3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Urine DNA Isolation Kit for Exfoliated Cells or Bacteria — Product Insert

Product # 47050

Norgen's Urine DNA Isolation Kit for Exfoliated Cells or Bacteria is designed for the rapid isolation of either: 1) human genomic DNA from exfoliated cells that have been shed into the urine from the urinary tract; or 2) bacterial genomic DNA from urine samples. The kit allows for the isolation of DNA from 1 to 50 mL of urine. The genomic DNA isolated from exfoliated cells can be used in a number of diagnostic and research applications including the diagnosis and monitoring of bladder, kidney, or other urinary-tract cancers. Bacterial genomic DNA from both human urine samples and urine samples from animals can be isolated with this kit in order to study the levels and types of bacteria that are present. The kit allows for the isolation of genomic DNA from both Gram negative and Gram-positive bacteria, including *E. coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomonas* spp, *Clostridial* ssp. and *Leptospirosis* spp., as well as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Typical yields of human genomic DNA from exfoliated cells will vary depending on the cell density of the urine sample, which is affected by a number of factors including health, diet and sex of the individual donating the urine. Typical yields of bacterial genomic DNA will vary depending on the urine sample and the bacterial species, if any, present in the urine. Healthy humans generally have < 10, 000 CFU of bacteria per mL of urine, and this kit is sufficiently sensitive to isolate and detect DNA from even this minimal number of bacteria. The genomic DNA purified with this kit is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications such as PCR, qPCR and Southern Blot analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography. The genomic DNA is preferentially purified from the other cellular components such as proteins and RNA. The process involves first obtaining the urine sample and pelleting the exfoliated cells/bacterial cells that are present through the use of centrifugation. The cells are then resuspended in the Resuspension Solution A by vortexing and then lysed using Proteinase K (for exfoliated cells) or Proteinase K and lysozyme (for bacterial cells) in the presence of a Lysis Buffer B. Isopropanol is then added to the lysate, and the lysate mixture is loaded onto a spin column. The spin column binds DNA in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while most of the RNA and proteins will be removed in the flowthrough. The bound DNA is then washed twice with the provided Wash Solution A in order to remove any remaining impurities, and the genomic DNA is eluted with the Elution Buffer B. The purified DNA is of the highest quality and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Minimum Urine Input	1 mL
Maximum Urine Input	50 mL
Time to Complete 10 Purifications	10 minutes (plus a 30 minute incubation - Bacteria) (plus a 15 minute incubation - Exfoliated)

Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate genomic DNA from human exfoliated cells found in as little as 1 mL of urine
- Isolate genomic DNA from Gram positive and Gram-negative bacteria found in as little as 1 mL of urine
- Isolate high quality DNA from urine – free from salts, metabolic wastes and proteins found in urine
- Purified DNA is of the highest quality and can be used in downstream application**.**

Kit Components

Component	Product# 47050 (50 samples)
Resuspension Solution A	20 mL
Lysis Buffer B	40 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Proteinase K in Storage Buffer	1.2 mL
Micro Spin Column	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions 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. The buffered Proteinase K is stable for up to 2 years after the date of shipment when stored at room temperature.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. 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*.

