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Urine RNA Concentration, Preservation and Isolation Kit – 25 Individual Devices

Product Insert **Product #: 38100**

Norgen's Urine RNA Concentration, Preservation and Isolation Kit provides a safe and rapid all-in-one procedure for the concentration, preservation and isolation of urine RNA at ambient temperatures.

INTENDED USE

Norgen's Urine RNA Concentration, Preservation and Isolation Kit is an all-in-one solution designed for 1) rapid and simple urine concentration; 2) preservation of RNA in urine samples at ambient temperature and 3) isolation of high quality RNA within a laboratory setting. The Urine RNA Concentration, Preservation and Isolation Kit contains 25 Individual Urine Concentration and Preservation Devices, as well as the required reagents for the subsequent laboratory isolation of the urine RNA from the preserved concentrated samples. Each of the 25 Individualized Urine Concentration and Preservation Kits consist of 2 key components: (1) a Urine Concentration Tube, (2) Norgen's Urine Preservative contained within sealed squeezable tubes. The urine RNA in preserved samples is stable for more than 2 years at room temperature. This kit is ideal for concentrating, preserving and isolating RNA samples for epidemiological and population studies.

URINE RNA PRESERVATIVE

Norgen's Urine Preservative is an aqueous storage buffer that prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely. In addition, the buffer eliminates the need to immediately process or freeze samples and allows the samples to be shipped to centralized testing facilities at ambient temperature. The components of the buffer allow samples to be stored for over 24 months without any detectable RNA degradation.

NUCLEIC ACID ISOLATION FROM PRESERVATIVE

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Briefly, concentrated and preserved urine samples are lysed using Norgen's Lysis Solution. The lysed concentrated and preserved urine samples are then loaded onto a provided Mini Filter Spin Column. This is followed by washing of the bound RNA to remove the remaining proteins or other impurities. Lastly, the purified total urine RNA is eluted into an RNA Elution Solution.

Urine RNA Concentration, Preservation and Isolation Kit Contents:

Component	Contents
Individual Urine Concentration and Preservation Devices	25
Lysis Solution	30 mL
Wash Solution	12 mL
RNA Elution Solution	10 mL
Mini-Filter Columns	25
Collection Tubes	25
Elution Tubes (1.7mL)	25
Product Insert	1

Individual Urine Concentration and Preservation Device Contents:

Component	Contents
Urine Concentration Tube	1
Urine Preservative within Sealed Ampules	1
Absorbent Pad	1
ID Label	1
Donor Instructions	1

Kit Specifications	
Volume of Urine Processed	30 mL
Preservation Temperature Range	-20°C to 50°C
Preservation Time	24 months at room temperature
Time to Complete RNA Isolation	30 minutes
Average Yield from 30 mL*	22 ng/µL
Average OD 260/280	1.7

* Average RNA yield will vary depending on the health status of the donor

MATERIALS REQUIRED BUT NOT SUPPLIED

1. For Sample Collection and Preservation

- Clean container to collect urine sample
- Scissors

2. For RNA Isolation

- Benchtop microcentrifuge
- Micropipettors
- 100% Isopropanol
- β - mercaptoethanol

SHELF LIFE AND HANDLING

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 RNA Concentration, Preservation and Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS – Urine Donor

1. If any of the Urine Preservative comes into contact with skin or eyes, wash thoroughly with water. Do not ingest the Urine Preservative
2. Use caution when handling urine samples. Take care not to spill any of the urine. Ensure that hands are washed well after procedure and that the area where the procedure was performed is wiped down and cleaned if any spills occurred.

WARNINGS AND PRECAUTIONS – RNA Isolation from Preserved, Concentrated Urine Samples

1 Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
3. Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the procedures.
4. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
5. Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
6. As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
7. Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.
8. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Procedures

A. SAMPLE COLLECTION AND PRESERVATON PROCEDURES

1. Open an Individual Urine Concentration and Preservation Device, and remove the Urine Concentration Tube, Urine Preservative and Donor Instructions.
2. Collect urine into any clean urine collection container.
3. Open the **Urine Concentration Tube** and remove the inner **Urine Concentrator** from the outer stand.
4. Transfer collected urine up to the marked line engraved at the top of the **Urine Concentrator.**
5. Replace the **Urine Concentrator** back into the outer stand and screw the cap back onto the tube tightly.
6. Mix well by shaking to allow the grey material at the bottom of the tube to be in a complete re-suspension with the urine sample.
7. Stand the **Urine Concentration Tube** vertically for 10 minutes to allow the grey material to completely precipitate.
8. After 10 minutes, remove the lid from the Urine Concentration Tube and carefully remove the inner **Urine Concentrator** from the outer stand. Carefully decant the supernatant back to the urine container by pouring. Please ensure that none of the grey resin material is decanted along with the liquid. A small amount of supernatant may be left behind to ensure no loss of the resin.
9. Replace the **Urine Concentrator** back inside the outer stand.
10. Cut the tip of the **Urine Preservative** ampule with scissors. Squeeze the ampule to empty the contents into the **Urine Concentration Tube**.
11. Replace the cap onto the **Urine Concentration Tube** and screw tightly in place to close tube.
12. Shake well for 10 seconds to mix.
13. If required, fill in information on the provided ID Label and affix to the **Urine Concentration Tube.** Each tube is also marked with a unique identifier code which can be used for traceability of samples.
14. Dispose of the empty **Urine Preservative** ampule and tip in the garbage. Rinse scissors under water and wipe dry.
15. Dispose of the urine supernatant by discarding in the lavatory.
16. Thoroughly wash your hands and wipe down area in which the procedure was performed.

