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Bacterial EV Isolation Kit

Product # 76000

Product Insert

Extracellular vesicles (EVs) are membrane vesicles which are secreted by both Gram-positive and Gram-negative bacteria. These vesicles are increasingly recognized as key players in mediating communication not only between bacterial cells but also between bacteria and their host organisms. Bacterial EVs contain a diverse range of biomolecules, including nucleic acids, proteins and lipids, which allow them to influence various biological processes. Through the delivery of their cargo, bacterial EVs can modulate host cell signaling pathways and cellular functions, contributing to pathogenesis and immune evasion. They also play a role in bacterial survival strategies such as horizontal gene transfer. Due to their inherent features, bacterial EVs are being investigated for potential clinical applications, such as targeted drug delivery systems. As research progresses, these vesicles may offer novel insights into bacterial pathophysiology and open up new avenues for diagnostic and therapeutic development.

Norgen's Bacterial EV Isolation Kit is designed for the rapid purification of extracellular vesicles (EVs) ranging in size from as small as 30 nm to larger vesicles that are 400 nm, from bacterial culture media sample volumes between 5 mL and 35 mL. The kit provides a clear advantage over other available kits, as it does not require any special instrumentation, protein precipitation reagents, extension tubes, phenol/chloroform or protease treatments since it uses a slurry-based EV capture method. Moreover, the large range of sample volumes that can be processed with this kit provides users with more flexibility for isolations. The purified EVs can be used in a number of downstream applications, including real time PCR and NGS, by using Norgen's Exosomal RNA Isolation Kit to isolate EV RNA from the purified EVs.

Component	Product # 76000 (50, 25 or 15 preps)
Slurry E	12.5 mL
ExoC Buffer	1.5 mL
ExoR Buffer	12 mL
Mini Filter Column	50
Product Insert	1

[¥] ***Please check page 4 for Average Yields and Common RNA Quantification Methods***

Advantages

- Purification and enrichment of intact bacterial cell culture media EVs for functional studies.
- Fast, reproducible and easy processing using a slurry-based system.
- Recovered EVs are compatible with various downstream applications.
- No time-consuming ultracentrifugation or special syringes required.
- No precipitation reagents nor overnight incubation required.
- Pure EVs are purified and free-from any other RNA-binding proteins.

Specifications

Kit Specifications	
Sample Type	Bacterial Cell-Free Culture Media
Sample Volume Range	5-35 mL
Size of RNA purified	All sizes, including miRNA and small RNA (<200nt)
Elution volume	200-600µL

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.

Precautions and Disclaimers
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. Wash hands thoroughly when finished performing the procedures.
Do not smoke, drink or eat in areas where kit reagents and/or bacterial specimens are being used. Dispose of unused kit reagents and bacterial specimens according to local, provincial, or federal regulations.

For more information, please consult the appropriate Safety Data Sheets (SDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

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.

Quality Control
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Bacterial EV Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Bacterial EV Isolation Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Customer-Supplied Reagents and Equipment
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- Vortex
- Nuclease-Free Water
- 15 mL tubes
- 50 mL tubes

Procedures

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 filter 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 Mini Filter Columns.
- Centrifuging Norgen's Filter Columns at a speed higher than recommended may affect EV yield.
- Centrifuging Norgen's Filter Columns at a speed lower than recommended will not affect EV yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the filter column.
- Ensure that all solutions are at room temperature prior to use.
- If any of the solutions do not go through the Filter 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 EV yield.
- This kit enables purification of Bacterial EV's. The number of samples processed depends on the input volume: up to 50 samples using 5-10 mL input; up to 25 samples using 10-20 mL input; and up to 15 samples using 20-35 mL input.

Preparation of Bacterial Cell-free Media Sample

Notes Prior to Use

- To avoid cellular contamination, it is important to have completely cell-free preparation of the media.

