using both automated and manual methods. The automation method provides guidance for using IsoPure™ instruments, but the protocols can also be easily adapted for other magnetic bead-based automation platforms, such as KingFisher, Hamilton, or Tecan.

**Section 1** details the preparation of lysates from various sample types, applicable to both automated and manual RNA extraction methods.
**Section 2** is divided into multiple subsections, offering detailed instructions for extracting RNA from the lysates prepared in **Section 1**, using either automated systems or manual procedures.

To prepare for Total RNA extraction using automated systems, please follow the steps outlined below.

**Using the IsoPure™ Mini or IsoPure™ 96 Automation System**
The procedure outlined below is for extraction of Total RNA using Automation platforms IsoPure™ 96 (Cat. AP1096) and IsoPure™ Mini (Cat. AP1016).

**Set-up the IsoPure™ Plates**

***Notes Prior to Use***

- The numbering of plates is based on where it should be placed on the carousel in the instrument.
- IsoPure™ 96 uses multiple plates for RNA extraction while IsoPure™ Mini uses single plate for RNA extraction.
- For IsoPure™ 96 most of the lysate preparation is performed in plate 2 while other plates are used for performing washing and elution steps.
- For IsoPure™ Mini the lysate preparation is performed in column 1 and/or 7 while washing and elutions steps could be performed in other columns of the same plate.
- For IsoPure™ 96, RNA elution step is always performed in plate 8.
- Prepare the plates and label them based on the sample type as mentioned in the tables below.

**Table 1. Set-up for Total RNA extraction from All Sample Types**

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2**.

| Component | IsoPure™ Mini | IsoPure™ 96 | Location on Carousel | Amount |
|---|---|---|---|---|
| **Tip Comb** | In the instrument | Plate 1 | 1 | Empty Plate |
| **Lysate** | Column 1 / Column 7 | Plate 2 | 2 | Variable |
| **Wash Solution A** | Column 3 / Column 9, Column 4 / Column 10 and Column 5 / Column 11 | Plate 4, Plate 5, & Plate 6 | 4, 5 & 6 | 600 µL |
| **Elution Solution A** | Column 6 / Column 12 | Elution Plate | 8 | 50 – 100 µL |

Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **DNA Removal Protocol** is provided 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. Prepare a DNase working solution as mentioned below when using DNase treatment with the extraction.

i. For every DNase reaction to be performed, prepare a **DNase I Mix** of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer A** 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/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each sample to be treated. Set-up the plates as per **Table 2** when performing DNase treatment of the samples.

**Table 2. Set-up for Total RNA extraction from All Sample Types – with DNase Treatment**

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

| Component | IsoPure™ Mini | IsoPure™ 96 | Location on Carousel | Amount |
|---|---|---|---|---|
| **Tip Comb** | In the instrument | Plate 1 | 1 | Empty Plate |
| **Lysate** | Column 1 / Column 7 | Plate 2 | 2 | Variable |
| **DNase I Mix** | Column 2 / Column 8 | Plate 3 | 3 | 100 µL |
| **Wash Solution A** | Column 3 / Column 9, Column 4 / Column 10 and Column 5 / Column 11 | Plate 4, Plate 5, & Plate 6 | 4, 5 & 6 | 600 µL |
| **Elution Solution A** | Column 6 / Column 12 | Elution Plate | 8 | 50 – 100 µL |

## Section 1. Lysate Preparation from Various Sample Types

***Notes Prior to Use***

- Fresh or frozen tissues may be used for the procedure.
- Tissues should be immediately frozen and stored at -20°C or -70°C. Tissues may be stored at -70°C for several months.
- It is recommended that no more than 20 mg of tissue be used.

