WILSONWOLF

G-REX® 500M-CS

Gas Permeable Cell Culture System

REF Catalog Number **G285500-CS / RUO5500-CS**

- INSTRUCTIONS FOR USE -

PRODUCT DESCRIPTION

The G-Rex®500M-CS is a single-use, closed system (sterile fluid pathway) product designed for the expansion and recovery of mammalian cells.

Cells reside undisturbed on the gas permeable membrane and divide until they reach a maximum density or harvest timepoint.

The final cell population is typically harvested via the GatheRex™ Cell Harvest Pump, followed by further processing prior to the final application.

INDICATIONS FOR USE

The G-Rex500M-CS cell culture system is intended for expansion and recovery of non-attachment dependent mammalian cells.

CONTRAINDICATIONS

1. Not to be used with peristaltic pumps or other liquid pumping systems not designed to recover and concentrate final cell populations.
2. Not suitable for use in conditions outside of those typically intended for the maintenance and expansion of mammalian cell lines.

WARNINGS

1. This IFU is not a comprehensive reference for the application of use in cell culture protocols.
2. Users should be familiar with appropriate application and aseptic techniques involved with the use of this product and the G-Rex cell culture platform technology.
3. Do not exceed a fill rate of 1 liter per minute.
4. Do not use if the product is damaged.
5. Do not use the product if there is an apparent breach to sterility.
6. Unauthorized modification and improper use may result in the inability to culture cells or maintain a sterile environment.

PRECAUTIONS

DO NOT USE the product if any of the following are observed:

1. Damage to the product or packaging that may have occurred during shipping or storage
2. Loose or misshaped luer fittings on the product.
3. Open-ended or unattached tubing are present on the exterior of the product.
4. The product was frozen with fluid inside, the product is not designed for freezing fluid.
5. The product Use By Date (YYYY-MM-DD) has been exceeded.
6. The product has been grossly overfilled.
7. Do NOT re-sterilize or reuse the product. All G-Rex products are single-use only.

PREPARATION

1. Remove G-Rex from the packaging.
2. Check and hand tighten each luer fitting on the product. Do not overtighten.
3. The vent filter should always remain open (unclamped) during fill, throughout the culture period and at time of harvest.

Notes:

A. The vent filter line should only be clamped as a precaution during transit when the product is filled with media or when resuspending the cells (i.e., mixing the fluid). This ensures the vent filter does not become wet and will function properly in its dual-purpose role:
    a. Allows air to be displaced when filling the product with fluid and acts to equalize headspace pressure and gas composition during the culture period
    b. Serves as a sterile air filter if pressurizing the product during cell harvest.

Contact Wilson Wolf Technical Support for further assistance (contact info located at end of this document).

ADDING MEDIA AND CELLS

Media and cells can be added through any of the following 3 ports:

1. Sample Port via sample line
2. Media Reduction Ports
    a. Reduction line 1
    b. Reduction line 2
3. Cell Harvest Ports
    a. Harvest line 1
    b. Harvest line 2

Notes:

A. Pay close attention to any clamps that need to be open to allow proper fluid flow.
B. Do not exceed a fill rate of 1 liter per minute, as this will result in fluid leakage due to over pressurization of the vessel. If uncertain, contact Technical Support for step by step guidance.
C. Ensure each port is adequately flushed with media or sterile air following fluid addition so cells or growth factors are not retained in the fluid lines.
D. To maintain product integrity, it is best practice to add and remove media/cells using thermal welds with the weld-compatible tubing. PVC thermal weld compatible tubing is present on the media reduction port, line 1 (red-stripe) and the cell harvest port, line 2 (clear). Each line is 30 inches long and terminates in a luer fitting with a cap or plug. Additionally, the large diameter C-Flex tubing comprising media reduction port line 2 is thermal weld compatible and 30 inches long, terminating in a female quick-connect (MPC) connector and plug. Use of the larger diameter tubing will speed up media fill and harvest compared to the smaller diameter PVC tubing.
E. Product Capacities:
    a. Working Volume: 5 liters
    b. Maximum Capacity: 5.5 liters

SAMPLING AND ALTERNATE MEDIA FILL METHODS

SAMPLE PORT *(one line)*

1. The sample port line ends in a Clave™ needleless septum (dark blue in color) with a female luer lock and a cap. This line can be used to introduce reagents or remove samples during the culture period.
    - The sample port line corresponds to the silicone tube on the inside of the product which terminates about 50% of the way into the vessel
    - Remove the clear cap from the dark blue Clave connecter, wipe the connector end with a sterile 70% alcohol wiper and allow to dry, dock a syringe onto the Clave connecter, withdraw the sample, clear the line with sterile air, and replace the clear cap.

