NORGEN BIOTEK CORP.

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

EXTRAClean Plasma/Serum Exosome and Free-Circulating RNA Isolation Mini Kit

Product # 73400

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

Component	Product # 73400 (50 preps)
Slurry E	12.5 mL
ExoC Buffer	8 mL
ExoR Buffer	12 mL
Lysis Buffer A	2 x 20 mL
Lysis Additive B	2 mL
Wash Solution A	2 x 18 mL
Elution Solution A	2 x 6 mL
Mini Filter Column	50
EXTRAClean Mini Spin Columns	100
Collection Tubes	100
Elution tubes (1.7 mL)	100
Product Insert	1

Storage Conditions and Product Stability

All solutions 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 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 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 bottles containing **18 mL** of concentrated Wash Solution A. This will give a final volume of **60 mL.** The labels on the bottles have 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.
- This kit is suitable for the purification of exosomes 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.
- Frozen plasma/serum samples should be centrifuged for 2 minutes at 400 x g (~2,000 RPM) before processing. Only clear supernatant should be processed otherwise abundant plasma/serum proteins may interfere with any downstream application.

Preparation of Cell-free Plasma/Serum from Frozen Sample

1. Place your frozen Plasma/Serum at 4ºC to thaw.

2. After thawing your plasma/serum sample, aliquot the volume to be processed and centrifuge for 2 minutes at 400 x g (~2,000 RPM).
3. After centrifugation, transfer the clear plasma/serum supernatant to a fresh tube

- Cell-Free Plasma/Serum is now ready for exosomes purification.

Section 1. Exosome Purification from 50 µL - 1 mL Cell-Free Plasma/Serum

- The procedure outlined below is for 1 mL input of plasma/serum. If processing a sample volume lower than 1 mL plasma/serum, simply bring the volume of your samples up to 1 mL using Nuclease-free water and proceed as outlined below.

1. To 1 mL plasma/serum add 3 mL Nuclease-free water followed by the addition of 100 µL of ExoC Buffer. **(Note: The final volume of any plasma/serum sample to be processed should be 4 mL before the addition of the specified 100 µL of ExoC Buffer)**
2. To the mixture from Step 1 add 200 µL of Slurry E. Mix well by vortexing for 10 seconds and let stand at room temperature for 5 minutes. **(Note: Mix Slurry E well prior to use. For optimal performance ensure that resin is completely resuspended).**
3. Mix well by vortexing for 10 seconds. Centrifuge for 2 minutes at 2,000 RPM. Discard the supernatant.
4. Apply 200 µL ExoR Buffer to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 200 µL ExoR Buffer at room temperature for 5 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 in 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 300 µL of Lysis Buffer A and 37.5 µL of Lysis Additive B to the 200 µL ExoR Buffer containing the purified Exosomes (Section 1, Step 7).
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 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 300 µL of Lysis Buffer A. Mix well by vortexing for 10 seconds then centrifuge for 2 minutes at 500 RPM.
2. Transfer the 300 µL of Lysis Buffer A supernatant to a 1.5 mL tube (not-provided) then add 300 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 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.
4. 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.
5. Repeat Step 4 one more time, for a total of two washes.
6. Spin the column, empty, for 1 minute at 13,000 *x g* (~14,000 RPM). Discard the collection tube.
7. 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.
8. 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