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EXTRAClean Urine Exosome and Free-Circulating RNA Isolation Midi Kit

Product #73510

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

Component	Product #73510 (25 preps)
Slurry E	12.5 mL
ExoC Buffer	30 mL
ExoR Buffer	12 mL
Lysis Buffer A	2 x 20 mL
Lysis Additive B	2 mL
Wash Solution A	18 mL
Elution Solution A	6 mL
Mini Filter Column	25
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. 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.

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.
- Urine samples stored at -70°C, -20°C or at 4°C will develop some precipitation due to the aggregation of some of the highly abundant proteins in urine. Eliminating these precipitates using centrifugation or filtration may cause the loss of exosomes. Furthermore, these precipitates may affect the quality of the purified nucleic acid. We recommend the use of Norgen's Urine Preservative when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. The components of the Urine Preservative allow samples to be stored for over 2 years at room temperature with no detected degradation of urine DNA, RNA or proteins. Norgen's Urine Preservative is available as a

liquid format in Norgen's Urine Preservative Single Dose Ampules, as well as in a dried format in Norgen's Urine Collection and Preservation Tubes (please see Related Products Table).

Preparation of Cell-free Urine Sample

1. Collect and transfer 15-50 mL of the urine into a conical tube and centrifuge at 200 *x g (*~1,000 RPM) for 10 minutes to remove urine exfoliated cells and debris. Decant cell-free urine into new 15-50 mL conical tube.
2. Centrifuge the cell-free urine at 1,800 *x g (*~3,000 RPM) for 10 minutes to remove any residual debris or bacterial cells.
3. Transfer cell-free urine into a fresh 15-50 mL conical tube.

Section 1. Exosome Purification from 2 mL - 10 mL Cell-Free Urine

- The procedure outlined below is for 10 mL inputs of urine. If processing a sample volume in the range of 2 mL - 10 mL urine, simply bring the volume of your sample up to 10 mL using Nuclease-free water and proceed as outlined below.

1. To 10 mL urine add 1/10 the initial urine volume of ExoC Buffer followed by the addition of 400 µL of Slurry E. (for example, to 1 mL urine sample add 9 mL Nuclease-free water followed by the addition of 100 µL of ExoC Buffer). **(Note: Mix Slurry E well prior to use. For optimal performance ensure that resin is completely resuspended).**
2. Mix well by vortexing for 10 seconds and let stand at room temperature for 10 minutes.
3. Mix well by vortexing for 10 seconds. Centrifuge for 2 minutes at 2,000 RPM. Discard the supernatant.
4. Apply 400 µL ExoR Buffer to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 400 µL ExoR Buffer at room temperature for 10 minutes.
6. After incubation, mix well by vortexing for 10 seconds then centrifuge for 2 minutes at 500 RPM.
7. Transfer the supernatant to a Mini Filter column assembled with an elution tube and centrifuge for 1 minute at 6,000 RPM. **Do not discard the flowthrough which contains your purified Exosomes. Do not discard the slurry pellet which contains your Free-Circulating RNA.**

    - Your exosomes are now ready for RNA isolation (Section 2) or any other downstream applications.
    - Your Free-Circulating, protein-bound, RNA is now ready for isolation (Section 3).

Section 2. Exosomal RNA Isolation

1. Add 600 µL of Lysis Buffer A and 75 µL of Lysis Additive B to the 400 µL ExoR Buffer containing the purified Exosomes (Step 6, Section 1).
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 an 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 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. Free-Circulating RNA Isolation

1. To the slurry pellet (Section 1, Step 7) add 600 µL of Lysis Buffer A. Mix well by vortexing for 10 seconds then centrifuge for 2 minutes at 500 RPM.
2. Transfer the 600 µL of Lysis Buffer A supernatant to a 1.5 mL tube (not-provided) then add 600 µL of 96-100% Ethanol and mix well by vortexing for 10 seconds.
3. Transfer 600 µL of the mixture from Step 2 into a fresh EXTRAClean 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.
4. Repeat Step 3 one more time to transfer the remaining mixture from Step 3 into the EXTRAClean Spin Column.
5. Apply 600 µL of Wash Solution A to the EXTRAClean Spin 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.
6. Repeat Step 5 one more time, for a total of two washes.
7. Spin the column, empty, for 1 minute at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
8. Transfer the EXTRAClean 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.
9. 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).

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