MEDIA REDUCTION PORT *(two lines)*

1. Reduction Line 1 is red-striped PVC tubing, terminating in a female luer lock and luer plug. This tubing is thermal weld compatible for functionally closed connections to a transfer bag. This tubing is contiguous with the tube on the inside of the product that terminates about 1cm above the bottom membrane.
    - The female luer lock fitting can be used to dock a media/cell bag or syringe. This operation should be performed in a biosafety cabinet. Replace luer plug.
2. Reduction Line 2 has a quick connect (MPC) fitting ending on the larger diameter C-Flex tubing. This tubing is thermal weld compatible for functionally closed connections to a media bag.
    - This line corresponds to the tube on the inside of the product which terminates about 1cm above the membrane (same as reduction line 1).
    - The quick connect fitting can be used to dock a media bag. Remove the end plug and aseptically connect a media bag to the quick connect fitting. Lift the bag above the G-Rex and allow media to gravity drain into the product. Replace the end plug.

CELL HARVEST PORT *(two lines)*

1. Harvest Line 1 is the short silicone tube that comes off a T-fitting and terminates in a male luer lock.
    - This line corresponds to the silicone tube on the inside of the product that is angled into and attached to the side wall and touches the bottom membrane.
    - Attachment to this line should be performed in a biosafety cabinet. To do this, aseptically remove the end cap, dock a media bag onto the male luer fitting and gravity drain fluid into the product. Replace the end cap fitting.
2. Harvest Line 2 is a clear PVC tubing line that terminates with a male luer lock and end cap. This tubing is thermal weld compatible which is necessary to maintain a functionally closed connection to a media transfer bag.
    - This line corresponds to the silicone tube on the inside of the product that is angled into and attached to the side wall and touches the bottom membrane (same as harvest line 1).
    - As described for media reduction line 1, this line can be used to fill the product prior to inoculation. If a sterile weld is not performed, the male luer lock fitting can be attached to a media bag and used to fill the product. This operation should be performed in a biosafety cabinet, followed by replacement of the end cap fitting.

HARVESTING

Cell harvest should be performed in conjunction with the GatheRex Liquid Handling, Cell Harvest Pump.

Below is a brief guide. Reference the GatheRex User Manual for additional information:

1. Dock a waste media collection bag (or other receptible) onto reduction line 1 or reduction line 2. Use either reduction line 1 (red-stripe PVC tubing) or reduction line 2 (C-Flex tubing) in conjunction with compatible thermal weld equipment for functionally closed processing.
2. Dock a cell collection bag (or other receptible) onto harvest line 1 or harvest line 2. Use harvest line 2 (clear PVC tubing) in conjunction with thermal weld equipment for functionally closed processing.
3. Lay reduction line 1 or reduction line 2 and harvest line 2 in the corresponding conduit pathway of the appropriate GatheRex Pump. Ensure the tubing is properly seated in the housing and close the clamps.
4. Connect the Tubing Assembly from the GatheRex to the Vent Filter. Ensure the clamps for the selected fluid pathways are open to the media and cell collection bags and turn on the GatheRex pump.
5. Press the red arrow for media removal, allowing the device to pressurize and move fluid out of the vessel down to the 1cm height of the internal media reduction line.
6. With 90% of the full working volume (waste media) removed, swirl the remaining ~500 mls to resuspend the cells residing on the gas permeable membrane. Tilt the device so the fluid collects/pools where the internal harvest line touches the base/side of the vessel. Press the blue arrow for cell harvest, allowing the device to pressurize and move the cell population and all remaining fluid into the cell harvest bag.
7. Clamp reduction line 1 or reduction line 2 and harvest line 2 in order to seal the waste media and cell collection bags. Press release clamps button on GatheRex Pump and remove the tubing.
8. Seal the collection bags with sterile tube sealer. Discard G-Rex vessel and waste media.

