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Product Information

GenElute™ Plasma/Serum RNA Purification Maxi Kit
Catalog number **RNB700**

TECHNICAL BULLETIN

Product Description

This kit is suitable for the isolation of RNA 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.

Cell-free circulating RNA, including exosomal RNA in plasma or serum, has the potential to provide biomarkers for certain cancers and disease states, and includes tumor-specific extracellular RNA in the blood. 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.

GenElute™ Plasma/Serum RNA Purification Kits provide a fast, reliable, reproducible and simple procedure for isolating circulating RNA and exosomal RNA from small plasma/serum inputs ranging from 50 µL and up to 5 mL, with various kit formats addressing different plasma/serum input volumes. Purification is based on spin column chromatography that uses proprietary resin separation matrix. The kit is designed to isolate all sizes of circulating RNA, including microRNA, as well as all sizes of exosomal RNA. These kits provide a clear advantage over other available kits in that they do not require phenol/chloroform or any protease treatments. RNA can be isolated from either fresh or frozen samples using these kits. Moreover, the kits allow the user to elute into a flexible elution volume ranging from 10 µL to 100 µL. Typical yields of free-circulating and exosomal RNA 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. Normally, the RNA yield from plasma or serum RNA 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.

Components

Component	Cat. No. RNB700 (10 samples)
Lysis Buffer A	1 x 130 mL 1 x 30 mL
Wash Solution A	1 x 38 mL
Elution Solution A	6 mL
Elution Buffer F	15 mL
Maxi Spin Columns	10
Mini Spin Columns	10
Collection Tubes	10
Elution tubes (1.7 mL)	10
Product Insert	1

Reagents and Equipment Required But Not Provided

You must have the following in order to use the GenElute™ Plasma/Serum RNA Purification Midi Kit:

- Benchtop microcentrifuge
- Swinging bucket centrifuges
- Vortexer
- Micropipettors
- 96 – 100% ethanol
- 100% Isopropanol
- Optional: β - Mercaptoethanol

Precautions and Disclaimer

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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.

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, ensure that they remain on ice during downstream applications.

Reagents to be prepared

Before beginning the procedure, prepare the following:

1) Prepare a working concentration of the Wash Solution A:
    a. Add 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 18 mL of the concentrated Wash Solution A. This will give a final volume of 60 mL.
    b. Add 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing 38 mL from the concentrated Wash Solution A. This will give a final volume of 128 mL.
    c. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

2) 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.

Storage/Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25ºC) for up to 2 years without showing any reduction in performance. It is recommended to warm Lysis Buffer A for 20 minutes at 60ºC if any salt precipitation is observed.

Procedure

Notes prior to use

- All centrifugation steps are performed at room temperature. Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required. The provided spin columns are optimized to be used with a benchtop centrifuges.
- Most standard swinging bucket centrifuges will accommodate the Maxi Spin Columns. Do not use a fixed-angle rotor. These Maxi Spin Columns are centrifuged in 50mL centrifuge tubes.
- When placing the Maxi Spin Columns into the swinging bucket centrifuge make sure that lids of the tubes are not tightly closed. Tightly closed lids may cause back pressure which may cause column clogging or disintegration.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to RNA degradation.
- Frozen plasma or serum samples should be centrifuged for 2 minutes at 400 x g (~2,000 RPM) before processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.
- If any of the solutions do not go through the spin columns within the specified centrifugation time, spin for an additional 1-2 minutes until the solution completely passes through the column. Do not exceed the centrifugation speed as this may affect RNA yield.

The procedure outlined below is for processing 2 mL to 5 mL inputs of Plasma/Serum. If the sample volume is lower than 5 mL Plasma/Serum, simply bring the volume of your sample up to 5 mL using Nuclease-free water and proceed as outlined below.

1. Place 5 mL of plasma/serum sample in a 50 mL tube (provided by the user) and add 15 mL of Lysis Buffer A. Mix well by vortexing for 10 seconds.
2. Add 10 mL of 100% Isopropanol (provided by the user). Mix well by vortexing for 10 seconds.
3. Transfer 15 mL of the mixture from Step 2 into a Maxi Spin column assembled with one of the provided collection tubes. Centrifuge for 3 minutes at 1,000 x g (~2,200 RPM). Discard the flowthrough and reassemble the spin column with its collection tube. (Note: Make sure that lid of the tubes is not tightly closed during centrifugation).
4. Repeat Step 3 one more time until all the mixture from Step 2 has been transferred to the Maxi Spin column.
5. Apply 5 mL of Wash Solution A to the column and centrifuge for 3 minutes at 1,000 x g (~2,200 RPM). Discard the flowthrough and reassemble the spin column with its collection tube. (Note: Make sure that lid of the tubes is not tightly closed during centrifugation).
6. Repeat step 5 one more time, for a total of two washes.
7. Spin the column, empty, for 3 minutes at 2,000 x g (~3,000 RPM). Discard the collection tube.
8. Transfer the Maxi Spin column to a fresh 50 mL tube (not provided). Apply 800 µL of Elution Buffer F to the column and let stand at room temperature for 2 minutes. Centrifuge for 2 minutes at 500 x g (~1,600 RPM).
9. Reload the eluted RNA from Step 8 back to the Maxi Spin column and let stand at room temperature for 2 minutes. Centrifuge for 2 minutes at 500 x g (~1,600 RPM).
10. To the elution from Step 9, add 600 µL of Lysis Buffer A and mix well by vortexing for 10 seconds;
11. Add 800 µL of 96-100% Ethanol (provided by the user). Mix well by vortexing for 10 seconds.
12. Transfer 750 µL of the mixture from Step 11 into a Mini Spin column assembled with one of the provided collection tubes. Centrifuge for 2 minutes at 3,300 x g (~6,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
13. Repeat Step 12 two more times to transfer the remaining mixture into the Mini Spin column.

