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

Product # 42800 **Product Insert**

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from various amounts of plasma/serum ranging from 0.25 mL to 5 mL. Free-circulating and exosomal RNA in plasma and serum has the potential to provide biomarkers for certain cancers and disease states, and includes tumor-specific extracellular RNA in the blood. Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) provides an efficient method for the purification of all sizes of these fragmented free-circulating and Exosomal RNAs from human plasma or serum.

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.

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. Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit 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, and the kit allows for the concentration of RNA that is present in low concentrations (1-100ng/mL circulating RNA in human plasma). Typical yields of free-circulating and Exosomal RNA will vary depending on the input sample, as the amount of RNA 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 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 a 10 samples is less than 40 minutes. The purified plasma/serum free-circulating RNA are eluted in an elution solution that is compatible with reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

This kit includes enough reagents to process 50 samples of up to 2 mL of Plasma/Serum, 40 samples of 3 mL of Plasma/Serum, 30 samples of 4 mL of Plasma/Serum and 20 samples of 5 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 0.25 mL and up to 5 mL of Plasma/Serum.

Kit Components:

Component	Contents
Slurry C2	12 mL
Lysis Buffer A	2 x 130 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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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 solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

It is recommended to warm Slurry C2 and Lysis Buffer A for 20 minutes at 60ºC if any salt precipitation is observed. Slurry C2 contains a grey resin which will not disappear with warming up.

Quality Control

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

Product Use Limitations

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification 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.

Slurry C2 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

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 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 the supplied bottle containing the 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.
- 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**.
- ***Slurry C2 contains resin and must be mixed well before every pipetting***.
- It is highly recommended to warm up **Slurry C2** and **Lysis Buffer A** at 60ºC for 20 minutes and mix well until the solutions become clear again if precipitates are present. **Slurry C2** contains grey resin which will not disappear after warming up.
- 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 1 mL inputs. To process different Plasma/Serum volumes please check Table 1 for the appropriate volumes of Lysis Buffer A (Step 1) and 96-100% Ethanol (Step 3) to be added to different Plasma/Serum sample volumes. The volume of Slurry C2 and Lysis Buffer A (Step 5) is fixed for all Plasma/Serum volumes**.

1. In a 50 mL tube (provided by the user), add 0.2 mL of **Slurry C2** and 1.8 mL **Lysis Buffer A** (after the addition of β-mercaptoethanol) to 1 mL plasma/serum sample. Mix well by vortexing for 15 seconds.
   Note 1*: *Slurry C2 contains resin and must be mixed well before every pipetting
   Note 2: To process different Plasma/Serum volumes please check Table 1 for the appropriate volumes of Lysis Buffer A (Step 1) and 96-100% Ethanol (Step 3) that should be added to different Plasma/Serum sample volumes. The volume of Slurry C2 and Lysis Buffer A (Step 5) is fixed for all Plasma/Serum volumes.

2. Incubate the mixture from **Step 1** for 10 minutes at 60ºC.
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 60ºC.
7. After incubation add 0.3 mL 96-100% Ethanol (provided by the user). Mix well by vortexing for 15 seconds.
8. Transfer 650 µL from the mixture from **Step 7** into a Mini Filter Spin column. Centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube;
9. Repeat step 8 until all the mixture from **Step 7** has been transferred to the Mini Filter Spin column.
10. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.

Optional Step:

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) isolates RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. 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. Repeat step 10 two more times, for a total of three washes.
12. Spin the column, empty, for **3 minutes at 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 Solution A** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **3 minute at 14,000 RPM**.

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

Table 1. Slurry C2, Lysis Buffer A and 96-100% Ethanol to be added to different Plasma/Serum sample volumes

Sample Volume (mL)	Slurry C2 (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
3	0.2	5.8	9
4	0.2	7.8	12
5	0.2	9.8	15

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) isolates RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. 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 A** 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 and **Step 9**
3. Apply 400 μL of **Wash Solution A** to the column and centrifuge for **1 minute at 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 RPM** for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at **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 - 30ºC for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in **Step 11**.

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 RNA or reduction in the total RNA 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 A** (Step 1) and **96-100% Ethanol** (Step 3) to be added to different Plasma/Serum sample volumes. The volume of **Slurry C2** and **Lysis Buffer A** (Step 5) 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 RNA.

6. What If I forgot to do a dry spin after my second wash?
- Your elution will be contaminated with the Wash Solution A that contains Ethanol. This will dilute the RNA 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. Why do my samples show low RNA yield?
- 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 is the A260:280 ratio of the purified RNA 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

10. 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 solution with the intended use.

11. 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)

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Total RNA Purification 96-well Kit	24300
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 and Exosomal RNA Purification 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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