WILSONWOLF

G-REX® 10M-CS
Gas Permeable
Cell Culture Product

REF Catalog Number **80110-CS / 80110S-CS**

INSTRUCTIONS FOR USE (IFU)

PRODUCT DESCRIPTION

The G-Rex®10M-CS is a single-use, closed system products designed for efficient, mid-scale expansion of mammalian cells. The engineered tubing system allows for fully closed system operation and recovery of concentrated cell suspensions.

The closed system G-Rex product line enables repeatable, efficient, and high throughput manufacturing of CAR-T or other immune cell therapies.

Use	REF	STERILITY
GMP	80110-CS	Sterile Fluid Path, Sterilized Using Irradiation (SAL=10^{-6})
RUO	80110S-CS	Gamma Irradiated, Sterility Assurance Level (SAL) has not been validated

INDICATIONS FOR USE

The G-Rex10M-CS cell culture system is intended for activation, transduction (if applicable), expansion, concentration, and recovery of mammalian cells, preferably suspension cells, for use within the field of cell and gene modified cell therapy.

Wilson Wolf makes no claims regarding the performance of this product for clinical treatment or therapeutic applications. It is the responsibility of the end user to assess its suitability for specific applications.

CONTRAINDICATIONS

1. Not to be used with peristaltic pumps or other liquid pumping systems not designed for use with closed system G-Rex.
2. Not suitable for use in conditions outside of those typical for the maintenance and expansion of mammalian cell lines.

WARNINGS

1. This IFU is not a comprehensive reference for cell culture protocols.
2. Users should be familiar with basic aseptic techniques.
3. Do not exceed a fill or extraction rate of 300 mL per minute.
4. Closed System functionality cannot be assured if the green cap of G-Rex is removed.
5. Do not use the product if there is an apparent breach to sterility.
6. Unauthorized modification and improper use may result in poor recovery or cell culture contamination.
7. Do not freeze; the product is not designed for freezing fluid.

PRECAUTIONS

Contact info@wilsonwolf.com and 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. Detached luer fittings, caps, plugs, or tubing inside the packaging.
3. The Use By Date (YYYY-MM-DD) has been exceeded.
4. The product has been grossly overfilled.

Do NOT re-sterilize or reuse the product. All G-Rex products are single-use only.

Do NOT over-sanitize workstations or incubator shelves prior to use of G-Rex products. Alcohol or other sanitizing vapors could permeate the G-Rex membrane and cause cell toxicity. Allow sufficient time for drying of cleaning reagents and ensure disinfectant residues are adequately removed from surfaces.

PREPARATION

- Ensure Media is pre-warmed to 37°C.
- Remove G-Rex from the packaging.
- Check and hand tighten each luer fitting on the product. Do not overtighten.
- Verify the internal harvest line (the tube that contacts the bottom membrane) is positioned against the side wall of the vessel where the wall meets the bottom surface. You may need to tilt the G-Rex at an angle and tap the vessel to move this tube into place.
- The vent filter should always remain open (unclamped) during fill, throughout the culture period, and at time of harvest.

DESCRIPTION OF TUBING LINES AND PORTS (APPENDIX 1)

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 supernatant 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.
    - Connections to the sample port must be performed inside a biosafety cabinet. Remove the clear cap from the dark blue Clave connector, wipe the connector end with a sterile 70% alcohol wipe and allow it to dry, dock a syringe onto the Clave connector, withdraw the sample, clear the line with sterile air, and place the clear cap back onto the needless septum. The clear cap is not integral to maintaining the sterile fluid path.
    - If using the sample port to add small volume reagents, ensure there is enough air in the syringe to clear the line into the device.

MEDIA REDUCTION LINES ***(two lines)***

1. Reduction Line 1 is the red-striped PVC tubing, terminating with a female luer lock and luer plug. This tubing is thermal weld compatible for closed connection to a media or processing bag. This tubing is contiguous with the tube on the inside of the product that terminates about 1 cm above the bottom membrane.

- The female luer lock fitting can be used to dock a media/cell processing bag or syringe. If using the luer connection, this operation should be performed in a biosafety cabinet.

