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Urine DNA Isolation Mini Kit (Slurry Format) Product Insert

Product # 27000

Norgen's Urine DNA Isolation Mini Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating DNA from various amounts of urine ranging from 5 mL to 25 mL. DNA found in urine (See Figure below) 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. 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.

This kit is designed to process various numbers of samples depending on sample volumes:

Number of samples	Urine volume/Sample
48 Samples	5 mL
24 Samples	10 mL
12 samples	20 mL
8 Samples	25 mL

Kit Components:

Component	Contents
Stabilizer	3 mL
Binding Solution I	40 mL
Proteinase K	1 mL
Pronase	1 mL
Binding Solution II	4 mL
Wash Solution I	8 mL
Wash Solution II	25 mL
Elution Buffer	15 mL
Mini Filter Spin Columns	48
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 x g
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator
- 15 mL tubes

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

Norgen's Urine DNA Isolation Kit (Mini Slurry Format) contains ready-to-use Proteinase K and Pronase solutions, which are dissolved in a specially prepared storage buffer. The Proteinase K and the Pronase are stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K and Pronase, storage at 2–8°C is recommended.

Quality Control

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

Product Use Limitations

Norgen's Urine DNA Isolation Mini 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.

The **Binding Solution I, Binding Solution II, Wash Solution I** and **Wash Solution II** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

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 buffers 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.

Notes prior to use:

- 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.
- Always **vortex** both the **Proteinase K** and the **Pronase** before use.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of **Binding Solution II and Wash Solution I** by adding the proper volume of 96-100% ethanol indicated in Table 1 below (provided by the user) to the supplied bottle containing the concentrated **Binding Solution II** and **Wash Solution I**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Table 1. Volume of Ethanol to be added to Binding Buffer II and Wash Buffer I

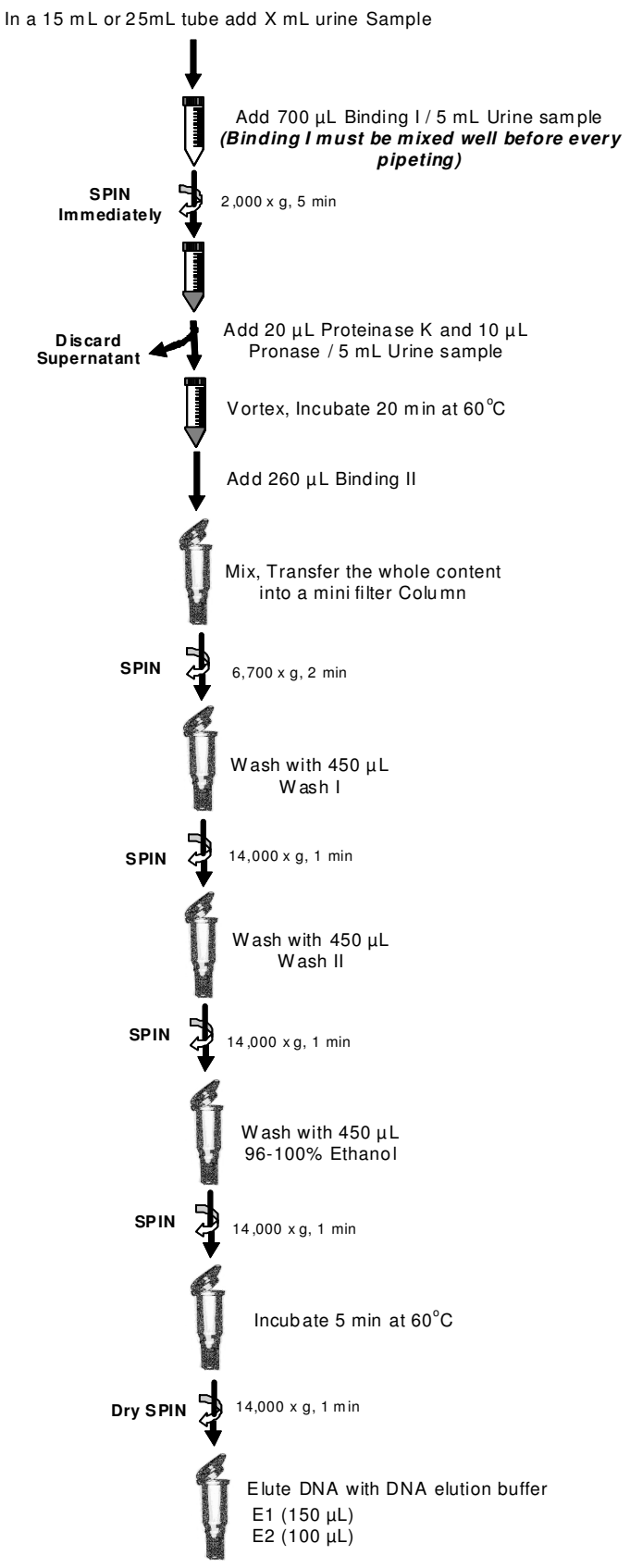
	Volume Provided	Ethanol (96-100%) Volume to Add	Final Volume
Binding Solution II	4 mL	11 mL	15 mL
Wash Solution I	8 mL	22 mL	30 mL

Detailed Procedure (Adjust the volume accordingly if you are processing different urine volume)