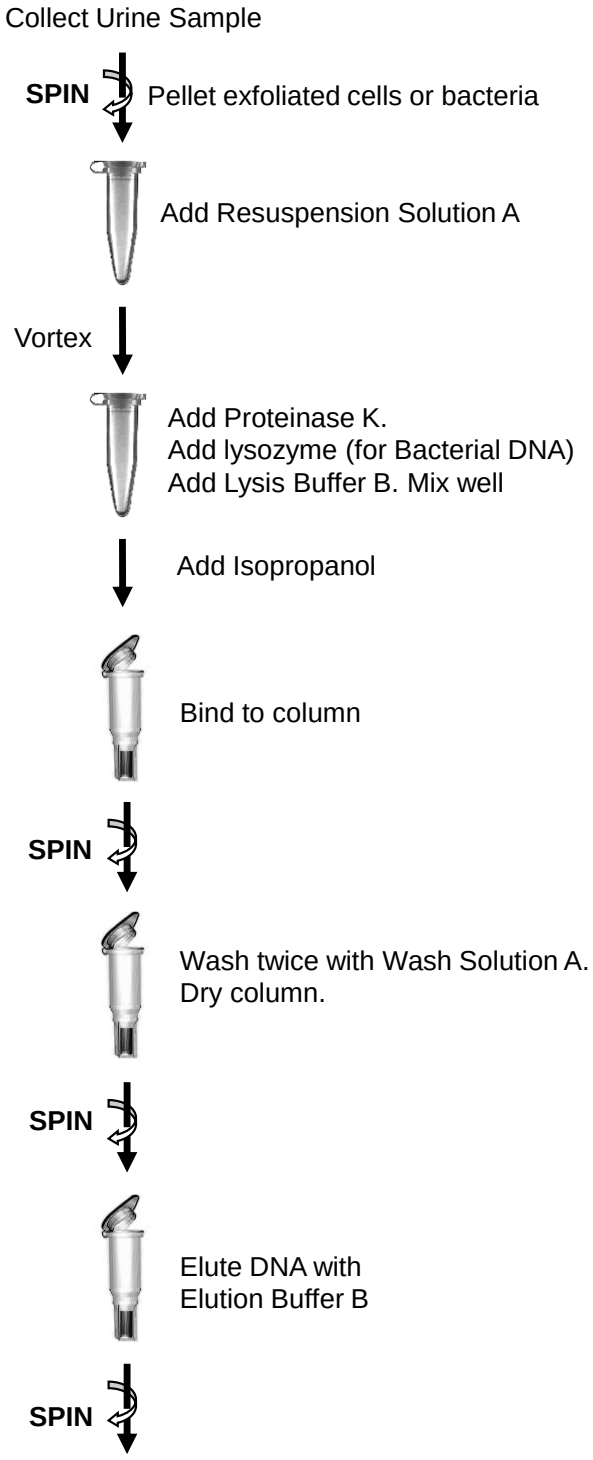
The **Lysis Buffer B** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 55^{o}C water bath or heating block or incubator
- 96 – 100% ethanol
- 100% Isopropanol
- Lysozyme (For Bacteria Genomic DNA Isolation)
- RNase A (optional)

Flow Chart

Procedure for Purifying DNA using
Norgen's Urine DNA Isolation Kit for Exfoliated Cells or Bacteria



Purified Urine Exfoliated Cell DNA or
Urine Bacterial Genomic DNA

Procedure

Notes prior to use:

- 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.
- It is recommended that no more than 50 mL of urine be used for each column.
- It is recommended that at least 1 mL of urine is used for each isolation.
- 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 **Proteinase K** before use.
- Prepare a working concentration of **Wash Solution A** by adding 42 mL of 96- 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- For Bacterial Genomic DNA Isolation: Prepare a 400 mg/mL stock solution (approximately 1.7 x10^7 units/mL) of lysozyme as per supplier's instructions.
- Preheat a water bath or incubator to 55°C.

1. Lysate Preparation

1.1 Lysate Preparation for Bacterial Genomic DNA Isolation

a. Transfer 1 - 1.5 mL of urine to a microcentrifuge tube and centrifuge at 14,000 RPM for 3 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For urine samples larger than 1.5 mL a swinging bucket centrifuge can be used to pellet the cells at 3,000 x g for 5 minutes. The maximum input of urine is 50 mL.

b. Add 200 µL of **Resuspension Solution A** to the cell pellet. Resuspend the cells by gentle vortexing.

Optional RNase A treatment: If RNA-free DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the cell suspension. Mix well and continue with Step **1c**.

c. Add 600 µL of **Lysis Buffer B,** 12 µL of previously prepared lysozyme stock solution and 10 µL of **Proteinase K (always vortex before use)** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 30 minutes.

d. Proceed to Step 2, Binding to Column.

