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EXTRAClean Exosomal RNA Isolation Kit
Product Insert

Product # 72800

Norgen's EXTRAClean Exosomal RNA Isolation Kit constitutes an all-in-one system for the isolation of exosomal RNA from exosomes previously purified using Norgen's Exosome Purification Kits or Norgen's EXTRAClean Exosome Purification Kits. This kit also allows for the isolation of RNA from intact extracellular vesicles (EVs) purified from different urine or plasma/serum sample volumes. The purification is based on spin column chromatography that employs Norgen's proprietary resin. The EXTRAClean columns undergo Norgen's stringent processing and rigorous quality control measures to minimize background traces, ensuring optimal results for sensitive applications such as NGS.

Norgen's Purification Technology

The kit is designed to isolate all sizes of RNA, including microRNA. The kit provides a clear advantage over other available kits in that it does not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Advantages

- Isolates all sizes of exosomal and extracellular vesicle RNA, including microRNA.
- Bind and elute all RNA irrespective of size or GC content, without bias.
- No phenol extractions.
- No Proteinase K treatment.
- No carrier RNA.
- Concentrate isolated RNA into a flexible elution volume ranging from 50 µL to 100 µL.
- Purify high-quality RNA in 15-20 minutes.
- Purified RNA is suitable for a variety of downstream applications, including Small RNA Sequencing.
- Purification is based on EXTRAClean spin column chromatography that uses Norgen's proprietary separation matrix.

Kit Components

Component	Product # 72800 (50 Preps)
Lysis Buffer A	20 mL
Lysis Additive B	2 mL
Wash Solution A	18 mL
Elution Solution A	6 mL
EXTRAClean Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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Specifications

Kit Specifications	
Sample type	Exosomes purified using Norgen's Purification Kits
Size of RNA Purified	All sizes, including miRNA and small RNA (< 200 nt)
Elution Volume	50-100 µL
Time to Complete 10 Purifications	35-40 minutes
Average Yields	Variable depending on specimen

[¥] ***Please check page 6 for Average Yields and Common RNA Quantification Methods***

Storage Conditions and Product Stability
All buffers 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 **Lysis Buffer A** for 20 minutes at 60ºC if any salt precipitation is observed.

Precautions and Disclaimers
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. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Lysis Buffer A contains guanidine thiocyanate, and should be handled with care. Guanidine thiocyanate forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

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

Customer-Supplied Reagents and Equipment
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- Vortex
- 96-100% Ethanol

Procedures
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.

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware are 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 defense 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.

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 centrifuge and not to be used on a vacuum apparatus.
- Most standard benchtop microcentrifuges will accommodate Norgen's EXTRAClean Mini Spin columns.
- Centrifuging Norgen's EXTRAClean Mini spin columns at a speed higher than recommended may affect RNA yield.
- Centrifuging Norgen's EXTRAClean Mini spin columns at a speed lower than recommended will not affect RNA yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the EXTRAClean Mini spin column.
- Ensure that all solutions are at room temperature prior to use.
- It is highly recommended to warm up **Lysis Buffer A** at 60°C for 20 minutes and mix well until the solutions become clear again if precipitates are present.
- Prepare a working concentration of the **Wash Solution A** by adding **42 mL** of 96 - 100% ethanol (provided by the user) to the supplied bottle containing **18 mL** of concentrated Wash Solution A. This will give a final volume of **60 mL.** The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- If any of the solutions do not go through the EXTRAClean 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.

Section 1. Exosomal RNA Isolation with exosome resuspended in about 200 µL of buffer.

Note: The procedure outlined below is for processing 200 µL of resuspended exosomes. If processing a sample volume less than this, simply bring the volume of your sample up to 200 µL by adding more ExoR or PBS. Then proceed as outlined below.

1. Add 300 µL of Lysis Buffer A and 37.5 µL of Lysis Additive B to the 200 µL of buffer containing the purified exosomes.
2. Mix well by vortexing for 10 seconds then incubate at room temperature for 10 minutes.
3. After incubation, add 500 µL of 96-100% Ethanol to the mixture from Step 2 and mix well by vortexing for 10 seconds.

4. Transfer 500 µL of the mixture from Step 3 into a EXTRAClean Mini Spin Column. Centrifuge for 1 minute at 3,300 *x g (*~6,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
5. Repeat Step 4 one more time to transfer the remaining mixture from Step 3 into the EXTRAClean Mini Spin Column.
6. 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 EXTRAClean Mini spin column with its collection tube.
7. Repeat Step 6 one more time, for a total of two washes.
8. Spin the column, empty, for 1 minute at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
9. Transfer the EXTRAClean Mini spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of Elution Solution A to the column and centrifuge for 1 minute at 2,000 RPM, followed by 2 minutes at 8,000 RPM.
10. 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).

Section 2. Exosomal RNA Isolation with exosome resuspended in about 400 µL of buffer.

Note: The procedure outlined below is for processing 400 µL of resuspended exosomes. If processing a sample volume in the range of 200 - 400 µL, simply bring the volume of your sample up to 400 µL by adding more ExoR or PBS. Then proceed as outlined below.

1. Add 600 µL of Lysis Buffer A and 75 µL of Lysis Additive B to the 400 µL of buffer containing the purified exosomes.
2. Mix well by vortexing for 10 seconds then incubate at room temperature for 15 minutes
3. After incubation add 1 mL of 96-100% Ethanol to the mixture from Step 2 and mix well by vortexing for 10 seconds.
4. Transfer 750 µL of the mixture from Step 3 into a EXTRAClean Mini Spin Column. Centrifuge for 1 minute at 3,300 *x g (*~6,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
5. Repeat Step 4 two more times to transfer the remaining mixture from Step 3 into the EXTRAClean Mini Spin Column.
6. 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 EXTRAClean Mini spin column with its collection tube.
7. Repeat Step 6 one more time, for a total of two washes
8. Spin the column, empty, for 1 minute at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
9. Transfer the EXTRAClean Mini spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of Elution Solution A to the column and centrifuge for 1 minute at 2,000 RPM, followed by 2 minutes at 8,000 RPM.
10. 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).