2. Reduction Line 2 is a silicone tube terminating with a female luer lock and luer plug.
   - This line also corresponds to the tube on the inside of the product which terminates about 1 cm above the membrane (same as Reduction Line 1).
   - Use of this line to add media or perform volume reduction is an alternative to welding the media source to Reduction Line 1 and attachment to this line should be performed in a biosafety cabinet. To perform media fill using this line, 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.

CELL HARVEST LINES ***(two lines)***

1. Harvest line 1 is a clear PVC tubing line terminating with a male luer lock and end cap. This tubing is thermal weld compatible for closed connection to a cell processing/harvest bag.
   - This line corresponds to the silicone tube on the inside of the product that is angled toward the side wall and touches the bottom membrane (same as Harvest Line 2).
   - This line should be used for closed system harvest operations in accordance with the **HARVESTING** instructions.
2. Harvest line 2 is a silicone tube terminating with a female luer lock and luer plug.
   - This line corresponds to the silicone tube on the inside of the product that is angled into 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 plug, dock a processing bag onto the female luer fitting and proceed to harvest concentrated cell product in accordance with the **HARVESTING** instructions.

SAMPLING

1. Daily cell counting samples are not required or recommended in GMP manufacturing.
   a. If a cell count sample is desired, remove 50-80% of the media volume and retain the media, re-suspend the cells, remove a sample for counts using a closed system compatible method, and return the media to the G-Rex 10M-CS.

2. Media can be sampled for tracking of metabolites in Process Development work.
   a. Lactate and glucose measurements trend closely with cell numbers.
   b. Process control is maintained with robust set-up procedures, engineering specification of the G-Rex membrane, and in process monitoring is obtained by monitoring the incubator environment for CO_2 and temperature.

GENERAL INSTRUCTIONS AND METHODS OF USE

The G-Rex 10M-CS is a closed system and does not require use of a biosafety cabinet if tubing connections are made via a thermal weld.

Ensure cells and media are well mixed once added to the G-Rex. A homogenous cell suspension will result in uniform distribution and settling of cells across the entire permeable gas membrane surface area. This is necessary to achieve maximum cell densities and consistent yields.

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 for pressurizing the product during cell harvest.

Wilson Wolf recommends the following thermal weld compatible processing bags for closed system G-Rex processing:

Supplier	Description	Catalog #
Wilson Wolf	G-Rex 250mL Processing Bag	WW-FP250ML
Wilson Wolf	G-Rex 1L Processing Bag	WW-SV1L444

For optimal T-Cell Expansion, Complete Media should be formulated with 3-5% HAB Serum, 10 ng/mL of IL-7 and 10 ng/mL of IL-15. Wilson Wolf recommends the following closed system compatible Media and Cytokine combination:

Supplier	Description	Catalog #
Bio-Techne	GMP Human T Cell Media	CCM038-GMP-1B
Bio-Techne	ProPakTM GMP Recombinant IL-7	PPK-007-GMP-010
Bio-Techne	ProPakTM GMP Recombinant IL-15	PPK-015-GMP-010

Please contact technical support at info@wilsonwolf.com to learn more about closed system media formulation and for standard closed system GMP cell manufacturing consultation and support.

T CELL EXPANSION IN G-REX

Day 0:

- Re-suspend at least 2.5 x 10^6 – 2.0 x 10^7 total activated T cells in Complete Media.
  - Create a cell suspension of activated T cells in Complete Media at a concentration of ~0.5 – 2.0 x 10^6 cells/mL (or higher) in a closed system sterile weld compatible processing bag.
    - Sterile weld the processing bag to Reduction Line 1 (refer to Appendix 1).
    - Gravity drain the Complete Media and cells into the G-Rex 10M-CS such that at least 2.5 x 10^6 – 2.0 x 10^7 cells reside in the G-Rex 10M-CS.
    - Heat seal the tubing and discard the processing bag.

