

Urine Exosome Purification Kits
Product # 57800**Product Insert**

Exosomes are 40 - 150 nm membrane vesicles which are secreted by most cell types. Exosomes can be found in 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 urine 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 biological fluids such as urine. 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 Urine Exosome Purification Kits constitute an all-in-one system for the purification of urinary exosomes from different urine sample volumes ranging from 250 µL to 30 mL. These kits also allow for the purification of intact extracellular vesicles (EVs) from different urine sample volumes, 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 Components

Component	Midi Kit (Cat# 57800) 2 mL - 10 mL
Number of Preps	25 preps
Slurry E	12.5 mL
ExoC Buffer	30 mL
ExoR Buffer	12 mL
Mini Filter Spin Columns inserted into 2 mL tubes	25
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Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature. These kits are stable for 2 years after the date of shipment.

Quality Control

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

Product Use Limitations

Norgen's Urine 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.

General Precautions

All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore be carried out when using this kit.

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.

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

Procedure

Important Notes

Notes Prior to Use

- 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).
- 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 centrifuges 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 Urine Sample

1. Collect and transfer 15-50 mL of 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 a 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.
- **Cell-Free Urine is now ready for Exosome purification**

Note: 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. 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 **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 10 minutes.

7. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minute at 500 RPM**.
8. 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 urine 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 accidentally some of the grey resin was 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 #
Urine Collection and Preservation Tubes (50 cc) – 1	18111
Urine Collection and Preservation Tubes (50 cc) – 50	18113
Urine Collection and Preservation Tubes (15 cc) – 1	18120
Urine Collection and Preservation Tubes (15 cc) – 50	18122
Urine Collection and Preservation Tubes (5 cc) – 1	18116
Urine Collection and Preservation Tubes (5 cc) – 50	18118
Urine Preservative Single Dose – 1 tube	18124
Urine Preservative Single Dose – 50 tubes	18126

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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