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Plasma/Serum Circulating and Exosomal RNA Purification 96-Well Kit (Slurry Format)

Product # 29500 **Product Insert**

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification 96-Well Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from plasma and serum samples ranging from 0.25 mL to 2 mL. Free-circulating RNA and exosomal RNA in plasma and serum can serve as both tumor- and fetal-specific markers for cancer detection and prenatal diagnosis. As well, free-circulating and exosomal RNAs have the potential to provide biomarkers for other disease states. Free-circulating RNA in plasma or serum are usually present as short fragments of less than 1000nt, and free-circulating miRNA (21nt) can also be found in plasma and serum. This kit is able to isolate all sizes of circulating and exosomal RNA, including microRNA, without the use of phenol or chloroform

Exosomes are 40 - 100 nm membrane vesicles, which are secreted by most cell types. Exosomes can be found in saliva, blood, urine, amniotic fluid and malignant ascitic fluids, among other biological fluids. Evidence has been accumulating recently that these vesicles act as cellular messengers, conveying information to distant cells and tissues within the body. The exosomes contain cell-specific proteins, lipids and RNAs, which are transported to other cells, where they can alter function and/or physiology. These exosomes may play a functional role in mediating adaptive immune responses to infectious agents and tumours, tissue repair, neural communication and transfer of pathogenic proteins. Recent work has demonstrated the presence of distinct subsets of microRNAs within exosomes which depend upon the tumour cell type from which they are secreted. For this reason exosomal RNAs may serve as biomarkers for various diseases including cancer. As the RNA molecules encapsulated within exosomes are protected from degradation by RNAses they can be efficiently recovered from biological fluids, such as plasma or serum.

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification 96-Well Kit (Slurry Format) provides an efficient method for the purification of free-circulating and exosomal RNA from human plasma or serum. Purification is based on the use of Norgen's proprietary resin as the separation matrix. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples using this kit. The kit also offers an elution volume of 100µL, allowing for the concentration of circulating and exosomal RNAs that are present in low concentrations (1-100ng/mL circulating RNAs in human plasma). Typical yields of free-circulating and exosomal RNAs purified will vary depending on the input sample, with more concentrated samples tending to yield more free-circulating and exosomal nucleic acids. ***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.***

Preparation time for 96 samples is approximately 1 hour. The purified plasma/serum free-circulating and exosomal RNA is eluted in an elution solution that is compatible with real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

This kit is optimized to process 96 plasma/serum samples ranging from 0.25 mL to 2 mL. 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 0.25 mL and up to 2 mL of Plasma/Serum.

Kit Components:

Component	Contents
Slurry C3	24 mL
Lysis Buffer A	4 x 100 mL
Wash Solution A	2 x 38 mL
Elution Solution A	20 mL
96-Well Filter Plate	1
Adhesive Tape	1
96-Well Collection Plate	1
96-Well Elution Plate	1
Product Insert	1

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.

Product Use Limitations
Norgen's Plasma/Serum Circulating and Exosomal RNA Purification 96-Well Kit (Slurry Format) is designed for research purposes only. It is not intended for human or diagnostic use.

Customer-Supplied Reagents and Equipment

- For ***Vacuum Format***:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads
- For ***Centrifuge Format***:
  - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)
- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- β - Mercaptoethanol
- 50 mL tubes

Quality Control
In accordance with Norgen's Quality Management System, each lot of Norgen's Plasma/Serum Circulating and Exosomal RNA Purification 96-Well Kit (Slurry Format) is tested against predetermined specifications to ensure consistent product quality.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK 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.

Slurry C3 and **Lysis Buffer A** 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 If liquid containing any 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.

