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Total RNA Purification Micro Kit

Product # 35300, 35350

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

Component	Product # 35300 (50 preps)	Product # 35350 (250 preps)
Buffer RL	40 mL	5 x 40 mL
Wash Solution A	38 mL	5 x 38 mL
Elution Solution A	6 mL	5 x 6 mL
Micro Spin Columns	50	250
Collection Tubes	50	250
Elution tubes (1.7 mL)	50	250
Product Insert	1	1

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

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. The **Buffer RL** contains guanidine salts, and should be handled with care. Guanidine salt forms 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 the supplied bottles 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, 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. Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **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. Add 200 µL of 96 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

1B. Lysate Preparation from Animal Tissues

- Norgen's Total RNA Purification 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. Also, to isolate total RNA from larger amounts of tissue Norgen also offers a Total RNA Purification Kit (Cat# 17200) and an Animal Tissue RNA Purification Kit (Cat# 25700).
- 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

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. Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix. **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.

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. Otherwise, proceed to **Step 1Ce.**
e. Add 300 µL of 70% ethanol (provided by the user) to the lysate. Vortex to mix. **Proceed to Step 2.**

Section 2. Total RNA Purification from All Types of Lysate

Note: 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.

2. Binding RNA to Column

a. Assemble a micro spin-column with one of the provided collection tubes
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 1**) 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).

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step **2b** and **2c** as necessary.

Optional Step:
Norgen's Total RNA Purification Micro Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix B 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.

3. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge 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 **3a** and **3b** 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.

4. 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 4b and 4c**).

5. 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

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.
- 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.
j. Add 450 µL of 96 - 100% ethanol (provided by the user) to the lysate. Vortex to mix. **Proceed to Step 2.**

Appendix B

Protocol for Optional On-Column DNA Removal
Norgen's Total RNA Purification Micro 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 15 µL of **DNase I** and 100 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 100 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 to Column**" (Steps 1 and 2 of all protocols)
3. Apply 400 µL of **Wash Solution A** to the micro spin column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 3c).

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