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Urine Cell-Free Circulating DNA Purification Maxi Kit — Product Insert

Product # 56800

Introduction

Recent evidence indicates that cell-free circulating DNA (cfc-DNA) contains valuable information for the discovery of biomarkers that can help with early detection of certain cancer types and for monitoring the disease status. The advantage for using urine as a source for cancer biomarkers is that it can be acquired in large quantities without using invasive procedures. In addition, repeated sampling from the same individual is applicable, which facilitates longitudinal studies. There are many advantages favouring the use of urinary nucleic acids for cancer biomarker discovery over blood, tissue samples or other bodily fluids, including: (1) urine is non-infectious for HIV and less infectious for many other pathogens; (2) the profile of urinary nucleic acid is similar to that in plasma or serum; (3) Nucleic acid purification from urine is technically much easier because of its low protein concentration (1000-fold lower than blood). Urine cell-free circulating DNA (cfc-DNA) has been utilized for the early diagnosis, prognosis and monitoring of therapy for several cancer types and autoimmune diseases, as well as for investigating fetal DNA that is normally present in the maternal blood. This cfc-DNA is usually present as short fragments, generally between 150 and 250 bp or its duplicates, and is derived either from the apoptotic process, necrotic process or from the fetus

Norgen's Urine Cell-Free Circulating DNA Purification Maxi Kit provides fast, reliable and simple procedures for isolating cell-free circulating DNA (cfc-DNA) from various amounts of urine ranging from 10 mL up to 30 mL. Purification is based on spin column chromatography that uses Norgen's proprietary resin separation matrix. The kit is designed to isolate all sizes of cfc-DNA from either fresh or frozen urine samples. Moreover, this kit allows the user to elute the purified cfc-DNA into a flexible elution volume ranging from 50 µL to 100 µL. The purified urine cfc-DNA is eluted in an elution buffer that is compatible with all downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis, and NGS.

Kit Components

Component	Maxi Kit (Cat.# 56800)
Number of Preps	10 preps
Binding Solution K	1 x 75 mL 1 x 25 mL
Lysis Buffer A	20 mL
Proteinase K	0.3 mL
Wash Solution A	38 mL
Elution Buffer B	15 mL
Mini Spin Columns	10
Maxi Spin Column	10
Collection Tubes	10
Elution tubes (1.7 mL)	10
Product Insert	1

This kit is suitable for the isolation of cfc-DNA from fresh urine samples frozen urine samples or urine samples collected on most urine preservative.

It has been noticed that urine samples stored at -70°C, -20°C or at 4°C will develop some precipitation due to the aggregation of some of the highly abundant proteins in urine. Eliminating these precipitates using centrifugation or filtration may cause the loss of some of the cfc protein-bound DNA. Furthermore, these precipitates may affect the quality of the purified nucleic acid. We recommend the use of Norgen's Urine Preservative when collecting urine samples. Norgen's

Urine Preservative is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures, therefore no protein precipitation will occur and the purified nucleic acids will be of a higher quality. 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 as 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.

Specifications

Kit Specifications	
Sample Type	Fresh or frozen urine
Sample volume Range	10 to 30 mL
Minimum Elution Volume	50 µL
Maximum Elution Volume	100 µL
Time to Complete 10 Purifications	40 - 45 minutes
Size of DNA Purified	≥ 50 bp
Average Yields ¥	Variable depending on specimen

¥ Please check page 6 for Average Urine cfc-DNA Yields and Common DNA Quantification Methods

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.

Quality Control
In accordance with Norgen's Quality Management System, each lot of Norgen's Urine Cell-Free Circulating DNA Purification Maxi Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Urine Cell-Free Circulating DNA Purification Maxi Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty
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.

Binding Solution K and **Lysis Buffer A** contain guanidium salt, and should be handled with care. Guanidium salt 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.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 15 mL Conical tube
- 50 mL Conical tube
- 1.5 mL eppendorf tube
- 96 – 100% ethanol

Procedure

Important Notes

Notes prior to Use

- First time users should read the entire manual before proceeding with the protocol.
- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The spin columns provided with Norgen's Urine Cell-Free Circulating DNA Purification Maxi Kit are optimized to be used with benchtop centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Mini Spin Columns.
- Most standard swinging bucket centrifuges will accommodate Norgen's Midi and Maxi Spin Columns. Do not use a fixed-angle rotor
- Norgen's Midi and Maxi Spin Columns are centrifuged in 15 mL and 50 mL centrifuge tubes, respectively.
- Centrifuging Norgen's spin columns at a speed higher than recommended may affect DNA yield.
- Centrifuging Norgen's spin columns at a speed lower than recommended will not affect DNA yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the spin column
- When placing Norgen's Midi and Maxi Spin Columns into the swinging bucket centrifuge make sure that lids of the tubes are not tightly closed. Tightly closed lids may cause back pressure which may cause column clogging or disintegration**.**
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- 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.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to each supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 128 mL. 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 55°C.
- Always **vortex** the **Proteinase K** before use
- ***If any of the solutions do not go through the Spin Columns within the specified centrifugation time, spin for an additional 1-2 minutes until the solution completely passes through the Column. Do NOT exceed the centrifugation speed as this may affect DNA yield.***

