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Cell Culture Media Exosome Purification Kits — Product Insert

Product # 60400, 60500, 60600

Exosomes are 40 - 150 nm membrane vesicles which are secreted by most cell types. Exosomes can be found in cell culture media, plasma, serum, saliva, urine, amniotic fluid and malignant ascite fluids, among other biological fluids. Evidence has been accumulating recently that these vesicles act as cellular messengers, conveying information to distant cells and tissues within the body. The exosomes contain cell-specific proteins, lipids and RNAs, which are transported to other cells, where they can alter function and/or physiology. These exosomes may play a functional role in mediating adaptive immune responses to infectious agents and tumours, tissue repair, neural communication and transfer of pathogenic proteins. Recent work has demonstrated the presence of distinct subsets of microRNAs within exosomes and other extracellular vesicles (EVs) which depend upon the tumour cell type from which they are secreted. For this reason exosomal RNA may serve as biomarkers for various diseases including cancer. Another subset of RNA that is found in cell culture media is the free-circulating RNA (fc-RNA). These fc-RNA are usually protein-bound RNA that are leaked from cells either during apoptosis or necrosis. As the RNA molecules encapsulated within exosomes or bound to proteins (fc-RNA) are protected from degradation by RNAses, they can be efficiently recovered from cell culture media. In general, these two RNA groups contain valuable information for the discovery of biomarkers that can help with early detection of certain cancer types and for monitoring the disease status.

Norgen's Cell Culture Media Exosome Purification Kits constitute an all-in-one system for the purification of cell culture media exosomes from different media volumes ranging from 5 mL to 35 mL. These kits also allow for the purification of intact extracellular vesicles (EVs) from cell culture media, and these EVs are ready for any downstream application. The purification is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require any special instrumentation, precipitation reagents or any protease treatments. More importantly, the purified exosomes will not be contaminated with any other RNA-bound proteins that may contaminate your exosomal RNA, which is essential if studying Exosomal RNA gene expression.

Kit Descriptions and Components			
	Mini Kit (Cat# 60400) 5 mL - 10 mL	**Midi Kit (Cat# 60500) 10 mL - 20 mL**	**Maxi Kit (Cat# 60600) 20 mL - 35 mL**
Number of Preps	50 preps	25 preps	15 preps
Slurry E	12.5 mL	12.5 mL	12.5 mL
ExoC Buffer	1.5 mL	1.5 mL	1.5 mL
ExoR Buffer	12 mL	12 mL	12 mL
Mini Filter Spin Columns inserted into 2 mL tubes	50	25	15
Product Insert	1	1	1

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25ºC) for up to 2 years without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2,000 RPM.
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- 1.5 mL tubes
- 15 mL conical tubes
- 50 mL conical tubes
- Nuclease-Free Water

General Precautions
Proper biosafety measures should be carried out when using this kit.

Quality Control
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Cell Culture Media Exosome Purification Kits are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen's Cell Culture Media Exosome Purification Kits are designed for research purposes only. They are not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). 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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Important Notes

- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The provided Mini Filter 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 Mini Filter Spin Columns.

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**

Please check the product # and proceed to the appropriate procedure

Section 1: Cell Culture Media Exosome Purification Mini Kit (Cat. #60400)

Note: 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 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 10 mL 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. 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 Spin column assembled with a 2 mL 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 any downstream applications.**

Section 2: Cell Culture Media Exosome Purification Midi Kit (Cat. #60500)

Note: 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 Spin column assembled with a 2 mL 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 any downstream applications.**

Section 3: Cell Culture Media Purification Maxi Kit (Cat. #60600)

Note: The procedure outlined below is for 35 mL inputs of cell-free media. If processing a sample volume in the range of 20 mL - 35 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 35 mL 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. Apply 600 µL **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 600 µL **ExoR Buffer** at room temperature for 15 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 Spin column assembled with a 2 mL 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 any downstream applications.**

Norgen's Exosomal RNA Isolation Kit (Cat# 58000) is highly recommended for the isolation of Exosomal RNA from ExoR Buffer.

Frequently Asked Questions

1. **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.

2. **What if I added more or less of Slurry E?**
- Adding less volume may reduce the amount of the purified exosomes. Adding more may not affect the exosome capture but may affect the release of the purified exosomes in the ExoR Buffer.

3. **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 exosomes.

4. **What if I added more or less of ExoR Buffer?**
- Adding less volume will reduce the release of the captured exosomes in the ExoR Buffer. Adding more will not affect the release of the captured exosomes but it will be more diluted.

5. **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 Spin Column and the flowthrough which contains the purified exosomes should not contain any grey resin.

Related Products	Product #
Exosomal RNA Isolation Kit	58000

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Cell Culture Media Exosome Purification Kits or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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

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