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Urine DNA Isolation Maxi Kit (Slurry Format) Product Insert

Product # 50100

Norgen's Urine DNA Isolation Maxi Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating DNA from various amounts of urine ranging from 25 mL to 80 mL. DNA found in urine can be divided into 2 basic categories. The larger species (genomic DNA) is generally greater than 1 kb in size, and appears to be derived mainly from cells shed into the urine. The second species is smaller, generally between 150 and 250 bp (apoptotic DNA), and derives, at least in part, from the circulation. The second species is also considered as an RNA/DNA hybrid as reported by Halicka *et al.,* 2000. Both types of DNA can be isolated reliably using this kit. Norgen's Urine DNA Isolation Maxi Kit (Slurry Format) can also be used for the isolation of viral DNA from urine. The viral DNA is isolated free from inhibitors and can be used directly as the template in a PCR reaction for viral DNA detection. The procedure can be used for the isolation of viral DNA from a broad range of DNA viruses. Typical yields of DNA isolated will vary depending on the input sample, with more concentrated samples tending to yield more DNA. Preparation time for a single sample is less than 30 minutes. The purified urine DNA is compatible with PCR and Southern Blot analysis.

The Urine DNA Isolation Maxi Kit (Slurry Format) contains sufficient materials for 50 DNA preparations from urine volumes ranging from 25 mL and up to 80 mL.

Kit Components:

Component	Contents
Slurry B1	18 mL
Binding Buffer A	50 mL
Lysis Buffer A	30 mL
Wash Solution A	38 mL
Elution Buffer B	15 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	100
Product Insert	1

Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator
- 50mL tubes
- Lysozyme (if bacterial gDNA isolation is needed)
- Proteinase K (20 mg/mL) (Optional)

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment. It is recommended to warm up Lysis Buffer A for 20 minutes at 60ºC if any salt precipitation is observed.

Quality Control

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

Product Use Limitations

Norgen's Urine DNA Isolation 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*.

Lysis Buffer A contains guanidine thiocyanate, and should be handled with care. Guanidine thiocyanate forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

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

General Precautions
- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents.
- Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.
- Use proper pipeting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers

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.
- Do not spin down or filter the urine sample before proceeding with the isolation, as this could decrease the DNA yield.
- 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. Slurry B1 contains grey resin that will not dissolve with warming.
- Prepare a 400 mg/mL stock solution (approximately 1.7 x10^7 units/mL) of lysozyme as per supplier's instructions if bacterial gDNA isolation is needed
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the Wash Solution A by adding 90 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 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Detailed Procedure

- Urine volumes from 25 – 80 mL can be processed with this kit

1. Transfer 25 mL of the urine sample to be processed to a 50 cc centrifuge tube and add 300 µL of **Slurry B1** to the urine sample. Mix well by vortexing for 10 seconds.
   ***(Note 1*: *Slurry B1 contains resin and must be mixed well before every pipeting*)**

2. Centrifuge for **2 minutes at 2,000 RPM**, then discard the supernatant carefully in order not to dislodge the precipitated slurry pellet.

3. To the slurry pellet from **Step 2** add up to an additional 25 mL of urine if the total urine input to be processed is more than 25 mL.
   (**Note**: Additional urine from 5 to 25 mL can be added at this step. If the total amount of urine to be processed is over 50 mL, the additional urine will be added in additional steps below)

4. For every **5 mL of urine added in Step 3, add 75 µL of Binding Buffer A and mix well by vortexing for 10 seconds.**

5. Centrifuge **for 2 minutes at 2,000 RPM,** then discard the supernatant carefully in order not to dislodge the precipitated slurry pellet**.**

6. **If additional urine over 50 mL is to be processed, repeat Steps 3, 4 and 5 to process an additional 25 mL (total of 75 mL). If a total of 80 mL is to be processed, repeat Steps 3, 4 and 5 with the remaining 5 mL of urine.**

7. **Option 1: To Isolate bacterial gDNA with the human DNA**
   - Add 12 µL of the previously prepared **lysozyme** to the precipitated slurry pellet. **Vortex for 10 seconds**. Incubate the mixture at **60°C for 20 minutes** then proceed to **Step 8**.

   Option 2 : Urine samples which contain large amounts of protein
   - In case your urine sample contains large amount of proteins a Proteinase K treatment can be done as this step. Add 50 µL from 20mg/mL Proteinase K to the precipitated slurry pellet. **Vortex for 10 seconds**. Incubate the mixture at **60°C for 20 minutes** then proceed to **Step 8**.

   Option 3: Proceed directly to **Step 8** if Option 1 or Option 2 is not required.

8. Add 500 µL **Lysis Buffer A** to the precipitated slurry pellet, mix well by vortexing for 10 seconds.

9. Add 500 µL of **96-100% Ethanol** to the mix from **Step 8**, mix well by vortexing for 10 seconds.

10. Transfer 650 µL from the previous mix into a Mini Filter Spin column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.

11. Repeat **Step 10** until the entire mixture from **Step 9** has been transferred to the Mini Filter Spin Column.

12. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.

13. Repeat Step 12 to wash column second time.

14. Wash the column a third time by adding another 400 µL of **Wash Solution A** and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.

