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RNA Clean-Up and Concentration Kit — Product Insert

Product # 23600, 43200

Norgen's RNA Clean-Up and Concentration Kit provides a rapid method for the purification, cleanup and concentration of up to 35 µg of RNA isolated using different methods including phenol/guanidine-based protocols, and from various upstream enzymatic reactions such as DNase treatment and labeling. This kit can also be used for the clean-up and concentration of RNA transcripts from *in vitro* transcription reactions by removing polymerases, unincorporated dNTPs, salts and DTT (please see Appendix B). The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other reaction components such as proteins, RNases and nucleotides, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the RNA samples or enzymatic reactions containing RNA with Buffer RL (please see the flow chart on page 4). Ethanol is then added and the mixture is loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound RNA is then washed three times with the provided Wash Solution A in order to remove any remaining impurities, and the purified RNA is eluted with the Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications. Norgen's RNA Clean-Up and Concentration Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications such quantitative PCR.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	35 µg
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material:	35 µg of RNA
Minimum Elution Volume	20 µL
Time to Complete 10 Purifications	20 minutes
Average Recovery	$\geq$ 90%

Advantages

- Efficient RNA cleanup from various enzymatic reactions – labeling, DNase treatment and *in vitro* transcription
- Cleanup of RNA isolated using different methods, including phenol/chloroform extractions
- Fast and easy processing using rapid spin-column format
- Suitable for all sizes of RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions

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.

Kit Components

Component	Product #23600 (50 preps)	Product # 43200 (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
Micro Spin Columns	50	100
Collection Tubes	50	100
Elution tubes (1.7 mL)	50	100
Product Insert	1	1

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 Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

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

Customer-Supplied Reagents and Equipment

For RNA Clean-Up and Concentration from Enzymatic Reactions or *Non-Phenol/Guanidine-Based RNA Isolation Methods*

- Benchtop microcentrifuge
- β-mercaptoethanol
- 96 - 100% ethanol
- RNase-free or DEPC-treated water

For RNA Clean-Up and Concentration from Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods

- Benchtop microcentrifuge
- 70% ethanol

Working with RNA

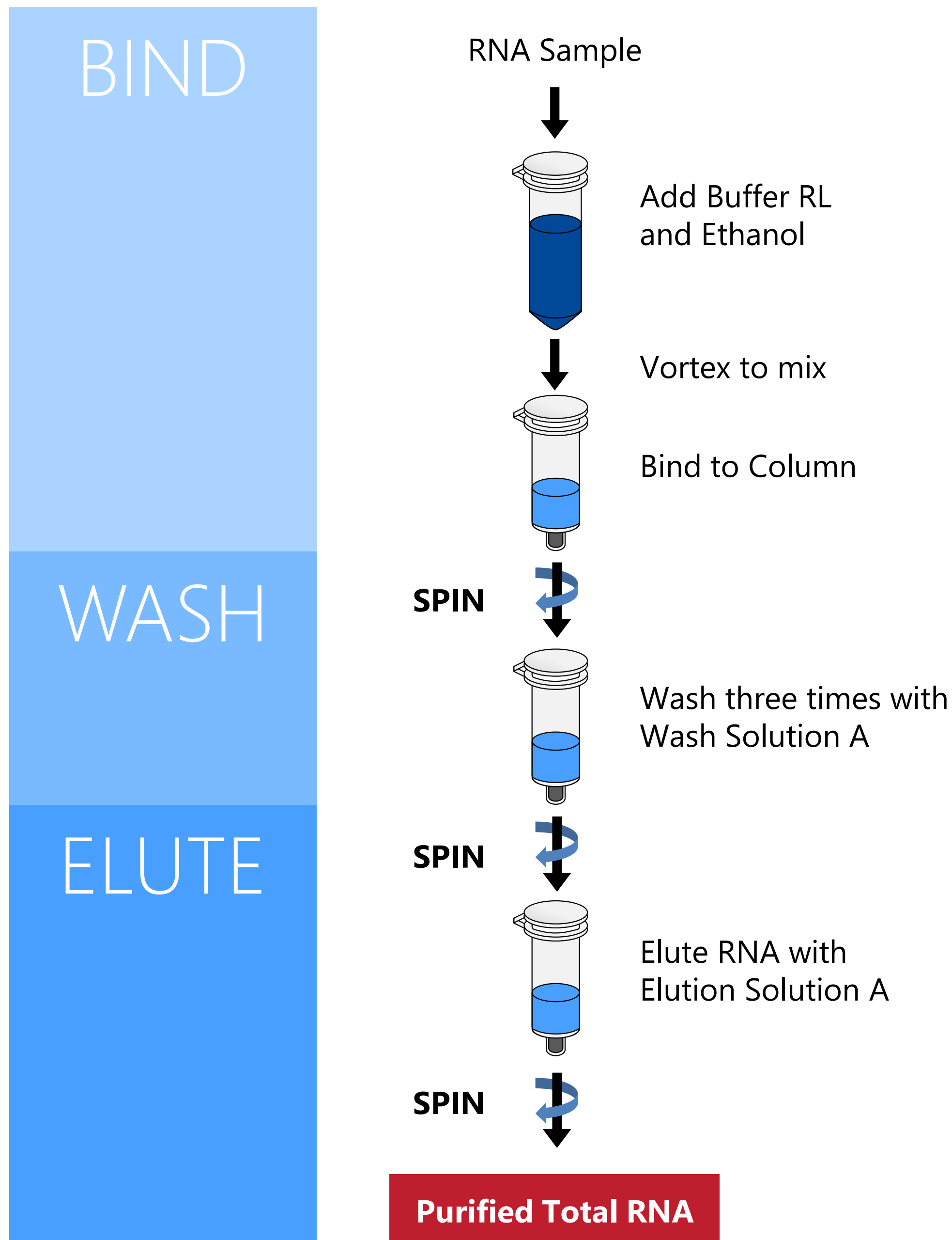
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- 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

