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

Product Insert

REF **Dx42800** CE IVD PIDx42800-1

Intended Use

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from plasma/serum samples for subsequent *in vitro* diagnostic use. Purification is based on the use of Norgen's proprietary resin as the separation matrix, and the kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to small RNAs.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated using the RNA isolated with Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including RNA isolation.

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format) does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

Component	Product #Dx42800 (50 samples)
PS Solution A	12 mL
PS Solution B	100 mL
PS Solution C	18 mL
Wash Solution	22 mL
RNA Elution Solution	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Label Legend

Do not reuse	Use by	Batch Code	Catalogue Number	Contains sufficient for <n> tests	Manu-facturer	In Vitro Diagnostic Medical Device	Consult instructions for use	Temper-ature limitation

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Advantages

- CE-IVD marked in accordance with EU Directive 98/79/EC
- Fits into *in vitro* diagnostic workflows
- Fast and easy processing using a rapid spin-column format
- Isolate high quality total RNA

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. All solutions and plastics can be used until the expiration date specified on their labels.

Precautions

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*.

PS Solution A, PS Solution B and **PS Solution C** contains guanidine hydrochloride, and should be handled with care. Guanidinium salts forms 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.

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

Procedure

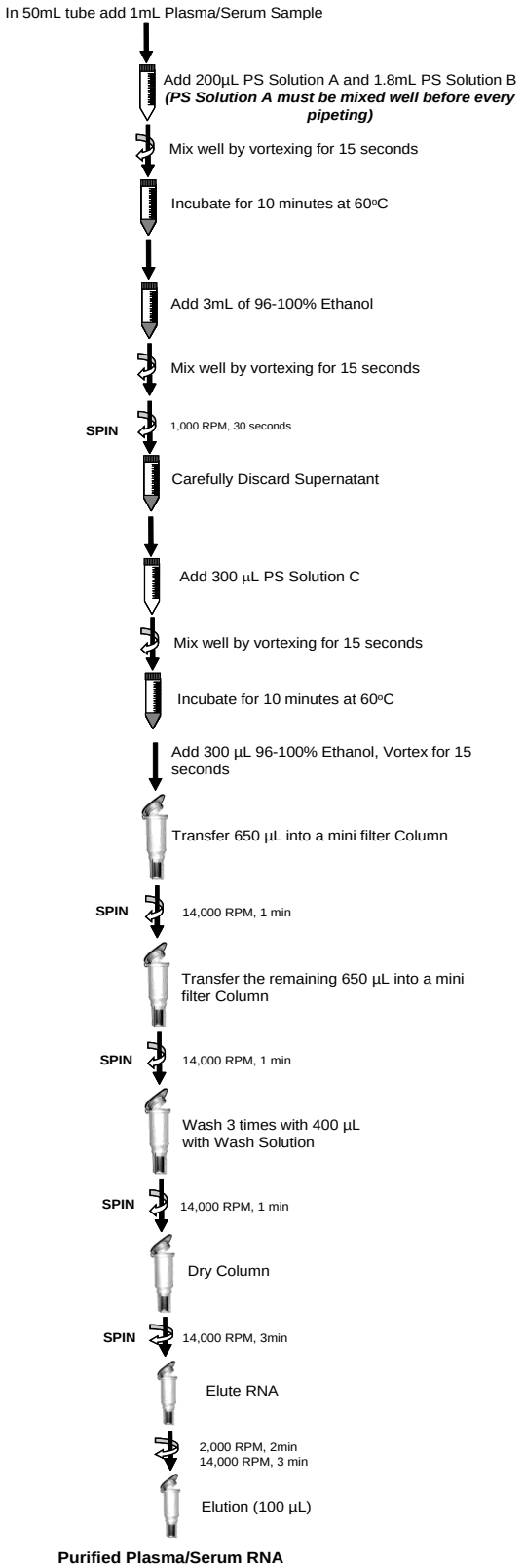
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can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

Flow Chart

Procedure for Purifying Circulating and Exosomal Total RNA using Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format)



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

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** by adding 50 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 72 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 PS Solution B..
- ***PS Solution A contains resin and must be mixed well before every pipetting***.
- It is highly recommended to warm up **PS Solution A**, **PS Solution B** and **PS Solution C** 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.***

Detailed Procedure

1. In a 50 mL tube (provided by the user), add 0.2 mL of **PS Solution A** and 1.8 mL **PS Solution B** (after the addition of β-mercaptoethanol) to 1 mL plasma/serum sample. Mix well by vortexing for 15 seconds. ***Note 1*: *PS Solution A contains resin and must be mixed well before every pipetting***
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 **PS Solution C**, 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. 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.

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

10. Apply 400 µL of **Wash Solution** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
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** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **3 minute at 14,000 RPM**.

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** 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** 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 - 30^{o}C 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 **PS Solution B** and **96-100% Ethanol** to be added to different Plasma/Serum sample volumes. The volume of **PS Solution A** and **PS Solution C** is fixed for all Plasma/Serum volumes.

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

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)

Technical Support

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.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit Dx (Slurry Format)) for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

Authorized Representative

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