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Urine Exfoliated Cell and Bacteria RNA Purification Kit — Product Insert

Product # 22550

Norgen's **Urine Exfoliated Cell and Bacteria RNA Purification Kit** provides a rapid method for the isolation and purification of total RNA from exfoliated cells that have been shed into the urine from the urinary tract. The kit is also designed for the rapid preparation of bacterial RNA from urine samples. RNA biomarkers from exfoliated cells can be used as a non-invasive tool for a number of diagnostic and research applications including the diagnosis and monitoring of bladder, kidney, or other urinary-tract cancers. 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 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 exfoliated cells or bacteria that are present in the urine sample through the use of centrifugation, followed by lysing the exfoliated cells or bacteria with the provided Buffer SK (please see the flow chart on page 4). Ethanol is then added to the 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 A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Buffer E. 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	1 – 50 mL
Maximum Input of Exfoliated Cells	1 x 10^6
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	20 minutes (for Exfoliated Cells) 30 minutes (for Bacteria)
Average Yield	(For Exfoliated Cells) ~ 1 μg RNA per 1 x 10^5 cells(Varies due to cell density sample) (For Bacteria) ~ 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)
- RNA can be isolated and detected from as little as 100 exfoliated cells
- Isolate high quality total RNA from urine
- 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 # 22500 (25 samples)
Buffer SK	15 mL
Wash Solution A	18 mL
Elution Buffer E	6 mL
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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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*.

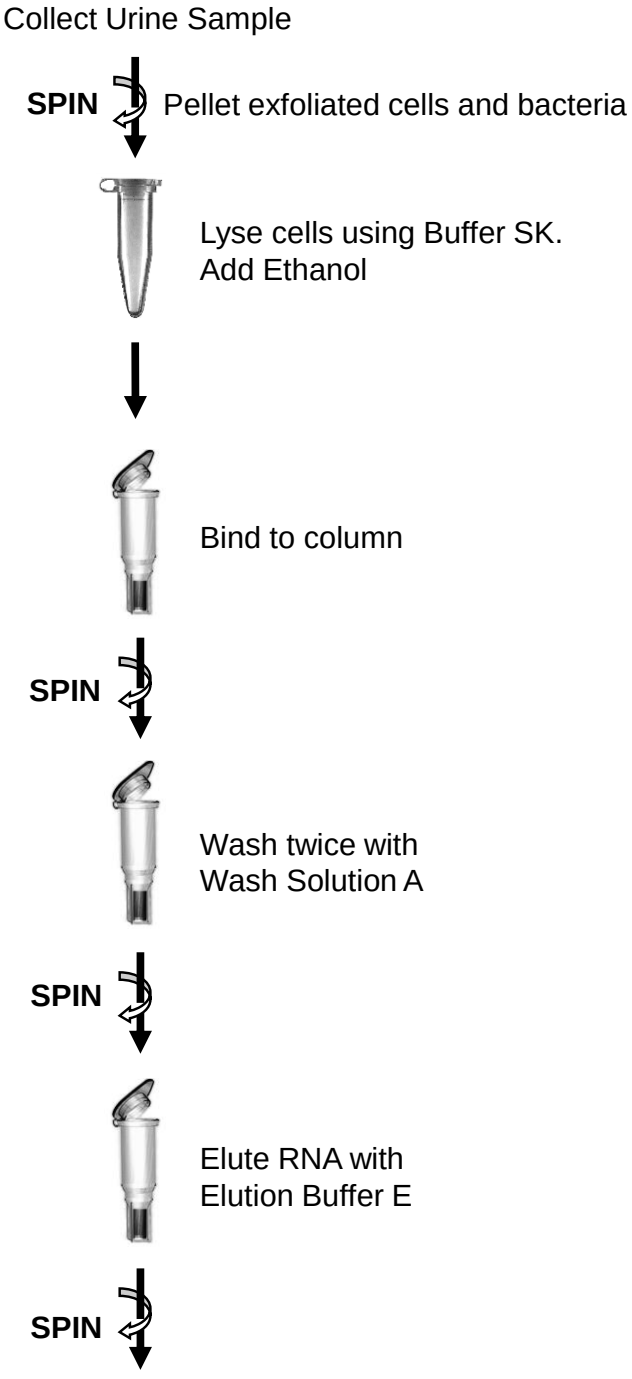
Customer-Supplied Reagents and Equipment

