

Urine DNA Isolation Kit

Product # 18100

Product Insert

Norgen's Urine DNA Isolation Kit provides a fast, reliable and simple procedure for isolating DNA from as low as 50 µL of urine. DNA found in urine can be divided into 2 basic categories. The larger species, genomic-DNA (gDNA), is generally greater than 1 kb in size, and appears to be derived mainly from exfoliated cells (**See Figure on page 6**). 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 about 30 minutes. The purified urine DNA is compatible with most molecular biological applications such as PCR, q-PCR, Southern Blot, etc.

This kit is designed to process 50 urine samples with a volume of 1.75 mL or less.

Kit Components:

Component	Contents
Binding Solution K	15 mL
Proteinase K in Storage Buffer	2 mL
Pronase in Storage Buffer	2 mL
Solution WN	9 mL
Wash Solution D	9 mL
Wash Solution B	30 mL
Elution Buffer B	15 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	100
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Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator

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. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer.

Quality Control

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

Product Use Limitations

Norgen's Urine DNA Isolation Kit is designed for research purposes only. It is not intended for 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 Safety Data Sheets (SDSs). 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 K**, **Solution WN**, **Wash Solution B** and **Wash Solution D** 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.

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.

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.
- First time users should read the entire manual before proceeding with the protocol.
- 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.
- Prepare a working concentration of **Solution WN** and **Wash Solution D** 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 **Solution WN** and **Wash Solution D**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat an incubator or heating block to 60°C.
- This protocol is outlined for an input of 1.75 mL of urine, however it is possible to process 50 µL – 2.6 mL of urine. Ensure that the volume of **Binding Solution K** added is adjusted accordingly based on the urine input.

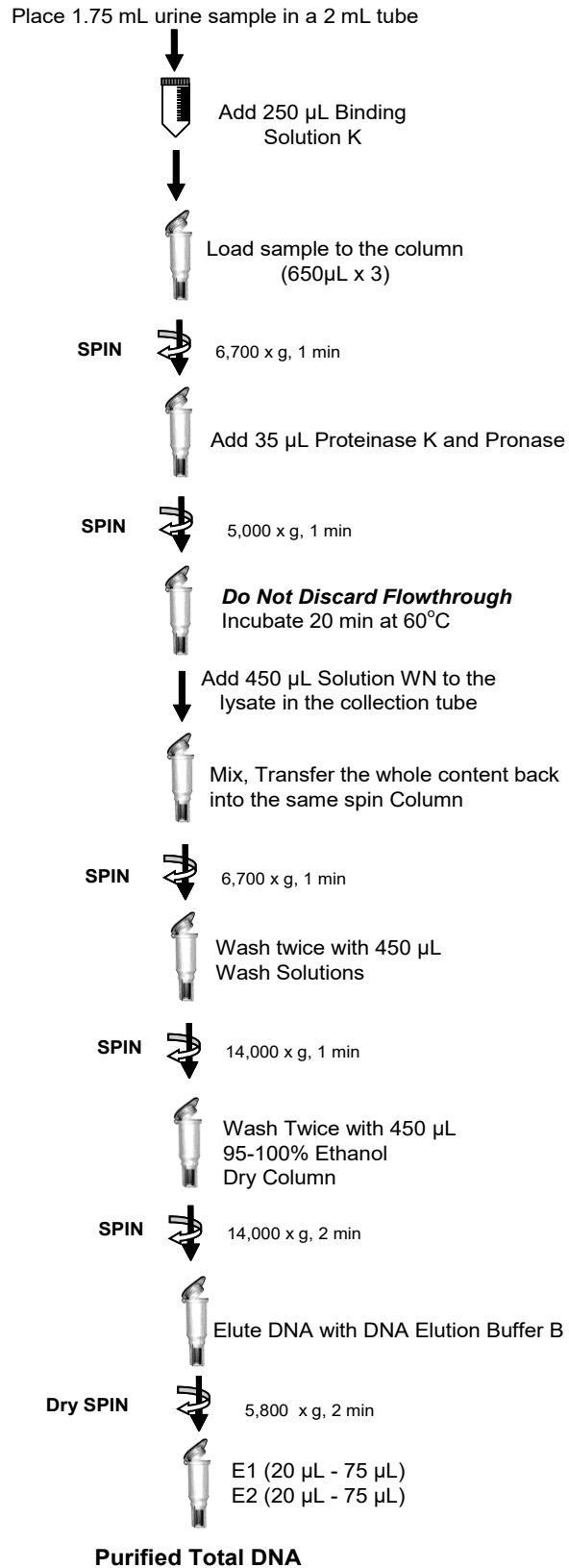
Table 1: Volume of Ethanol to be added to Solution WN and Wash Solution D

	Volume Provided	Ethanol Added (96-100%)	Final Volume
Solution WN	9 mL	21 mL	30 mL
Wash Solution D	9 mL	21 mL	30 mL

