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 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 (Slurry 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 140 mL to 280 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 Descriptions and Components

<table>
<thead>
<tr>
<th></th>
<th>FBS Exosome Depletion Kit I (Slurry Format) Cat# 61100 Up to 140 mL</th>
<th>FBS Exosome Depletion Kit II (Slurry Format) Cat# 61400 Up to 280 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Preps</td>
<td>6 preps</td>
<td>12 preps</td>
</tr>
<tr>
<td>ExoC Buffer</td>
<td>2 x 1.5 mL</td>
<td>8 mL</td>
</tr>
<tr>
<td>Slurry E</td>
<td>12.5 mL</td>
<td>2 x 12.5 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</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
- Swinging bucket centrifuges.
- Vortexer
- Sterile pipette tips with filters
- 50 mL conical tubes
- 0.2µ Filter
- DMEM, RPMI or other base media
**General Precautions**
Proper biosafety measures should 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 FBS Exosome Depletion Kits (Slurry Format) are tested against predetermined specifications to ensure consistent product quality.

**Product Use Limitations**
Norgen’s FBS Exosome Depletion Kits (Slurry 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.

**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.

**Important Notes**
- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.

### 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 µL ExoC Buffer, 100 µL Slurry E and 250 µL 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 µL of ExoC Buffer and 2 mL 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 2,000 RPM. Do Not Discard the supernatant. *(The supernatant from step 3 contains your Exosome-depleted FBS).*
4. Transfer the supernatant into a fresh 50 cc tube (not provided) without disturbing the slurry pellet.
5. Filter the supernatant using a 0.2 µM Filter (not provided).
6. Aliquot your Exosome-depleted FBS filtrate and store it at -20°C for future use
   - **Your FBS is now Exosome-depleted and ready to be used as a as a growth supplement in your culture medium.**

### Section 2: Media Preparation with Exosome-Depleted FBS

1. Thaw the Exosome-Depleted FBS for overnight at 4°C.
2. Combine 63.5 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)*
<table>
<thead>
<tr>
<th>Related Products</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exosomal RNA Isolation Kit</td>
<td>58000</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification Mini Kit</td>
<td>60400</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification Midi Kit</td>
<td>60500</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification Maxi Kit</td>
<td>60600</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification and RNA Isolation Mini Kit</td>
<td>60700</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification and RNA Isolation Midi Kit</td>
<td>60800</td>
</tr>
<tr>
<td>Cell-Culture Media Exosome Purification and RNA Isolation Maxi Kit</td>
<td>60900</td>
</tr>
</tbody>
</table>

**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 FBS Exosome Depletion Kits (Slurry Format) 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