

Urine DNA/Protein Isolation Kit
Product # 27400

Product Insert

Norgen's Urine DNA/Protein Isolation Kit provides a fast, reliable and simple procedure for isolating DNA and proteins sequentially from a single urine sample. Due to the fact that urine can be collected non-invasively in large amounts, it provides an attractive alternative to blood plasma as a potential source of disease biomarkers. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The urinary DNA and urinary proteins are isolated from a 1 – 3 mL urine sample using 2 separate spin columns in 15 minutes using this kit.

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. 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. The kit also 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*. Therefore this kit can isolate all 3 types of DNA: exfoliated cell gDNA, apoptotic DNA and urine bacterial DNA. Furthermore, each spin column is able to concentrate and desalt up to 200 µg of urine proteins.

The genomic DNA isolated from exfoliated cells in urine 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 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. Urine protein analysis can be used for the identification of potential biomarkers in urine, and to diagnose and/or monitor renal and other diseases. The purified urine DNA is compatible with most molecular biological applications such as PCR, q-PCR, Southern Blot and Methylation-sensitive PCR. Purified urine proteins can be used in downstream proteomic applications including SDS-PAGE, 2D SDS-PAGE gels, whole protein mass spectrometry, and protein microarrays.

This kit is designed to process 25 urine samples with a volume of 1 - 3 mL urine

Specifications

Kit Specifications	
Minimum Urine Input	1 mL
Maximum Urine Input	3 mL
Maximum Input of Exfoliated/Bacterial Cells	1 x 10 ⁶
Protein Yield	Up to 200 µg
Time to Complete 12 Purifications	15 minutes (plus a 20 minute incubation)

Kit Components:

Component	Contents
Stabilizer	1 mL
Resuspension Buffer	8 mL
Lysis Solution	8 mL
DNA Binding Solution	2 mL
Wash Solution I	3.5 mL
Wash Solution II	15 mL
DNA Elution Buffer	6 mL
Proteinase K	1 vial
Protein pH Binding Buffer	4 mL
Column Activation and Wash Buffer	60 mL
Protein Elution Buffer	3 mL
Neutralizer	1 mL
Protein Mini Spin Columns	25
DNA Micro Spin Columns	25
Collection Tubes	50
Elution tubes (1.7 mL)	75
Product Insert	1

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)
- Protease Inhibitor Cocktail (optional)

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.

Quality Control

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

Product Use Limitations

Norgen’s Urine DNA-Protein Isolation Kit 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 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:

- First time users should read the entire manual before proceeding with the protocol.
- 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 μ L of molecular biology grade water, aliquot in 100 μ L fractions and store the unused portions at -20°C until needed.
- Prepare a working concentration of **Wash Solution I** by adding 22 mL of 96-100% ethanol to the supplied bottle containing the concentrated **Wash Solution I**. This will give a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a 400 mg/mL Lysozyme stock solution (approximately 1.7×10^7 units/mL) as per supplier's instructions
- Preheat an incubator or heating block to 55°C.

Detailed Procedure

Section 1: Urine DNA Isolation

1. Obtain a urine sample from 1 mL to 3 mL. Add 10 μ L of **Stabilizer** for every 1 mL urine sample 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. **Retain the supernatant for Urinary Protein Purification (Section 2);**
 - **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
2. Add 250 μ L of **Resuspension Solution** and 12 μ L of previously prepared Lysozyme stock solution to the cell pellet. Resuspend the cells by gentle vortexing;
 - **Note: Optional RNase A treatment:** If RNA-free DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 μ L) to the cell suspension
3. Add 250 μ L of **Lysis Solution** and 12 μ L of **Proteinase K** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 20 minutes.
4. Add 60 μ L of **DNA Binding Solution** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
5. Apply 650 μ L 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;
 - Discard the flowthrough and reassemble the spin column with its collection tube.
6. Apply 500 μ L of **Wash Solution I** 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;
7. Apply 450 μ L of **Wash Solution II** 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;
8. Apply 500 μ L 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;
9. Repeat Step 8 a second time;
10. Spin the column, empty, for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the collection tube;
11. Incubate the column horizontally for 3 minutes at 55°C;
12. Transfer the spin column to a fresh Elution Tube. Apply 100 μ L of **DNA 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)**;
13. Transfer the spin column to a second fresh Elution Tube. Apply 100 μ L of **DNA 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.**



