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Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format)

Product # 53400 **Product Insert**

Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating nucleic acid (mRNA, miRNA, exosomal RNA, viral RNA, DNA and viral DNA) from various amounts of plasma/serum ranging from 2 mL to 5 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 nucleic acid fragments and fetal DNA in maternal blood. Free-circulating DNA is usually present as short fragments of less than 1000 bp, and free-circulating RNA and exosomal RNA in plasma and serum are usually present as short fragments of less than 1000 nt. In addition, plasma or serum also contains free-circulating miRNA that are as small as 21 nt. Furthermore, circulating viral DNA or viral RNA may be present in the plasma or the serum of viral-infected patients. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) provides an efficient method for the purification of all sizes of these fragmented free-circulating nucleic acids 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 RNA, including microRNA, as well as all sizes of exosomal RNA and circulating DNA. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating NA from large sample volumes. Free-circulating nucleic acids can be isolated from either fresh or frozen samples using this kit, and the kit allows for the concentration of nucleic acids that are present in low concentrations (1-100ng/mL in human plasma). Typical yields of free-circulating nucleic acids will vary depending on the input sample, as the amount of nucleic acids present in plasma and serum will depend upon the health status of the individual and the level of nucleases present in the blood. ***This kit is suitable for the purification of nucleic acids from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.***

Preparation time for 10 samples is less than 40 minutes. The purified plasma/serum free-circulating Nucleic Acid are eluted in an elution solution that is compatible with PCR, qPCR, reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays, methylation-sensitive PCR and Southern Blot analysis.

Kit Components:

Component	Contents
PSNA Lysis Buffer	2 x 125 mL
PSNA Solution A	6 mL
PSNA Solution B	9 mL
Wash Solution	11 mL
NA Elution Solution	3 mL
Mini Filter Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- β - Mercaptoethanol
- 50 mL tubes
- 15 mL tubes

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25^{o}C) for up to 1 year without showing any reduction in performance.

It is recommended to warm PSNA Solution A, PSNA Solution B and PSNA Lysis Buffer for 20 minutes at 60^{o}C if any salt precipitation is observed.

Quality Control

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

Product Use Limitations

Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) is designed for research purposes only. Not for use in diagnostic procedures.

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.

PSNA Solution A, **PSNA Solution B** and **PSNA Lysis Buffer** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions

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.

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defence against these enzymes.

- The RNA area should be located away from microbiological work stations
- 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
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Purification of Circulating Nucleic Acid from 2mL Serum or Plasma

Notes Prior to Use
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

- 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.
- Prepare a working concentration of the **Wash Solution** by adding 25 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The use of β-mercaptoethanol in lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of PSNA Solution B.
- ***PSNA Solution A contains resin and must be mixed well before every pipetting***.
- It is highly recommended to warm up **PSNA Solution A**, **PSNA Solution B** and **PSNA Lysis Buffer** at 60^{o}C for 20 minutes and mix well until the solutions become clear again if precipitates are present.
- It is important to work quickly during this procedure.
- ***This kit is suitable for the isolation of RNA from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.***

- **The procedure is outlined for 2 mL inputs. To process different Plasma/Serum volumes please check Table 1 for the appropriate volumes that should be added of PSNA Lysis Buffer, PSNA Solution A and 96-100% Ethanol to the different Plasma/Serum sample volumes. The volume of PSNA Solution B is fixed for all Plasma/Serum volumes**.

