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Total RNA Purification Plus Kit

Product # 48300, 48400

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

Component	Product # 48300 (50 preps)	Product # 48400 (100 preps)
Buffer RL	40 mL	2 x 40 mL
Wash Solution A	38 mL	2 x 38 mL
Elution Solution A	6 mL	2 x 6 mL
RNA Purification Columns	50	100
Genomic DNA Removal Columns	50	100
Collection Tubes	100	200
Elution tubes (1.7 mL)	50	100
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 1 year 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. For the isolation of total RNA from plasma/serum samples please download the full manual from our website and refer to Appendix A.**
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to each supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL.
- **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.

Section 1. Preparation of Lysate From Various Cell Types

1A. Lysate Preparation from Cultured Animal Cells

- 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 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

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 a microcentrifuge tube.
e. **Proceed to Step 2.**

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 onto the column.

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. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
d. **Proceed to Step 2.**

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 onto the column.

1B. Lysate Preparation from Animal Tissues

- Norgen's Total RNA Purification Plus Kit is designed for isolating RNA from small amount of tissue sample (up to 20 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.
- 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 RNA*later*® 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 1 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 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain	25 mg
Heart	5 mg
Kidney, Liver, Lung, Spleen	20 mg

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing. Please refer to Table 1 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. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
h. **Proceed to Step 2.**

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 download the full protocol from our website and refer to Appendix A.***
- 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 to prevent clogging of the column.
- 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 µL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
b. Add 350 µL of **Buffer RL** to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. **Proceed to Step 2.**

1D. Lysate Preparation from Nasal or Throat Swabs

- 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.

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.
d. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube (not provided). Note the volume of the lysate.

e. **Proceed to Step 2.**

1E. Lysate Preparation from Bacteria

- Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 2. 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 1Ec)**.

a. Pellet bacteria by centrifuging at 14,000 *x g* (~14,000 RPM) for 1 minute.
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 (see Table 2) by vortexing. Incubate at room temperature for the time indicated in Table 1.
d. Add 300 µL of **Buffer RL** and vortex vigorously for at least 10 seconds.
e. **Proceed to Step 2.**

Table 2: 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

1F. Lysate Preparation from Yeast

- 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 1Fc)**.

a. Pellet yeast by centrifuging at 14,000 *x g* (~14,000 RPM) for 1 minute.
b. Decant supernatant, and carefully remove any remaining media by aspiration.
c. Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37ºC for 10 minutes.
d. Add 300 µL of **Buffer RL** and vortex vigorously for at least 10 seconds.
e. **Proceed to Step 2.**

1G. 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 50 mg of fungi be used for this procedure in order to prevent clogging of the column.

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 the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
g. **Proceed to Step 2.**

1H. Lysate Preparation from Plant

- 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.

a. Transfer ≤50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen.

Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.

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).
d. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
e. **Proceed to Step 2.**

1I. Lysate Preparation from Viruses

- For the isolation of integrated viral RNA, follow Section **1A** if the starting material is cell culture, follow Section **1B** if the starting material is tissue, 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 prevent clogging of the column.

1I. Cell Lysate Preparation from Viral Suspension

a. Transfer up to 100 µL of viral suspension to an RNase-free microcentrifuge tube (not provided).
b. Add 350 µL of **Buffer RL**. Lyse viral cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
c. **Proceed to Step 2.**

Section 2. Genomic DNA Removal from All Types of Lysate

Notes:

- The following steps of the procedure are the same for all of the different types of lysate.
- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
    - gDNA Removal Columns - column has blue and white contents
    - RNA Purification Columns – column has grey and white contents

2. Genomic DNA Removal

a. Assemble a gDNA Removal Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate prepared from Section 1 onto the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

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

c. Retain the flowthrough for RNA Purification (Section 3). The flowthough contains the RNA and should be stored on ice or at -20°C until the RNA Purification protocol is carried out.
d. Dispose of the gDNA Removal Column with the bound gDNA.

Section 3. Total RNA Purification from All Types of Lysate

3. Binding RNA to Column

a. To every 100 µL of flowthrough from Step 2c, add 60 µL of 96 – 100 % Ethanol. Mix by vortexing.

Note: For example, for 300 µL of flowthrough, add 180 µL of 96 – 100 % Ethanol

b. Assemble an RNA Purification Column with one of the provided collection tubes.
c. Apply up to 600 µL of the lysate with the ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x *g* (~6,000 RPM)**.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x *g* (~14,000 RPM)**.

d. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.

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

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **4a** and **4b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Solution A** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Technical Support

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@norgenbiotek.com.

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