NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Plasma/Serum RNA/DNA Purification Mini Kit Product Insert

Product# 55200

Norgen's Plasma/Serum RNA/DNA Purification Mini Kit provides a fast, reliable reproducible and simple procedure for the sequential purification of circulating/exosomal RNA and cell-free circulating DNA (cfc-DNA) from a single plasma/serum sample. Norgen's Plasma/Serum RNA/DNA Purification Mini Kit can purify RNA/DNA from various amounts of plasma/serum ranging from 10 µL to 200 µL. Purification is based on spin column chromatography that uses Norgen's proprietary resin separation matrix. The kit is designed to isolate all sizes of circulating RNA (including microRNA), all sizes of exosomal RNA, and all sizes of cfc-DNA. Norgen's Plasma/Serum RNA/DNA Purification Mini Kit provides a clear advantage over other available kits in that it does not require phenol/chloroform or any protease treatments. RNA/DNA can be isolated from either fresh or frozen samples using this kit, and the kit allows the user to elute into a flexible elution volume ranging from 10 µL to 25 µL. Typical yields of free-circulating, exosomal RNA and cfc-DNA vary depending on the input sample, as the amount of RNA/DNA present in plasma and serum will vary depending upon the health status of the individual. Normally, the RNA/NA yield from plasma or serum is highly variable (ranging from 1 to 100 ng/mL). Variability is also observed between samples collected from the same donor at different times during the day. ***This kit is suitable for the isolation of RNA and DNA 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.***

Kit Specifications	
Minimum Plasma/Serum Input Volume	10 µL
Maximum Plasma/Serum Input Volume	200 µL
Minimum Elution Volume	10 µL
Maximum Elution Volume	25 µL
Size of RNA Purified	All sizes, including miRNA and small RNA (<200 nt)
Size of DNA Purified	≥ 50 bp
Time to Complete 10 Purifications	15 to 20 minutes
Average Yields	Variable depending on specimen

Note: Do not exceed the recommended sample input volume of 200 µL.

Component	Product # 55200
Lysis Buffer A	30 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Elution Buffer B	8 mL
Micro Spin Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	100
Product Insert	1

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- β - Mercaptoethanol

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25^{o}C) for up to 2 year without showing any reduction in performance. It is recommended to warm Lysis Buffer A 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 RNA/DNA Purification Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Plasma/Serum RNA/DNA Purification Mini Kit 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/55200.*

Lysis Buffer A contains 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.

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.
- 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, ensure that they remain on ice during downstream applications

Notes Prior to Use

- All centrifugation steps are performed at room temperature.
- The Micro spin columns in this kit are for use with a benchtop microcentrifuge and not for use on a vacuum apparatus
- A variable speed centrifuge should be used for maximum kit performance. 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**.
- It is important to work quickly during this procedure.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to RNA/DNA degradation.
- ***This kit is suitable for the isolation of RNA and DNA from fresh or frozen 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 200 µL inputs, however the kit can be used to process 10 µL to 200 µL of Plasma/Serum. Simply add 3X the Plasma/Serum input volume of Lysis Buffer A to your sample, mix by vortexing for 10 seconds and proceed with Step 2 below. (For example, to process 100 µL of Plasma/Serum, add 300 µL of Lysis Buffer A).

Section A: Cell-Free Circulating DNA Purification Procedure

1. Place 200 µL of plasma/serum sample in a 2 mL tube (provided by the user) and add 3 times the plasma volume (600 µL) of **Lysis Buffer A**. Mix well by vortexing for 10 seconds.
2. Transfer the mixture into a Micro Spin Column assembled with a collection tube. Centrifuge for **2 minutes at 5,800 *x g* (~8,000 RPM)**. Ensure the entire volume has passed through into the collection tube. If not spin for an additional minute.
3. **Retain the flowthrough for RNA Purification (Section B). The flowthough contains the RNA and should be stored on ice or at -20°C until the RNA Purification procedure is carried out.**
4. Apply 600 µL of **Wash Solution A** to the Micro Spin Column and centrifuge for **1 minute at 5,800 *x g* (~8,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
5. Repeat **Step 4** one more time, for a total of two washes.
6. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
7. Transfer the Micro Spin Column to a fresh 1.7 mL Elution tube. Apply from 10 µL up to 25 µL of **Elution Buffer B** to the column and centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**. See Table 1 below for guidelines on choosing an elution volume.
8. **Retain the Micro Spin Column for Circulating RNA and Exosomal RNA Purification**. Proceed to Section B for RNA Purification.

