

Urine Exosome Purification Kits
Product # 57700, 57800, 57900

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 Descriptions and Components			
	Mini Kit (Cat# 57700) 250 µL - 1 mL	Midi Kit (Cat# 57800) 2 mL - 10 mL	Maxi Kit (Cat# 57900) 11 mL - 30 mL
Number of Preps	50 preps	25 preps	15 preps
Slurry E	12.5 mL	12.5 mL	12.5 mL
ExoC Buffer	8 mL	30 mL	50 mL
ExoR Buffer	12 mL	12 mL	12 mL
Mini Filter Spin Column	50	25	15
Elution tubes (1.7 mL)	50	25	15
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Important Note

- 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).

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) 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

All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore 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 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.

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 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**

Please check the product # and proceed to the appropriate procedure

Section 1: Urine Exosome Purification Mini Kit (Cat. #57700)

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

1. To 1 mL urine add 100 μ L of **ExoC Buffer** followed by the addition of 200 μ L 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 μ L **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 200 μ L **ExoR Buffer** at room temperature for 5 minutes.
6. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minute at 500 RPM**.
7. Transfer the supernatant to a Mini Filter Spin 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 any downstream applications.**

Section 2: Urine Exosome Purification Midi Kit (Cat. #57800)

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 μ L of **Slurry E**. (for example, to 1 mL urine sample add 9 mL Nuclease-free water followed by the addition of 100 μ L 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. Apply 400 μ L **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 400 μ L **ExoR Buffer** at room temperature for 10 minutes.
6. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minute at 500 RPM**.
7. Transfer the supernatant to a Mini Filter Spin 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 any downstream applications.**

Section 3: Urine Purification Maxi Kit (Cat. #57900)

Note: The procedure outlined below is for 30 mL inputs of urine. If processing a sample volume in the range of 11 mL - 30 mL urine, simply bring the volume of your samples up to 30 mL using Nuclease-free water and proceed as outlined below.

1. To 30 mL urine add 1/10 the initial urine volume of **ExoC Buffer** followed by the addition of 600 μ L of **Slurry E**. (for example, to 15 mL urine sample add 15 mL Nuclease-free water followed by the addition of 1.5 mL 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. Apply 600 μ L **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
5. Incubate the slurry pellet resuspended in the 600 μ L **ExoR Buffer** at room temperature for 15 minutes.
6. After incubation, mix well by vortexing for 10 seconds then centrifuge for **2 minute at 500 RPM**.
7. Transfer the supernatant to a Mini Filter Spin 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 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 tube	18111
Urine Collection and Preservation Tubes (50 cc) – 50 tubes	18113
Urine Collection and Preservation Tubes (15 cc) – 1 tube	18120
Urine Collection and Preservation Tubes (15 cc) – 50 tubes	18122
Urine Collection and Preservation Tubes (5 cc) – 1 tube	18116
Urine Collection and Preservation Tubes (5 cc) – 50 tubes	18118
Urine Preservative Single Dose – 1 tube	18124
Urine Preservative Single Dose – 50 tubes	18126

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 Urine 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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