B. INSTRUCTIONS FOR STORAGE OF PRESERVED URINE SAMPLES

1. Preserved urine samples can be stored at room temperature (20-26℃) for more than 2 years without significant loss of RNA quality.
2. RNA has also been successfully isolated from samples stored at 55℃ for 4 months.
3. Storage at -80℃ is recommended for archival samples and will provide optimal preservation. The preservation buffer will freeze at -80℃. Samples can be stored indefinitely at -80℃.

C. ISOLATION OF RNA FROM CONCENTRATED AND PRESERVED URINE SAMPLES

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

- 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 33 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 45 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 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.

1. Mix the preserved urine sample in the **Urine Collection Tube** by inversion and gentle shaking for a few seconds.
2. To the preserved urine add **1 mL Lysis Solution** (with β-mercaptoethanol added) and mix well by vortexing for 15 seconds.
3. Add **1 mL of 100% Isopropanol** and mix well by vortexing for 15 seconds.
4. Allow the resin to settle down completely to the bottom of the **Urine Collection Tube** either by gravity or by centrifugation for **3 minutes at 2,000 RPM**. Decant the supernatant by pouring or pipetting.
5. Add **800 µL Wash Solution** and mix well by vortexing.
6. Transfer **750 µL** of the grey **Slurry**/**Lysate** into a Mini Filter Spin column. Centrifuge for **1 minute at 10,000 rpm** and discard the flow-through.
7. Repeat step 6 until you have completely transferred the entire **Slurry**/**Lysate** into a Mini Filter Spin column.
8. Apply **400 µL of Wash Solution** to the column and centrifuge for **1 minute at 14,000 rpm**. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Apply **400 µL of Wash Solution** to the column and centrifuge for **1 minute at 14,000 rpm**. Discard the flow-through and reassemble the spin column with its collection tube.
10. Spin the column for **3 minutes at 14,000 rpm** in order to thoroughly dry the resin. Discard the collection tube.
11. Transfer the spin column to a provided Elution tube. Depending on the downstream application **apply 100 - 300 µL of RNA Elution Solution** to the column and centrifuge for **2 minutes at 2000 rpm**, followed by **2 minute at 14,000 rpm**.
12. The purified RNA sample may be stored at 4°C up to 2 months. It is recommended that samples be placed at –20°C for long term storage.

Frequently Asked Questions – Sample Concentration and Preservation

1. What type of container should I use to collect my urine sample?

- Urine should be collected in a clean urine collection cup.

2. What should I do if the some of the grey resin is transferred out of the Urine Concentration Tube when I am decanting the urine supernatant?

- Pour the urine sample back into the Urine Concentration Tube, remix and allow to stand for another 10 minutes to precipitate. After 10 minutes decant the urine supernatant into the urine collection cup.

3. What if I do not have a large enough urine sample to reach the indicated line on the Urine Concentrator?

- Urine samples can be pooled from urine collected the same day in order to reach the indicated line.

4. What if the grey resin has not precipitated fully after the 10 minutes?

- Wait for an additional 5 minutes and decant the urine supernatant. Once you see a clear separation between the precipitated resin and the urine supernatant, decant the urine supernatant even if it was still turbid.

5. What if I spill some of the Urine Preservative when I cut open the tube?

- At least half of the Urine Preservative must be added to the concentrated urine. If less than half of the Urine Preservative was spilt, you can still add the remainder to the concentrated urine. If you have lost more than half of the Urine Preservative you will have to use a new Urine Preservative.

Frequently Asked Questions – RNA Isolation

1. What If a variable speed centrifuge is not available?

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

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

- This may lead to the degradation of the genomic RNA or reduction in the total RNA yields.

3. 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.

4. My centrifuge speeds are defined in rpm and not in *g*-force, how can I convert *g-force* to rpm?

- A 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.

5. What If I added more or less of 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 higher volumes of RNA Elution Solution will result in diluting your RNA.

6. What If I forgot to do a dry spin after my second wash?

- Your first RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your first elution and it may interfere with your down stream applications.

7. Can I perform a second elution?

- Yes, you can. A third elution is possible, but it is recommended that this elution is performed in a smaller volume (50 µL).

8. Why do my samples show very low RNA yield?

- 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.

9. Why does my RNA does 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.

10. 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.

11. 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 DNA Concentration, Preservation and Isolation Kit	38000
Urine Protein Concentration, Preservation and Isolation Kit	38200
Urine Collection and Preservation Tube	18111

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. We reserve the right to change, alter, or modify any product to enhance its performance and design.

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 RNA Collection, Preservation and Isolation Kits 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.

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