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

Urine microRNA Purification Kit — Product Insert

Product # 29000

Norgen's Urine microRNA Purification Kit provides a rapid method for the isolation and purification of small RNA molecules (< 200 nt) from urine samples. 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. Typically miRNAs are 20-25 nucleotides long, and regulate gene expression by binding to mRNA molecules and affecting their stability or translation. Several recent studies have shown 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 concentration. The small RNA molecules are preferentially purified from other cellular components such as ribosomal RNA without the use of phenol or chloroform. The small RNA molecules isolated using Norgen's Urine microRNA Purification Kit can be used in various downstream applications relating to gene regulation and functional analysis, including RT-PCR, northern blotting and microarray analysis.

This kit is designed to process 25 urine samples with a volume of 1.5 mL Urine

Kit Components:

Component	Contents
Stabilizer	1 mL
Lysis Solution	15 mL
Wash Solution	11 mL
Elution Solution	2.5 mL
Mini Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 15 mL conical tubes
- 95 – 100% ethanol
- β-mercaptoethanol (optional)

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 microRNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Urine microRNA Purification Kit 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.

The **Stabilizer** contains sodium azide. Sodium azide is highly toxic, and may be fatal if swallowed or absorbed through skin. Careful handling of this material must be followed, and should include the use of protective eye wear, gloves and lab coats.

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

Procedure

Notes prior to use:

- First time users should read the entire manual before proceeding with the protocol.
- 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 **Wash Solution** by adding 25 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The volume of the urine sample to be processed can vary from 0.5 mL – 3 mL, however the all the solution included in this kit are optimized for an input of 1.5 mL of urine. All the solutions included in this kit are in a linear relationship to the volume of urine sample processed; therefore the volumes of solutions can be adjusted accordingly for the urine input. Ensure that you do not deviate from the ratio specified in the product manual.

Detailed Procedure

1. Add 15 µL of Stabilizer to each 1.5 mL urine sample.

2. Aliquot the 1.5 mL urine into a 15 mL conical tube. Add 500 µL of **Lysis Solution** directly to the urine. Lyse cells by **vortexing** for 15 seconds.

3. Add 1 mL of **95 - 100% ethanol** (provided by the user) to the lysate. Mix by **vortexing** for 10 seconds.

4. Assemble a column with one of the provided collection tubes.

5. Apply up to 650 µL of the lysate with the ethanol (Step 3) onto the column and centrifuge for **1 minute at 5,200 x g** (~ 8,000 RPM). Discard the flowthrough.

   ❖ Note: Ensure the entire lysate 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.

6. Reassemble the spin column with its collection tube. Repeat **Step 5** twice to load the entire lysate.

7. Apply 400 µL of **Wash Solution** to the column and centrifuge for 1 minute. Discard the flowthrough and reassemble the spin column with its collection tube.

   ❖ 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.

