

Urine (Exfoliated Cell) DNA Isolation Kit

Product # 22300

Product Insert

Norgen's **Urine (Exfoliated Cell) DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from exfoliated cells that have been shed into the urine from the urinary tract. The genomic DNA isolated from these 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. The kit allows for the isolation of genomic DNA from exfoliated cells present in 1 to 50 mL of urine. Typical yields of DNA 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. The purified genomic DNA 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 using Norgen's proprietary resin as the separation matrix. The kit preferentially purifies DNA from other cellular proteinaceous components, as well as from the contaminating species found in urine such as glucose and salts. The process first involves pelleting the exfoliated cells in the urine, and resuspending the cell pellet in Resuspension Solution and Proteinase K (please see the flow chart on page 3). Next, the cells are lysed using the provided Lysis Solution. Binding Solution is then added to the lysate, and the solution is loaded onto a spin column. Norgen's resin 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 Solutions in order to remove any remaining impurities, and the purified 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	50 mL
Maximum Input of Exfoliated Cells	1×10^6
Time to Complete 10 Purifications	15 minutes (plus a 30 minute incubation)

Advantages

- Fast and easy processing using rapid spin-column format
- DNA can be isolated and detected from the exfoliated cells 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

Kit Components

Component	Product # 22300 (20 samples)
Resuspension Solution	8 mL
Lysis Solution	8 mL
Binding Solution	4 mL
Wash Solution I	7.5 mL
Wash Solution II	15 mL
Elution Buffer	8 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.

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
- 96 – 100% ethanol
- RNase A (optional)
- Molecular biology grade water

Flow Chart

Procedure for Purifying Genomic DNA from the Exfoliated Cells in Urine using Norgen's Urine (Exfoliated Cell) DNA Isolation Kit

Collect Urine Sample

SPIN  Pellet exfoliated cells



Add Resuspension Solution and Proteinase K

Vortex



Add Lysis Solution.
Incubate for 30 minutes.
Add Binding Solution



Bind to column

SPIN 



Wash twice with Wash Solutions

SPIN 



Elute DNA with Elution Buffer

SPIN 

Purified Urine 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:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5}) (r)}}$$

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:

- 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.
- 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 μL of molecular biology grade water, aliquot in 100 μL fractions and store the unused portions at -20°C until needed.
- Prepare a working concentration of **Wash Solution I** by adding 22.5 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution**. This will give a final volume of 30 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Preheat a water bath or heating block to 55°C
- Maximum urine input per column is 50 mL and the minimum input per column is 1 mL.
- We recommend that the number of cells present in the urine be determined using standard cytological methods prior to beginning the protocol. The maximum input per column is 1×10^6 exfoliated cells.

1. Lysate Preparation

- a. Transfer 1 - 1.5 mL of urine to a microcentrifuge tube and centrifuge at $650 \times g$ ($\sim 2,000$ 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 $650 \times g$ for 5 minutes. The maximum input of urine is 50 mL or 1×10^6 cells per column. We recommend that the cell count be determined using standard cytological methods.

- b. Add 300 μL of **Resuspension Solution** and 10 μL of **Proteinase K** 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 300 μL of the **Lysis Solution** to the cell suspension. Mix well by gentle vortexing and incubate at 55°C for 30 minutes.

2. Binding to Column

- a. Add 60 μL of **Binding Solution** to the lysate and mix well with gentle vortexing. Ensure that a homogeneous mixture is obtained.
- b. Assemble a 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 x g (~ 8,000 RPM).

Note: If any liquid does not pass through the column after the 3 minute spin, centrifuge the unit for an additional 1 minute at 14,000 x g (~14,000 RPM).

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

3. Washing Bound DNA

- a. Apply 500 μL of **Wash Solution I** to the column, and centrifuge the unit for 1 minute at 14,000 x g (~14,000 RPM).

Note: Ensure the entire wash solution 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 II** to the column, and centrifuge the unit for 1 minute at 14,000 x g (~14,000 RPM).
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

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 μL of **Elution Buffer** to the center of the resin bed. Centrifuge for 1 minute at **3,000 x 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 x g (~14,000 RPM)** for an additional minute to collect the total elution volume.
- d. **(Optional):** An additional elution may be performed if desired. Another 100 μL of **Elution Buffer** may be added to the column and centrifuged at 3,000 x g for 1 minute into a new elution tube. Then, centrifuge the column at 14,000 x 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 spin column is clogged	The sample is too large	Too many cells were applied to the column. Ensure that the amount of cells used is less than 1×10^6 cells, and that no more than 50 mL of urine is pelleted and applied to the column. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
The lysate is very gelatinous prior to loading onto the column	The lysate/binding solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
	The sample is too large	Too many cells are in the lysate preparation. We recommend that the number of cells present in the urine be determined using standard cytological methods prior to beginning the protocol. Ensure that the amount of cells used is less than of 1×10^6 cells, and that no more than 50 mL of urine is pelleted and applied to the column.
The yield of genomic DNA is low	The sample does not contain a lot of exfoliated cell	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. It is possible that the genomic DNA isolated is not 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.
	The cells are old	Older samples contain prematurely lysed cells which release endonucleases and can degrade DNA. Fresh urine samples are recommended.
	Incomplete lysis of cells	Extend the incubation time of the Proteinase K digestion or reduce the amount of exfoliated cells used for lysis.
	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 cells are old	Older samples contain prematurely lysed cells which release endonucleases and can degrade DNA. Fresh urine samples are recommended.

Related Products	Product #
Urine DNA Isolation Kit	18100
Urine (Exfoliated Cell) RNA Isolation Kit	22500
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100

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) or through email at techsupport@norgenbiotek.com.

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