Section B: Circulating RNA and Exosomal RNA Purification Procedure

1. Add 800 µL of 96-100% ethanol (provided by the user) to the 800 µL flowthrough retained from **Step 3 (Section A)**. Mix well by vortexing for 10 seconds.
2. Assemble the Micro Spin Column from **Step 8** (**Section A**) with a new collection tube.
3. Transfer 650 µL of the mixture into the Micro Spin column. Centrifuge for **2 minutes at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the Micro Spin Column with its collection tube.
4. Repeat Step 3 two more times until all the mixture from Step 2 has passed through the Micro Spin Column.
5. Apply 600 µL of **Wash Solution A** to the column and centrifuge for **30 seconds at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the Micro Spin Column with its collection tube.
6. Repeat **Step 5** one more time, for a total of two washes.
7. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
8. Transfer the Micro Spin Column to a fresh 1.7 mL Elution tube. Apply from 10 µL up to 25 µL of **Elution Solution A** to the column and centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**. See table below for guidelines on choosing an elution volume.

Table 1. DNA Elution Guidelines

Plasma Volume	***200 µL EDTA Plasma***		
Input Elution Volume	10 µL Elution	15 µL Elution	25 µL Elution
Recovered Elution Volume	8 µL Elution	13 µL Elution	22 µL Elution
Percentage of Recovery (%)*	95	93	91

* **Note:** The table above is to show the percentage of recovery of cell-free circulating DNA purified from 200µL Plasma and eluted at different elution volumes.