1. Harvest the bacterial cell culture in its exponential phase. It is crucial to collect the culture during the exponential growth phase (not the stationary phase) to minimize the presence of dead or lysed cells, which can release intracellular contents and interfere with the accuracy and purity of EV isolation. For optimal results, process the sample immediately after collection.
2. Transfer the required bacterial cell culture media volume into a conical tube and centrifuge at **4000 *x g* at 4°C for 15 minutes** to remove any cells and debris.
3. Transfer supernatant (cell-free media) into a fresh 15-50 mL conical tube.
    - **Bacterial Cell-Free Media is now ready for EV purification.**

Section 1: EV Purification of 5-10 mL of Bacterial Cell-free Media (50 Preps)

Notes Prior to Use

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

1. To 10 mL of cell-free media add 25 µL of **ExoC Buffer** followed by the addition of 200 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 5 minutes.
3. Mix well by vortexing for 10 seconds. Centrifuge for **2 minutes at 2,000 RPM**. Discard the supernatant.

4. Centrifuge again to collect any liquid sticking to the walls of the tube and then remove this liquid using a pipette carefully without disturbing the slurry pellet.
5. Apply 200 µL of **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 200 µL of **ExoR Buffer** at room temperature for 5 minutes.
7. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minutes at 500 RPM**.
8. 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 EVs.**
    - **Your EVs are now ready for any downstream applications.**

Section 2: EV Purification of 10-20 mL of Bacterial Cell-free Media (25 Preps)

Notes Prior to Use

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

1. To 20 mL of 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. Centrifuge again to collect any liquid sticking to the walls of the tube, and then remove this liquid using a pipette carefully without disturbing the slurry pellet.
5. Apply 400 µL of **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 400 µL of **ExoR Buffer** at room temperature for 10 minutes.
7. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minutes at 500 RPM**.
8. 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 EVs.**
    - **Your EVs are now ready for any downstream applications.**

Section 3: EV Purification of 20-35 mL of Bacterial Cell-free Media (15 preps)

Notes Prior to Use

- The procedure outlined below is for 35 mL inputs of cell-free media. If processing a sample volume lower than 35 mL media, simply add 2.5 µL of ExoC Buffer for every 1 mL of cell-free media. The volume of Slurry E and ExoR Buffer is constant for any volume to be processed.

1. To 35 mL of cell-free media add 87.5 µL of **ExoC Buffer** followed by the addition of 600 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. Centrifuge again to collect any liquid sticking to the walls of the tube, and then remove this liquid using a pipette carefully without disturbing the slurry pellet.
5. Apply 600 µL of **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 400 µL **ExoR Buffer** at room temperature for 15 minutes.
7. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minutes at 500 RPM**.
8. 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 EVs.**
   - **Your EVs are now ready for any downstream applications.**

Norgen's Exosomal RNA Isolation Kit (Cat# 58000) is highly recommended for the isolation of Extracellular Vesicle RNA from ExoR Buffer and cell-free RNA from the slurry pellet obtained in Step 8 of Sections 1, 2, 3.

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 EV. Eluting your EV 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 should I do if some of the grey resin is transferred out when I am decanting the media supernatant?**
   - Simply remix and recentrifuge. After centrifuging decant the supernatant.
5. **What if I added more or less of Slurry E?**
   - Adding less volume may reduce the amount of the purified EVs. Adding more may not affect the EV capture but may affect the release of the purified EV in the ExoR Buffer
6. **What if I added more or less of ExoC Buffer?**
   - Adding a different volume from the specified optimum volume will significantly reduce the amount of the purified EVs
7. **What if I added more or less of ExoR Buffer?**
   - Adding less volume will reduce the release of the captured EVs in the ExoR Buffer. Adding more will not affect the release of the captured EVs but it will be more diluted
8. **What will happen if some of the grey resin was accidently transferred with the ExoR buffer?**
   - Any grey resin will be filtered through the Mini Filter Column and the flowthrough which contains the purified EVs should not contain any grey resin.

Related Products	Product #
Exosomal RNA Isolation Kit	58000

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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