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Plasma/Serum Cell-Free Circulating DNA Purification Kits Product Insert

Product # 55500

Introduction

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 (cf-mtDNA) 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.

Norgen's 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 Norgen's 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.

Kit Components

Component	Micro Kit Cat# 55500
Number of Preps	50 preps
Binding Buffer B	40 mL
Proteinase K	0.6 mL
Solution WN	18 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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These kits are suitable for the isolation of cfc-DNA from fresh or frozen serum/plasma prepared from blood collected on either Norgen's cf-DNA Preservative Tubes (Cat. 63950, 63960), Cell-Free DNA BCT® (Streck), Heparin, EDTA or Citrate.

Specifications

Kits Specifications	
Sample Type	Plasma/Serum
Anti-coagulant (for Plasma)	EDTA, Citrate or Heparin
Plasma Preservative	Norgen's cf-DNA Preservative Tubes (Cat. 63950, 63960) Cell-Free DNA BCT ® (Streck)
Sample Volume Range	10 to 200 µL
Minimum Elution Volume	25 µL
Maximum Elution Volume	50 µL
Time to Complete 10 Purifications	15 - 20 minutes
Size of DNA Purified	≥ 50 bp
Average Yields ¥	Variable depending on specimen

¥ Please check page 6 for Average Plasma/Serum 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 Plasma/Serum Cell-Free Circulating DNA Purification Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Kits is designed for research purposes only. It is not intended for 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 PDF files online at *www.norgenbiotek.com*.

Binding Buffer B and **Solution WN** contain 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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Swinging bucket centrifuge (Midi and Maxi)
- Micropipettors
- 15 mL and/or 50 mL tubes
- 96 – 100% ethanol

Procedure

Notes Prior to Use

- 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 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 benchtop microcentrifuges will accommodate Norgen's Micro and 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 in the procedure may affect DNA yield.
- Centrifuging Norgen's spin columns at a speed lower than recommended in the procedure 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 **Solution WN** by adding 24 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated Solution WN. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding:
    - **42 mL of 96 - 100% ethanol** (provided by the user) to the supplied bottle containing the **18 mL** 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
    - **90 mL of 96 - 100% ethanol** (provided by the user) to the supplied bottle containing the **38 mL** 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
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation.
- Always **vortex** the **Proteinase K** before use.
- ***This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on Heparin, EDTA or citrate.***

- ***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 (from Blood collected on Heparin, EDTA or Citrate Tubes) 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.***

- ***VERY IMPORTANT! Frozen plasma recovered from Norgen's cf-DNA Preservative Tubes (Cat. 63950, 63960) may contain some precipitates upon thawing. DO NOT discard any precipitates before cfc-DNA purification. Briefly vortex the plasma and proceed immediately for cfc-DNA purification. Discarding any precipitates may significantly lower cfc-DNA yield.***

Note: The procedure outlined below is for 200 µL inputs of Plasma/Serum. If processing a sample volume lower than 200 µL Plasma/Serum, simply bring the volume of your samples up to 200 µL using 1X PBS and proceed as outlined below.

1. Place 200 µL of plasma/serum sample in a 2 mL tube (provided by the user). Add 10 µL **Proteinase K** and mix well by vortexing for 10 seconds, then incubate for **10 minutes at room temperature** while slowly inverting the tube (end-over-end) for the span of incubation.
2. After incubation, add 600 µL of **Binding Buffer B,** and mix well by vortexing for 10 seconds.
3. Transfer 400 µL of the mixture from **Step 2** into a Micro Spin column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,800 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Repeat **Step 3** to transfer the remaining mixture into the Micro Spin column.
5. Apply 500 µL of **Solution WN** to the column and centrifuge for **1 minute at 3,800 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
6. Apply 500 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 3,800 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat **Step 6** one more time.
8. Spin the column, empty, for **3 minutes at 20,800 *x g* (~14,000 RPM)**. Discard the collection tube.
9. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 25 - 50 µL of **Elution Buffer B** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 420 x *g* (~2,000 RPM),** followed by **2 minutes at 6,800 x g (~8,000 RPM)**.
10. 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 420 x *g* (~2,000 RPM),** followed by **2 minutes at 6,800 x g (~8,000 RPM)**.

- ***Plasma/Serum 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

Plasma/Serum 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 plasma or serum. 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 plasma or serum unless large plasma/serum volumes have been processed***. This would only be applicable if plasma/serum 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 plasma or serum. Plasma/Serum DNA is short fragmented DNA which is usually present in less than 1000 bp. Purified plasma/serum 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 plasma/serum DNA purification. ***The only reliable method that can assess the quality and the relative quantity of the purified plasma/serum 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**

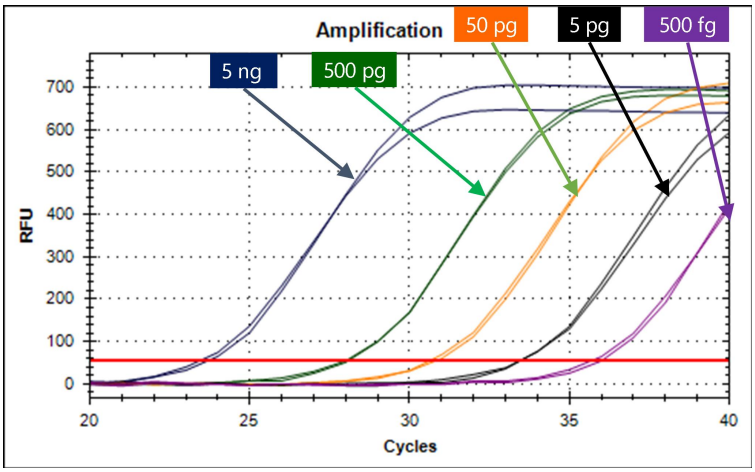
2) NanoDrop 2000

- Detection Limit: 2 ng/µl (dsDNA)

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

- Detection Limit: 25 pg/mL

4) qPCR DNA 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 time varied from what is specified in the product manual?
- Varying the incubation time will result in a reduction in your DNA yields.

7. 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.

8. 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.

9. Do I need to do an RNase treatment for my DNA Elution?
- Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Kits do not co-purify plasma/serum circulating RNA along with circulating DNA, therefore an RNase step is not required.

10. 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.

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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