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# Product Information

**GenElute™ Urine Cell-Free DNA Purification Mini Kit**

Catalog number **DNB300**

## TECHNICAL BULLETIN

### Product Description

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

GenElute™ Urine Cell-Free DNA Purification Kits provide fast, reliable and simple procedures for isolating cell-free circulating DNA (cfc-DNA) from various amounts of urine ranging from 250 µL up to 30 mL. Purification is based on spin column chromatography that uses proprietary resin separation matrix. The kits are designed to isolate all sizes of cfc-DNA from either fresh or frozen urine samples. Moreover, these kits allow the user to elute the purified cfc-DNA into a flexible elution volume ranging from 25 µL to 50 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.

These kits are suitable for the isolation of cfc-DNA from fresh urine samples frozen urine samples or urine samples collected on any urine preservative.

### Components

| Materials Provided | 50 preps |
|---|---|
| Number of Preps | 50 preps |
| Binding Solution K | 25 mL |
| Proteinase K | 1.2 mL |
| Wash Solution A | 38 mL |
| Elution Buffer B | 8 mL |
| Mini Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

### Reagents and Equipment Required But Not Provided.

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

### Precautions and Disclaimer

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). This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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.

**Reagents to be prepared**
Before beginning the procedure, prepare the following:

Prepare a working concentration of Wash Solution A by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

**Storage/Stability**
All buffers should be kept tightly sealed and stored at room temperature (15-25ºC) for up to 2 years without showing any reduction in performance.

The Urine Cell-Free Circulating DNA Purification Kits contain ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2 years after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

**Procedure**

**Note:**
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 Urine Preservative when collecting urine samples. 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.

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.

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.

**Procedure**

**A. 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.

**Proceed to Step B.**

**B. Urine cfc-DNA Purification Mini Kit**

**Note:** The procedure outlined below is for 2 mL inputs of cell-free urine. If processing a sample volume lower than 2 mL cell-free urine, simply bring the volume of your samples up to 2 mL using 1X PBS and proceed as outlined below.

1. Place 2 mL of cell-free urine in a 15 mL tube. Add 20 µL **Proteinase K** and mix well by vortexing for 10 seconds, and then incubate at 55°C for 10 minutes.
2. After incubation, add 400 µL **Binding Solution K** and mix well by vortexing for 10 seconds.
3. Transfer 800 µL of the mixture from Step 2 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.

4. Repeat Step 3 two more times to transfer the remaining mixture into the Mini Spin column.
5. 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.
6. Repeat Step 5 one more time, for a total of two washes.
7. Spin the column, empty, for 2 minutes at 14,000 x g (~14,000 RPM). Discard the collection tube.
8. 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).
9. 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).

## 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

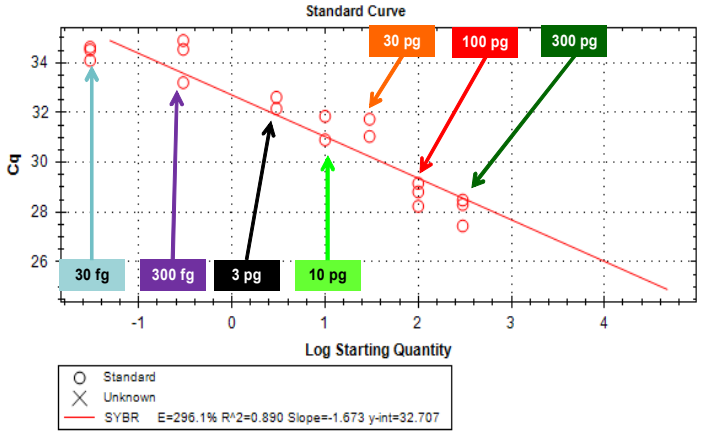
- Detection Limit: 2 ng/µl (dsDNA)

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

- Detection Limit: 25 pg/mL

### 4) qPCR Standard Curve

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.




## 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 2$^{nd}$ elution be in a smaller volume (50% of 1$^{st}$ Elution). It is also recommended to perform the 2$^{nd}$ elution into a separate elution tube to avoid diluting the 1$^{st}$ 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?**
GenElute™ Urine Cell-Free DNA Purification Mini 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.

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