You must have the following in order to use the Urine (Exfoliated Cell) RNA Purification Kit:

- 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

Flow Chart

Procedure for Purifying Urine RNA using Norgen's Urine Exfoliated Cell and Bacteria RNA Purification Kit



Purified Total Urine RNA

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

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.

Protocol for Total RNA Purification from Exfoliated Cells in Urine

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

- 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.
- 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 42 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash**

Solution A. This will give a final volume of 60 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 SK by adding 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Buffer SK required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- It is important to work quickly during this procedure.
- The maximum input of urine per column is 50 mL or 1 x 10^6 exfoliated cells.

1. A) Lysate Preparation for Exfoliated cells

a. Transfer 30 mL of urine to a 50 mL conical tube. Centrifuge the samples in a swinging bucket centrifuge at 650 x g for 5 minutes. The maximum input of urine is 50 mL or 1 x 10^6 cells per column. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For samples less than 1.5 mL, transfer urine to a micro centrifuge tube and centrifuge at 250 x g (~2,000 RPM) for 5 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 350 μL of **Buffer SK** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step. Transfer the lysate to an RNase-free microcentrifuge tube.

c. Add 200 μL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

B) Lysate Preparation for Bacteria

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 **Buffer SK** and vortex vigorously for at least 10 seconds. Transfer the lysate to a microcentrifuge tube.

d. Add 200 μL of 96 – 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.

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 Exfoliated Cell and 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 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 50 µL of **Elution Buffer E** to the column.

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *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 x g (~14,000 RPM) for 1 additional minute.

Note: A smaller volume of Elution Buffer E may be used in order to obtain a more concentrated sample. A minimum volume of 20 µL is recommended

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 Exfoliated Cell and 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 A** 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 RNA isolation procedure up to and including "**Binding to Column**" (Steps 1 and 2 of protocol).

3. Apply 400 µL of **Wash Solution A** 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 of the "**Column Wash**" section (Step 3c of protocol).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells	Ensure that the appropriate amount of Buffer SK was added to the exfoliated cell pellet. Ensure that the appropriate amount of lysozyme-containing TE buffer and Buffer SK are added to the bacterial pellet in order to completely lyse the cells.
	Column has become clogged	Do not exceed the recommended amounts of 50 mL of urine or 1 x 10^6 cells. 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 E 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 42 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	Low cell density in the sample	The cell number in different urine samples vary. While individuals with various diseases have >1000 exfoliated cells per mL of urine, a healthy male may have a number much lower than the 1000 cells per mL limit. It is possible that the total RNA isolated is not visible when resolved on an agarose gel or detected by spectrophotometry. In such cases, a larger input volume may be used. Alternatively, a more sensitive method such as BioAnalyzer or RT-PCR may be used for detection.
	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.

Problem	Possible Cause	Solution and Explanation
Clogged Column	Insufficient solubilization of cells	Ensure that the appropriate amount of Buffer SK was added to the exfoliated cell pellet. Ensure that the appropriate amount of lysozyme-containing TE buffer and Buffer SK are added to the bacterial pellet in order to completely lyse the cells.
	Maximum number of cells exceeds kit specifications	Do not exceed the recommended amounts of 50 mL of urine or 1 x 10^6 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
	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 20°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
	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.
	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.
	The cells are old	Older samples contain prematurely lysed cells which release RNase and can degrade RNA. Fresh urine samples are recommended.

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

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.

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

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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