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Urine Total RNA Purification Maxi Kit (Slurry Format) Product Insert

Product # 29600

Norgen's Urine Total RNA Purification Maxi Kit (Slurry Format) provides a rapid method for the isolation and purification of total RNA from 5 – 10 mL of urine. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to small RNAs. These small RNAs include regulatory RNA molecules such as microRNA (miRNA) as well as tRNA and 5S rRNA. Small RNA molecules are often studied due to their ability to regulate gene expression. miRNAs are typically 20-25 nucleotides long, and regulate gene expression by binding to mRNA molecules and affecting their stability or translation. Several recent studies showed that miRNA regulates cell growth and apoptosis. Furthermore, clinical and experimental analyses suggested that miRNAs may function as a novel class of oncogenes or tumor suppressor genes. MicroRNA expression profiles of different tumor types, relative to their normal tissues, have recently been shown to provide phenotypic signatures for particular cancer types. Unique patterns of aberrant miRNA expression may serve as molecular biomarkers for tumor diagnosis, prognosis of disease specific outcomes, and prediction of therapeutic responses

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds RNA in a manner that depends on ionic concentrations. The RNA is preferentially purified from other cellular components such as proteins, 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 real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

This kit includes enough reagents to process 50 samples of 5 mL of urine, 40 samples of 6 mL of urine, 35 samples of 7 mL of urine, 30 samples of 8 mL of urine and 25 samples of 9-10 mL of urine. A single protocol is provided with the volumes optimized for 5 mL inputs; however the volumes can be adjusted for inputs of as high as 10 mL urine.

Kit Components:

Component	Contents
Slurry C3	20 mL
Lysis Buffer A	2 x 130 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 50 mL conical tubes
- 96 – 100% ethanol
- β-mercaptoethanol

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.

Quality Control
In accordance with Norgen's Quality Management System, each lot of Norgen's Urine Total RNA Purification Maxi Kit (Slurry Format) is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Urine Total RNA Purification Maxi Kit (Slurry Format) is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information
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*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

If liquid containing these solutions is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

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 defence 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

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.
- 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 RNA Wash Solution by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 128 mL. The bottle label contains a box to check to indicate that the ethanol has been added.
- The use of β-mercaptoethanol in lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Buffer A required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- If precipitates (crystallization) are present in the Lysis Buffer A or in Slurry C3 it is highly recommended to warm up the Lysis Buffer A or in Slurry C3 at 60°C for 20 minutes and mix well until the solution becomes clear again. (***Note: Slurry C3 contains grey resin that will not disappear by warming up***)
- The minimum recommended urine input is 5 mL and the maximum recommended urine input is 10 mL.
- It is important to work quickly during this procedure.

Detailed Procedure

Note: This procedure as written is for processing 5mL urine samples. To process different urine volumes (up to 10mL) please check Table 1 for the appropriate volumes of Lysis Buffer A and 96-100% Ethanol to be added to different urine sample volumes. The volume of Slurry C3 is fixed for all urine volumes

1. Aliquot a 5 mL urine sample into a 50 mL conical tube. Add 0.35 mL **Slurry C3** and 4.65 mL of **Lysis Buffer A** directly to the urine. Lyse cells by **vortexing** for 15 seconds. ***(Note: Slurry C3 contains resin and must be mixed well before every pipeting)***

2. Add 5 mL of **96 - 100% ethanol** (provided by the user) to the lysate. Mix by **vortexing** for 10 seconds.

3. Centrifuge for **5 minutes at 2,000 x g**, Discard the supernatant.

4. Add 500 µL **Wash Solution A**, mix well by pipeting or vortexing and then transfer the entire contents into a Mini Filter Spin column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.

Optional Step:

Norgen's Urine Total RNA Purification Maxi Kit (Slurry Format) isolates 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