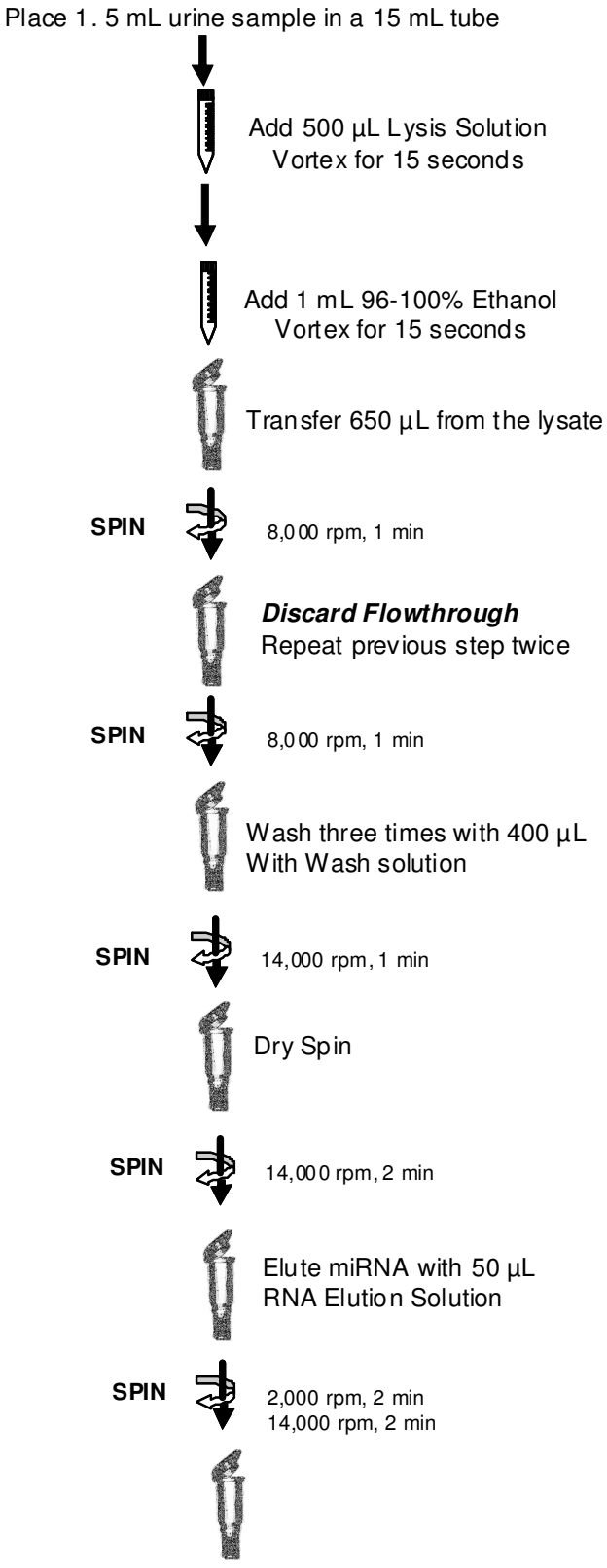
8. Repeat **Step 7** twice**.** Discard the flowthrough and reassemble the spin column with its collection tube.

9. Spin the column, empty, for **2 minutes at 14,000 x g (~14,000 RPM)**. Discard the collection tube.

10. Place the column into a fresh 1.7 mL Elution tube provided with the kit. Add 50 µL of **Elution Solution** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by additional **2 minutes at 14,000 x g (~14,000 RPM)**.

11. 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.

Rapid Flow Chart Procedure



Purified Urinary miRNA

Frequently Asked Questions

1. What if I forgot to add the urine Stabilizer upon sample collection?

- The urine stabilizer should be added immediately upon sample collection. The urine sample may not be stable for more than 3 days without adding the stabilizer. Make sure you add the provided urine stabilizer within one day of sample collection.

2. I am not going to process my samples immediately. Are there any additional preservative for long term storage?

- If the urine sample is not going to be processed within a day of collection, protease inhibitors must be added. We recommend that Sigma's Protease Inhibitor Cocktail is used (Product Number P2714). This product contains a mixture of protease inhibitors known to be very effective with our kit. The cocktail includes AEBSF, EDTA, Bestatin, E-64, Leupeptin and Aprotinin. Add 20 µL of this cocktail to the 1.75 mL sample of urine, as per manufacturer's instructions.

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

- Urine samples in the presence of the stabilizer and the preservative should be stored at room temperature. Turbidity or precipitation may be observed if the urine samples are stored at either 4°C or at -20°C. **DO NOT** discard this precipitate and/or spin down your samples to get rid of the turbidity; this will significantly reduce your DNA yields. Make sure to mix your samples thoroughly before processing.

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

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

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

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

6. 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.

7. 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.

8. Can I process a different urine volume?

- Yes, you can. All the buffers included in this kit are in a linear relationship to the volume of urine sample processed. Make sure that you do not deviate from the ratio specified in the product manual. The minimum recommended urine input is 0.5 mL, and the maximum recommended input is 3 mL. Please note that the buffers are optimized for an input of 1.5 mL of urine.

9. What if I added more or less of the specified reagents' volume?

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

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

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

11. Why did my samples show very low miRNA yields?

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

12. Why does my miRNA not perform well in downstream applications?

- If a different Elution Buffer 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.

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

- The centrifugation speed may be 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.

14. Why my miRNA 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.

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 DNA Isolation Kit 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.

Related Products	Product #
Urine DNA Isolation Kit (Mini slurry Format)	27000
Urine (Exfoliated Cell) DNA Purification Kit	22300
Urine (Exfoliated Cell) RNA Purification Kit	22500
Urine Bacteria DNA Purification Kit	22400
Urine Bacteria RNA Purification Kit	23400
Urine Protein Concentration Micro Kit	17400
Urine Protein Concentration Maxi Kit	21600

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362