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Exosome Purification Mini Kit — Product Insert

Product # 75700

The kit is intended to purify exosomes from Urine, Plasma, Serum, Cell Culture Medium and Saliva.

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 ascites 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 tumors, 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 tumor 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 plasma or serum are 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 being bound to proteins (fc-RNA) are protected from degradation by RNAses, they can be efficiently recovered from biological fluids, such as plasma or serum. 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 Exosome Purification Kits provide an all-in-one solution for the purification of exosomes from various sample types, including plasma/serum, urine, cell culture media, and saliva. These kits also allow for the purification of intact extracellular vesicles (EVs) from different plasma/serum 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.

This universal kit replaces the following kits, Plasma/Serum Exosome Purification Mini Kit cat. 57400, Urine Exosome Purification Mini Kit cat. 57700, Cell Culture Media Exosome Purification Mini Kit cat. 60400 and Saliva Exosome Purification Kit cat. 65300

Kit Components

Component	Mini Kit (Cat# 75700)
Slurry E	12.5 mL
ExoC Buffer	8 mL
ExoR Buffer	12 mL
Mini Filter Columns	50
Product Insert	1

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.

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

Product Use Limitations
Norgen's Plasma/Serum Exosome Purification Kits are 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.

General Precautions
The user should exercise the following precautions while using these kits:

- 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 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.

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

1. All centrifugation steps are performed at room temperature.
2. Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
3. The provided Mini Filter Columns are optimized to be used with a benchtop centrifuge and not to be used on a vacuum apparatus.
4. Ensure that all solutions are at room temperature prior to use.
5. Most standard benchtop microcentrifuges will accommodate Norgen's Mini Filter Columns.

Section 1. Cell-Free Sample Preparation: Plasma/Serum, Urine, Cell Culture Media, and Saliva

1A. Preparation of Cell-free Plasma/Serum Sample

- ***This kit is suitable for the purification of exosomes from fresh or frozen serum or plasma prepared from blood collected on either EDTA or Citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.***
- ***It is required to make the plasma/serum cell-free before freezing in order to avoid contamination from cellular exosomes.***

1. Once the plasma/serum is separated from the blood, centrifuge plasma/serum for additional 10 minutes at 2000 x g before processing/freezing.
2. Transfer the clear supernatant into a new 15 mL conical tube (provided by user), leaving cells and other debris in the pellet. Only clear supernatant should be processed otherwise abundant plasma/serum proteins may interfere with any downstream application.
3. At this point, the cell-free plasma/serum can be used to purify exosomes or can be frozen until further use.
4. If not frozen, proceed to **Section 2A** for purifying exosomes. If the samples are required to be stored, freeze them at -80ºC until further use.
5. Place your frozen Plasma/Serum at 4ºC to thaw.
6. After thawing your plasma/serum sample, aliquot the volume to be processed and centrifuge for **2 minutes at 400 x g (~2,000 RPM)**.
7. After centrifugation, transfer the clear plasma/serum supernatant to a fresh tube. Proceed to **Section 2A** for purifying exosomes from cell-free plasma/serum.

Cell-Free Plasma/Serum is now ready for Exosome Purification

1B. Preparation of Cell-free Urine Sample

- ***This kit is suitable for the purification of exosomes from fresh, frozen or preserved urine using Norgen's Urine Sample Collection and Preservation Devices.***
- ***It is required to make the urine cell-free before freezing in order to avoid contamination from cellular exosomes.***

1. Collect and transfer 15-50 mL of urine into a new conical tube (provided by the user) and centrifuge at 200 x g (~1,000 RPM) for 10 minutes to remove urine exfoliated cells and debris.

2. Decant cell free urine into a new 15-50 mL conical tube (provided by the user).
3. Centrifuge the cell-free urine at 1,800 x g (~3,000 RPM) for 10 minutes to remove any residual debris or bacterial cells.
4. Transfer cell-free urine into a new 15-50 mL conical tube (provided by the user).
5. At this point, the cell-free urine can be used to purify exosomes or can be frozen until further use.
6. If not frozen, proceed to **Section 2B** for purifying exosomes. If the samples are required to be stored, freeze them at -80ºC until further use.
7. Place your frozen urine at 4ºC to thaw.
8. Proceed to **Section 2B** for purifying exosomes from cell-free urine.

Cell-Free Urine is now ready for Exosome Purification

1C. Preparation of Cell-free Cell Culture Media Sample

- ***This kit is suitable for the purification of exosomes from fresh or frozen cell culture medium.***
- ***It is required to make the cell culture medium cell-free before freezing in order to avoid contamination from cellular exosomes.***

1. Harvest and transfer the required cell culture media volume into a new conical tube (provided by the user) 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 new 15-50 mL conical tube (provided by the user).
3. At this point, the cell-free cell culture medium can be used to purify exosomes or can be frozen until further use.
4. If not frozen, proceed to **Section 2C** for purifying exosomes. If the samples are required to be stored, freeze them at -80ºC until further use.
5. Place your frozen cell-free cell culture medium at 4ºC to thaw.
6. Proceed to **Section 2C** for purifying exosomes from cell-free cell culture medium.

