

## Urine Bacteria Genomic DNA Isolation Kit

Product # 22400

## Product Insert

Norgen's **Urine Bacteria Genomic DNA Isolation Kit** is designed for the rapid preparation of bacterial genomic DNA from 1 to 20 mL of urine. Bacterial genomic DNA can be isolated from both human urine samples and urine samples from animals 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 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 amount of bacteria. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with PCR and Southern Blot analysis

### Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The bacterial 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 bacterial cells that are present through the use of centrifugation. The bacterial cells are then resuspended in the Resuspension Buffer by vortexing. The bacterial cells are then lysed using lysozyme, Proteinase K and Lysis Solution, and the lysate is applied to one of the supplied spin columns containing resin. Norgen's resin binds DNA in a manner that depends on ionic concentrations. Thus, only the DNA while bind to the column while most of the RNA and digested proteins will be removed in the flowthrough. The bound DNA is then washed twice with the two provided Wash Solutions in order to remove any remaining impurities, and the genomic DNA is eluted with the Elution Buffer. 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	20 mL
Time to Complete 10 purifications	45 minutes

### Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate genomic DNA from both Gram positive and Gram negative urine bacteria
- High sensitivity – isolate and detect DNA from only 1 mL of healthy human urine
- Purified DNA is of the highest quality and can be used in downstream application

## Kit Components

Component	Product # 22400 (20 samples)
Resuspension Solution	8 mL
Lysis Solution	8 mL
Binding Solution	2 mL
Wash Solution I	3 mL
Wash Solution II	12 mL
Elution Buffer	5 mL
Proteinase K	10 mg
Micro Spin Columns	20
Collection Tubes	20
Elution tubes (1.7 mL)	20
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## Storage Conditions and Product Stability

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. The lyophilized Proteinase K should be stored at -20°C upon arrival and after reconstitution.

## 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](http://www.norgenbiotek.com).

The **Binding Solution**, **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 this solution.

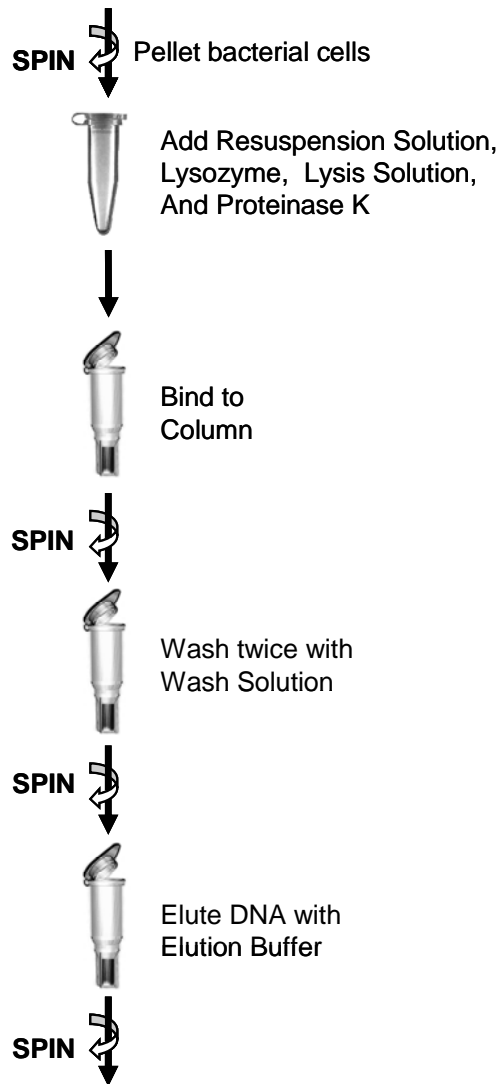
## Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 55°C water bath or heating block or incubator
- 96 – 100% ethanol
- Lysozyme
- RNase A (optional)

## Flow Chart

Procedure for Purifying Bacterial Genomic DNA from Urine using  
Norgen's Urine Bacteria Genomic DNA Isolation Kit

Collect urine sample and transfer to a centrifuge tube



**Pure Bacterial Genomic DNA**

## Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. 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.

