

FBS Exosome Depletion Kit I (Column Format)
Product # 61200

Product Insert

Exosomes are 40 - 150 nm membrane vesicles which are secreted by most cell types. Exosomes can be found in cell-culture media, 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 cell-culture media 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.

Most culture medium used for the growth and propagation of cells in culture require the addition of fetal bovine serum (FBS) as a growth complement to media. FBS is obtained from bovine (cow) serum, and therefore contains large quantities of cow exosome vesicles. These exosomes may interfere with some types of studies, or may lead to unreliable results when studying the exosomes shed from your cells of interest in normal culture conditions. Therefore, the use of exosome-depleted FBS is highly recommended for many types of studies.

Norgen's FBS Exosome Depletion Kits (Column Format) constitute an all-in-one system for the depletion of cow's exosomes from FBS prior to using it as a growth supplement in your culture medium. The FBS recovered from the depletion process is exosome-depleted and does not contain any quantifiable bovine miRNAs. Moreover, the exosome-depleted FBS will support the growth of your cells of interest similar to the non-depleted FBS. Norgen's kits allows for the depletion of different FBS volumes with a maximum volume ranging from 120 mL to 240 mL. The depletion is based on Norgen's proprietary resin. These kits provide a clear advantage over other available kits in that they do not require ultracentrifugation, any special instrumentation, precipitation reagents or any protease treatments. More importantly, the depletion process is an inexpensive method for depletion of your own FBS, as compared to the current ready-to-use exosome-depleted media available on the market.

Kit Components

Component	FBS Exosome Depletion Kit I (Column Format) Cat# 61200 Up to 120 mL
Number of Preps	6 preps
ExoC Buffer	2 x 1.5 mL
Maxi Spin Column	6
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 FBS Exosome Depletion Kits (Column Format) are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's FBS Exosome Depletion Kits (Column Format) 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

Proper biosafety measures should 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
- Swinging bucket centrifuges.
- Vortexer
- Sterile pipette tips with filters
- 50 mL conical tubes
- DMEM, RPMI or other base media

Procedure

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 Maxi Spin Columns are optimized to be used with a benchtop centrifuge and not to be used on a vacuum apparatus
- Most standard swinging bucket centrifuges will accommodate Norgen's Maxi Spin Columns. Do not use a fixed-angle rotor.
- Centrifuging Norgen's Spin Columns at a speed higher than recommended may affect the depletion process
- Centrifuging Norgen's Spin Columns at a speed lower than recommended will not affect the depletion process. However, centrifugation at a lower speed may require longer time for the solutions to pass through the spin column
- When placing Norgen's Maxi Spin Columns into the swinging bucket centrifuge make sure that lids of the tubes are not tightly closed. Tightly closed lids may cause back pressure which may cause column clogging or disintegration.

Section 1. FBS Exosome Depletion

Note: The procedure outlined below is for 20 mL inputs of FBS. If processing a sample volume lower than 20 mL media, simply add 25 μ L ExoC Buffer and 250 μ L from your media of interest for every 1mL of FBS.

1. To 20 mL FBS add 5 mL media of your interest followed by the addition 400 μ L of **ExoC Buffer**.
2. Mix well by vortexing for 10 seconds and let stand at room temperature for 10 minutes.
3. After incubation, transfer 15 mL of the mixture from **Step 2** into a Maxi Spin column assembled with one of the provided collection tubes. Centrifuge for 15 minutes at 500 x g (~1,000 RPM). Do Not Discard the flowthrough. **(Note: Make sure that lid of the tubes is not tightly closed during centrifugation).**
4. Transfer the flowthrough into a fresh 50 cc tube (not provided) and reassemble the spin column with its collection tube. **(Note: The flowthrough from step 3 contains your Exosome-depleted FBS).**
5. Transfer the remainder of the mixture from Step 2 into the Maxi Spin Column assembled with its collection tube. Centrifuge for 15 minutes at 500 x g (~1,000 RPM). Do Not Discard the flowthrough. **(Note: Make sure that lid of the tubes is not tightly closed during centrifugation).**
6. Transfer the flowthrough into the 50cc tube (**Step 4**) that contains the flowthrough from **Step 3**. **(Note: The flowthrough from Step 3 contains your Exosome-depleted FBS).**
7. Aliquot your Exosome-depleted FBS and store it at -20°C for future use
 - **Your FBS is now Exosome-depleted and ready to be used as a growth supplement in your culture medium.**

Section 2. Media Preparation with Exosome-Depleted FBS

1. Thaw the Exosome-Depleted FBS overnight at 4°C.
2. Combine 62.5 mL of your Exosome-Depleted FBS and 5 mL of your Antibiotic - Antimycotic stock of interest in 500 mL media of interest (Note: your media of interest should be the same media used to dilute your FBS during the depletion process in **Section 1, Step 1**)

Related Products	Product #
Exosomal RNA Isolation Kit	58000
Cell-Culture Media Exosome Purification Mini Kit	60400
Cell-Culture Media Exosome Purification Midi Kit	60500
Cell-Culture Media Exosome Purification Maxi Kit	60600
Cell-Culture Media Exosome Purification and RNA Isolation Mini Kit	60700
Cell-Culture Media Exosome Purification and RNA Isolation Midi Kit	60800
Cell-Culture Media Exosome Purification and RNA Isolation Maxi Kit	60900

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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