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Urine Exosome RNA Isolation Kit — Product Insert

Product #47200

Norgen's Urine Exosome RNA Isolation Kit provides a rapid procedure for the isolation of exosomal RNA from urine samples.

Exosomes are 40 - 100 nm membrane vesicles, which are secreted by most cell types. Exosomes can be found in saliva, blood, 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 which depend upon the tumour cell type from which they are secreted. For this reason exosomal RNAs may serve as biomarkers for various diseases including cancer. As the RNA molecules encapsulated within exosomes are protected from degradation by RNAses they can be efficiently recovered from biological fluids, such as urine. Norgen's Urine Exosome RNA Isolation Kit then makes exosomal RNA discoveries simple, rapid and reliable. Users can simultaneously concentrate and isolate high quality exosomal RNA, including microRNA, for use in sensitive downstream assays.

INTENDED USE

Norgen's Urine Exosome RNA Isolation Kit constitutes an all-in-one system for the concentration and isolation of exosomal RNA from biological samples. The isolation is based on spin column chromatography and employs Norgen's proprietary resin. The exosomal RNA is isolated free from inhibitors and can be used as template in a number of sensitive downstream assays. The kit is designed to allow for the isolation of exosomal RNA from **50 urine samples**. The kit is designed to allow for the isolation of exosomal RNA from **1 mL** to **10 mL** urine samples.

Although the intended use of this kit is for the concentration and isolation of exosomal RNA, the procedure can be slightly modified for the investigation of exosomal protein markers (Appendix A) or for analysis using NanoSight® or Electron Microscopy for assessing the approximate exosome size range and concentration (Appendix B).

Kit Components:

Component	Contents
Slurry B1	22 mL
Binding Buffer A	20 mL
Lysis Buffer A	2 x 20 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Filter Spin Columns	50
Collection Tubes	50
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 (15-25^{o}C) for up to 1 year without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2500 RPM.
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- 67% Isopropanol
- 15 mL conical tubes
- 96-100% Ethanol
- NuPAGE® LDS Sample Buffer (4X) (Catalog number: NP0007 - NP0008, Thermo Fisher Scientific) - Western Blot
- 1X PBS pH 7.4 - NanoSight® or Electron Microscopy

General Precautions

The user should exercise the following precautions while using the kit:

- 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 RNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Urine Exosome RNA Isolation Kit is designed for research purposes only. It is 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*.

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

Lysis Buffer A contains guanidine thiocyanate, and should be handled with care. Guanidine thiocyanate forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

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.

Protocol

Notes:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Slurry B1** must be mixed well before each use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **This procedure is outlined for the isolation of exosomal RNA from 1 - 5 mL urine samples. However urine samples up to 10 mL may also be processed. To isolate RNA from samples over 5 mL of urine, 50 µL of Binding Buffer A will need to be added for each 1 mL being processed over 5 mL. (For example, 300 µL Slurry B1 + 50 µL Binding Buffer A will be added to a 6 mL urine sample whereas ONLY 300 µL Slurry B1 will be added to urine volumes from 1 – 5 mL without any addition of Binding Buffer A**)

1. Collect a urine sample and transfer 15-50 mL of the urine into a conical tube and centrifuge at **1,000 rpm for 10 minutes** to remove cells and debris. Decant cell-free urine into new 15-50 mL conical tube.

2. Centrifuge the cell-free urine at **2,500 rpm for 10 minutes** to remove any residual debris or bacterial cells. Transfer 1 to 10 mL of cell-free urine into a new 15 mL conical tube.

3. To each 1 mL to 10 mL sample to be processed add **300 µL of Slurry B1 (Note: Mix Slurry B1 well prior to use. For optimal performance ensure that resin is completely resuspended).**

   Note: For every 1 mL of urine sample to be processed over 5 mL, add 50 µL Binding Buffer A. For example, to process a 10 mL sample of urine add 250 µL of Binding Buffer A at this point.

4. Mix well by vortexing for **10 seconds**.

5. Centrifuge the sample at **2,500 rpm for 2 minutes** to pellet the resin and decant the supernatant. Decant carefully in order not to dislodge the slurry pellet.

6. Resuspend the pellet in **300 µL of Lysis Buffer A** by pipetting up and down and then incubate for **15 minutes** at room temperature.

7. After the 15 minute incubation at room temperature, add **300 µL 67% Isopropanol** (provided by the user). Mix well by vortexing for **10 seconds**.

8. Transfer the lysate to a mini filter spin column assembled with a collection tube. Centrifuge at **14,000 rpm for 1 minute** and decant flowthrough.

9. Reassemble filter spin column with collection tube and add **400 µL of Wash Solution A** and again centrifuge at **14,000 rpm for 1 minute**. Decant flowthrough and reassemble column.

10. Repeat step 9 two more times for a total of three washes.

11. Spin the column, empty, for **3 minutes at 14,000 RPM**. Discard the collection tube;

12. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **Elution Solution A** to the column and centrifuge for **2 minutes at 2,000 RPM,** followed by **2 minute at 14,000 RPM**.

Rapid Flow Chart Procedure
Urine Exosome RNA Isolation Kit

Collect Urine Sample

SPI N 2x Transfer cell-free urine

Add Slurry B1. If processing over 5 mL of urine add appropriate amount of Binding Buffer A

SPIN Decant supernatant

Resuspend pellet in Lysis Buffer A.
Incubate at room temperature.
Add isopropanol.

Vortex well to mix

Transfer to Filter Spin Column

SPIN

Wash three times.
Perform dry spin.

