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Product Information

GenElute™ Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit

Catalog number **DNB600**

TECHNICAL BULLETIN

Product Description

It has been suggested recently that Plasma/Serum cell-free circulating DNA (cfc-DNA) can be utilized as a biomarker. CFC-DNA has the potential to provide biomarkers for certain cancers and disease states as well as fetal DNA in maternal blood. Currently, significant advancements are being made in utilizing cfc-DNA as biomarkers for the early diagnosis, prognosis and monitoring of therapy for several cancer types and autoimmune diseases. Cell-free mitochondrial DNA (cfmtDNA) is also under investigation for its clinical significance. This cfc-DNA is usually present as short fragments of less than 1000 bp. In addition, cell-free fetal DNA has been widely used as a non-invasive method for prenatal diagnosis including early identification of fetal sex, genetic studies for families at high risk for inherited genetic disorders, screening for Rhesus factor, screening for aneuploidy and identification of preeclampsia.

GenElute™ Plasma/Serum Cell-Free Circulating DNA Purification Kits provide fast, reliable and simple procedures for isolating cell-free circulating DNA (cfc-DNA) from various amounts of plasma/serum ranging from 10 µL up to 10 mL, where various kit formats address different plasma/serum input volumes. 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 plasma/serum 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 plasma/serum 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, microarrays and NGS.

These kits are suitable for the isolation of cfc-DNA from fresh or frozen serum/plasma prepared from blood collected on Heparin, EDTA or Citrate.

Components

Materials Provided	50 preps
Number of Preps	20 preps
Binding Buffer B	2 x 85 mL
Proteinase K	1 mL
Wash Solution A	18 mL
Elution Buffer B	30 mL
Mini Spin Columns	20
Midi Spin Column	20
Collection Tubes	20
Elution tubes (1.7 mL)	20
Product Insert	1

Reagents and Equipment Required But Not Provided.

- Benchtop microcentrifuge
- Micropipettors
- 15 mL and/or 50 mL tubes
- 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.

Binding Buffer B contains guanidine hydrochloride (GnHCl), and should be handled with care. GnHCl forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing this buffer 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.

Plasma or serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.

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

1) Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 – 100 % ethanol 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 (15-25°C) for up to 2 year without showing any reduction in performance. The kit contains a ready-to-use **Proteinase K** solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 2.5 years after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Procedure

Note:
The spin columns provided with GenElute™'s Plasma/Serum Cell-Free Circulating DNA Purification Kits are optimized to be used with benchtop centrifuges and not to be used on a vacuum apparatus.

Most standard swinging bucket centrifuges will accommodate the Midi Spin Columns provided. Do not use a fixed-angle rotor. The Midi Spin Columns are centrifuged in 15 mL centrifuge tubes. When placing the 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 samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.

Preheat an incubator or heating block to 55ºC. 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.

Frozen plasma or serum samples should be centrifuged for 2 minutes at 400 x g (~2,000 RPM) before processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.

Procedure

- 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.
- The procedure outlined below is for processing 5 mL to 10 mL inputs of Plasma/Serum. If the sample volume is lower than 10 mL Plasma/Serum, simply bring the volume of your sample up to 10 mL using 1X PBS and proceed as outlined below.

1. Place 4 mL of plasma/serum sample in a 15 mL tube (provided by the user). Add 7 mL of **Binding Buffer B**, and mix well by vortexing for 10 seconds.
2. Transfer up to 5.5 mL of the mixture from **Step 1** into a Midi 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. (Note: Make sure that lid of the tubes is not tightly closed)
3. Repeat **Step 2** to transfer the remaining mixture from Step 1 into the Midi Spin column.
4. Transfer the Midi Spin column to a fresh 15 mL tube (not provided). Apply 0.25 mL of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x g** (~1,600 RPM).
5. Apply an additional 1 mL of **Elution Buffer B** to the column and let stand at room temperature for 3 minutes. Centrifuge for 3 minutes at 500 x g (~1,600 RPM).

6. To the elution from Step 5, add 47µL of **Proteinase K** and mix well by vortexing for 10 seconds, then incubate the mixture at 55ºC for 25 minutes.
7. After incubation, add 1.25 mL of **Binding Buffer B** to the mixture and mix well by vortexing for 10 seconds.
8. Transfer 700 µL of the mixture from **Step 7** into a Mini Spin column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,300 x g (~6,000 RPM).** Discard the flowthrough and reassemble the spin column with its collection tube.
9. Repeat **Step 8** three more times to transfer the remaining mixture into the Mini Spin column.
10. Apply 600 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 3,300 x g (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
11. Repeat **Step 10** one more time, for a total of two washes.
12. Spin the column, empty, for **2 minutes at 13,000 x g (~14,000 RPM)**. Discard the collection tube.
13. 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 400 x g (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

Quantification of DNA

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 plasma or serum unless large plasma/serum volumes have been processed.

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

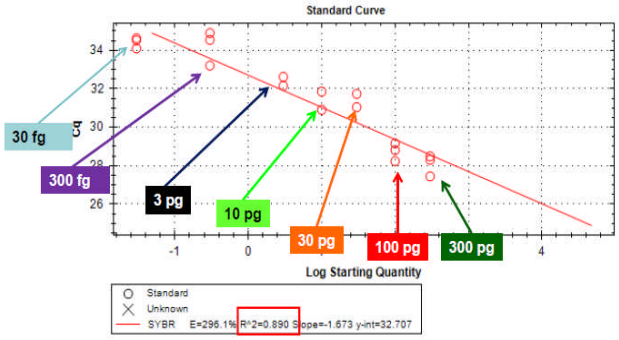
2) NanoDrop 2000

- Detection Limit: 2 ng/µl (dsDNA)

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

- Detection Limit: 25 pg/mL

4) qPCR DNA Standard Curve



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 55ºC?
The incubation temperature can be in the range of 55ºC - 60ºC. 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?
Plasma/Serum samples contain very little cfc-DNA. This varies from individual to individual. In order to increase the yield, the amount of Plasma/Serum 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™'s Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit doesn't co-purify plasma/serum 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 Plasma/Serum Cell-Free Circulating DNA is present in short fragments. The low A260:280 ratio and the low A260:230 ratio will not affect any downstream applications.

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