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EXTRAClean RNA Clean-Up and Concentration Micro-Elute Kit

Product # 73600

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

Component	Contents
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Column Activation Solution	30 mL
EXTRAClean Micro-Elute RNA Spin Columns	50
Collection Tubes	50
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 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 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

A. Protocol for RNA Clean-Up and Concentration from Enzymatic Reactions or Previously Isolated RNA

Notes Prior to Use

- 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.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare an appropriate amount of Buffer RL by adding 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.
- It is recommended that no more than 10 µg of RNA be used per cleanup.
- The maximum volume of RNA sample that can be processed is 200 µL.
- It is important to work quickly during this procedure.
- This kit purifies RNA with minimal amounts of DNA contamination. However, an optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR. The procedure in Appendix A is to be carried out prior to performing the kit procedure below.

1. Sample Preparation

a. Adjust the volume of the RNA sample to 100 µL by adding RNase-free or DEPC-treated water. It is recommended that no more than 10 µg of RNA be used for each column.
   Note: If an input volume between 100 and 200 µL is used, adjust the sample volume to 200 µL (maximum allowable) with RNase-free or DEPC-treated water. In this case, use the volumes indicated in bold in the bracket in Steps 1b and 1c**.**

b. Add 250 µL **(or 500 µL)** of Buffer RL to the RNA sample. Mix by vortexing

c. Add 200 µL **(or 400 µL)** of 96 – 100% ethanol (provided by the user) to the mixture from Step 1b**.** Mix by vortexing for 10 seconds.
   Note: If the sample being processed is highly enriched for small RNA (such as microRNA, siRNA, Piwi-interacting RNA, etc.) increase the amount of 96 – 100% ethanol added to the mixture from Step 1b. In this case, add 350 µL (or 700 µL for 200 µL original input volume) of 96 – 100% ethanol in Step 1c. RNA isolated from bodily fluids (serum, plasma,

urine, etc.) and exosomes are considered to belong to this category. If the sample contains a heterogeneous mixture of a range of RNA sizes, including large RNA, follow the procedure as outlined in **Step 1c** without adjustments.

2. Column Activation and Sample Binding to EXTRAClean Micro-Elute Spin Column

a. Assemble a column with one of the provided collection tubes.
b. Apply 500 µL of Column Activation Solution onto the column and centrifuge for 1 minute.
c. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
d. Apply up to 600 µL of the RNA sample with the ethanol (from Step 1c) onto the activated column and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
f. If the volume of the RNA sample is greater than 600 µL, repeat Steps 2d and 2e until all the remaining RNA sample has passed through the column.

3. EXTRAClean Micro-Elute Spin 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 EXTRAClean Micro-Elute Spin Column with its collection tube.
c. Repeat steps 3a and 3b to wash the 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 EXTRAClean Micro-Elute 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 EXTRAClean Micro-Elute Spin Column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 8 – 15 µL of Elution Solution A to the column.

Note: For maximum concentrations of RNA, the 8 elution µL volume may be used. For maximum recovery of RNA the 15 µL volume 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

a. 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.

B. Protocol for RNA Clean-up and Concentration from Aqueous Phase (RNA fraction) of Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods

Notes Prior to Use

- 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.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 10 µg of RNA to be used per cleanup.
- It is important to work quickly during this procedure.

1. Sample Preparation

a. Isolate RNA using a phenol/guanidine-based reagent such as Trizol or Tri Reagent, according to manufacturer's instruction. After the separation of the aqueous and organic phases, collect the upper (aqueous) fraction containing the RNA into a new RNase-free microcentrifuge tube (not provided). Note the volume.
b. Add one volume of 70% ethanol (provided by the user) to the fraction from step 1a. Mix by vortexing for 10 seconds.
Note: If the RNA sample being processed is highly enriched for small RNA (such as microRNA, siRNA, Piwi-interacting RNA, etc.) increase the amount of ethanol added to the fraction from Step 1a. In this case, add one volume of 96 – 100% ethanol in Step 1b. RNA isolated from bodily fluids (serum, plasma, urine, etc.) and exosomes are considered to belong to this category. If the sample contains a heterogeneous mixture of a range of RNA sizes, including large RNA, follow the procedure as outlined in Step 1b without adjustments.

2. Column Activation and Sample Binding to EXTRAClean Micro-Elute Spin Column

a. Assemble a column with one of the provided collection tubes.
b. Apply 500 µL of Column Activation Solution onto the column and centrifuge for 1 minute.
c. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
d. Apply up to 600 µL of the RNA sample with the ethanol (from Step 1b) onto the activated column and centrifuge for 1 minute.
e. Discard the flowthrough. Reassemble the EXTRAClean Micro-Elute Spin Column with its collection tube.
f. If the volume of the RNA sample is greater than 600 µL, repeat Steps 2d and 2e until all the remaining RNA sample has passed through the column.

3. EXTRAClean Micro-Elute Spin 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 EXTRAClean Micro-Elute Spin Column with its collection tube.
c. Repeat steps 3a and 3b to wash the 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 EXTRAClean Micro-Elute 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 EXTRAClean Micro-Elute Spin Column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 8 – 15 µL of Elution Solution A to the column.
Note: For maximum concentrations of RNA, use the 8 µL elution volume. For maximum recovery of RNA the 15 µL volume 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

a. 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.

This kit purifies RNA with minimal amounts of DNA contamination. However, an optional protocol is provided on our website for maximum removal of residual DNA that may affect sensitive downstream applications such as quantitative PCR.

Technical Support

Contact our Technical Support Team between the hours of 9:00 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