NORGEN BIOTEK CORP.

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Total RNA Purification Plus Micro Kit
Product # 48500

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

Component	Product # 48500 (50 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Genomic DNA Removal Column	50
RNA Purification Micro Column	50
Collection Tubes	100
Elution tubes (1.7 mL)	50
Product Insert	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.

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).**
- 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), including LCM samples. 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 5 x 10^5. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a each well of a confluent 12-well plate of HeLa cells will contain 5 x 10^5 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.**

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.

Note: For inputs of over 10^5 cells, 5-10 μL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged. For inputs of fewer than 10^5 cells, 30-50 μL of media may be left behind in order to ensure that the pellet, which could be invisible, 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.**

1B. Lysate Preparation from Animal Tissues

- Norgen's Total RNA Purification Plus Micro Kit is designed for isolating RNA from small amounts of non-fibrous tissue samples (up to 3 mg in most cases). If a larger amount of starting material or fibrous tissue is desired, an additional Proteinase K treatment is required. Please refer to Appendix A for instruction.
- 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 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 3 mg.

Table 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Brain, Kidney, Liver, Lung, Spleen	3 mg
Heart, Muscle	Refer to Appendix A

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 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 3 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 400 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 Laser-Captured Microdissection (LCM)

- LCM samples obtained from frozen sections are recommended. Formalin-Fixed, Paraffin-Embedded sections may also be used. However, RNA isolated from FFPE samples generally has poorer quality than that from frozen sections.

1C. Cell Lysate Preparation from Laser-Captured Microdissection (LCM)

a. Aliquot 300 μL of **Buffer RL** to an RNase-free microcentrifuge tube.
b. Remove the thermoplastic film containing the captured cells using sterile fine forceps. Carefully submerge the sample into the aliquoted **Buffer RL**. Close the microcentrifuge cap.
c. Incubate the sample at 42ºC for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
d. At the end of the incubation, vortex the tube one more time for 15 seconds. The thermoplastic film may be removed at this point using sterile fine forceps.
e. **Proceed to Step 2.**

Section 2. Genomic DNA Removal from All Types of Lysate

Notes

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

2. Genomic DNA Removal

a. Assemble a gDNA Removal Column with a provided collection tube.
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 Micro Column with one of the provided collection tubes. Apply up to 600 µL of the lysate with the ethanol onto the column and centrifuge 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)**.

c. 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 centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure the entire Wash Solution A 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. Repeat steps **4a** and **4b** to wash column a third time.
e. 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 40 µL of **Elution Solution A** to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 µL is recommended

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 volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

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

Appendix A

Lysate Preparation from Animal Tissues with the use of Proteinase K

Customer-Supplied Reagent

- RNase-Free Proteinase K
- RNase-Free Water
- β-mercaptoethanol

Notes Prior to Use

- We recommend the use of Norgen's **Proteinase K** (Cat # 17904) for this step. Reconstitute each of the **Proteinase K** vials in 600 μL of molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free). For every isolation, 20 μL of the reconstituted Proteinase K is needed. Aliquot the remainder into small fractions and store the unused portions at -20°C until needed.
- If using another source of **Proteinase K,** reconstitute in molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free) to give a 20 mg/mL final concentration. For every isolation, 20 μL of the reconstituted Proteinase K is needed. Aliquot the remainder into small fractions and store the unused portions at -20°C until needed.
- 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.
- 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 RNA*later*® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry any excessive liquid.
- This protocol is particularly suitable for isolating RNA from up to 7.5 mg of tissues including fibrous, connective tissues.

Cell Lysate Preparation

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing.
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.

   Note: The use of liquid nitrogen is recommended. However, if homogenization without flash-freezing is preferred, proceed to Step **e**.

d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e. Add 300 μL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.

   Note: Maximum homogenization may be achieved 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. Add 600 μL of **RNase-Free Water** (not provided) to the lysate. Vortex to mix.
h. Add 20 μL of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.
i. Spin the lysate for 2 minutes to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume.
j. **Proceed to Step 2.**

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

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