5. Apply 500 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.

6. Repeat Step 5.

7. Apply 500 µL of **96-100% ethanol** (provided by the user) and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

8. Spin the column, empty, for **3 minutes at 14,000 x g (~14,000 RPM)**. Discard the collection tube.

9. Incubate the column horizontally, with the lid open, at 60°C for 3 minutes.

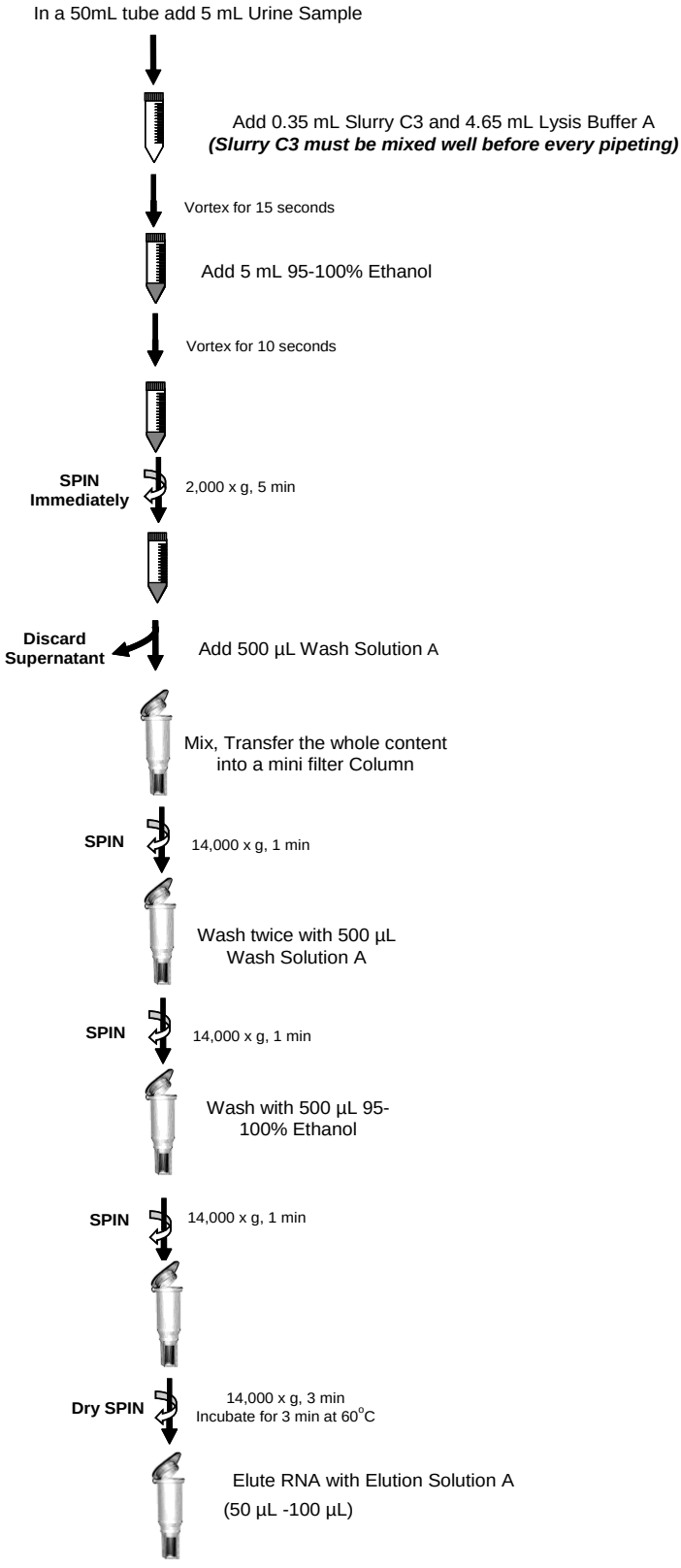
10. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50-100 µL of **Elution Solution A** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **3 minute at 14,000 x g (~14,000 RPM)**.

- **Urine Total RNA is now ready for downstream applications.**

Table 1. Slurry C3, Lysis Buffer A and 96-100% Ethanol to be added to different Urine Sample Volumes

Sample Volume (mL)	Slurry C3 (mL) (Step 1)	Lysis Buffer A (mL) (Step 1)	96-100% Ethanol (mL) (Step 2)
5	0.35	4.65	5
6	0.35	5.65	6
7	0.35	6.65	7
8	0.35	7.65	8
9	0.35	8.65	9
10	0.35	9.65	10

Rapid Flow Chart Procedure



Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Urine Total RNA Purification Maxi Kit (Slurry Format) 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 urine total RNA isolation procedure up to and including Step 3 of protocol.

3. Apply 500 µL **Wash Solution A**, mix well by pipeting or vortexing and then transfer the entire contents into a Mini Filter Spin column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and 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 - 30ºC for 15 minutes.

7. Without any further centrifugation, proceed directly to the second wash step (Step 5 of protocol).

Frequently Asked Questions

1. If I am not going to process my samples immediately is there any additional preservative for long term storage?

- We recommend the use of Norgen's Urine Preservative, 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.

2. If I am not going to process my samples immediately, how should I store my samples?

- We recommend the use of Norgen's Urine Preservative for storage at ambient temperatures. Please see Question 1 above.

3. What if a variable speed centrifuge is not available?

- A fixed speed centrifuge can be used, however reduced yields may be observed.

4. What will happen if my centrifugation speed varied from the recommended speed?

- This may decrease the binding of the RNA to the column.

5. At what temperature should I centrifuge my samples?

- All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.

6. My centrifuge speeds are defined in rpm and not in *g*-force. How can I convert *g-force* to rpm?

- 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.

7. Can I process a different urine volume?

- Yes, you can. To process different urine volumes (up to 10mL) please check Table 1 for the appropriate volumes of Lysis Buffer A and 96-100% Ethanol to be added to different urine sample volumes. The volume of Slurry C3 is fixed for all urine volumes. The minimum recommended urine input is 5 mL, and the maximum recommended input is 10 mL.

8. What if I added more or less from the specified reagents' volume?

- Adding less volume may reduce your RNA yields. Adding more may not affect the RNA yields EXCEPT if more Elution Solution A was added. Eluting RNA in a higher volume of Elution Solution A will result in diluting your RNA.

9. What if I forgot to do a dry spin after my third wash?

- Your RNA elution will be contaminated with traces of the Wash Solution A. This may dilute the RNA yield in elution. Also, it may interfere with your downstream applications. Re-isolate the eluted RNA using the same procedure as you initially isolated the RNA from urine but using your elution as your input.

10. Why did my samples show very low RNA yields?

- Some urine samples contain very little RNA. This varies from individual to individual based on numerous variables. In order to increase the yield, the amount of urine input could be increased.

11. Why does my RNA not perform well in downstream applications?

- If a different Elution solution was used other than the one provided in the kit, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

12. What if the solutions did not flow through the column?

- The centrifugation speed was too low. Check the centrifuge to ensure that it is capable of generating a sufficient centrifugal force that is required to move the liquid phase through the resin. You may also spin an additional two minutes to ensure that the liquid is able to flow completely through the column.

13. Why my 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.
- The cells are old: Older samples contain prematurely lysed cells which release RNase and can degrade RNA. Fresh urine samples are recommended.

Related Products	Product #
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 Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Urine Total RNA Purification Maxi Kit (Slurry) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please 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. or call one of the NORGEN local distributors (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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