Preparation of Cell-free Urine Sample

1. Collect and transfer 15-50 mL of the urine into a conical tube and centrifuge at **200 *x g* *(*~1,000 RPM) for 10 minutes** to remove urine exfoliated cells and debris. Decant cell-free urine into new 15-50 mL conical tube.
2. Centrifuge the cell-free urine at **1,800 *x g* *(*~3,000 RPM) for 10 minutes** to remove any residual debris or bacterial cells.
3. Transfer cell-free urine into a fresh 15-50 mL conical tube.

- **Cell-Free Urine is now ready for the purification of cell-free DNA**

Note: The procedure outlined below is for 30 mL inputs of cell-free urine. If processing a sample volume lower than 30 mL cell-free urine, simply bring the volume of your samples up to 30 mL using 1X PBS and proceed as outlined below.

1. Place 30 mL of cell-free urine in a 50 mL tube (provided by the user). Add 9 mL **Binding Solution K** and mix well by vortexing for 10 seconds.
2. Transfer 13 mL of the mixture from **Step 1** into a Maxi Spin column assembled with one of the provided collection tubes. Centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
3. Repeat **Step 2** two more times to transfer the remaining mixture into the Maxi Spin column.
4. Apply 5 mL of **Wash Solution A** to the column and centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
5. Repeat **Step 4** one more time, for a total of two washes.
6. Apply 800 µL of **Elution Buffer B** to the column let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x *g* (~1,600 RPM)**.
7. Reload the eluted 800 µL **Elution Buffer B** from **Step 6** back to the column let stand at room temperature for 2 minutes. Centrifuge for **3 minutes at 500 x *g* (~1,600 RPM)**
8. To the 800 µL **Elution Buffer B** from **Step 7,** add 16 µL **Proteinase K** and mix well by vortexing for 10 seconds, then incubate at **55°C for 10 minutes**.
9. After incubation, add 600 µL of **Lysis Buffer A,** and mix well by vortexing for 10 seconds.
10. Add 800 µL of **96-100% Ethanol,** and mix well by vortexing for 10 seconds.
11. Transfer 750 µL of the mixture from **Step 10** into a Mini Spin column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,300 *x g* (~7,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
12. Repeat **Step 11** two more times to transfer the remaining mixture into the Mini Spin column.
13. Apply 600 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 3,300 *x g* (~7,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
14. Repeat **Step 13** one more time, for a total of two washes.
15. Spin the column, empty, for **2 minutes at 14,000 *x g* (~14,000 RPM)**. Discard the collection tube.
16. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 200 x *g* (~2,000 RPM),** followed by **2 minutes at 5,200 x g (~8,000 RPM)**.
17. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
    - ***Cell-Free Circulating DNA is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the DNA, please refer to Appendix A***

Appendix A

Cell-Free Circulating DNA Yield

Urine Cell-free circulating DNA (cfc-DNA) is normally found in very low amounts (1 - 100 pg/µL), therefore measuring cfc-DNA concentration using common DNA quantification methods is very difficult and challenging. Typical yields of cfc-DNA vary significantly from sample to sample. Variability is also observed between samples collected from the same donor at different times during the day and therefore there is no absolute yield for cfc-DNA purified from bodily fluids including urine. Cell-free circulating DNA yield varies depending on a number of factors including age, sex, diet, exercise and most importantly the health status of the donor.

Below is a list of the most common DNA quantification methods, as well as the limit of detection for each of these methods. ***Unfortunately, none of these methods can be used reliably for measuring the concentration of DNA purified from urine unless large urine volumes have been processed***. This would only be applicable if urine contains the maximum amount of DNA that can fit within the specification range of these quantification tools. It should be noted that the specifications outlined below are based on measuring a pure dsDNA, which will not be the case for the DNA purified from urine. urine cfc-DNA is short fragmented DNA which is usually present in less than 1000 bp. Purified urine cfc-DNA usually contains traces of proteins which will interfere with most quantification methods, leading to the overestimation of the purified DNA concentration. Therefore purified DNA contaminated with more proteins will be presented at a higher concentration as compared to DNA purified with less protein contaminants, which in this case will depend on the method used for urine DNA purification. ***The only reliable method that can assess the quality and the relative quantity of the purified urine DNA is qPCR amplification of a standard DNA using a small DNA amplicon such as the 5S rRNA housekeeping gene***.