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

Procedure for Purification of Circulating and Exosomal RNA from 1mL Serum or Plasma

Notes Prior to Use

- 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 A by adding 90 mL of 96-100% ethanol (provided by the user) to each supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 128 mL in each bottle. The labels on the bottles have 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 **Lysis Buffer A** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- ***Slurry C3 contains resin and must be mixed well before every pipetting.***
- It is highly recommended to warm up **Slurry C3** and **Lysis Buffer A** at 60^{o}C for 20 minutes and mix well until the solutions become clear again if precipitates are present. Slurry C3 contains grey resin that will not disappear by warming up.
- It is important to work quickly during this procedure.
- The purification of total RNA can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section A. For purification using centrifugation, please follow the procedure outlined in Section B.
- ***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 1 mL inputs. To process different Plasma/Serum volumes please check Table 1 for the appropriate volumes that should be added of Slurry C3, Lysis Buffer A (Step 1) and 96-100% (Step 3) Ethanol to different Plasma/Serum sample volumes. The volume of Lysis Buffer A (Step 5) is fixed for all Plasma/Serum volumes**.

Table 1. Slurry C3, Lysis Buffer A and 96-100% Ethanol to be added to different Plasma/Serum sample volumes

Sample Volume (mL)	Slurry C3 (mL) (Step 1)	Lysis Buffer A (mL) (Step 1)	96-100% Ethanol (mL) (Step 3)
0.25	0.2	0.3	0.75
0.5	0.2	0.8	1.5
1	0.2	1.8	3
1.5	0.2	2.8	4.5
2	0.2	3.8	6

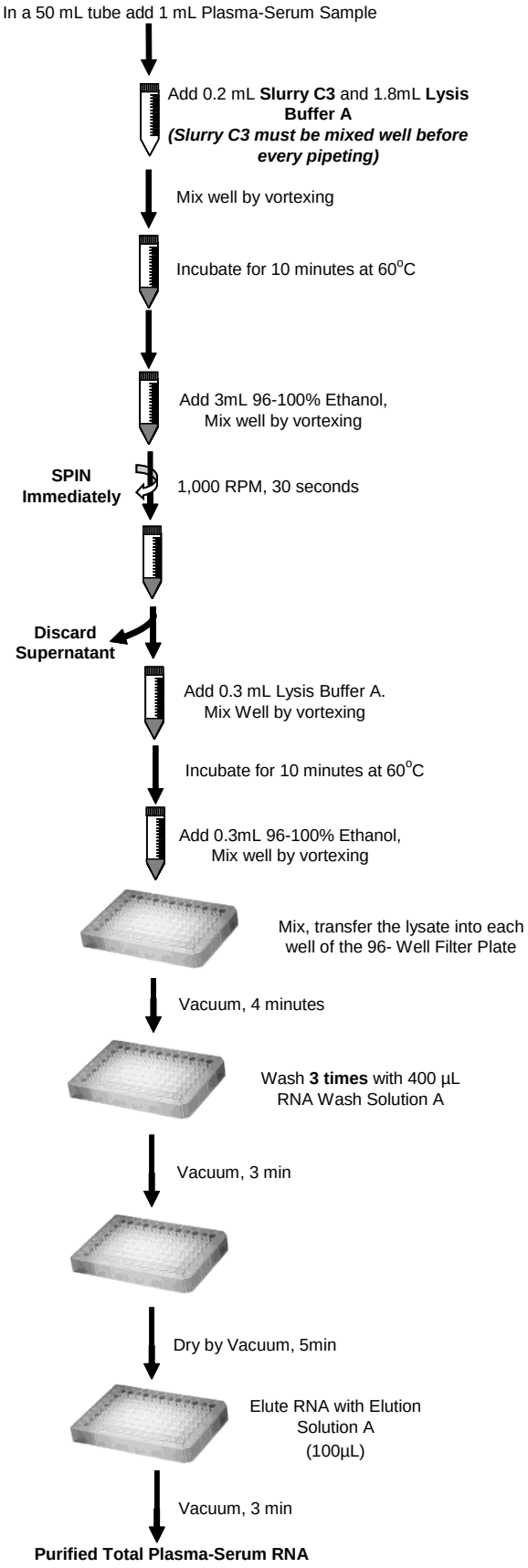
A. Detailed Procedure Using Vacuum Manifold for Purification of Circulating and Exosomal RNA from 1mL Serum or Plasma (Adjust according to Table 1 if you are processing different input volumes)