MANUFACTURED BY

Wilson Wolf Manufacturing Corporation
St. Paul, Minnesota 55112 USA
Made in USA

TECHNICAL SUPPORT / ORDERING

Tel (+1) 651.628.9259
Email info@wilsonwolf.com
Web www.wilsonwolf.com

FIGURE 1: External Lines

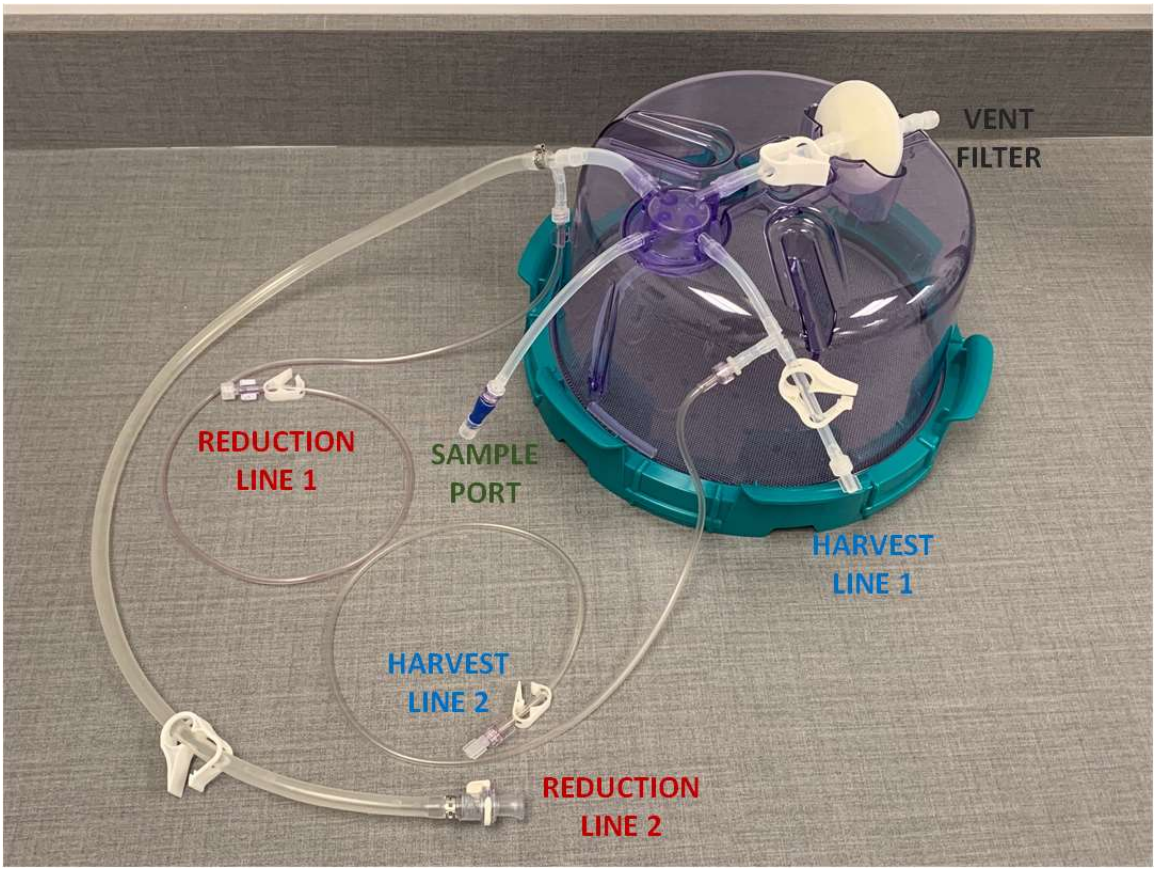

FIGURE 2: Internal Lines