Detailed Procedure - (Adjust the volume of **Binding Solution K** accordingly if you are processing a different urine volume)

1. Add 250 μL of **Binding Solution K** for every 1.75 mL urine sample. **Mix** well by inverting a few times;
2. Apply 650 μL of the urine sample onto a column and centrifuge for **1 minute at 6,700 x g**. Discard the flowthrough and reassemble the spin column with its collection tube. **Note:** If the entire volume does not pass through into the collection tube the column can be spun for an additional minute;
 - Repeat Step 2 until the entire urine sample has passed through the column.
3. Add 35 μL of **Proteinase K** and 35 μL of **Pronase** to each column and centrifuge for **1 minute at 6,700 x g**. **Do Not Discard the Flowthrough**, as it contains the DNA;
4. Incubate the column and collection tube at **60°C for 20 minutes**;
5. After the 20 minute incubation, add 450 μL of **Solution WN** to the lysate in the collection tube, mix well by pipeting and then transfer the entire contents of the collection tube back onto the spin column;
6. Centrifuge for **1 minute at 6,700 x g**. Discard the flow-through;
7. Apply 450 μL of **Wash Solution D** 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;
8. Apply 450 μL of **Wash Solution B** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flow-through and reassemble the spin column with its collection tube;
9. Apply 450 μL of 96-100% ethanol (supplied by the user) to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flow-through and reassemble the spin column with its collection tube;
10. Repeat Step 9 a second time;
11. Spin the column, empty, for **2 minutes at 14,000 x g (~14,000 RPM)**. Discard the collection tube;
12. Transfer the spin column to a fresh Elution Tube. Apply 20 μL - 75 μL of **Elution Buffer B** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM)**, followed by **2 minutes at 5,800 x g (~8,000 RPM)**;
13. Transfer the spin column to a second fresh Elution Tube. Apply 20 μL - 75 μL of **Elution Buffer B** to the column and centrifuge for **2 minutes at 5,800 x g (~8,000 RPM)**.
 - ❖ **Urine DNA is now ready for downstream applications.**

Rapid Flow Chart Procedure



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. 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.

6. Can I process a different urine volume?

- Yes, you can. This protocol is outlined for an input of 1.75 mL of urine, however it is possible to process 50 µL – 2.6 mL of urine. Ensure that the volume of Binding Solution K added is adjusted accordingly based on the urine input.

7. What if I added more or less from 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 a higher volume of Elution Buffer B will result in diluting your DNA.

8. Can I pass more than 1.75 mL of urine through this column?

- Yes, you can pass up to 2.6 mL of urine (650 µL x 4 spins) through the column. If more than 2.6 mL of urine is passed through the column the column will be saturated. If more samples need to be processed, you can use the Urine DNA Isolation Mini or Maxi Kit (Slurry format) (see related products).

9. What if my incubation temperature varied from the specified 60°C?

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

10. 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.

11. What if I forgot to do a dry spin after my second wash?

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

12. 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 μ L).

13. Why did my samples show very low DNA yields?

- 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 incubation time at 65°C (up to overnight) could also result in increased yields.

14. 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.

15. 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 μ g/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. (See Figure below)

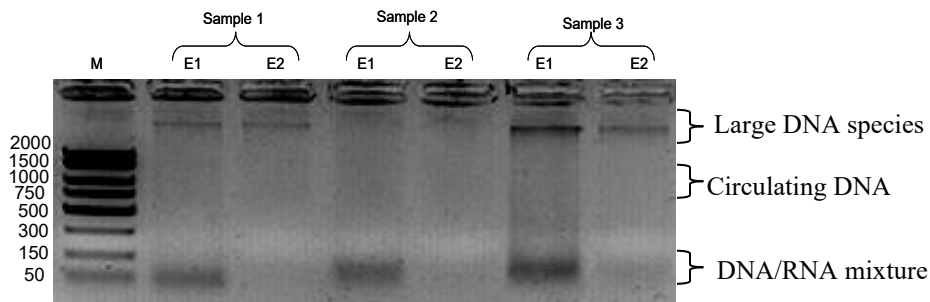


Figure. A typical 1.2% agarose gel showing total urinary DNA isolated from different urine samples using Norgen's Urine DNA Isolation Micro Kit. This gel shows the variability in the DNA yield which can be observed with different urine samples. This gel also shows the different DNA types which can be isolated from urine. Each lane shows one tenth from each elution (i.e. E1: 7.5 μ L out of 75 μ L were loaded on the gel, E2: 7.5 μ L out of 75 μ L were loaded on the gel). M: 10 μ L Norgen's FastRunner DNA Ladder.

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 9:00 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 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

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 downloads/Application Notes/Application Note 10 - Urine DNA.pdf](http://www.norgenbiotek.com/tech%20downloads/Application%20Notes/Application%20Note%2010%20-%20Urine%20DNA.pdf)

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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