1. In a 50 mL tube (provided by the user), add 3.8 mL **PSNA Lysis Buffer** (after the addition of β-mercaptoethanol) for 2 mL plasma/serum sample. Mix well by vortexing for 15 seconds.
2. Add 0.2 mL of **PSNA Solution A** to the previous mixture ***(Note: PSNA Solution A contains resin and must be mixed well before every pipeting)***
3. Incubate the mixture from **Step 2** for 10 minutes at 60°C.
4. After incubation add 6 mL of 96-100% ethanol (provided by the user). Mix well by vortexing for 15 seconds.
5. Centrifuge for **30 seconds at 1,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
6. To the slurry pellet add 0.3 mL **PSNA Solution B**, and mix well by vortexing for 15 seconds
7. Incubate the mixture from **Step 6** for 10 minutes at 60°C.
8. After incubation add 0.3 mL 96-100% ethanol (provided by the user). Mix well by vortexing for 15 seconds.
9. Transfer 650 µL from the mixture from **Step 8** into a Mini Filter Spin column. Centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube
10. Repeat **Step 9** until all the mixture from **Step 8** has been transferred to the Mini Filter Spin column.

Optional Step:

Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) isolates RNA and DNA from plasma and serum samples in a single elution. However, an optional protocol is provided in Appendix A for the removal of plasma/serum DNA If only plasma/serum RNA is required. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol.

11. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
12. Repeat step 10 two more times, for a total of three washes.
13. Spin the column, empty, for **3 minutes at 14,000 x g (~14,000 RPM)**. Discard the collection tube.
14. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **NA Elution Solution** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM),** followed by **2 minute at 14,000 x g (~14,000 RPM)**.

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

Table 1. PSNA Lysis Buffer, PSNA Solution A and 96-100% Ethanol to be added to different Plasma/Serum sample volumes

Sample Volume (mL)	PSNA Lysis Buffer (mL) (Step 1)	PS Solution A (mL) (Step 2)	96-100% Ethanol (mL) (Step 3)
2	3.8	0.2	6
3	5.8	0.2	9
4	7.8	0.2	12
5	9.8	0.2	15

Appendix A

Protocol for Optional On-Column DNA Removal
Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) isolates RNA and DNA from plasma and serum samples in a single elution. However, an optional protocol is provided below for the maximum removal of plasma/serum DNA If only plasma/serum RNA is required. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

   Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated.

2. Perform the appropriate RNA Isolation Procedure for your starting material up to **Step 10**
3. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube
4. Apply 100 µL of the **RNase-free DNase I solution** prepared in **Step 1** to the column and centrifuge at **14,000 x g (~14,000 RPM)** for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at **14,000 x g (~14,000 RPM)** for an additional minute.

5. After the centrifugation in **Step 4**, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure **Step 5** is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in **Step 12**.

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 a decrease in overall yield of the purified nucleic acids.

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 that should be added from **PSNA Lysis Buffer**, **PSNA Solution A** and **96-100% Ethanol** to different Plasma/Serum sample volumes. The volume of **PSNA Solution B** is fixed for all Plasma/Serum volumes.

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

- Adding more or less from the specified volumes outlined in Table 1 may affect both the quality and quantity of the isolated nucleic acid.

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

- Your elution will be contaminated with the Wash Solution that contains Ethanol. This will dilute the nucleic acid yield and it will interfere with your downstream applications.

7. 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).

8. Can I elute in a smaller volume?

- Yes, you can. The minimum volume that can be used for elution is 50 μL. Elute in 50 μL then re-load the eluted solution back into the column and re-elute. The final elution volume should be 50 μL.

9. Why do my samples show low NA yield?

- Plasma/Serum samples contain very little NA. 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.

10. Why do the A260:280 ratio of the purified NA is lower than 2.0?

- Most of the Free-Circulating Plasma/Serum NA is degraded and present in short fragment. The A260:280 ratio is normally between 1 – 1.6. This low A260:280 ratio will not affect any downstream application

11. Why does my isolated NA not perform well in downstream applications?

- If a different Elution Solution 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.

12. Do I need to do a DNase or RNase treatment for my Elution?

- You may need to do a DNase or RNase treatment to your isolated Plasma/Serum Circulating NA if you need to work with either pure RNA or DNA.

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Blood Genomic DNA Isolation Kit	18200

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 Nucleic Acid Purification Maxi 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.

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