1. Add 50 µL of **Stabilizer** for every 5 mL urine sample.
2. Add 700 µL of **Binding Solution I** for every 5 mL urine sample. Mix well by inversion. ***(Note: Binding Solution I must be mixed well before every pipeting)***
3. Centrifuge for **5 minutes at 2,000 x g**, then discard the supernatant.
4. Add 20 µL from both **Proteinase K** and **Pronase** to the precipitated slurry pellet resulting from 5 mL of urine sample. **Vortex for 10 seconds**.
5. Incubate the mixture at **60°C for 20 minutes**.
6. After the 20 minute incubation, add 260 µL **Binding Solution II**, mix well by pipeting and then transfer the entire contents into a Mini Filter Spin column.
7. Centrifuge for **2 minutes at 6,700 x g**, and discard the flow-through.
8. Apply 450 µL of **Wash Solution I** to the column and centrifuge for **1 minute**. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Apply 450 µL of **Wash Solution II** to the column and centrifuge for **1 minute**. Discard the flow-through and reassemble the spin column with its collection tube.
10. Apply 450 µL of ethanol (96-100%) to the column and centrifuge for **1 minute**.
11. Discard the flow-through and place the column (without collection tube) into a 60°C incubator for 5 minutes. Remove column and reassemble the spin column with its collection tube.
12. Spin the column for 1 minute in order to thoroughly dry the resin. Discard the collection tube.
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 150 µL of **Elution Buffer** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **1 minute at 14,000 x g (~14,000 RPM)**.
14. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM).**

    ❖ **Urine DNA is now ready for downstream applications.**

Rapid Flow Chart Procedure



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 be stable for not more than 3 days without adding the stabilizer. Make sure you add the provided urine stabilizer within one day from sample collection.

2. I am not going to process my samples immediately. Is there any additional preservative that can be added for a 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 50 µL of this cocktail to the 5 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 were stored at either 4°C or at -20°C. **DO NOT** discard this precipitate and/or spin down your samples to remove 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 lead to the degradation of the genomic DNA or reduction in the total DNA yields.

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?

- 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.

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 buffers are optimized per 5 mL of urine sample.

9. 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 was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.

10. What If my incubation temperature varied from the specified 60°C?

- The incubation temperature can be in the range of 55°C - 65°C. At other temperatures the activity of both the Proteinase K and the Pronase will be reduced. This will result in a reduction in your DNA yields.

11. What If my incubation varied from the 20 minutes specified in the product manual?

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

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

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

13. Can I perform a third elution?

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

14. Why am I eluting my DNA into two elutions?

- The first elution will mainly contain the low nucleic acid species and traces from the large DNA species. However, your second elution will mainly contain the large DNA species and traces from the low nucleic acid species.

15. 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. Increasing the first incubation time at 60ºC (up to overnight) could also result in increased yields.

16. Why does my DNA 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.

17. What is the expected DNA yield from urine?

- The urinary DNA yield varies between individual samples. 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 of the downstream applications including PCR and Southern hybridization. (See Figure 1below).

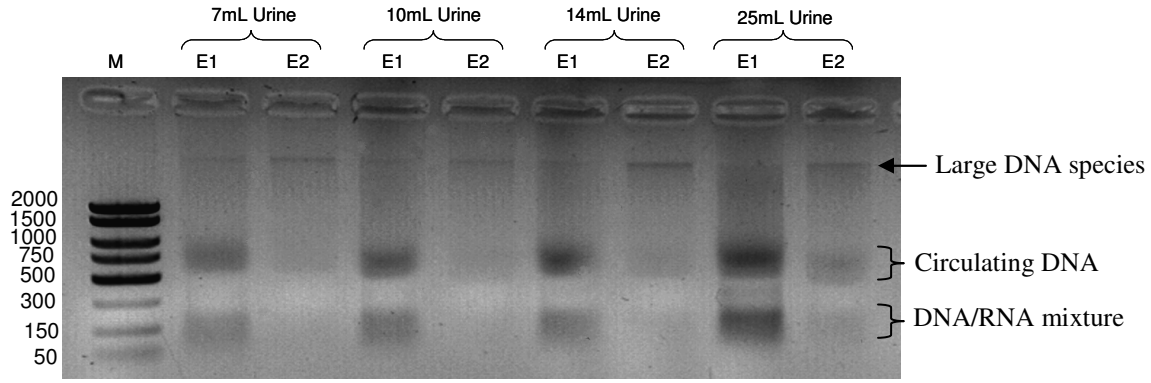


Figure 1. A typical 1.2% agarose gel showing total urinary DNA isolated from different urine volumes using Norgen Urine DNA Isolation Kit. Each lane shows one tenth from each elution (i.e. E1: 15 µL out of 150 µL were loaded on the gel, E2: 10 µL out of 100 µL were loaded on the gel). M: 10 µL Norgen's FastRunner DNA Ladder.

Related Products	Product #
Urine DNA Isolation Kit	18100
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

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 Mini 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 (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

References

Halicka, H. D., Bedner, E. and Darzynkiewicz, Z. (2000). Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. *Exp Cell Res*. **260**, 248-256.

M. Abdalla and Y. Haj-Ahmad. (2006). Urinary Proteomic and Genomic Profiles from Hepatitis C virus, Hepatitis B Virus, and Hepatocellular Carcinoma Patients. *Molecular & Cellular Proteomics*. ASBMB, S369

Application Note:
http://www.norgenbiotek.com/tech%20downloads/Application%20Notes/Application%20Note%2010%20-%20Urine%20DNA.pdf

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