Section 3. Exosomal RNA Isolation with exosome resuspended in about 600 µL of buffer.

Note: The procedure outlined below is for processing 600 µL of resuspended exosomes. If processing a sample volume in the range of 400 - 600 µL, simply bring the volume of your sample up to 600 µL by adding more ExoR or PBS. Then proceed as outlined below.

11. Add 900 µL of Lysis Buffer A and 125 µL of Lysis Additive B to the 600 µL of buffer containing the purified exosomes.
12. Mix well by vortexing for 10 seconds then incubate at room temperature for 20 minutes
13. After incubation add 1.5 mL of 96-100% Ethanol to the mixture from Step 2 and mix well by vortexing for 10 seconds.
14. Transfer 750 µL of the mixture from Step 3 into a EXTRAClean Mini Spin Column. Centrifuge for 1 minute at 3,300 *x g (*~6,000 RPM). Discard the flowthrough and reassemble the EXTRAClean Mini Spin Column with its collection tube.
15. Repeat Step 4 two more times to transfer the remaining mixture from Step 3 into the EXTRAClean Mini spin column.
16. Apply 600 µL of Wash Solution A to the EXTRAClean Mini Spin Column and centrifuge for 30 seconds at 3,300 *x g (*~6,000 RPM). Discard the flowthrough and reassemble the EXTRAClean Mini Spin Column with its collection tube.
17. Repeat Step 6 one more time, for a total of two washes.
18. Spin the EXTRAClean Mini Spin Column, empty, for 1 minute at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
19. Transfer the EXTRAClean Mini Spin Column to a fresh 1.7 mL Elution tube. Apply 50 µL of Elution Solution A to the column and centrifuge for 1 minute at 2,000 RPM, followed by 2 minutes at 8,000 RPM.
20. 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).

Appendix A

Exosomal RNA Yield

Exosomal RNA, like RNA in other cell-free bodily fluids, is normally found in very low amounts (1 - 100 pg/µL), therefore measuring exosomal RNA concentration using common quantification methods is very difficult and challenging. Typical yields of exosomal RNA vary significantly from sample to sample. Variability is also observed between samples collected from the same donor at different times during the day and therefore there is no absolute yield for RNA purified from bodily fluids. Exosomal RNA yield varies depending on a number of factors including age, sex, diet, exercise and most importantly the health status of the donor.

Below is a list of the most common RNA quantification methods, as well as the limit of detection for each of these methods. ***Unfortunately, none of these methods can be used reliably for measuring the concentration of RNA purified from exosomes unless large volumes have been processed***. This would only be applicable if exosomes contain the maximum amount of RNA that can fit within the specification range of these quantification tools. It should be noted that the specifications outlined below are based on measuring a pure RNA sample, which will not be the case for exosomal RNA. Exosomal RNA is short fragmented RNA which is usually present in less than 1000 bp. Purified exosomal RNA usually contains traces of proteins which will interfere with most quantification methods, leading to the overestimation of the purified RNA concentration. Therefore, purified RNA contaminated with more proteins will be presented at a higher concentration as compared to RNA purified with less protein contaminants. ***The only reliable method that can assess the quality and the relative quantity of the purified exosomal RNA***

is RT-qPCR amplification of a standard RNA using a small RNA amplicon such as the 5S rRNA housekeeping gene.

Common RNA Quantification 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	----	------

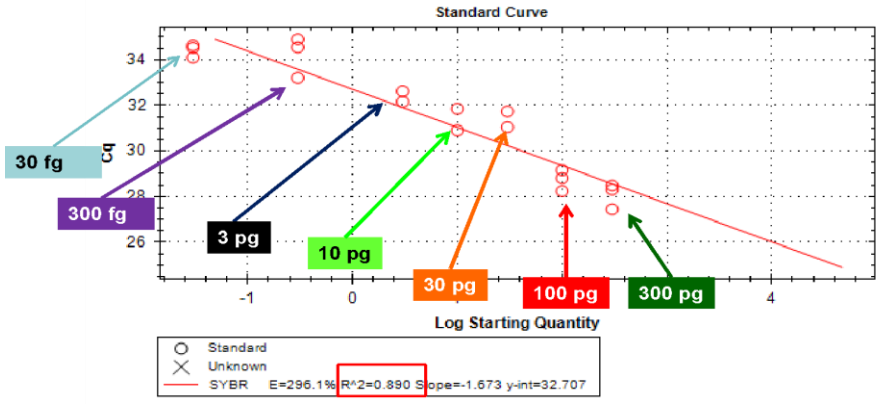
2) NanoDrop 2000

- Detection Limit: 2 ng/µl (dsDNA)

3) Quant-iT™ RiboGreen® RNA Assay Kit

- Quantitation Range: 1-200 ng

4) qPCR Standard Curve (generated by Norgen)



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. **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 RNA will be contaminated with the Wash Solution A. This may reduce the quality of your purified RNA 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?**
   Exosomes contain very little RNA. This varies from individual to individual based on numerous variables. In order to increase the yield, the amount of urine, plasma or serum input could be increased.
7. **Why do the A260/280 ratio of the purified RNA is lower than 2.0?**
   Most of the Exosomal RNA is short RNA fragments with a very low concentration, where the A260/280 ratio tends to decrease with the decrease in the RNA concentration. 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.

Technical Support

Contact our Technical Support Team between the hours of 9:00 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 support@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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