

Urine Bacteria RNA Purification Kit

Product # 23400

Product Insert

Norgen's **Urine Bacteria RNA Purification Kit** is designed for the rapid preparation of bacterial RNA from urine samples. Bacterial RNA can be isolated from both human urine samples and urine samples from animals in order to study the levels and types of bacteria that are present, as well as to study the stage of bacterial pathogenesis through the use of RNA biomarkers. The kit allows for the isolation of RNA from both Gram negative and Gram positive bacteria, including *E. coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomonas* spp., *Clostridial* ssp. and *Leptospirosis* spp., as well as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. The Urine Bacteria RNA Purification 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 cellular components such as proteins, as well as from the contaminating species found in urine such as glucose and salts, without the use of phenol or chloroform. Typical yields of RNA will vary depending on the urine sample and the bacterial species, if any, present in the urine. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, 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 pelleting the bacteria that are present in the urine sample through the use of centrifugation, followed by lysing the bacteria cells with the provided Lysis Solution (please see the flow chart on page 4). Binding solution and ethanol are then added to the bacterial lysate, and the solution 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 will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed twice with the provided Wash Solution in order to remove any remaining impurities, and the purified total 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.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 µg
Volume of Urine Processed	10 – 50 mL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	30 minutes
Average Yield	~ 0.5 µg RNA per 1 x 10 ⁷ cells (Varies due to cell density of sample)

Advantages

- Fast and easy processing using rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- Isolate high quality total RNA from urine
- No phenol or chloroform extractions

Kit Components

Component	Product # 23400 (20 samples)
Lysis Solution	9 mL
Wash Solution	10 mL
Elution Buffer	6 mL
Micro Spin Columns	20
Collection Tubes	20
Elution tubes (1.7 mL)	20
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

- Benchtop microcentrifuge
- Swinging bucket centrifuge
- 96 – 100% ethanol
- β -mercaptoethanol
- 50 mL conical tubes
- Microcentrifuge tubes
- Lysozyme-containing TE Buffer:
 - 3 mg/mL lysozyme in TE Buffer

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 Bacterial RNA using Norgen's Urine Bacteria RNA Purification Kit

Collect Urine Sample


SPIN  Pellet bacterial cells

 Lyse cells using TE Buffer with Lysozyme and Lysis Solution. Add Ethanol




 Bind to column

SPIN 

 Wash twice with Wash Solution

SPIN 

 Elute RNA with Elution Buffer

SPIN 

Purified Total Urine Bacteria RNA

Procedure

Centrifugation steps are carried out in both a benchtop microcentrifuge and a swinging bucket centrifuge. 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:

- We recommend the use of **Norgen's Urine Preservative** when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. The components of the Urine Preservative allow samples to be stored for over 2 years at room temperature with no detected degradation of urine DNA, RNA or proteins. Norgen's Urine Preservative is available in 2 convenient formats: in a liquid format in Norgen's Urine Preservative Single Dose Ampules, as well as in a dried format in Norgen's Urine Collection and Preservation Tubes. Please see the Related Products table below.
- It is recommended that at least 20 – 30 mL of urine be used as the input for each column. The maximum urine input should not exceed 50 mL.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Wash Solution** by adding 20 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 30 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 Lysis Solution by adding 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Prepare an appropriate amount of TE Buffer containing 3 mg/mL of lysozyme. This solution should be prepared with sterile, RNase-free TE Buffer and RNase-free lysozyme. Keep the solution on ice until needed. These reagents are to be provided by the user.
- The use of fresh urine is recommended for this procedure. Since RNA is a biomolecule that undergoes rapid turnover within a bacterial cell, a delay in isolation may result in purified RNA that does not reflect its true state at the time of sample collection.

1. Lysate Preparation

- a. Transfer 20 - 30 mL of urine to a 50 mL conical tube and centrifuge at 3,000 x g for 5 minutes in a swinging bucket centrifuge to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.
- b. Resuspend the bacteria thoroughly in 100 µL of the lysozyme-containing TE Buffer (prepared by user) by vortexing. Incubate at room temperature for 10 minutes.