## 1A. Lysate Preparation from Cultured Animal Cells

***Notes Prior to Use***

- Cells grown in suspension or monolayer may be used.
- The maximum recommended input of cells is 3 x $10^6$. 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.
- 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 cell pellets should not be subjected to repeated cycles of freeze and thaw prior to beginning the protocol.
- 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**

a) Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
b) Add 350 µL of **Buffer RL** directly to culture plate.
c) Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
d) Transfer lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
e) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

**Note:** For input amounts greater than $10^6$ cells, it is recommended that the lysate is passed through a 25-gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading into the well.

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

a) 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.
b) 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.
c) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d) Transfer lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
e) Proceed to **Section 2:** RNA Isolation from all Types of Lysates.

**Note:** For input amounts greater than $10^6$ cells, it is recommended that the lysate is passed through a 25-gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading into the well.

If the volume of cell suspension is 1 mL or less, **steps a – c** can be performed in **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit**.** Make sure to seal the plate with an Adhesive Film. In this case, proceed to **Section 2** directly after performing **step c**.

## 1B. Lysate Preparation from Animal Tissues

***Notes Prior to Use***

- Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) is designed for isolating RNA from small amount of tissue sample (up to 10 mg in most cases). If a larger amount of starting material is desired, Norgen's Animal Tissue RNA Purification Kit (Cat. #25700) should be used.
- 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 grinding with the mortar and pestle.
- Tissues stored in RNA stabilization reagents such as RNAlater® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid.

- The maximum recommended input of tissue varies depending on the type of tissue being used. Please refer to **Table 3** below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below, we recommend starting with an input of no more than 10 mg.

**Table 3. Recommended Maximum Input Amounts of Different Tissues**

| Tissue | Maximum Input Amount |
|---|---|
| Brain | 25 mg |
| Heart | 5 mg |
| Kidney | 10 mg |
| Liver | 10 mg |
| Lung | 10 mg |
| Spleen | 10 mg |

**1B. Cell Lysate Preparation from Animal Tissues**

a) Excise the tissue sample from the animal.
b) Determine the amount of tissue by weighing. Please refer to **Table 3** for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.
c) 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.
d) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e) Add 600 µL of **Buffer RL** 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.
f) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
g) Spin the lysate for 2 minutes to pellet any cell debris and transfer 500 µL lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
h) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 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 Section 1J.***
- 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 in order not to saturate the magnetic beads.
- 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-coagulated blood to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
b) Add 350 µL of **Buffer RL** to the blood.
c) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 1D. Lysate Preparation from Nasal or Throat Swabs

***Notes Prior to Use***

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

**1D. Lysate Preparation from Nasal or Throat Swabs**

a) Add 600 µL of **Buffer RL** to an RNase-free microcentrifuge tube (not provided).
b) Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.
c) Using sterile techniques cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the **Buffer RL**. Close the tube. Vortex gently and incubate for 5 minutes at room temperature.
i) Transfer 500 µL lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.

***Note:*** In case of swab samples collected in Norgen's Total Nucleic Acid Preservation Tubes (Cat# 69200) transfer 500 µL preserved sample directly to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit and proceed to **Section 2**.

j) Proceed to **Section 2**: RNA Isolation from All Types of Lysates.

## 1E. Lysate Preparation from Bacteria

***Notes Prior to Use***

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in **Table 4.** 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 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.
- 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 1E. c).**

**1E. Cell Lysate Preparation from Bacteria**

a) Pellet bacteria by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute. (***Note:*** Centrifuge at 4000 RPM for 3 minutes while centrifuging bacterial suspension in a 96-well plate).
b) Decant supernatant, and carefully remove any remaining media by aspiration.

c) Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer by vortexing. Incubate at room temperature for the time indicated in **Table 4**.
d) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
e) Transfer lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.