- Gently rock G-Rex back and forth, and side to side, to ensure a homogeneous cell suspension.
- Weld a bag of Complete Media to Reduction Line 1 (refer to Appendix 1) and gravity drain the Complete Media into the G-Rex 10M-CS (do not exceed 110 mL fill volume).
- Gravity filling should take approximately 1 minute.
- Gently rock G-Rex back and forth and side to side periodically while draining the Complete Media into the G-Rex to ensure a homogeneous cell suspension.
- Heat seal the tubing and discard the media bag.

Alternatively, 2.5 x 10^6 – 2.0 x 10^7 activated T cells can be suspended in 100 mL of media in a closed system sterile weld compatible processing bag and gravity drained into the G-rex in one step. Ensure the culture is well mixed before adding to the G-Rex to ensure even distribution.

- Place G-Rex in a standard incubator for seven (7) to ten (10) days without disturbing the cells or the culture.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

T CELL ACTIVATION AND EXPANSION IN G-REX

Day 0:

- Create a cell suspension (PBMC or selected T cells) in Complete Media at a concentration of 0.5 – 2.0 x 10^6 cells/mL in a closed system sterile weld compatible processing bag and mix well.
- Add suitable activation reagent at standard concentrations or ratios according to the manufacturer's recommendation to the processing bag and mix well.
  - An activation volume of 10 mL or 1 mL/cm^2 is recommended.
- Sterile weld the processing bag to Reduction Line 1 (refer to Appendix 1).
- Gravity drain the Complete Media and cells with activation reagent into the G-Rex 10M-CS.
- Gently rock G-Rex back and forth, and side to side, to ensure a homogenous cell suspension.
- Heat seal the tubing and discard the processing bag.
- Place G-Rex in a standard incubator for 48-72 hours.
  - **Note:** In high throughput, closed system, GMP manufacturing settings, it may be advisable to batch prepare an activation Mastermix and standardize upstream operations. Contact technical support at info@wilsonwolf.com to learn more.

Day 2 or 3:

- Gently rock and swirl the G-Rex to resuspend the cells in the Complete Media to ensure a homogeneous cell suspension.
- Weld a bag of Complete Media to Reduction Line 1 (refer to Appendix 1) and gravity drain the Complete Media into the G-Rex 10M-CS. Do not exceed 110 mL fill volume.
- Gravity filling should take approximately 1 minute.
- Heat seal the tubing and discard the media bag.
- Place G-Rex in a standard incubator for four (4) to seven (7) more days without disturbing the cells or the culture.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

ACTIVATION, TRANSDUCTION, AND EXPANSION IN G-REX

Day 0:

- Create a cell suspension (PBMC or selected T cells) in Complete Media at a concentration of 0.5 – 2.0 x 10^6 cells/mL in a closed system sterile weld compatible processing bag and mix well.
- Add suitable activation reagent at standard concentrations or ratios according to the manufacturer's recommendation to the processing bag and mix well.
  - An activation volume of 10 mL or 1 mL/cm^2 is recommended.
- Sterile weld the processing bag to Reduction Line 1 (refer to Appendix 1).
- Gravity drain the Complete Media and cells with activation reagent into the G-Rex 10M-CS.
- Heat seal the tubing and discard the processing bag.
- Gently rock G-Rex back and forth, and side to side, to ensure a homogenous cell suspension.
- Place G-Rex in a standard incubator overnight.
  - **Note**: If starting cell material is selected T-Cells, Activation and Transduction can proceed simultaneously for 48-72 hours. Contact technical support at info@wilsonwolf.com for more detailed Day 0 simultaneous Activation and Transduction instructions.

Day 1:

- Gently rock G-Rex to ensure cells are evenly distributed in the media, and obtain a cell count to determine virus needed to achieve desired MOI.
- Add viral vector and choice of transduction enhancer (optional) at desired MOI to the G-Rex using a closed system compatible method.
  - Alternatively, the virus can be added via syringe through the sample port.
  - Contact technical support at info@wilsonwolf.com for process specific assistance.
- Rock the G-Rex back and forth to mix cells and virus to ensure sufficient distribution of cells and virus within the media.
- Place in standard cell culture incubator overnight.

Day 2 or 3:

- Gently rock the G-Rex back and forth, and side to side, to resuspend the cells in the Complete Media to ensure a homogeneous cell suspension.

