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Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format)

Product # 51200 **Product Insert**

Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating DNA from various amounts of plasma/serum ranging from 400 µL to 2 mL. Free-circulating nucleic acids in plasma and serum have the potential to provide biomarkers for certain cancers and disease states, and include tumor-specific extracellular DNA fragments and fetal DNA in maternal blood. These free-circulating nucleic acids are usually present as short fragments of less than 1000bp (DNA)

Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) provides an efficient method for the purification of these fragmented free-circulating DNA from human plasma or serum. Purification is based on the use of Norgen's proprietary resin as the separation matrix. The kit is able to isolate all sizes of circulating DNA. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating DNA from large sample volumes. DNA can be isolated from either fresh or frozen samples using this kit. Typical yields of free-circulating DNA purified will vary depending on the input sample (1-100ng/mL circulating DNA in human plasma), with more concentrated samples tending to yield more free-circulating nucleic acids. Preparation time for a single sample is less than 45 minutes. The purified plasma/serum free-circulating DNA is eluted in an elution solution that is compatible with PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis.

This kit includes enough reagents to process 20 samples of up to 2 mL of Plasma/Serum. A single protocol is provided with the volumes optimized for 1 mL inputs; however the volumes can be adjusted for inputs of as low as 400 µL and up to 2 mL of Plasma/Serum.

Kit Components:

Component	Contents
Lysis Buffer D	125 mL
Slurry A1	6 mL
Binding Buffer B	6 mL
Wash Solution A	2 x 38 mL
Binding Buffer C	6 mL
Elution Buffer B	8 mL
Mini Filter Spin Columns	20
Collection Tubes	40
Spin Columns	20
Elution tubes (1.7 mL)	40
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Centrifuge with a swinging bucket rotor capable of at least 2000 RPM
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator
- 15 mL tubes

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.

Quality Control
In accordance with Norgen's Quality Management System, each lot of Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) is designed for research purposes only. It is not intended for human or 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 convenient PDF files online at *www.norgenbiotek.com*.

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

The **Lysis Buffer D, Slurry A1, Binding Buffer B** and **Binding Buffer C** 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 these solutions.

If liquid containing these buffers 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

Procedures

Purification of Circulating DNA from 1mL Serum or Plasma

Notes prior to use:

- 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. **Slurry A1** contains grey resin which will not dissolve after warming.
- 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.
- All centrifugation steps are performed at room temperature.
- 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.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottles containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The labels on the bottles have 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.
- ***Slurry A1 contains resin and must be mixed well before every pipetting***.
- ***This kit is suitable for the isolation of DNA from fresh or frozen serum or plasma prepared from blood collected on either Heparin, EDTA or citrate.***
- **The procedure is outlined for 1mL inputs. To process different Plasma/Serum volumes please check Table 1 for the appropriate volume of Lysis Buffer D that should be added for inputs from 400 µL to 2 mL of Plasma/Serum.**