1. In a 50 mL tube (provided by the user), add 0.2 mL of **Slurry C3** and 1.8 mL **Lysis Buffer A** (after the addition of β-mercaptoethanol) for every 1 mL plasma/serum sample. Mix well by vortexing for 15 seconds. ***(Note*: *Slurry C3 contains resin and must be mixed well before every pipeting)***
2. Incubate the mixture from **Step 1** for 10 minutes at 60^{o}C.
3. After incubation add 3 mL of 96-100% Ethanol (provided by the user). Mix well by vortexing for 15 seconds.
4. Centrifuge for **30 seconds at 1,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
5. To the slurry pellet add 0.3 mL **Lysis Buffer A**, and mix well by vortexing for 15 seconds
6. Incubate the mixture from Step 5 for 10 minutes at 60^{o}C.
7. After incubation add 0.3 mL 96-100% Ethanol (provided by the user). Mix well by vortexing for 15 seconds.
8. Assemble the 96-Well Filter Plate and the vacuum manifold according to manufacturer's recommendations. (***Note:*** *The provided 96-Well Collection Plate can be used as the collection/waste tray if desired).*
9. Transfer the entire mixture from **Step 7**, including all resin, into a well of the 96- Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for **4 minutes**. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the vacuum manifold.
10. Apply 400 µL of **Wash Solution A** to each used well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for **3 minutes**. Turn off vacuum and ventilate the manifold. Discard the flowthrough;
11. Repeat **Step 10 twice**;
12. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the vacuum manifold. Apply vacuum for an additional **5 minutes** in order to completely dry the plate. Turn off vacuum and ventilate the manifold;
13. Replace the collection/waste tray in the vacuum manifold with a provided 96-Well Elution Plate. Complete the vacuum manifold assembly with the 96-Well Filter Plate. Add 100 µL of **Elution Solution A** to each used well of the plate. Apply vacuum for **3 minutes**.

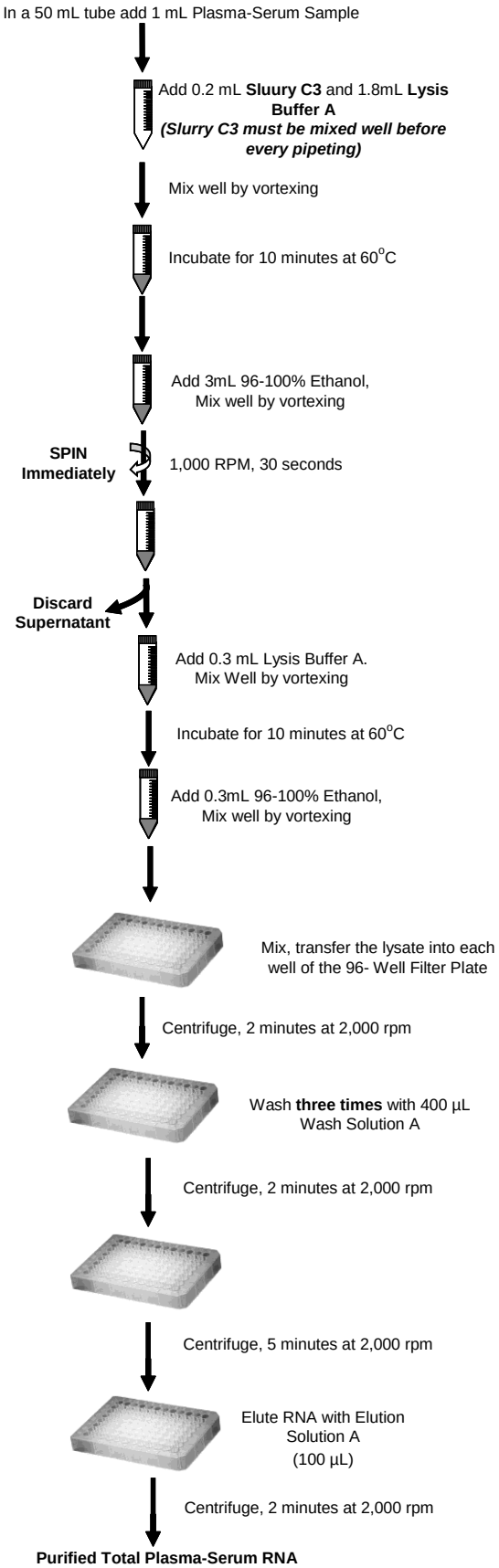
B. Detailed Procedure Using Centrifugation for Purification of Circulating and Exosomal RNA from 1mL Serum or Plasma (Adjust according to Table 1 if you are processing different input volumes)