- Weld a bag of Complete Media to Reduction Line 1 (refer to Appendix 1) and gravity drain the Complete Media into the G-Rex 10M-CS. Do not exceed 110 mL fill volume.
- Gravity filling should take approximately 1 minute.
- Heat seal the tubing and discard the media bag.
- Place G-Rex in a standard incubator for four (4) to seven (7) more days without disturbing the cells or the culture.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

Processing 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 300 mL per minute, as this may result in fluid leakage due to over-pressurization of the vessel. Gravity flow is generally sufficient.
C. It may be advisable to flush tubing lines 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 PVC tubing that consists of the Reduction Line 1 (red-striped PVC tubing) and the Harvest Line 1 (clear PVC tubing). Each line is 30 inches long and terminates in a luer fitting with a cap or plug.
E. Product Capacities:

Working Volume	Maximum Capacity
100 ml (0.1 liter)	110 ml (0.11 liter)

Contact technical support at info@wilsonwolf.com for closed system downstream processing recommendations or more detailed instructions for other cell culture or cell manufacturing applications including but not limited to:

- T cells (including CAR/TCR gene-modified T cells)
  - α/β & γδ T cell subsets
  - Regulatory T cells
- Tumor Infiltration Lymphocytes (TIL)
- Natural Killer (NK) cells (including CAR/TCR gene-modified NK cells)
- Hematopoietic Stem Cells (HSCs)
- Red Blood Cells (RBCs)

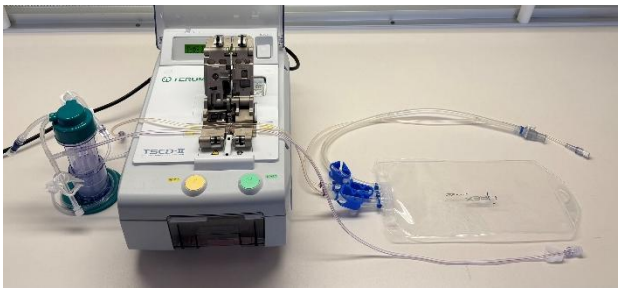
HARVESTING

A 90% volume reduction and cell concentration followed by concentrated cell harvest can be performed in approximately 5 minutes, using the GatheRex Liquid Handling, Cell Harvest Pump.

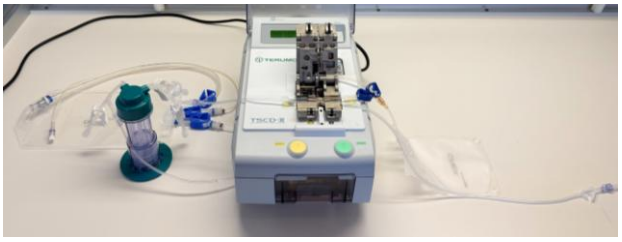
Supplier	Description	Catalog #
Wilson Wolf	GatheRex Liquid Handling, Cell Harvest Pump	80000E

Below is a guide to closed system, semi-automated, volume reduction and concentrated cell harvest from the G-Rex 10M-CS. Reference the GatheRex User Manual for additional information:

1. Carefully remove the G-Rex 10M-CS from the incubator taking care not to disturb the cell layer. If the cell layer is inadvertently disturbed in transit, allow the G-Rex to rest for at least 20 minutes to allow the cells to resettle to the bottom.
2. Sterile weld a 1 L waste media collection bag onto Reduction Line 1 (red striped PVC tubing). Weld should be approximately 20 cm from Luer on G-Rex and 20 cm from fitting on 1L bag. Refer to recommended processing bags above.



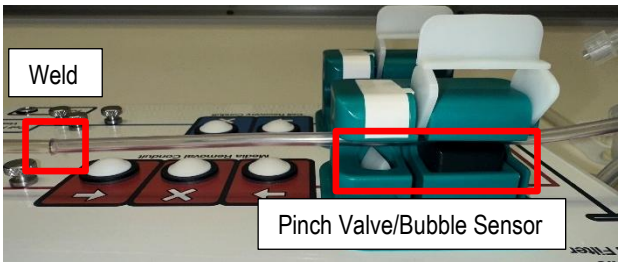
3. Sterile weld a cell harvest bag onto Harvest Line 1 (clear PVC tubing). Weld should be approximately 20 cm from Luer on G-Rex and 20 cm from 250 mL bag. Refer to recommended processing bags above.