***Note:*** If the volume of bacterial suspension is 1 mL or less, **steps a – d** can be performed in **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit***.*** Make sure to seal the plate with an Adhesive Film. In this case proceed to **Section 2** directly after performing **step d**.

f) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

**Table 4: 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 |

## 1F. Lysate Preparation from Yeast

***Notes Prior to Use***

- Prepare the appropriate amount of Lyticase-containing Resuspension Buffer, considering that 100 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 for this procedure.
- 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 1F. c).**

**1F. Cell Lysate Preparation from Yeast**

a) Pellet yeast by centrifuging at 14,000 x g (~14,000 RPM) for 1 minute. (***Note:*** Centrifuge at 4000 RPM for 3 minutes while centrifuging bacterial suspension in a 96-well plate).
a) Decant supernatant, and carefully remove any remaining media by aspiration.
b) Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing.
c) Incubate at 37°C for 10 minutes.
d) Add 350 µL of **Buffer RL** to the pellet and dissolve the pellet by mixing with a pipette. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
e) Transfer lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.

***Note:*** If the volume of yeast suspension is 1 mL or less, **step a – e** can be performed in **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit**.** Make sure to seal the plate with an Adhesive Film. In this case proceed to **Section 2** directly after performing **step e**.

f) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 1G. 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 50 mg of fungi be used for this procedure in order not to saturate the magnetic beads.

**1G. Cell Lysate Preparation from Fungi**

a) Determine the amount of fungi by weighing. It is recommended that no more than 50 mg of fungi be used for the protocol.
b) Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle.

**Note:** At this stage the ground fungus may be stored at -70°C, such that the RNA purification can be performed at a later time.

c) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
d) Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
e) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
f) Spin the lysate for 2 minutes to pellet any cell debris. Transfer 500 µL lysate to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
g) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 1H. Lysate Preparation from Plant

***Notes Prior to Use***

- The maximum recommended input of plant tissue is 50 mg or 5 x $10^6$ plant cells.
- 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.

**1H. Cell Lysate Preparation from Plant**

a) Determine the amount of plant by weighing and transfer ≤50 mg 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.

b) Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
c) Add 600 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.
d) Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided). Spin the lysate for 2 minutes to pellet any cell debris. Transfer up to 500 µL supernatant to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
e) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 1I. Lysate Preparation from Viral Suspension

***Notes Prior to Use***

- For the isolation of integrated viral RNA, follow **Section 1A** if the starting material is tissue, follow **Section 1B** if the starting material is cell culture, follow **Section 1C** if the starting material is blood, or follow **Section 1D** if the starting material is a nasal or throat swab.
- For the isolation of RNA from free viral particles, follow the procedure below.
- It is recommended that no more than 100 µL of viral suspension be used in order to perform effective isolation.
- It is important to work quickly during this procedure.

**1I. Cell Lysate Preparation from Viral Suspension**

a) Transfer 100 µL of viral suspension to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
b) Add 350 µL of **Buffer RL**.
c) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## 1J. Lysate Preparation 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.
- Plasma prepared from fresh blood using heparin as an anti-coagulant is not suitable for use with this protocol. For heparin-prepared samples follow the protocol in section 1C, Lysate Preparation from Blood.
- It is recommended that no more than 200 µL of plasma or serum be used in order to prevent saturation of magnetic bead.
- Avoid multiple freeze-thaw cycle of the plasma or serum sample. Aliquot to the appropriate volume for usage prior to freezing.
- It is important to work quickly during this procedure.

### 1J. Lysate Preparation from Plasma or Serum

a) Transfer 100 µL of plasma or serum to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or the **96-Well Plate** (Manual extraction) provided in the kit.
b) Add 350 µL of **Buffer RL.**
c) **Optional:** Add 0.7 µL of 0.8 µg/µL MS2 RNA per sample.

***Note:*** The use of MS2 RNA could increase the consistency of downstream applications such as RT-PCR. However, the use of MS2 RNA is not recommended for applications involving global gene expression analysis such as microarrays or sequencing.

d) Proceed to **Section 2:** RNA Isolation from All Types of Lysates.