Common DNA Quantification Methods

1) 2100 Bioanalyzer DNA Quantification kits

	DNA 1000 Kit	DNA 7500 Kit	DNA 12000 Kit	High Sensitivity DNA Kit
Size Range	**25–1000 bp**	**100–7500 bp**	**100–12000 bp**	**50-7000 bp**
Quantitation accuracy	**20% CV***	**20% CV***	**25% CV***	**20% CV**
Quantitative range	**0.5-50 ng/µL**	**0.5-50 ng/µL**	**0.5-50 ng/µL**	**5-500 pg/µL**

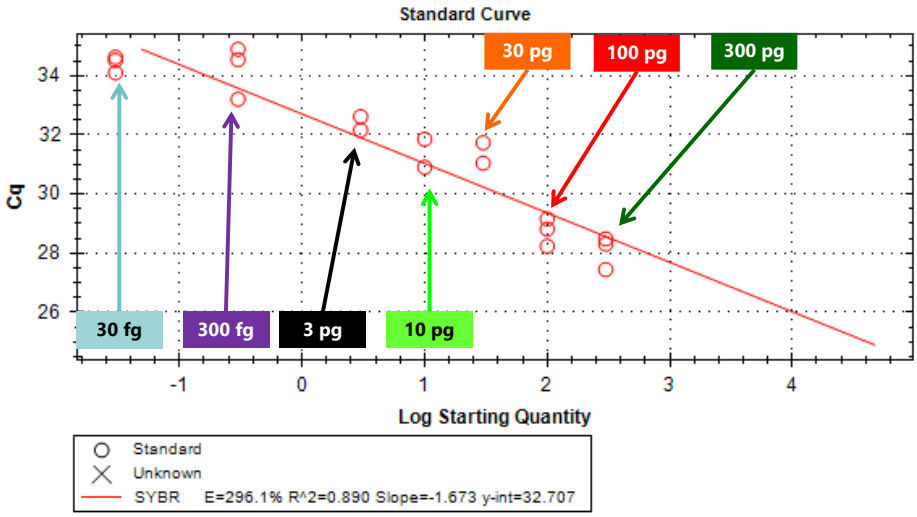
2) NanoDrop 2000

- Detection Limit: 2 ng/µl (dsDNA)

3) Quant-iT™ Pico Green® dsDNA Assay Kit

- Detection Limit: 25 pg/mL

4) qPCR Standard Curve (generated using Norgen's Low Abundance DNA Quantification Kit, Cat# 57200)



Frequently Asked Questions

1. What if a variable speed centrifuge is not available and the speed differs from the recommended?

- A fixed speed centrifuge can be used, however reduced yields may be observed.

2. 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.

3. What if I added more or less of the specified reagents' volume?

- Adding more or less than the specified volumes may reduce both the quality and the quantity of the purified DNA. Eluting your DNA in high volumes will increase the yield but will lower the concentration. Eluting in small volumes will increase the concentration but will lower the overall yield.

4. What if I forgot to do a dry spin before my final elution step?

- Your purified DNA will be contaminated with the Wash Solution A. This may reduce the quality of your purified DNA and will interfere with your downstream applications.

5. Can I perform a second elution?

- Yes, but it is recommended that the 2nd elution be in a smaller volume (50% of 1st Elution). It is also recommended to perform the 2nd elution into a separate elution tube to avoid diluting the 1st elution.

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

- The incubation temperature can be in the range of 55°C - 60°C. If the temperature is outside of that range the activity of the Proteinase K will be reduced. This will result in a reduction in your DNA yields.

7. What if my incubation time varied from what is specified in the product manual?

- Varying the incubation time will result in a reduction in your DNA yields.

8. Why do my samples show very low DNA yield?

- Urine samples contain very little cfc-DNA. This varies from individual to individual. In order to increase the yield, the amount of urine input could be increased.

9. Why does my purified cfc-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.

10. Do I need to do an RNase treatment for my DNA Elution?

- Norgen's Urine Cell-Free Circulating DNA doesn't co-purify urine circulating RNA along with circulating DNA, therefore an RNase step is not required.

11. Why are the A260:280 ratio and the A260:230 ratio of the purified DNA low?

Most of the urine DNA is present in short fragments as well as in a very low concentration. The low A260:280 ratio and the low A260:230 ratio will not affect any downstream applications.

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 support@norgenbiotek.com.

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

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