

Plasma/Serum Circulating RNA Purification Kit
Product # 30000

Product Insert

Norgen's Plasma/Serum Circulating RNA Purification Kit provides a fast, reliable and simple procedure for isolating circulating RNA from various amounts of plasma/serum ranging from 1 mL to 5 mL. Free-circulating 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. Free-circulating RNA is usually present as short fragments of less than 1000 nt, and includes free-circulating miRNA (21nt). Norgen's Plasma/Serum Circulating RNA Purification Kit provides an efficient method for the purification of all sizes of these fragmented free-circulating RNAs 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. Norgen's Plasma/Serum Circulating 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 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.

Preparation time for a single sample is less than 30 minutes. The purified plasma/serum free-circulating RNA is eluted in a 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 sufficient reagents to process 12 samples of up to 5mL of plasma/serum. All the reagents provided are optimized to be in a linear relationship to the input sample volume. A single protocol is provided with the volumes optimized for 1 mL input; however the volumes can be adjusted for inputs of 2mL, 3mL, 4mL and 5mL of plasma/serum.

Kit Components:

Component	Contents
Lysis Solution	100 mL
Wash Solution I	5.5 mL
Elution Solution I	10 mL
Binding Solution	15 mL
Wash Solution II	4 mL
Elution Solution II	2 mL
Maxi Filter Columns	12
Mini Spin Columns with Collection Tubes	12
Elution tubes (1.7 mL)	12
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Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 3000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 100% isopropanol
- β - Mercaptoethanol
- 50 mL tubes

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. If any precipitates are observed in the Lysis Solution or Binding Solution it is recommended to warm the solutions for 20 minutes at 60°C.

Quality Control

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

Product Use Limitations

Norgen's Plasma/Serum Circulating RNA Purification Kit 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 convenient PDF files online at www.norgenbiotek.com.

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

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: Purification of Circulating 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 I** by adding 14.5 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 20 mL. The bottle label contains a box to check to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution II** by adding 10 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 14 mL. The bottle label contains a box to check to indicate that the ethanol has been added.

- The use of β -mercaptoethanol during lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 μ L of β -mercaptoethanol (provided by the user) to each 1 mL of RNA Lysis Solution required. β -mercaptoethanol is toxic and should be dispensed in a fume hood.
 - If any precipitates are observed in the Lysis Solution or Binding Solution it is recommended to warm the solutions for 20 minutes at 60°C.
 - It is important to work quickly during this procedure.
 - The procedure is outlined for 1 mL inputs. Adjust the volumes of the Lysis Solution and Isopropanol accordingly if processing different plasma/serum volumes. Inputs of 1 mL to 5 mL may be processed.
1. Add 1.5 mL of **Lysis Solution** (after the addition of β -mercaptoethanol) for every 1 mL plasma/serum sample. **Vortex for 15 seconds.**
 2. Add 1.5 mL of **100% Isopropanol** (provided by the user) for every 1 mL plasma/serum sample. **Mix by vortexing for 10 seconds.**
 3. Transfer 4 mL of lysate to the provided Maxi Column assembly and securely close the lid. Vortex the column assembly for 15 seconds. **Do Not Over Vortex.** If processing more than 1 mL of plasma/serum the total lysate volume from Steps 1 and 2 may exceed 4 mL. In this case transfer up to 7 mL of lysate to the Maxi Column assembly.

Note: During vortexing, the grey resin that is contained within the column will mix with the lysate to form grey colored slurry.
 4. After the vortexing step centrifuge the Maxi Column assembly for **2 minutes at 2,000 RPM.** Remove the collection tube and discard the flowthrough. Reassemble the Maxi Column with the collection tube.

Note: The entire lysate must go through the Maxi Column. If liquid remains in the column, vortex the column assembly for an additional 15 seconds and centrifuge for an additional 2 minutes at 2,000 RPM.
 5. If processing more than 1 mL plasma/serum, repeat **Steps 3 and 4** as required in order to process the entire lysate volume.
 6. After the entire lysate volume has been applied to the Maxi Column, apply 700 μ L of **Wash Solution I** to the column and centrifuge for **1 minute at 3,000 RPM.** Discard the flowthrough and reassemble the Maxi Column with the collection tube.
 7. Repeat **Step 6** a second time.
 8. Spin the column, empty, for **2 minutes at 3,000 RPM.** Discard the collection tube.
 9. Transfer the spin column to a fresh 50 mL conical tube (not provided). Apply 700 μ L of **Elution Solution I** to the column and centrifuge for **2 minutes at 500 RPM,** followed by **3 minutes at 3,000 RPM.** Remove the Maxi Column from the conical tube and discard.
 10. Add 1 mL of **Binding Solution** to the eluted RNA contained within the conical tube from **Step 9** above. Vortex for 15 seconds.
 11. Add 1 mL of 100% isopropanol (provided by the user) to the mixture from **Step 10.** Mix by vortexing for 10 seconds.
 12. Apply up to 700 μ L of the mixture from **Step 11** onto the provided Mini Spin Column and centrifuge for 1 minute **at 14,000 RPM.** Discard the flowthrough. Reassemble the spin column with its collection tube.
 13. Repeat **Step 12** as necessary to load the rest of the sample that results from **Step 11.**
 14. Apply 400 μ L of **Wash Solution II** to the column and centrifuge for 1 minute **at 14,000 RPM.** Discard the flowthrough and reassemble the spin column with its collection tube.
 15. Repeat **Step 14** to wash column a second time.
 16. Spin the column for 2 minutes **at 14,000 RPM** in order to thoroughly dry the resin. Discard the collection tube.

17. Place the column into a fresh 1.7 mL Elution tube provided with the kit. Add 100 μ L of **Elution Solution II** to the column. Centrifuge for **2 minutes at 2,000 RPM**, followed by **2 minute at 14,000 RPM**. Note the volume eluted from the column. If the entire 100 μ L has not been eluted, spin the column at **14,000 RPM for an additional 1 minute**.

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

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. Can I process a different plasma/serum volume?

- Yes, you can. All the buffers included in this kit are in a linear relationship to the volume of Plasma/Serum sample processed. Make sure that you do not deviate from the ratio specified in the product manual. The buffers are optimized per 1 mL of Plasma/Serum sample.

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

- Adding less volume may reduce your RNA yields. Adding more may not affect the RNA yields EXCEPT if more Elution Buffer was added. Eluting your nucleic acids in higher volumes of Elution Buffer/solution will result in diluting your nucleic acids.

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

- Your elution will be contaminated with the Wash Solution. This may dilute the nucleic acid yield in your 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 μ L).

8. Why does my isolated RNA 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 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 that Norgen's RNase-Free DNase I Kit (Cat# 25710) is used.

Related Products	Product #
Plasma-Serum Viral DNA Isolation Kit	29700
Plasma-Serum Circulating DNA Isolation Kit	29900
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Total RNA Purification 96-Well Kit	24300
Blood Genomic DNA Isolation Micro Kit	18200
Blood Genomic DNA Isolation Mini Kit	31000
Blood Genomic DNA Isolation Midi Kit	31100
Blood Genomic DNA Isolation Maxi Kit	31200

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 Acids Purification Kit 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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