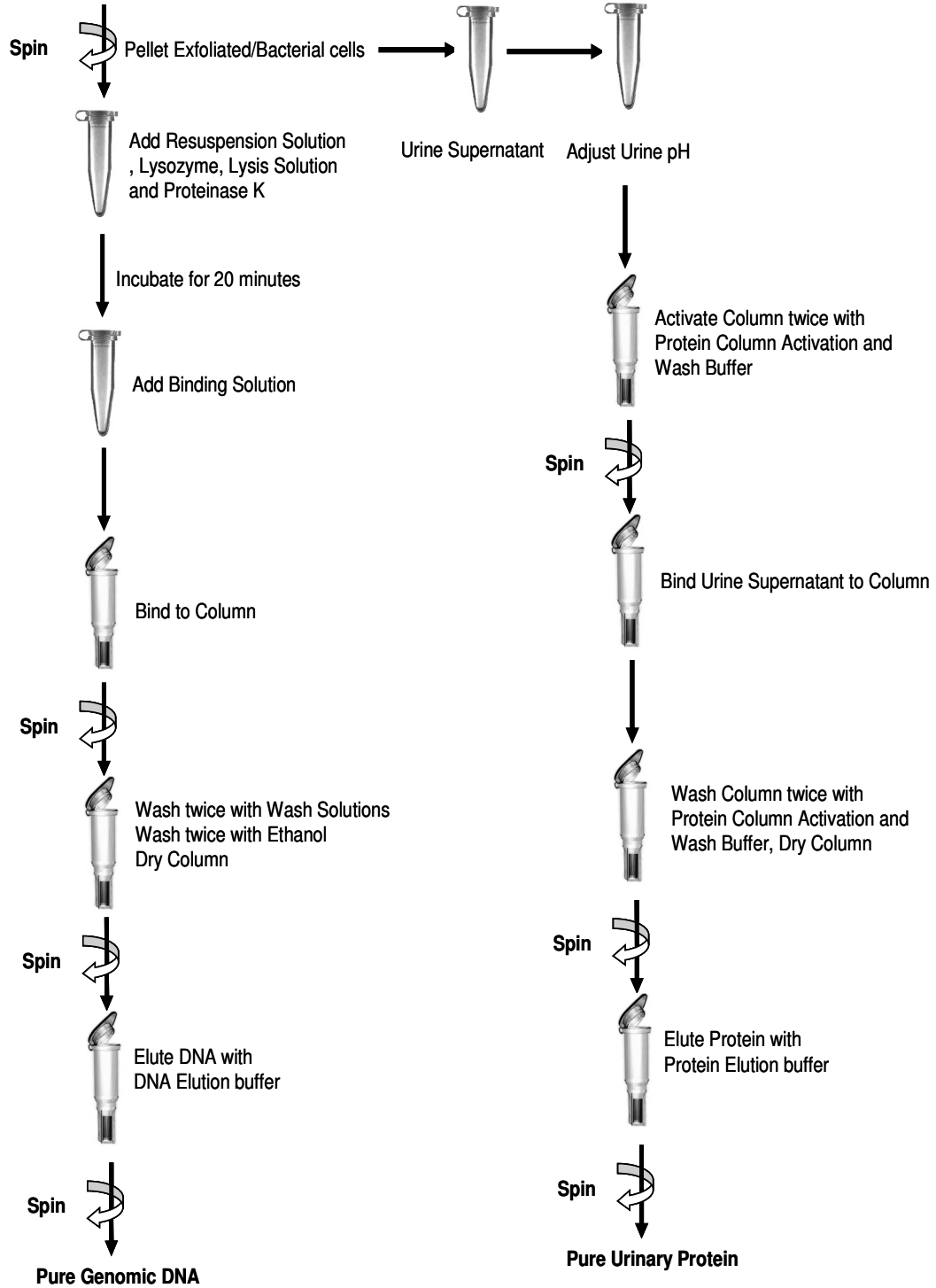
Section 2: Urine Protein Purification

1. Add 40 μL of **Protein pH Binding Buffer** to every 1 mL urinary supernatant retained from **Step 1, Section 1** above. Mix contents well, verify that the pH is 3.5, and add more pH Binding Buffer if necessary;
 - **Note:** In some concentrated urine samples, precipitation may occur with the addition of the pH Binding Buffer. This precipitate includes urine proteins, and thus should not be discarded. The precipitate should be resuspended as much as possible, and loaded onto the column with the rest of the sample.
2. Assemble a spin column with a provided collection tube. Add 500 μL of **Column Activation and Wash Buffer** to the column. Centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**;
3. Repeat **step 2**;
4. Apply up to 650 μL of the **pH-adjusted urine sample** onto the column, and centrifuge for **1 minute at 3,300 x g (~7,000 RPM)**. Inspect the column to ensure that the entire sample has passed through into the collection tube. If necessary, spin for an additional 1 minute;
5. Discard the flowthrough. Reassemble the spin column with its collection tube. Repeat **step 4** until the entire protein sample has been loaded onto the column,
 - **Note:** You can save the flowthrough in a fresh tube for assessing your protein's binding efficiency.
6. Apply 500 μL of **Column Activation and Wash Buffer** to the column. Centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**;
7. Repeat **Step 6**;
8. Add 9.3 μL of **Neutralizer** to a fresh 1.7 mL Elution Tube. Transfer the spin column from the Column Wash procedure into the Elution Tube.
9. Apply 100 μL of **Protein 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)**;

❖ **Urine Proteins are now ready for downstream applications.**

Rapid Flow Chart Procedure

Collect Urine Sample and transfer to a Centrifuge tube



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

2. I am not going to process my samples immediately, Is there any additional preservative 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 20 μ L of this cocktail to the 1.75 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 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.

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?

- 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 minimum recommended urine input is 500 μ L, and the maximum recommended input is 3 mL. Please note that the buffers are optimized for an input of 1 mL of urine.

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