4. Turn on the GatheRex pump and wait for the green power light to turn on.

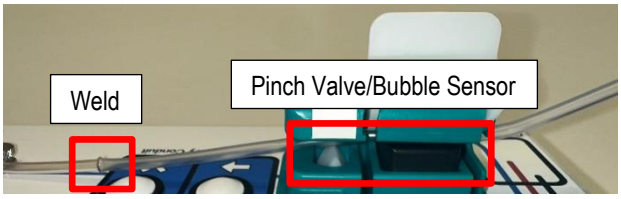
5. Position the GatheRex in the center of the workstation, G-Rex on the left approximately 2.5 cm away from the GatheRex, and the processing bags on the right. Thread the welded Reduction Line 1 tubing through the Media Removal Conduit's pinch valve housing on the GatheRex.

6. Verify the tubing weld is outside of the pinch valve housing and the tubing is oriented above the bubble sensor and the pinch valve.

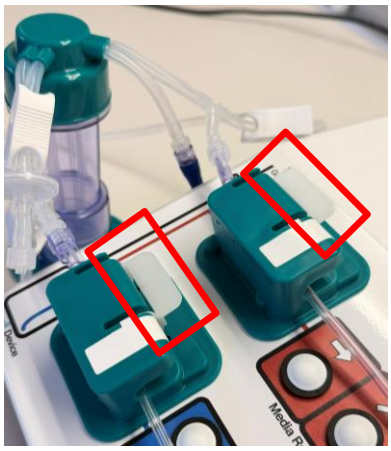


7. Thread the welded Harvest Line 1 tubing through the Cell Recovery Conduit's pinch valve housing on the GatheRex.

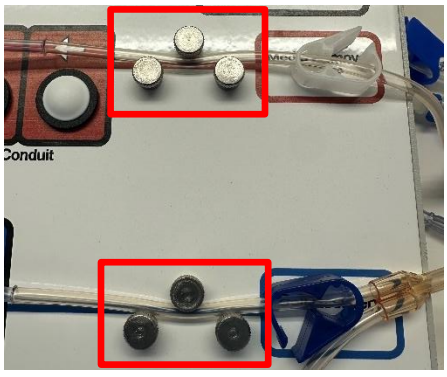
8. Verify the tubing weld is outside of the pinch valve housing and the tubing is oriented above the bubble sensor and the pinch valve.



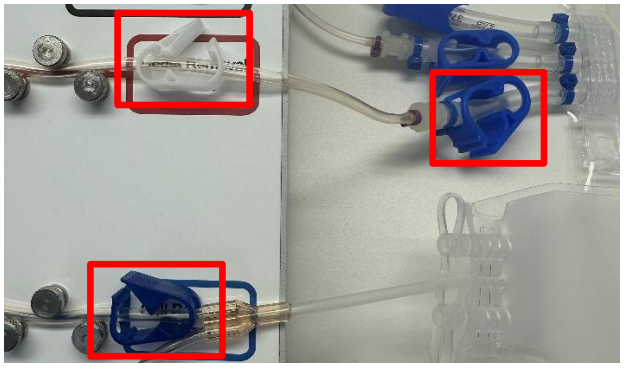
9. Close the Pinch Valve Doors



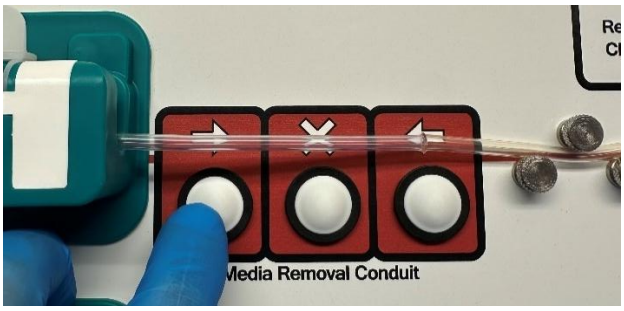
10. Route the tubing lines through the cleats on GatheRex.



