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EXTRAClean Exosomal RNA Isolation Kit

Product # 72800

Please note that a more detailed protocol is available online at www.norgenbiotek.com/product/

Component	Contents
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
Product Insert	1

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. **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. All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore be carried out when using this kit.

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 EXTRAClean Mini 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 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 spin column.
- Ensure that all solutions are at room temperature prior to use.
- It is highly recommended to warm up Lysis Buffer A at 60ºC 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 Mini 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.
- For an explanation of expected yields and recommendations for quantification of the RNA, please refer to Appendix A of the full version manual available on our website.

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

- 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 EXTRAClean Mini 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 EXTRAClean Mini Spin 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 µL of Elution Solution A to the EXTRAClean Mini Spin 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.

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

- 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 EXTRAClean Mini 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 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.
7. Repeat Step 6 one more time, for a total of two washes
8. Spin the EXTRAClean Mini Spin 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 EXTRAClean Mini Spin 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 EXTRAClean Mini Spin 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.

- 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.

1. Add 900 µL of Lysis Buffer A and 125 µL of Lysis Additive B to the 600 µL of buffer containing the purified exosomes.
2. Mix well by vortexing for 10 seconds then incubate at room temperature for 20 minutes
3. After incubation add 1.5 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 EXTRAClean Mini 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 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.
7. Repeat Step 6 one more time, for a total of two washes
8. Spin the EXTRAClean Mini Spin 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 EXTRAClean Mini Spin 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 ExtraClean Mini Spin 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).

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 or through email at techsupport@norgenbiotek.com.

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