Flow Chart

Procedure for Purifying RNA using Norgen's RNA Clean-up and Concentration Kit



Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\ (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes Prior to Use:

- **The steps for RNA clean-up are different depending on the starting material. Please ensure that you use the proper procedure for your starting material:**
    - Section A: For RNA Clean-Up and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods.
    - Section B: For RNA Clean-up and Concentration from Phenol/Guanidine-Based RNA (Trizol or Tri Reagent) Isolation Methods.
    - Appendix B: For RNA Transcript Clean-up and Concentration from *in vitro* Transcription Reactions.
- 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 35 μg of RNA to 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.

A. Protocol for RNA Clean-Up and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods

Notes Prior to Use

- 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.

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 35 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.

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

    Note: Ensure the entire sample 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. If the volume of the RNA sample is greater than 600 μL, repeat Step **2b** and **2c** until all the remaining RNA sample has passed through the column.

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 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 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 50 μL of **Elution Solution A** to the column and let stand for 2 minutes at room temperature.

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

    c. Centrifuge for 1 minute at **200 x *g* (~2,000 RPM)**, followed by 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at **14,000 x g (~14,000 RPM)** for 30 additional seconds.
    d. **For Maximum Recovery:** Transfer the eluted solution back to the column and let stand for 2 minutes at room temperature. Centrifuge for 1 minutes at **200 x g (~2,000 RPM)**, followed by 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at **14,000 x g (~14,000 RPM)** for 30 additional seconds.

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.

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

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.

2. **Binding to Column**
   a. Assemble a column with one of the provided collection tubes.
   b. Apply up to 600 μL of the RNA mixed with the ethanol (from **Step 1b**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

   Note: Ensure the entire sample 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. If the volume of RNA mix is greater than 600 μL, repeat Steps **2b** and **2c** until all the remaining RNA mix has passed through the column.

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 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 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 50 μL of **Elution Solution A** to the column and let stand for 2 minutes at room temperature.

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

c. Centrifuge for 1 minute at **200 x *g* (~2,000 RPM)**, followed by 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at 14,000 x g (~14,000 RPM) for 30 additional seconds.

d. **For Maximum Recovery:** Transfer the eluted solution back to the column and let stand for 2 minutes at room temperature. Centrifuge for 1 minutes at **200 x g (~2,000 RPM)**, followed by 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at **14,000 x g (~14,000 RPM)** for 30 additional seconds.

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: Optional DNA Removal in Solution Followed by RNA Clean-Up and Concentration

Norgen's RNA Clean-Up and Concentration Kit purifies RNA with minimal amounts of DNA contamination. An optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications such quantitative PCR. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This procedure is to be performed prior to starting the kit protocol.

1. Adjust the volume of the RNA sample to be treated to 50 μL with RNase-free water.

2. Add 45 μL of the provided Enzyme Incubation Buffer.

3. Add 5 μL of Norgen's DNase I to the RNA sample. **Gently mix by pipeting the sample up and down using a pipet. Do not vortex.**

4. Incubate at 25 to 30ºC for 15 minutes.

5. Proceed directly to Protocol A "**Protocol for RNA Clean-up and Concentration from Enzymatic Reactions or Non-Phenol/Guanidine-Based RNA Isolation Methods**".

Appendix B: Supplementary Protocol for RNA Transcript Clean-up and Concentration from *in vitro* Transcription Reactions

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- β-mercaptoethanol
- Isopropanol
- 96 - 100% ethanol
- RNase-free or DEPC-treated water

Notes Prior to Use:

- 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 35 µg of RNA transcripts be used per clean-up.
- The kit is robust with transcripts ≥ 100b.
- Digestion of the DNA template used in the *in vitro* transcription reaction should be performed before cleaning the RNA transcript. Ensure that RNase-free DNase is used for the digestion step.

1. Sample Preparation

a. Adjust the volume of the sample to 100 μL by adding nuclease-free water.
b. Add 250 μL of **Buffer RL** to the sample. Mix by vortexing for 10 seconds.
c. Add 250 μL of isopropanol. Mix by vortexing for 10 seconds.

2. Binding to Column

a. Assemble a column with one of the provided collection tubes.
b. Apply up to 600 μL of the sample with the isopropanol (from **Step 1c**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

Note: Ensure the entire sample 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. Repeat steps **2b** and **2c** as required to bind the entire sample to the column.

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 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 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. 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 and let stand for 2 minutes at room temperature.

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 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at 14,000 x g (~14,000 RPM) for 30 additional seconds.

d. **For Maximum Recovery:** Transfer the eluted solution back to the column and let stand for 2 minutes at room temperature. Centrifuge for 1 minutes at **200 x g (~2,000 RPM)**, followed by 2 minutes at **5,800 x g (~8,000 RPM)**. Spin the column at **14,000 x g (~14,000 RPM)** for 30 additional seconds.

5. Storage of Purified RNA Transcripts

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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
Clogged Column	High amounts of RNA in the input	Ensure that no more than 35 µg of RNA is used as the input for each column.
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.

Problem	Possible Cause	Solution and Explanation
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
DNA or Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample prior to starting the protocol as outlined in Appendix A. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
1kb RNA Ladder	15003

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 (www.norgenbiotek.com) 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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