Optional Step: An optional On-Column DNA Removal Protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

14. Apply 400 µL of Wash Solution A to the column and centrifuge for 1 minute at 3,300 x g (~6,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
15. Repeat Step 14 two more times, for a total of two washes.
16. Spin the column, empty, for 2 minutes at 13,000 x g (~14,000 RPM). Discard the collection tube.
17. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of Elution Solution A to the column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x g (~2,000 RPM), followed by 2 minutes at 5,800 x g (~8,000 RPM).
18. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x g (~2,000 RPM), followed by 2 minutes at 5,800 x g (~8,000 RPM).

For an explanation of expected yields and recommendations for quantification of the RNA, please refer to **RNA Quantification Methods** below.

Protocol for Optional On-Column DNA Removal

GenElute™ Plasma/Serum RNA Purification Kits isolate 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 a RNase-Free DNase Kit 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 with a RNase-Free DNase I Kit. 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 procedure up to Step 13 (Maxi Format).
3. Apply 400 µL of Wash Solution A to the column and centrifuge for 30 seconds at 6,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.

4. Apply 50 µL of the RNase-free DNase solution prepared in Step 1 to the column and centrifuge at 8,000 x g (~10,000 RPM) for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 13,000 x g (~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 15 (Maxi Format).

RNA Quantification Methods

Below is a list of the most common RNA quantification methods, as well as the limit of detection for each of these methods:

1) Bioanalyzer RNA Quantification Kits

	RNA 6000 Nano Kit		RNA 6000 Pico Kit		Small RNA kit
	Total RNA	**mRNA**	**Total RNA**	**mRNA**	**Total RNA**
Quantitative range	25 - 500 ng/µL	25 - 250 ng/µL	----	----	50-2000 pg/µL
Qualititative range	5 - 500 ng/µL	5 - 250 ng/µL	50 - 5000 pg/µL	250 - 5000 pg/µL	50-2000 pg/µL
Quantitation accuracy	20% CV	20% CV	30% CV	----	------

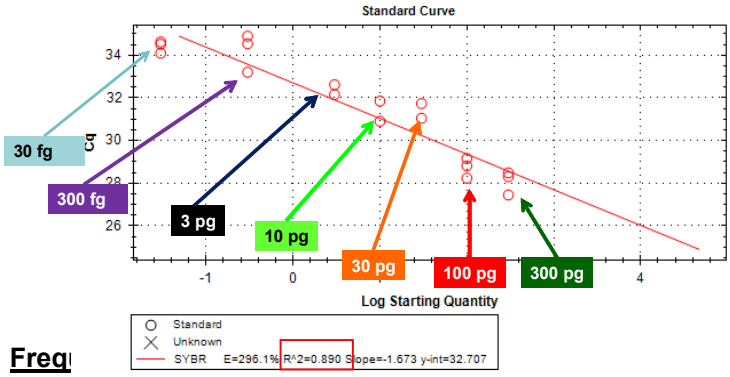
2) NanoDrop 2000

- Detection Limit: 2 ng/µL (dsDNA)

3) Quant-iT™ RiboGreen® RNA Assay Kit

- Quantitation Range: 1-200 ng

4) qPCR DNA Standard Curve



Freq

1. What If a variable speed centrifuge is not available?
A fixed speed centrifuge can be used, however reduced yields may be observed.

2. 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.

3. What if I added more or less of the specified reagents' volume?
Adding more or less than the specified volumes may reduce both the quality and the quantity of the purified RNA. Eluting your RNA in high volumes will increase the yield but will lower the concentration. Eluting in small volumes will increase the concentration but will lower the overall yield.

4. What If I forgot to do a dry spin before my final elution step?
Your purified DNA will be contaminated with the Wash Solution A. This may reduce the quality of your purified DNA and will interfere with your downstream applications.

5. Can I perform a second elution?
Yes, but it is recommended that the 2nd elution be in a smaller volume (50% of 1st Elution). It is also recommended to perform the 2nd elution into a separate elution tube to avoid diluting the 1st elution.

6. 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.

7. **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.

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 miRNA. It is recommended to use a RNase-Free DNase I Kit. Also please refer to the protocol for optional on-column DNA removal.

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