

## Saliva Exosome Purification Kit

### Product # 65300

## 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 saliva 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 saliva. 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 Saliva Exosome Purification Kit allows for the purification of saliva exosomes from 500  $\mu$ L to 2 mL of fresh saliva samples or saliva samples collected using Norgen's Saliva Exosome Collection and Preservation Kit (Cat 65400). This kit also allows for the purification of intact extracellular vesicles (EVs) from different saliva 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	Product #65300 (50 preps)
Slurry E	12.5 mL
ExoC Buffer	1.5 mL
ExoR Buffer	12 mL
Mini Filter Spin Columns inserted into 2 mL tubes	50
Product Insert	1

### Important Note

We recommend the use of Norgen's Saliva Exosome Collection and Preservation Kit (Cat. 65400) when collecting saliva samples, which is designed for the preservation of exosomes in fresh saliva specimens at ambient temperatures. The components of the buffer allow samples to be stored for 2 years without any detectable exosome lysing. The preservative is present in the saliva collection tube in a dried format, and will preserve the saliva exosomes once mixed thoroughly with the collected saliva sample. The buffer prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely.

### **Storage Conditions and Product Stability**

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

### **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
- Nuclease-Free Water
- 0.2 $\mu$  - Filtered 1X PBS (pH 7.4)

### **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 Saliva Exosome Purification Kit is tested against predetermined specifications to ensure consistent product quality.

### **Product Use Limitations**

Norgen's Saliva Exosome Purification 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](http://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.**

### **Notes Prior to Use:**

- 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.
- Ensure you have prepared 0.2 $\mu$  - Filtered 1X PBS (pH 7.4) before starting procedure

## Preparation of Cell-free Saliva Sample

1. Transfer the collected saliva (fresh saliva or saliva collected using 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.
  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 fresh 15 mL conical tube.
- ❖ **Cell-Free diluted saliva is now ready for exosome purification**

## Saliva Exosome Purification Procedure

**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 Spin assembled with a 2 mL tube and centrifuge for 1 minute at **600 xg (~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 saliva 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 #
Saliva Exosome Collection and Preservation Kit	65400
Exosome RNA Purification 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 Saliva Exosome Purification 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 9:00 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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

Norgen's purification technology is patented and/or patent pending. See [www.norgenbiotek.com/patents](http://www.norgenbiotek.com/patents)

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