1. Aliquot 1 mL of Plasma/Serum sample into a 15 mL tube.
2. To each 1 mL Plasma/Serum sample add 3mL of **Lysis Buffer D**. Mix well by vortexing for 15 seconds.
3. Incubate the mixture from **Step 2** for 10 minutes at 60°C.
4. After incubation add 200 µL of **Slurry A1** and mix well by vortexing for 15 seconds. ***(Note*: *Slurry A1 contains resin and must be mixed well before every pipetting**)*.
5. Centrifuge the mixture from **Step 4** for **1 minute at 50 x g (500 RPM)**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
6. To the slurry pellet add 180 µL of **Binding Buffer B** followed by the addition of 420 µL of **96-100% Ethanol** (provided by the user). Mix well by vortexing for 15 seconds.
7. Centrifuge the mixture for **1 minute at 50 x g (500 RPM)**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
8. To the slurry pellet add 1 mL **Wash Solution A**, mix well by vortexing for 15 seconds, and then centrifuge for **1 minute at 800 x g (2,000 RPM)**. Carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
9. Repeat **Step 8** to wash the slurry pellet for a second time.
10. To the slurry pellet from **Step 9** add 500 µL **Wash Solution A,** and mix well by vortexing for 15 seconds.
11. Transfer the entire mixture from **Step 10** into a Mini Filter Spin column (black O-ring). Centrifuge for **1 minute at 10,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
12. Spin the column, empty, for **2 minutes at 10,000 x g (~14,000 RPM)**. Discard the collection tube.
13. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer B** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **2 minutes at 10,000 x g (~14,000 RPM)**.
14. Reload the elution back onto the Mini Filter Spin Column, and centrifuge the samples for 2 minutes at **10,000 x g (~14,000 RPM)**.
15. To the eluate obtained in **Step 14**, add 200 µL of **Binding Buffer C**. Apply the entire volume of the sample to a Spin Column (white O-ring) assembled with its collection tube and centrifuge for 1 minute at **8,000 x g (~8,000 RPM)**.
16. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **10,000 x g (~14,000 RPM)**.
17. Discard the flowthrough and reassemble the spin column with its collection tube.
18. Repeat **steps 16 and 17** to wash the column for a second time.
19. Spin the column for 2 minutes at **10,000 x g (~14,000 RPM).**in order to thoroughly dry the column. Discard the collection tube.
20. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Buffer B** to the center of the column bed. It is important that the Elution Buffer B be placed directly onto the column bed, and not onto the side of the column to obtain the best DNA recovery.
21. Let stand at room temperature for 1 minute.
22. Centrifuge for **2 minutes at 10,000 x g (~14,000 RPM).**

❖ **Free-circulating plasma/serum DNA is now ready for downstream applications.**

Table 1. Lysis Buffer D to be added to different Plasma/Serum sample volumes

Sample Volume (mL)	Lysis Buffer D (mL) (Step 2)
0.4	1.2
0.5	1.5
0.6	1.8
0.7	2.1
0.8	2.4
0.9	2.7
1	3
2	6

Frequently Asked Questions

1. What If a variable speed centrifuge is not available?

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

2. What will happen if my centrifugation speed varied from the recommended speed?

- This may lead to the degradation of the isolated DNA or reduction in the total DNA yields.

3. 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.

4. Can I process a different Plasma/Serum volume?

- Yes, you can. To process different Plasma/Serum volumes please check Table 1. for the appropriate volumes of **Lysis Buffer D** that should be added to different Plasma/Serum sample volumes. The volume of **Slurry A1, Binding Buffer B, Binding Buffer C** and **Wash Solution A** is fixed for all Plasma/Serum volumes.

6. What If I added more or less of the specified reagents' volume?

- Adding less volume may reduce both the quality and the quantity of the purified DNA. Adding more may not affect the nucleic acid yields EXCEPT if more Elution Buffer B was added. Eluting your DNA in higher volumes will result in diluting your nucleic acids.

7. What If my incubation varied from the 10 minutes specified in the product manual?

- Less than 10 minutes will result in a lower DNA yields and poor DNA quality. More than 10 minutes may not affect your DNA yields.

8. What If I forgot to do a dry spin after my second wash?

- Your elution will be contaminated with the Wash Solution A. This may dilute the nucleic acid yield in your first elution and may interfere with your down stream applications.

9. Can I perform a second elution?

- Yes, you can. A second elution is possible, but it is recommended that this elution is performed in a smaller volume (50 μL).

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

- Plasma/Serum samples contain very little DNA. This varies from individual to individual based on numerous variables. In order to increase the yield, the amount of Plasma/Serum input could be increased.

11. Why do my isolated nucleic acids not perform well in downstream applications?

- If a different Elution Buffer B 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 B with the intended use.

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

- Unlike other kits, Norgen's Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) doesn't co-purify plasma/serum circulating RNA along with circulating DNA, therefore an RNase step is not required.

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

- Most of the free-circulating Plasma/Serum DNA is present in short fragment. This low A260:280 ratio and the low A260:230 ratio will not affect any downstream application

Related Products	Product #
Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format)	50600
Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format)	51300
Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format)	42800
Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit (Slurry Format)	51000
Plasma/Serum Circulating and Exosomal RNA Purification Maxi Kit (Slurry Format)	50900

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please 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 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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

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