Note: The length of the incubation step may be decreased to as little as 5 minutes if the urine bacteria of interest from which RNA is being isolated is known to be Gram-negative.

- c. Add 300 μL of **Lysis Solution** and vortex vigorously for at least 10 seconds. Transfer the lysate to a microcentrifuge tube.
- d. Add 200 μL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

2. Binding to Column

- a. Assemble a column with one of the provided collection tubes
- b. Apply the lysate with the ethanol onto the column and centrifuge for 1 minute at 14,000 $\times g$ (~14,000 RPM).

Note: Ensure the entire lysate 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. Discard the flowthrough. Reassemble the spin column with its collection tube.

Optional Step:

Norgen's Urine Bacteria RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A 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** to the column and centrifuge for 1 minute at 14,000 $\times g$ (~14,000 RPM).

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 column a second time.
- d. Wash column a third time by adding another 400 μL of **Wash Solution** and centrifuging for 1 minute at 14,000 RPM (~14,000 RPM).
- 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.
- c. Centrifuge for 2 minutes at **200 $\times g$ (~2,000 RPM)**, followed by 1 minute at **14,000 $\times g$ (~14,000 RPM)**. Note the volume eluted from the column. If the entire 50 μL has not been eluted, spin the column at 14,000 $\times g$ (~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

Protocol for Optional On-Column DNA Removal

Norgen's Urine Bacteria RNA Purification 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 procedure up to and including "**Binding to Column**" (Steps 1 and 2 of Protocol).
3. Apply 400 μL of **Wash Solution** to the 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 of Protocol).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of bacterial cells	Ensure that the appropriate amount of lysozyme-containing TE buffer and Lysis Solution are added to the bacterial pellet in order to completely lyse the cells.
	Column has become clogged	Do not exceed the recommended amount of 50 mL of urine. 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 35 mL of 95% ethanol is added to the supplied Wash Solution prior to use.
	There is very little or no bacteria in the urine	The expected amount of bacteria in a urine sample is very little. A healthy individual usually has < 10,000 CFU/mL, therefore it is possible that the urine sample has very little bacteria present. The isolated RNA may not be visible when resolved on a gel. In such cases, a larger input volume may be used. Alternatively, a more sensitive method such as BioAnalyzer or PCR amplification may be used for detection.
Clogged Column	Insufficient solubilization of cells	Ensure that the appropriate amount of lysozyme-containing TE buffer and Lysis Solution are added to the bacterial pellet in order to completely lyse the cells.
	Too many bacteria present in the urine	The urine sample that was applied to the column contained too many bacterial cells. Reduce the amount of urine used. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the urine passes through the column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20 °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 “ <i>Working with RNA</i> ” 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.
	Lysozyme used may not be RNase-free	Ensure that the lysozyme being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.
	The urine sample is old.	The use of fresh urine samples is recommended. Proteases and RNases may be present in the sample, and storing the sample for too long before RNA isolation increases the chances of recovering degraded RNA.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed twice with 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.
Genomic DNA contamination	Large amounts of starting material used	Perform RNase-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination (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
Urine Bacteria Genomic DNA Isolation Kit	22400
Urine DNA Isolation Kit	18100
Urine (Exfoliated Cell) DNA Isolation Kit	22300
Urine (Exfoliated Cell) RNA Purification Kit	22500
ProteoSpin™ Urine Protein Concentration 96-Well Kit	23100
ProteoSpin™ Urine Protein Concentration Kit	17400
ProteoSpin™ Urine Protein Concentration Maxi Kit	21600
1kb RNA Ladder	15003
Urine Collection and Preservation Tubes (50 cc) – 1 tube	18111
Urine Collection and Preservation Tubes (50 cc) – 50 tubes	18113
Urine Collection and Preservation Tubes (15 cc) – 1 tube	18120
Urine Collection and Preservation Tubes (15 cc) – 50 tubes	18122
Urine Collection and Preservation Tubes (5 cc) – 1 tube	18116
Urine Collection and Preservation Tubes (5 cc) – 50 tubes	18118
Urine Preservative Single Dose – 1 tube	18124
Urine Preservative Single Dose – 50 tubes	18126

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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