### Notes prior to use:

- It is recommended that no more than 20 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.
- Reconstitute the **Proteinase K** in 500  $\mu$ L of molecular biology grade water, aliquot in 100  $\mu$ L fractions, and store the unused portions at -20°C until needed.
- Prepare a working concentration of **Wash Solution I** by adding 8.4 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution I**. This will give a final volume of 11.4 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Prepare a 400 mg/mL stock solution (approximately  $1.7 \times 10^7$  units/mL) of lysozyme as per supplier's instructions.
- Preheat a water bath or incubator to 55°C.

### 1. Lysate Preparation

- a. Transfer 1 - 1.5 mL of urine to a micro centrifuge tube and centrifuge at 14,000 x g (~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 650 x g for 5 minutes. The maximum input of urine is 20 mL per column.

- b. Add 250  $\mu$ L of **Resuspension Solution** to the cell pellet. Resuspend the cells by gentle vortexing.
- c. Add 12  $\mu$ L of previously prepared lysozyme stock solution and mix well.

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

- d. Add 250  $\mu$ L of **Lysis Solution** and 12  $\mu$ L of **Proteinase K** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 30 minutes.

## 2. Binding to Column

- a. Add 60  $\mu\text{L}$  of **Binding Solution** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
- b. Assemble a micro spin column with a provided collection tube. Apply the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 3 minutes at 5,200  $\times g$  (~ 8,000 RPM).
- c. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.

## 3. Washing Bound DNA

- a. Apply 500  $\mu\text{L}$  of **Wash Solution I** to the column, and centrifuge the unit for 2 minutes at 14,000  $\times g$  (~14,000 RPM).
- b. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
- c. Apply 500  $\mu\text{L}$  of **Wash Solution II** to the column, and centrifuge the unit for 2 minutes at 14,000  $\times g$ .
- d. Carefully detach the spin column from the collection tube and discard the collection tube and flowthrough.

**Note:** If any liquid is left on the side of the spin column, discard the flowthrough and reassemble the spin column with its collection tube. Spin for an additional 1 minute at 14,000  $\times g$  in order to completely dry the column.

## 4. Elution of Clean DNA

- a. Assemble the spin column (with DNA bound to the resin) with a provided 1.7 mL **Elution tube**.
- b. Add 100  $\mu\text{L}$  of **Elution Buffer** to the center of the resin bed. Centrifuge for 1 minute at **3,000  $\times g$  (~6,000 RPM)**. A portion of the **Elution Buffer** will pass through the column which allows for hydration of the DNA to occur.
- c. Centrifuge at **14,000  $\times g$**  for an additional minute to collect the total elution volume.
- d. **(Optional):** An additional elution may be performed if desired. Another 100  $\mu\text{L}$  of **Elution Buffer** may be added to the column and centrifuged at 3,000  $\times g$  for 1 minute into a new elution tube. Then, centrifuge the column at 14,000  $\times g$  for an additional minute. The yield can be improved by an additional 20-30% when this second elution is performed.

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 micro spin column is clogged.	Too many cells present in the urine.	The urine sample that was applied to the column contained too many cells. Reduce the amount of urine used. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the urine passes through the column.
The yield of genomic DNA is low.	There is very little or no bacteria in the urine.	The expected amount of bacteria in a urine sample is very little. A healthy individual usually has < 10,000 CFU/mL, therefore it is possible that the urine sample has very little bacteria present. 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 amount of bacterial cells used for lysis (reduce the urine input).
	The DNA elution is incomplete.	Ensure that centrifugation at 14,000 x g is performed after the 3,000 x g 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 with Proteinase K (Step 1b), pellet the cells again. Then, resuspend the pellet in another 300 $\mu$ L of Resuspension Solution with Proteinase K and proceed with lysis. This extra wash will aid in reducing the inhibitors present on the cells.

<b>Related Products</b>	<b>Product #</b>
HighRanger 1kb DNA Ladder	11900
Urine DNA Isolation Kit	18100
Urine (Exfoliated Cell) DNA Isolation Kit	22300
Urine (Exfoliated Cell) RNA Isolation Kit	22500
ProteoSpin™ Urine Protein Concentration Kit	17400
ProteoSpin™ Urine Protein Concentration Maxi Kit	21600

### **Technical Support**

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.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto: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