1. In a 50 mL tube (provided by the user), add 0.2 mL of **Slurry C3** and 1.8 mL **Lysis Buffer A** (after the addition of β-mercaptoethanol) for every 1 mL plasma/serum sample. Mix well by vortexing for 15 seconds. ***(Note: Slurry C3 contains resin and must be mixed well before every pipeting)***
2. Incubate the mixture from **Step 1** for 10 minutes at 60^{o}C.
3. After incubation add 3 mL of 96-100% Ethanol (provided by the user). Mix well by vortexing for 15 seconds.
4. Centrifuge for **30 seconds at 1,000 RPM**, then carefully decant the supernatant in order to ensure that the slurry pellet is not dislodged.
5. To the slurry pellet add 0.3 mL **Lysis Buffer A**, and mix well by vortexing for 15 seconds
6. Incubate the mixture from Step 5 for 10 minutes at 60^{o}C.
7. After incubation add 0.3 mL 96-100% Ethanol (provided by the user). Mix well by vortexing for 15 seconds.
8. Place the 96-Well Filter Plate on top of a provided 96-Well Collection Plate. (***Note:*** *The provided 96-Well Collection Plate can be used as the collection/waste tray if desired).*
9. Transfer the entire mixture from **Step 7**, including all resin, into a well of the 96- Well Filter Plate. Centrifuge the assembly at maximum speed or **2,000 RPM for 2 minutes**. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the bottom plate;
10. Apply 400 µL of **Wash Solution A** to each used well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or **2,000 RPM for 2 minutes**. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the bottom plate;
11. Repeat **Step 10 <u>twice</u>**;
12. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the bottom plate. Centrifuge the assembly at **2,000 RPM for 5 minutes** in order to completely dry the plate.
13. Stack the 96-Well Filter Plate on top of the provided 96-Well Elution Plates. Add 100 µL of **Elution Solution A** to each used well of the plate. Centrifuge the assembly at **maximum speed** or **2,000 RPM for 2 minutes**.

Rapid Flow Chart Procedure for the Isolation of Plasma/Serum Circulating and Exosomal RNA Using Vacuum Manifold



Rapid Flow Chart Procedure for the Isolation of Plasma/Serum Circulating and Exosomal RNA Using Centrifugation



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 nucleic acids or reduction in the total nucleic acid 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. Why are the wells that I am using clogged?

- The wells of the 96-well plate will clog if;
    1. There was an insufficient vacuum. Ensure that a vacuum pressure of at least -650 mbar or - 25 in. Hg is developed
    2. The centrifuge temperature was too low. Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.

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 RNA.

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

- Your first elution will be contaminated with the Wash Solution. This may dilute the nucleic acid yield in your first elution and it may interfere with your down stream 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. Why do my samples show very low RNA yield?

- Some Plasma/Serum samples contain very little RNA. 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.

9. Why does my isolated RNA 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.

10. Do I need to do a DNase treatment for my RNA Elution?

- You may need to do a DNase treatment to your isolated Plasma/Serum Circulating RNA. It is recommended to use Norgen's RNase-Free DNase I Kit (Cat# 25710)

11. Why is my RNA degraded?

- RNase contamination: RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "Working with RNA" at the beginning of this user guide.
- Procedure not performed quickly enough: In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.

12. Why do the A260:280 ratio of the purified RNA is lower than 2.0?

- Most of the Free-Circulating Plasma/Serum RNA 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

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Total RNA Purification 96-Well Kit	24300
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 Plasma/Serum Circulating and Exosomal RNA Purification 96-Well 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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