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Exosomal RNA Isolation Kit (for the extraction of RNA from EVs that have been isolated using IZON's qEV columns)
Product Insert

Product #68910

Exosomes are 40 - 150 nm membrane vesicles, which are secreted by most cell types. Exosomes can be found in urine, saliva, plasma, serum, amniotic fluid and malignant ascite 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 and other extracellular vesicles (EVs) which depend upon the tumour cell type from which they are secreted. For this reason, exosomal RNA may serve as biomarkers for various diseases including cancer.

Norgen's Exosomal RNA Isolation Kit (for the extraction of RNA from EVs that have been isolated using IZON's qEV columns) constitutes an all-in-one system for the isolation of exosomal RNA from exosomes previously isolated using IZON's qEV columns or concentrated using IZON's EV Concentration kit. This kit allows for the isolation of RNA from intact extracellular vesicles (EVs) where the purification is based on spin column chromatography that employs Norgen's proprietary resin. The kit is designed to isolate all sizes of extracellular vesicle 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.

Kit Components

Component	Product #68910 (50 preps)
Lysis Buffer A	60 mL
Lysis Additive B	7 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

¥ ***Please check page 5 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 (15-25ºC). 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

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.

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, 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 centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Micro Spin Columns.
- Centrifuging Norgen's spin columns at a speed higher than recommended may affect RNA yield.

- Centrifuging Norgen's 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 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 **90 mL** of 96 - 100% ethanol (provided by the user) to the supplied bottle containing **38 mL** of 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.
- 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.

Section 1. Exosomal RNA Isolation

1. For lysate preparation add 1.5X of **Lysis Buffer A** and 0.2X of **Lysis Additive B** to the volume of qEV isolate. (E.g. For 100 µL of qEV isolate add 150 µL of **Lysis Buffer A** and 20 µL of **Lysis Additive B** this will make up the total volume of 270 µL).
2. Mix well by vortexing for 10 seconds then incubate at room temperature for 20 minutes
3. After incubation add 0.93X of **96-100% Ethanol** to the mixture from **Step 2** and mix well by vortexing for 10 seconds (use the total volume of Lysate as a reference. For e.g. for 270 µL of Lysate, add 251 µL of Ethanol).
4. Transfer up to 750 µL of the mixture from **Step 3** into a **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 **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 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 spin column to a fresh 1.7 mL Elution tube. Apply 50-100 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)**.

    - **Exosomal 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. **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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