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EXTRAClean Cell Culture Media Exosome Purification and RNA Isolation Midi Kit

Product # 73310

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

Component	Product # 73310 (25 preps)
Slurry E	12.5 mL
ExoC Buffer	1.5 mL
ExoR Buffer	12 mL
Lysis Buffer A	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	25
Collection Tubes	25
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.

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 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

Preparation of Cell-free Cell Culture Media Sample

1. Harvest and transfer the required cell culture media volume into a conical tube and centrifuge at 200 *x g (*~1,000 RPM) for 15 minutes to remove any cells and debris.
2. Transfer cell-free media into a fresh 15-50 mL conical tube.

- Cell-Free Cell Culture Media is now ready for Exosome purification.

Section 1. Exosome Purification from 10 mL - 20 mL of Cell Culture Media

- The procedure outlined below is for 20 mL inputs of cell-free media. If processing a sample volume in the range of 10 mL - 20 mL media, simply add 2.5 µL ExoC Buffer for every 1mL of cell-free media. The volume of Slurry E and ExoR Buffer is constant for any volume to be processed.

1. To 20 mL cell-free media add 50 µL of ExoC Buffer followed by the addition of 400 µL of Slurry E. **(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.**

- Your exosomes are now ready for RNA isolation or any other downstream applications.

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 7, 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 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).

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