## Section 2: Total RNA Purification from All Types of Lysates

### 2.1 Total RNA Purification using IsoPure™ 96 system

a) Setup plates according to **Table 1.** Add 600 µL of **Wash Solution A** to **Plate 4, 5 and 6** and keep it at **position 4, 5 and 6** respectively on the carousel.
b) Add 50 – 100 µL of **Elution Solution A** to the **Elution plate** and place it at **position 8** on the carousel.
c) Place sample plate **(Plate 2)** in the machine at **position 2**.
d) Place a clean tip comb in an empty plate **(Plate 1)** and keep it at **position 1.**
e) After setting up, run the program **TOTRNA96**.
f) Prepare a mixture of **Isopropanol** and 40 µL **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Plate 2**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
g) After few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step f**. Open the cover and remove the plate from the instrument and add appropriate volume of **Isopropanol** (not provided) and **Magnetic Beads C** mixture that was prepared in **step f**.
h) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.
i) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the RNA is now ready for further downstream processing.
j) The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **Elution plate** for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

### 2.2 Total RNA Purification using IsoPure™ Mini system

a) Setup plates according to **Table 1.** Add 600 µL of **Wash Solution A** to **Column 3 and/or Column 9, Column 4 and/or Column 10, Column 5 and/or Column 11**.
b) Add 50 – 100 µL of **Elution Solution A** to **Column 6 and/or Column 12.**
c) Place a clean tip comb into IsoPure™ Mini.
d) After setting up, place the plate into the instrument and run the program **TOTRNA16**.
e) Prepare a mixture of **Isopropanol** and 40 µL **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate

volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Colum 1 and/or Column 7**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.

f) After few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step e**. Open the cover and remove the plate from the instrument and add appropriate volume of **Isopropanol** (not provided) and **Magnetic Beads C** mixture that was prepared in **step e** to **Column 1 and/or Column 7**.

g) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.

h) The instrument will prompt after the procedure is complete. Remove the plate from the instrument and the RNA is now ready for further downstream processing.

i) Transfer the elution from **Column 6 and/or Column 12** and transfer it to a fresh plate for storage. The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the plate for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

**Table 5. Isopropanol and Magnetic Beads C Volume for Each Sample Type**

| Section | Sample Type | Lysate Volume (µL) | Isopropanol (µL) | Magnetic Beads C (µL) |
|---|---|---|---|---|
| 1A (i) | Cells (Monolayer) | 350 | 350 | 40 |
| 1A (ii) | Cells (Suspension) | 350 | 350 | 40 |
| 1B | Animal Tissues | 500 | 500 | 40 |
| 1C | Blood | 450 | 450 | 40 |
| 1D | Nasal or Throat Swabs | 500 | 500 | 40 |
| 1E | Bacteria | 350 | 350 | 40 |
| 1F | Yeast | 350 | 350 | 40 |
| 1G | Fungi | 500 | 500 | 40 |
| 1H | Plant | 500 | 500 | 40 |
| 1I | Viral Suspension | 450 | 450 | 40 |
| 1J | Plasma or Serum | 450 | 450 | 40 |

### 2.3 Total RNA Purification using IsoPure™ 96 system – with DNase Treatment

a) Setup plates according to **Table 2.** Add 100 µL of **DNase I Mix** to **Plate 3** and place it at **position 3.**

b) Add 600 µL of **Wash Solution A** to **Plate 4, 5 and 6** and place it at **position 4, 5 and 6** respectively on the carousel.

c) Add 50 – 100 µL of **Elution Solution A** to the **Elution plate** and place it at **position 8** on the carousel.

d) Place sample plate **(Plate 2)** in the machine at **position 2**.

e) Place a clean tip comb in an empty plate **(Plate 1)** and keep it at **position 1.**

f) After setting up, run the program **TOTRNA96DNA**.

g) Prepare a mixture of **Isopropanol** (not provided) and 40 µL of **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** (not provided)

with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Plate 2**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.