10. What if my incubation temperature varied from the specified 55°C?

- The incubation temperature can be in the range of 50°C - 60°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.

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 traces of the Wash Solution. 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.

13. 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)

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 as well as traces from the bacterial genomic DNA. However, your second elution will mainly contain both the large DNA species as well as the bacterial genomic DNA.

15. 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 55°C (up to overnight) could also result in increased yields.

16. Why does my DNA 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 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.

18. What if the Protein solution did not flow through the column?

- The centrifugation speed may be too low. Check the centrifuge to ensure that it is capable of generating 3,300 x g. sufficient centrifugal force is required to move the liquid phase through the resin. Centrifugation speeds may be increased to 6,700 x g, but this speed should not be exceeded. You may also spin an additional two minutes to ensure that the liquid is able to flow completely through the column.

19. Why am I getting a very poor protein recovery?

- Poor protein recovery is possibly due to an incorrect pH adjustment of urine sample. Depending on a person's acid-base status, the starting pH of the urine may range from 4.5 to 8. Therefore, it is important that the proper amount of the Protein pH Binding buffer be added to the urine sample in order to adjust the pH to 3.5 prior to loading onto the column.

20. What will happen if I forgot to neutralize the eluted proteins?

- If eluted proteins are not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH. Some proteins are sensitive to high pH, such as the elution buffer at pH 12.5

21. The elution from the protein purification is colored, Is this will have an effect on any of the proteomic-based downstream applications?

- The observed color in the protein elution comes from the pigments that are naturally present in urine and will not interfere with any of the proteomic-based downstream applications.

Related Products	Product #
Urine DNA Isolation Mini Kit (Slurry Format)	27000
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 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 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

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