1.2 Lysate Preparation for Exfoliated Cell Genomic DNA Isolation

a. Transfer 1 - 1.5 mL of urine to a microcentrifuge tube and centrifuge at 2,000 RPM for 5 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For urine samples larger than 1.5 mL a swinging bucket centrifuge can be used to pellet the cells at 2,000 RPM for 5 minutes. The maximum input of urine is 50 mL or 1 x 10^6 cells per column. We recommend that the cell count be determined using standard cytological methods.

b. Add 200 µL of **Resuspension Solution A** to the cell pellet. Resuspend the cells by gentle vortexing.

Optional RNase A treatment: If RNA-free DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 μL) to the cell suspension. Mix well and continue with step **1c**.

c. Add 600 μL of the **Lysis Buffer B** and 10 μL of **Proteinase K (always vortex before use)** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 15 minutes.

d. Proceed to Step 2, Binding to Column.

2. Binding to Column

a. Add 160 μL of **100% Isopropanol** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.

b. Apply 500 μL from the lysate mixture to the spin column. Cap the column, and centrifuge the unit for 2 minutes at 8,000 RPM.

Note: If any liquid does not pass through the column after the 2-minute spin, centrifuge the unit for an additional 1 minute at 14,000 RPM.

c. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.

d. Repeat Steps **2b** and **2c** to load the remaining lysate mixture.

3. Washing Bound DNA

a. Apply 500 μL of **Wash Solution A** to the column, and centrifuge the unit for 1 minute at 14,000 RPM.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.

c. Apply 500 μL of **Wash Solution A** to the column, and centrifuge the unit for 1 minute at 8,000 RPM.

d. Discard the flowthrough and reassemble the spin column with its collection tube.

e. Spin the column, empty, for 2 minutes at 14,000 RPM. Carefully remove the spin column from the collection tube and discard the collection tube and the flowthrough.

4. Elution of Clean DNA

a. Assemble the spin column with a provided 1.7 mL **Elution tube.**

b. Add 20 μL - 100 μL of **Elution Buffer B.** Centrifuge for 2 minutes at **8,000 RPM**.

c. Centrifuge at **14,000 RPM** for an additional minute to collect the total elution volume.

The purified DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The yield of genomic DNA is low.	There is very little or no cells in the urine.	The expected number of bacteria in a urine sample is typically low. A healthy individual usually has < 10,000 CFU/mL, therefore it is possible that the urine sample has very little bacteria present. Cell number in a urine sample varies. While individuals with various diseases have > 1000 exfoliated cells per mL of urine, a healthy male may have a number much lower than the 1000 cells per mL limit. The genomic DNA isolated may not be visible when resolved on an agarose gel. In such cases, a larger input volume may be used. Alternatively, a more sensitive method such as BioAnalyzer or PCR amplification may be used for detection.
	Incomplete lysis of cells.	Extend the incubation time of Proteinase K digestion or reduce the number of bacterial cells used for lysis (reduce the urine input).
	The cells are old	Older samples contain prematurely lysed cells which release endonucleases and can degrade DNA. Fresh urine samples are recommended.
	The DNA elution is incomplete.	Ensure that centrifugation at 14,000 RPM is performed after the 8,000 RPM centrifugation cycle, to ensure that all the DNA is eluted.
The genomic DNA is sheared.	The genomic DNA was handled improperly.	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).
	The urine sample is old.	Proteases and DNAses may be present in the sample. Storing the sample for too long before DNA isolation increases the chances of recovering sheared DNA. The use of fresh urine samples is recommended.
The DNA does not perform properly in downstream applications such as PCR.	There are inhibitors present within the urine.	After the cell pellet obtained from the urine is resuspended in the Resuspension Solution A with Proteinase K (Step 1b), pellet the cells again. Then, resuspend the pellet in another 200 μL of Resuspension Solution A with Proteinase K and proceed with lysis. This extra wash will aid in reducing the inhibitors present on the cells.

Related Products	Product #
HighRanger 1kb DNA Ladder	11900
Urine DNA Isolation Kit	18100
Urine Exfoliated Cell and Bacteria RNA Purification Kit	22550
ProteoSpin™ Urine Protein Concentration Kit	17400
ProteoSpin™ Urine Protein Concentration Maxi Kit	21600

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 techsupport@norgenbiotek.com.

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