Cell-Free Cell Culture Medium is now ready for Exosome Purification

1D. Preparation of Cell-free Saliva Sample

- ***This kit is suitable for the purification of exosomes from fresh or frozen saliva.***
- ***It is required to make the saliva cell-free before freezing in order to avoid contamination from cellular exosomes.***

1. Transfer the collected saliva (fresh saliva or saliva collected using suitable collection device such as Norgen's Saliva Exosome Collection and Preservation Device) into a fresh 15 mL conical tube (provided by the user).
2. Dilute saliva samples by adding 0.5X the saliva volume of 1X PBS (pH 7.4) (provided by the user). Mix well by pipetting up and down until the saliva is completely mixed.
3. Centrifuge the diluted saliva sample at 200 x g (~1,000 RPM) for 10 minutes to remove exfoliated cells and debris. Decant cell-free diluted saliva into a new 15 mL conical tube (provided by the user).
4. Centrifuge the cell-free diluted saliva at 1,800 x g (~3,000 RPM) for 10 minutes to remove any residual debris or bacterial cells.
5. Transfer cell-free diluted saliva into a new 15 mL conical tube (provided by the user).
6. At this point, the cell-free saliva can be used to purify exosomes or can be frozen until further use.

7. If not frozen, proceed to **Section 2D** for purifying exosomes. If the samples are required to be stored, freeze them at -80ºC until further use.
8. Place your frozen saliva at 4ºC to thaw.
9. Proceed to **Section 2D** for purifying exosomes from cell-free saliva.

Cell-Free Saliva is now ready for Exosome Purification

Section 2. Exosome purification from Cell-Free samples: Plasma/Serum, Urine, Cell Culture Media, and Saliva

2A. Exosome Purification from Plasma/Serum Samples Ranging in Volume from 50 µL to 1 mL

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

1. To 1 mL plasma/serum add 3 mL Nuclease-free water, followed by the addition of 100 µL of **ExoC Buffer**.

   Note: The final volume of any plasma/serum sample to be processed should be 4 mL before the addition of the specified 100 µL of **ExoC Buffer**.

2. To the mixture from Step 1 add 200 µL of **Slurry E**. Mix well by vortexing for 10 seconds and let stand at room temperature for 5 minutes.

   Note: Mix **Slurry E** well prior to use. For optimal performance ensure that resin is completely resuspended.

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 carefully remove this liquid using a pipette without disturbing the slurry pellet.
5. Apply 200 µL **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 200 µL **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 a 2 mL tube, and centrifuge for 1 minute at 6,000 RPM.

   Note: Do Not Discard the flowthrough which contains your purified Exosomes.

➢ **Exosomes are now ready for downstream applications.**

2B. Exosome Purification from Urine Samples Ranging in Volume from 250 µL to 1 mL.

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 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 **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 200 µL **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 a 2 mL tube and centrifuge for 1 minute at 6,000 RPM.

   Note: Do Not Discard the flowthrough which contains your purified Exosomes.

➢ **Exosomes are now ready for downstream applications.**

2C. Exosome Purification from Cell Culture Media Samples Ranging in Volume from 5 mL to 10 mL.

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 culture 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. 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 **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 200 µL **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 a 2 mL tube and centrifuge for 1 minute at 6,000 RPM.

   Note: Do Not Discard the flowthrough which contains your purified Exosomes.

➢ **Exosomes are now ready for downstream applications.**

2D. Exosome Purification from Saliva Samples Ranging in Volume from 500µL to 2mL.

Note: The procedure outlined below is for 3 mL inputs of diluted saliva which is equivalent to 2 mL undiluted saliva. If processing a sample volume lower than 3 mL diluted saliva, simply bring the volume of your samples up to 3 mL using 1X PBS (pH 7.4) and proceed as outlined below.

1. To 3 mL cell-free diluted saliva, add 6 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 10 minutes.
3. Mix well by vortexing for 10 seconds. Centrifuge for 2 minutes at 200 xg (~ 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 **ExoR Buffer** to the slurry pellet and mix well by vortexing for 10 seconds.
6. Incubate the slurry pellet resuspended in the 200 µL **ExoR Buffer** at room temperature for 10 minutes.
7. After incubation, mix well by vortexing for 10 seconds then centrifuge for 2 minutes at 100 xg (1,000 RPM).
8. Transfer the supernatant to a **Mini Filter Column** assembled with a 2 mL tube and centrifuge for 1 minute at 600 xg (~6,000 RPM).

   Note: Do Not Discard the flowthrough which contains your purified Exosomes.

➢ **Exosomes are now ready for downstream applications.**

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

Frequently Asked Questions

1. **What should I do if some of the grey resin is transferred out when I am decanting the plasma/serum supernatant?**
   - Simply remix and re-centrifuge. After centrifuging decant the supernatant.

2. **What if I added more or less from 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 from 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 from 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 accidently some of the grey resin was transferred with the ExoR buffer?**
   - Any grey resin will be filtered through the Mini Filter Column and the flowthrough which contains the purified exosomes should not contain any grey resin.

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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