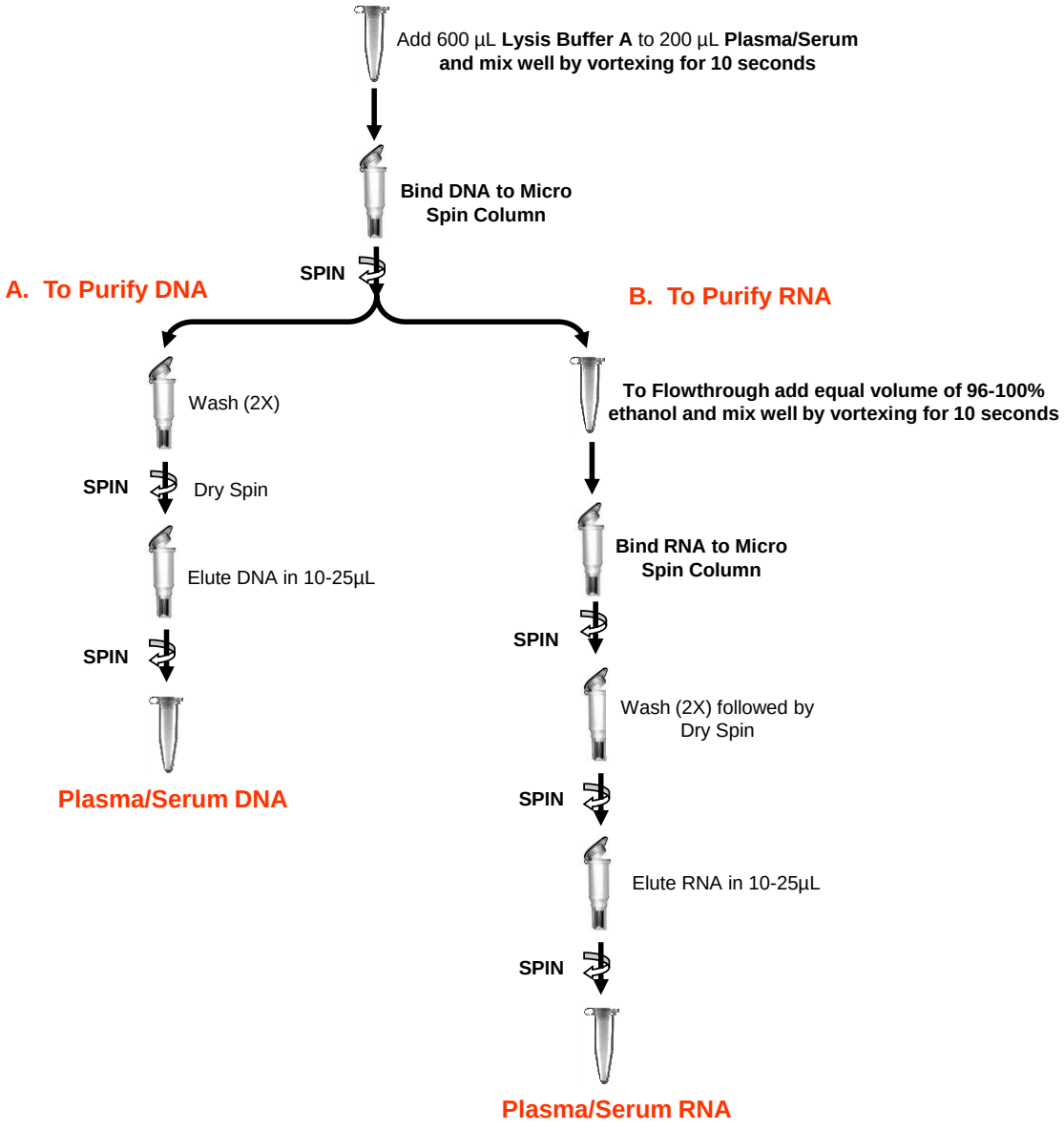
Table 2. RNA Elution Guidelines

Plasma Volume	***200 µL EDTA Plasma***			***200 µL Citrate Plasma***		
Input Elution Volume	10 µL Elution	15 µL Elution	25 µL Elution	10 µL Elution	15 µL Elution	25 µL Elution
Recovered Elution Volume	8 µL Elution	13 µL Elution	22 µL Elution	8 µL Elution	13 µL Elution	22 µL Elution
Percentage of Recovery (%)*	87	91	89	85	96	90

***Note:** The table above shows the percentage of recovery of RNA purified from 200 µL Plasma and eluted at different elution volumes. The percentage of recovery was calculated based on RT-qPCR amplification of a spike-in control (cel-miR-39) of a known amount. The cel-miR-39 was added to the plasma sample after the addition of **Lysis Buffer A** (step 1).

Flow Chart

Simplified Procedure for Norgen's Plasma/Serum RNA/DNA Purification Mini Kit



Flow Chart: Add 3 volumes of Lysis Buffer A to 1 volume Plasma/Serum, mix well by vortexing for 10 seconds. Load the entire plasma/serum lysate onto the Micro Spin Column and centrifuge. ***Keep the flowthrough plasma/serum for RNA Purification***.
To Purify DNA: Wash twice with Wash Solution A followed by dry spin. Elute Plasma/Serum DNA in 10 µL - 25 µL.
To Purify RNA: Add an equal volume of 96-100% ethanol to the plasma/serum flowthrough and mix well by vortexing for 10 seconds. Load the plasma/serum lysate to the Micro Spin Column and centrifuge. Wash twice with Wash Solution A followed by dry spin. Elute Plasma/Serum RNA in 10 µL - 25 µL.
For more details, please refer to procedure outlined on Page 3

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/RNA or reduction in the total DNA/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 less than 200 µL of Plasma/Serum, add Nuclease-free water to your sample to obtain a final volume of 200 µL and proceed with the procedure outlined on **Page 3** above.

5. 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/RNA. Adding more may not affect the nucleic acid yields EXCEPT if more Elution Buffer B or Elution Solution A was added. Eluting your DNA/RNA in higher volumes 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 A. This may dilute the nucleic acid yield in your first elution and 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 (25 µL).

8. Why do my samples show very low DNA yield?
- Plasma/Serum samples contain very little Cell-Free Circulating 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.

9. 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.

10. Why my isolated nucleic acids do not perform well in downstream applications?
- If a different Elution Buffer or 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.

11. Do I need to do an RNase treatment for my DNA Elution?
- Unlike other kits, Norgen's Plasma/Serum RNA/DNA Purification Mini Kit doesn't co-purify plasma/serum circulating RNA along with circulating DNA, therefore an RNase step is not required.

12. Why do the A260:280 ratio and the A260:230 ratio of the purified DNA are low?
- Most of the Plasma/Serum Cell-Free Circulating DNA is present in short fragment. This low A260:280 ratio and the low A260:230 ratio will not affect any downstream application

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

Most of the Free-Circulating Plasma/Serum RNA is short RNA fragments. The A260:280 ratio is normally between 1 – 1.6. This low A260:280 ratio will not affect any downstream application

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 RNA/DNA Purification Mini 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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