

Urine Total RNA Purification Maxi Kit Dx (Slurry Format)

Product Insert

REF Dx29600



IVD

 PIDx29600-7

Intended Use

Norgen's Urine Total RNA Purification Maxi Kit Dx (Slurry Format) provides a fast, reliable and simple procedure for isolating total RNA from urine for subsequent *in vitro* diagnostic use. Purification is based on the use of Norgen's proprietary resin as the separation matrix, and the kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to small RNAs.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated using the RNA isolated with Urine Total RNA Purification Maxi Kit Dx (Slurry Format) in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Urine Total RNA Purification Maxi Kit Dx (Slurry Format) is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including RNA isolation.










Norgen's Urine Total RNA Purification Maxi Kit Dx (Slurry Format) does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

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 (see Table 1).

Kit Components

Component	Product #Dx29600 (50 samples)
RNA Lysis Solution	3 x 90 mL
RNA Wash Solution	24 mL
RNA Elution Solution	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Label Legend

								
Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manufacturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temperature limitation

Advantages

- CE-IVD marked in accordance with Regulation (EU) 2017/746
- Fits into *in vitro* diagnostic workflows
- Sample collection is non-invasive and painless
- Fast and easy processing using a rapid spin-column format
- Isolate high quality total RNA
- Compatible with preserved urine samples collected using Norgen's Urine Preservative (please see Related Products Table)

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable until the expiration date specified on the label.

Precautions

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.

The **RNA Lysis Solution** contains guanidine hydrochloride, and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Urine of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with urine.

Should any serious incident, such as injury or impairment of the user, occur during the use of this device, or because the use of this device, please contact Norgen Biotek to report the incident. Additionally, report the incident to the competent authority in the country in which the user and/or patient is established.

Customer-Supplied Reagents and Equipment

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

Procedure

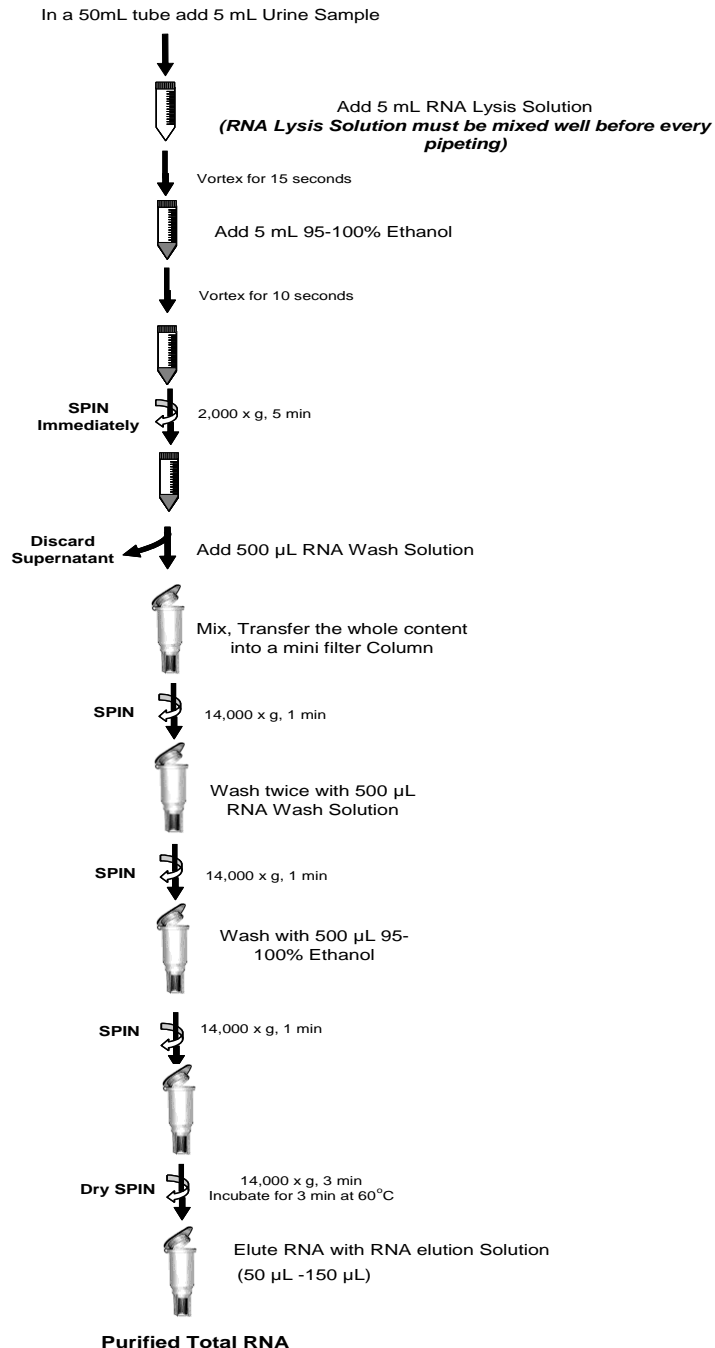
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can 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

Flow Chart

Procedure for Purifying Urine Total RNA using Norgen's Urine Total RNA Purification Maxi Kit Dx (Slurry Format)



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 66 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 90 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 μ L of β -mercaptoethanol (provided by the user) to each 1 mL of RNA Lysis Solution required. β -mercaptoethanol is toxic and should be dispensed in a fume hood.
- If precipitates are present in the RNA Lysis Solution it is highly recommended to warm up the RNA Lysis Solution at 60°C for 20 minutes and mix well until the solution becomes clear again.
- It is important to work quickly during this procedure.

Detailed Procedure

1. Aliquot a 5 mL urine sample into a 50 mL conical tube. Add 5 mL of **RNA Lysis Solution** directly to the urine. Lyse cells by **vortexing** for 15 seconds. (**Note: RNA Lysis Solution 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 μ L **RNA Wash Solution**, mix well by pipeting 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.
5. Apply 500 μ L of **RNA Wash Solution** 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 μ L of **95-100% 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, with the lid open, at 60°C for 3 minutes.
10. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 μ L of **RNA Elution Solution** 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)**.

Table 1. RNA Lysis Solution and 96-100% Ethanol to be added to different Urine Sample Volumes.

Sample Volume (mL)	RNA Lysis Solution (mL) (Step 1)	96-100% Ethanol (mL) (Step 2)
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

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. 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 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 Buffer was added. Eluting RNA in a higher volume of Elution Buffer 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. 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 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.

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

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Total RNA Purification Maxi Kit Dx (Slurry Format) for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Technical Support

Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

Authorized Representative



EMERGO EUROPE
Westervoortsedijk 60,
6827 AT Arnhem
The Netherlands



Norgen Biotek Corp.
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