h) After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step g**. Open the cover and remove the plate from the instrument and add appropriate volume of **Isopropanol** (not provided) and **Magnetic Beads C** mixture that was prepared in **step g**.

i) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.

j) To rebind the sample to the magnetic beads, prepare a mixture of 200 µL **Buffer RL** and 300 µL Isopropanol (not provided) for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and mix 2 mL of **Buffer RL** with 3 mL **Isopropanol**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.

k) After a few minutes, the instrument will pause and allow the user to add mixture prepared in **step j**. Open the cover and remove the plate from the instrument and 500 µL of **Buffer RL** and **Isopropanol** mixture that was prepared in **step j** to each well of **Plate 3**.

l) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.

m) The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the RNA is now ready for further downstream processing.

n) The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **Elution plate** for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

### 2.4 Total RNA Purification using IsoPure™ Mini system – with DNase Treatment

a) As per the sample being isolated, setup plates according to **Table 2.** Add 100 µL of **DNase I Mix** to **Column 2 and/or Column 8.**

b) Add 600 µL of **Wash Solution A** to **Column 3 and/or Column 9, Column 4 and/or Column 10, Column 5 and/or Column 11**.

c) Add 50 – 100 µL of **Elution Solution A** to **Column 6 and/or Column 12**.

d) Place a clean tip comb into IsoPure™ Mini.

e) After setting up, place the plate into the instrument and run the program **TOTRNA16D**.

f) Prepare a mixture of **Isopropanol** (not provided) and 40 µL **Magnetic Beads C** as per **Table 5** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and if the lysate volume obtained in **Section 1** is 500 µL, mix 5 mL of **Isopropanol** with 400 µL **Magnetic Beads C** and add 540 µL of the mixture to each well of **Colum 1 and/or Column 7**). A fresh bulk mixture can be made every time RNA isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.

g) After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step f**. Open the cover and remove the plate from the instrument and add appropriate volume of **Isopropanol** (not provided) and **Magnetic Beads C** mixture that was prepared in **step f** to **Column 1 and/or Column 7**.

h) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.

i) To rebind the sample to the magnetic beads, prepare a mixture of 200 µL **Buffer RL** and 300 µL **Isopropanol** for the number of samples extracted. (For example, if RNA is to be extracted from 10 samples, and mix 2 mL of **Buffer RL** with 3 mL **Isopropanol**). Make 10% extra volume to avoid volume loss due to pipetting.

j) After a few minutes, the instrument will pause and allow the user to add mixture prepared in **step i**. Open the cover and remove the plate from the instrument and 500 µL of **Buffer RL** and **Isopropanol** mixture that was prepared in **step i** to **Column 2 and/or Column 8**.
k) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.
l) The instrument will prompt after the procedure is complete. Remove the elution plate from the instrument and the RNA is now ready for further downstream processing.
m) Transfer the elution from **Column 6 and/or Column 12** and transfer it to a fresh plate for storage. The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the plate for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

**2.5 Total RNA Purification – Manual method**

a) Cover the **96-Well Plate** with the Adhesive Tape and incubate the plate on an orbital shaker at 1000 RPM for 5 minutes. Alternatively mix by pipetting at least 10 times and incubate for 5 minutes.
b) Remove the Adhesive Tape, add **Isopropanol** to the lysate and 40 µL of **Magnetic Bead C** as per table 5 in section 2.2.
c) Cover the **96-Well Plate** with the Adhesive Tape, and incubate the plate on an orbital shaker at 1000 RPM for 5 minutes. Alternatively mix by pipetting at least 10 times and incubate for 5 minutes.
d) Remove the Adhesive Tape and place the **96-Well Plate** on the magnetic rack. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
e) Aspirate and discard supernatant without touching the magnetic beads.