SPIN

Elute

SPIN

Pure Urine Exosomal RNA

Appendix A

Western Blot Analysis

Notes:

- **Slurry B1** must be mixed well before each use.
- Prepare a working solution of **1x NuPAGE LDS Sample Buffer**.
- Resolve Exosomal proteins on SDS-PAGE under ***non-reducing conditions***.

1. Collect a urine sample and transfer 15-50 mL of the urine into a conical tube and centrifuge at **1,000 rpm for 10 minutes** to remove cells and debris. Decant cell-free urine into new 15-50 mL conical tube.

2. Centrifuge the cell-free urine at **2,500 rpm for 10 minutes** to remove any residual debris or bacterial cells. Transfer 1 to 10 mL of cell-free urine into a new 15 mL conical tube.

3. To each 1 mL to 10 mL sample to be processed add **300 µL of Slurry B1 (Note: Mix Slurry B1 well prior to use. For optimal performance ensure that resin is completely resuspended).**

4. **Note: For every 1 mL of urine sample to be processed over 5 mL, add 50 µL Binding Buffer A. For example, to process a 10 mL sample of urine add 250 µL of Binding Buffer A at this point.**

5. Mix well by vortexing for **10 seconds**.

6. Centrifuge the sample at **2,500 rpm for 2 minutes** to pellet the resin and decant the supernatant. Decant carefully in order not to dislodge the slurry pellet.

7. Resuspend the pellet in **200 µL of 1x NuPAGE LDS Sample Buffer (Thermo Fisher Scientific)** by vortexing for **10 seconds**.

8. Centrifuge at **2,500 rpm for 2 minutes** to pellet the resin and collect the 1x NuPAGE LDS Sample Buffer

9. Resolve exosomal proteins in 1X NuPAGE LDS Sample Buffer for western blot analysis on SDS-PAGE under non-reducing conditions.

This procedure is based on the following reference: Royo F, Zuñiga-Garcia P, Sanchez-Mosquera P, et al. Different EV enrichment methods suitable for clinical settings yield different subpopulations of urinary extracellular vesicles from human samples. *Journal of Extracellular Vesicles*. 2016;5:10.3402/jev.v5.29497. doi:10.3402/jev.v5.29497.

Appendix B

NanoSight® or Electron Microscopy Analysis

Notes:

- **Slurry B1** must be mixed well before each use.

1. Collect a urine sample and transfer 15-50 mL of the urine into a conical tube and centrifuge at **1,000 rpm for 10 minutes** to remove cells and debris. Decant cell-free urine into new 15-50 mL conical tube.

2. Centrifuge the cell-free urine at **2,500 rpm for 10 minutes** to remove any residual debris or bacterial cells. Transfer 1 to 10 mL of cell-free urine into a new 15 mL conical tube.

3. To each 1 mL to 10 mL sample to be processed add **300 µL of Slurry B1 (Note: Mix Slurry B1 well prior to use. For optimal performance ensure that resin is completely resuspended).**

4. **Note: For every 1 mL of urine sample to be processed over 5 mL, add 50 µL Binding Buffer A. For example, to process a 10 mL sample of urine add 250 µL of Binding Buffer A at this point.**

5. Mix well by vortexing for **10 seconds**.

6. Centrifuge the sample at **2,500 rpm for 2 minutes** to pellet the resin and decant the supernatant. Decant carefully in order not to dislodge the slurry pellet.

7. Resuspend the pellet in **200 µL of 1x PBS pH 7.4** by vortexing for **10 seconds**.

8. Centrifuge at **2,500 rpm for 2 minutes** to pellet the resin and collect the 1x PBS containing intact exosomes for NanoSight® or Electron Microscopy Analysis

This procedure is based on the following reference: Crossland RE, Norden J, Bibby LA, Davis J, Dickinson AM. Evaluation of optimal extracellular vesicle small RNA isolation and qRT-PCR normalisation for serum and urine. *J Immunol Methods. 2016 Feb;429:39-49. doi: 10.1016/j.jim.2015.12.011*.

Frequently Asked Questions

1. **What should I do if some of the grey resin is transferred out of the 15 mL conical tube when I am decanting the supernatant?**

   Simply remix and re-centrifuge. After centrifuging decant the supernatant.

2. **What if I added more or less of the specified reagents volume?**

   Adding less volume may reduce RNA yields. Adding more may not affect the RNA yields except when performing the RNA elution step. Eluting RNA in higher volumes of Elution Solution A will result in diluting your RNA.

3. **Can I perform a second elution?**

   Yes, you can. A second elution is possible, but it is recommended that this elution be performed in a smaller volume (50 µL).

4. **Why do my samples show very low RNA yield?**

   Some biological samples will contain very little exosomal RNA. This will vary from individual to individual based on a number of variables such as how dilute the sample is before processing. In order to increase the RNA yield it may be necessary to isolate RNA from a larger volume of sample or isolate from several samples and then pool the RNA eluted from the samples.

5. **Why does my RNA not perform well in downstream applications?**

   If a different elution solution was used from the one provided in the kit, the buffer should be checked for components that may interfere with the application. Common components know to interfere with downstream assays include high salts, EDTA, detergents and other denaturants. Check the compatibility of your elution buffer with its intended use.

6. **What if my elution was contaminated with the precipitated resin?**

   Let the elution stand vertically for 10 minutes to precipitate the resin and then use the clear elution supernatant for downstream applications. This will neither decrease the RNA yield nor interfere with any downstream application.

Related Products	Product #
Urine Collection and Preservation Tubes (single dose)	18126
Urine Collection and Preservation Tubes (5 cc)	18118
Urine Collection and Preservation Tubes (15 cc)	18122
Urine Collection and Preservation Tubes (50 cc)	18113

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 RNA Isolation Kit 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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