NORGEN BIOTEK CORPORATION

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

RNA Clean-Up and Concentration Micro Kit Product Insert

Product # 23600

Norgen's RNA Clean-Up and Concentration Micro 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, labeling and *in vitro* transcription. 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 Binding Solution (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 in order to remove any remaining impurities, and the purified RNA is eluted with the Elution Buffer. 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 Micro 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	
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	≥ 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

Kit Components

Component	Product #23600 (50 preps)
Binding Solution	40 mL
Wash Solution	22 mL
Elution Buffer	6 mL
Micro 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. These reagents should remain stable for at least 1 year in their unopened containers.

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*.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the RNA Clean-up and Concentration Micro Kit:

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

- Benchtop microcentrifuge
- β-mercaptoethanol
- 95 - 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

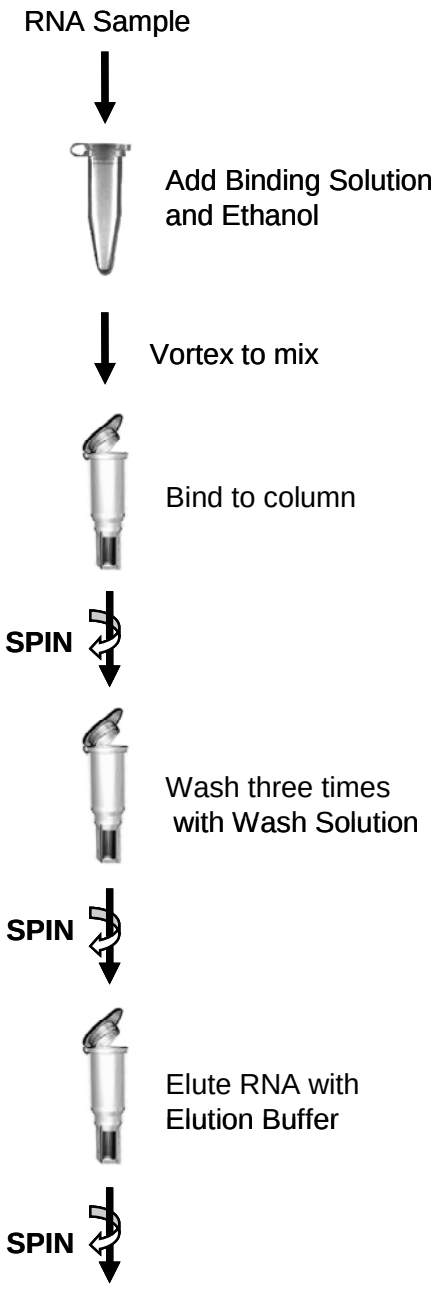
Working with RNA

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 Micro 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.

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

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.

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** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 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 Binding Solution by adding 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Binding Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- 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.

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 **Binding Solution** to the RNA sample. Mix by vortexing
c. Add 200 μL **(or 400 μL)** of 95 – 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.
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** 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 **3a** and **3b** to wash the column a second time.
d. Wash column a third time by adding another 400 μL of **Wash Solution** 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 Buffer** 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.

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

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.

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** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 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.
- 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.

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.
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** 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.

c. Discard the flowthrough and reassemble the spin column with its collection tube.
d. Repeat steps **3a** and **3b** to wash the column a second time.
e. Wash column a third time by adding another 400 μL of **Wash Solution** and centrifuging for 1 minute.
f. Discard the flowthrough and reassemble the spin column with its collection tube.
g. 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 Buffer** 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: Optional DNA Removal in Solution Followed by RNA Clean-Up and Concentration

Norgen's RNA Clean-Up and Concentration Micro 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 an RNase-free DNase I be used. This procedure is to be preformed prior to starting the kit protocol.

1. Adjust the volume of the RNA sample to be treated to 80 µL with RNase-free water.

2. Prepare a working stock of 0.5 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM $MgCl_2$ and 3 mM $CaCl_2$, made RNase-free) to give a final concentration of 0.5 Kunitz unit/µL.

3. Add 20 µL of 0.5 Kunitz unit/µL DNase I to the RNA sample.

4. Incubate at 25 to 30^{o}C 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**".

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 Buffer 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	Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution 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.
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.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed three times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution. 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.

Related Products	Product #
Total RNA Purification Kit	17200
CleanAll DNA/RNA Clean-Up and Concentration Micro Kit	23800
100b RNA Ladder	15002
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.

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