**Optional DNase Treatment**

Norgen's Total RNA Purification 96-Well Kit (Magnetic Bead System) isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **DNA Removal Protocol** for manual extraction 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.

a) Remove the sample plate from the magnetic rack and add 600 µL of **Wash Solution A (ensure ethanol was added).**
b) Resuspend by pipetting and shake the plate 2 minutes on an orbital shaker at 1000 RPM. Alternatively mix by pipetting 10 times.
c) Place the **96-Well Plate** on the magnetic rack. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
d) Aspirate and discard supernatant without touching the magnetic beads.
e) Repeat **steps 2f to 2i** two more times, for a total of three washes.
f) Remove as much of the wash solution as possible and air-dry the beads at room temperature for 5 to 10 min. (Do not let the magnetic beads to over-dry as this may affect the RNA yield).
g) Remove the plate from the magnetic rack and add 50 – 100 µL of **Elution Solution A**.
h) Mix by pipetting and incubate at 50˚C for 2 minutes.
i) Resuspend by pipetting and shake the plate 2 minutes on an orbital shaker at 1000 RPM. Alternatively mix by pipetting 10 times and incubate for 2 minutes at room temperature.

j) Place the sample plate on the magnetic rack. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
k) Carefully transfer the elution to a fresh **96-Well Elution Plate** without touching the magnetic beads.
l) The purified RNA sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **96-Well Elution Plate** for storage of the RNA. It is recommended that samples be placed at -70°C for long-term storage.

## Appendix A

**Protocol for Optional DNA Removal**

Norgen's 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 Norgen's RNase- Free DNase I Kit (Product # 25710) be used for this step.

1. For every DNase reaction to be performed, prepare a **DNase I Mix** of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer A** 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 100 µL aliquot is required for each sample to be treated.

2. Perform appropriate binding up to **step e** of the manual extraction and remove the plate from the magnetic rack.
3. Add 100 µL of **DNase I Mix** that to each sample in the **96-Well plate** and mix by pipetting and incubate at 25-30°C for 15 minutes.
4. Add 200 µL **Buffer RL** and 300 µL **Isopropanol** to each sample.
5. Cover the **96-Well Plate** with the Adhesive Tape and incubate the plate on an orbital shaker at 1000 RPM for 5 minutes. Alternatively mix by pipetting and incubate for 5 minutes.
6. Remove the Adhesive Tape and place **96-Well Plate** on the magnetic rack. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
7. Aspirate and discard supernatant without touching the magnetic beads.
8. Proceed to **step f** of **section 2.5** to continue manual extraction.

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Magnetic beads were accidentally pipetted up with the supernatant. | The pipette tip was placed too close to the magnetic beads while pipetting | Return the magnetic beads and the supernatant back into the sample tube. Mix well, and place the tube back onto the magnetic separation rack for the specified time. Carefully remove the supernatant without touching the magnetic beads. |
| The yield of RNA is low | Incomplete lysis of cells | Ensure that correct lysis protocol was applied to the sample. |
| | Amount of magnetic beads added was not sufficient | Ensure that the Magnetic Bead C is mixed well prior to use to avoid any inconsistency in RNA isolation. |
| | RNA concentration in the cell or tissue sample being used is low. | Some samples contain very little target RNA. This varies from individual to individual based on numerous variables. |
| Very gelatinous prior to adding the Magnetic bead and Isopropanol | The lysate solution mixture is not homogeneous | To ensure a homogeneous solution, vortex for 10-15 seconds before adding the magnetic beads to the lysate. |
| | 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. |
| RNA does not perform well in downstream applications. | RNA was not washed with Wash A | Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with Wash A. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads. |
| | Ethanol carryover | Ensure that the drying step after the last wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |
| DNA is present in eluted RNA. | DNA is coeluted with the RNA | Carry out a digestion with DNase I on the elution if the DNase present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature. |

| Related Products (Required for Automated Workflow) | Product # |
|---|---|
| Adhesive Tape | 28394 |

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