11. Connect the gas line from the GatheRex to the vent filter on the G-Rex 10M-CS. Ensure the clamps for the selected fluid pathways are open to the waste media collection and cell harvest bags.

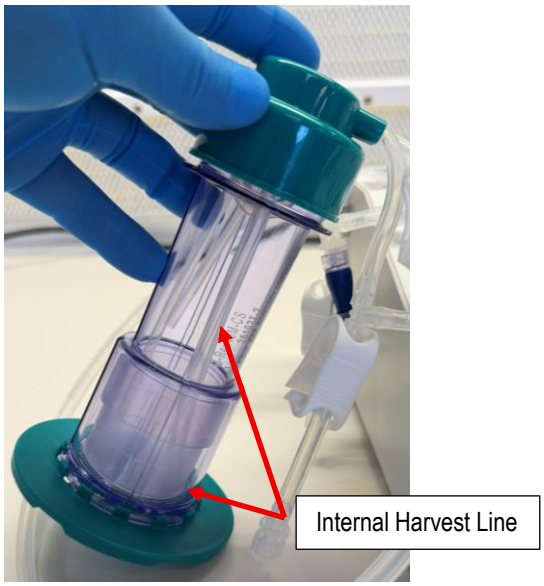


12. Press the red arrow pointed towards the waste media collection bag to begin volume reduction, to pressurize the device and move fluid out of the G-Rex and into the waste media collection bag. The cell layer will remain undisturbed.

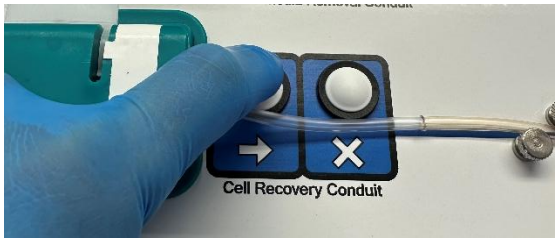


13. When the liquid level reaches the opening of the internal Reduction Line, the bubble sensor will detect an air bubble in the Media Removal Conduit and automatically stop fluid flow.
14. Approximately 90% of the full working volume will have been removed (waste media) leaving approximately 10 mL of media and highly concentrated cells in G-Rex. Rock and swirl the remaining ~10 mLs to resuspend the cells residing on the gas permeable membrane.

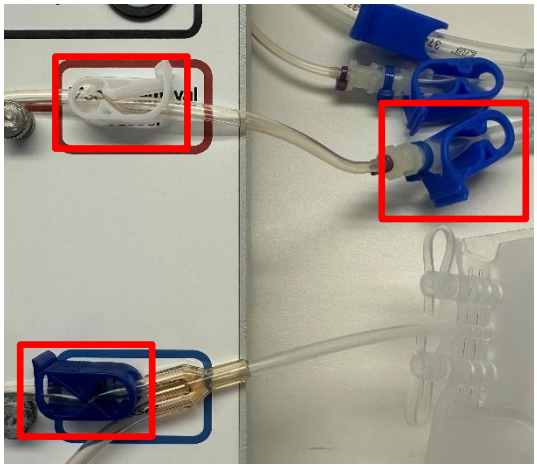
15. Tilt the device so the concentrated cell suspension collects/pools where the internal harvest line touches the base/side of the vessel.



16. Press the blue arrow for cell harvest, to pressurize the device and move the concentrated cell suspension and all remaining fluid into the cell harvest bag.



17. Clamp reduction line 1 and harvest line 1 to seal the waste media and cell collection bags. Press release clamps button on GatheRex Pump and remove the tubing.



18. Heat seal the waste collection and cell harvest bag with tubing sealer.
19. Proceed with downstream processing.

MANUFACTURED BY

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TECHNICAL SUPPORT / ORDERING

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Appendix 1: Reduction, Harvest, Sample Ports and All Three Internal Lines