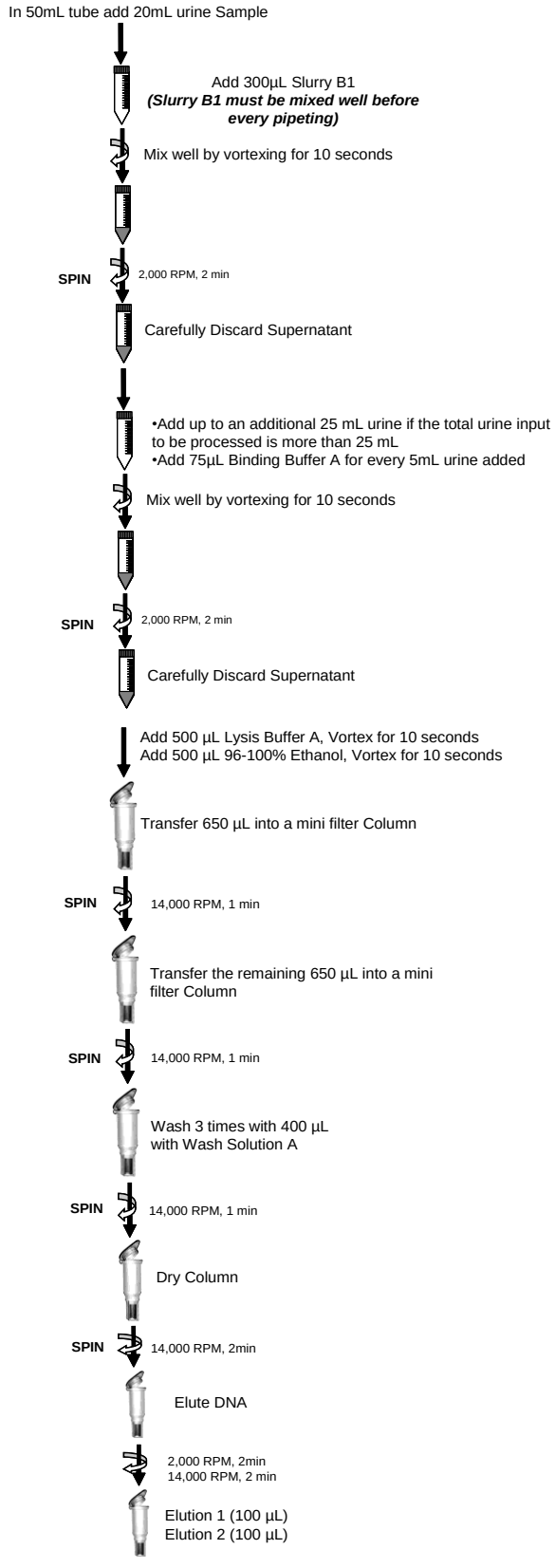
15. Spin the column for **2 minutes** empty at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.

16. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer B** to the column and centrifuge for **2 minutes at 2,000 RPM**, followed by **2 minutes at 14,000 RPM**.

17. Transfer the spin column to a second fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer B** to the column and centrifuge **2 minutes at 14,000 RPM**.

Note: The majority of the DNA will be eluted in the first elution, however additional DNA will be eluted in the second elution. We do not recommend pooling the 2 elutions as this will dilute the final concentration of the DNA.

Rapid Flow Chart



Frequently Asked Questions

1. 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** when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. Urine samples in 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.

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

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

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

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

4. 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.

5. Can I process a different urine volume?

- Yes, you can process different urine volumes ranging from 25 mL and up to 80 mL without changing the volumes of Slurry B1 outlined in the detailed procedure. However, for every 5 mL of urine input over 25 mL (up to 80 mL) 75 µL of Binding Buffer A needs to be added.

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

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

7. What if my incubation time varied from the 20 minutes specified in the product manual?

- Less than 20 minutes will result in lower bacterial DNA yields. More than 20 minutes may not affect your bacterial DNA yields but may lower human DNA yields.

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

- Your DNA elution will be contaminated with the Wash Solution A. This may dilute the DNA yield in your first elution and it may interfere with your downstream applications.

9. Can I perform a third elution?

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

10. Why do my samples show very low DNA yield?

- Some urine samples contain very little DNA. 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 DNA not perform well in downstream applications?

- If a different Elution Buffer B 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 B with the intended use.

12. What is the expected DNA yield from urine?

- Urinary DNA yields vary sample to sample. Generally the DNA yield ranges between 50 ng – 2 µg/mL of urine sample. In many cases, DNA yields from urine are too low to be visualized on an agarose gel; however, the DNA yield is sufficient for most downstream applications including PCR and Southern hybridization.

13. I am noticing a white precipitate in my elution. What should I do?

- This white precipitate may appear in the elution depending on the nature of the sample. This white precipitate will not affect your downstream application. Simply mix your elution well before using.

14. **Can I process frozen urine sample?**
    - Frozen samples can be processed. You may notice some precipitation after thawing the urine sample. DO NOT discard any precipitates by either centrifugation or filtration. After thawing mix the urine sample very well before processing.

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 Maxi Kit (Slurry Format) 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. You can also visit our website (www.norgenbiotek.com) or email us at techsupport@norgenbiotek.com.

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

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