

SPECTRUM LABS
LEADING THE WAY IN BIOSEPARATION

CellMax[®] Duo Hollow Fiber Cell Culture System

Instructional Manual

www.spectrumlabs.com

Table of Contents

Table of Contents.....	3
Safety and Intended Use Information	5
CellMax Duo System Components	6
Specifications	6
System Description	7
ECU Operation	7
Front Panel Features.....	7
Power Supply Features	8
Cell Culture Recommendations	9
Aseptic Technique.....	9
Cell Lines.....	10
Startup Supplies.....	11
CellMax Duo System – System Setup	12
CellMax Duo System – Module Set-up	13
Sterilization of the Reservoir Cap/Bottle Assembly	13
Reservoir Cap to Flow Path Connections	14
Flow Path to Bioreactor Connections	16
Removal of Bioreactor Storage Solution	16
Preculture.....	17
Filling the Bioreactor ECS with Preculture Media.....	17
Priming the Flow Path	20
Attaching the Hollow Fiber Bioreactor to the Pump Station	20
Cell Inoculation	21
Guidelines	21
Removing Preculture Media from the ECS	21

Inoculating Cells into the Bioreactor	21
General Protocol	22
Post Inoculation Flow rate Settings.....	24
Variable Flow rate	24
Daily Maintenance	26
Media Changes and Feeding Schedule	26
Media Utilization	27
Harvesting from the ECS	29
Low Volume ECS Harvest.....	29
Cell/Secreted Product Harvest	30
Post-harvest ECS Wash.....	30
High Volume ECS Harvest	31
Final Comments	32
Reference Guide.....	33
Overview of Hollow Fiber Bioreactor Cell Culture	33
Cell Lines List.....	36
Selecting a Growth Media.....	37
General Considerations.....	37
Serum-Free or Protein-Free Formulations	38
CO ₂ - Independent Media.....	38
Calculation of Glucose and Lactate Rates	39
Background	39
Glucose Consumption Rate	39
Lactic Acid Production Rate	41
Bibliography	42

Safety and Intended Use Information

- Please read this entire Instruction Manual before using the system.
- The CellMax Duo operates on 100-240 volts, 50/60 Hz.
- Do not open the CellMax Duo or ECU. THERE ARE NO USER SERVICEABLE PARTS INSIDE THE CELLMAX DUO SYSTEM.
- Do not submerge either the CellMax Duo or ECU in water
- The ECU is connected to the CellMax Duo by an 8ft motor cable. Do not place the ECU inside the incubator. The ECU is designed to operate outside the incubator environment. The ECU box contains a magnetic strip to allow the user to fix the box to the outer surface of an incubator.
- The CellMax Duo Hollow Fiber Cell Culture System is designed for the continuous culture of mammalian cells. This product is for laboratory use only. It is not intended for diagnostic or therapeutic use in humans or animal.
- Hollow Fiber Bioreactors are single use only disposables and can be used for culture of a single cell line. When the experimental protocols are completed, the hollow fiber bioreactor module should be decontaminated and discarded. Consult local institutional procedures for approved disposal methods of potentially hazardous waste. All disposal methods should comply with federal, state, and local requirements.
- To protect laboratory personnel, handle and dispose of all materials that have come into contact with biological materials as if they contain infections agents.

CellMax Duo System Components

Compare and identify the components of the CellMax Duo system with Figure 1 below and verify that you have everything before proceeding. The CellMax Duo System includes

- ✓ CellMax Duo Base
- ✓ Electronic Control Unit (ECU) and Power Supply
- ✓ Power Cord
- ✓ Teflon Pump Bars (2)
- ✓ Instruction Manual

Specifications

Dimensions:	10 x 13 x 6.5 inches
Weight:	5 lbs
Power:	100-240 VAC 50/60 Hz Input 0.8A
Flow rates:	5* - 120 ml/min (w/o bioreactor)
Drive Motor:	Brushless DC Motor
Environment:	20 - 42°C, 100% Relative Humidity CO ₂ Incubator (BASE ONLY)
Motor Cable:	Insulated Cat5 Tray Cable with Connector

*Flow rates below 5 ml/min can be achieved with the CellMax Duo Perfusion Kit, sold separately.

System Description

The CellMax Duo base consists of a two-position pump station and bottle holders to mount up to two bioreactor modules and reservoir bottles simultaneously.

As the pump motor rotates, a cam on the motor shaft forces the Teflon pump bars to depress the thick-walled pump tubing on the hollow fiber bioreactor module. This action forces culture media to flow from the media reservoir bottle through the gas permeable silicone flow path tubing into the hollow fiber bioreactor module.

The Teflon pump bars are asymmetric to allow different flow curves to be generated. One side of the Teflon pump bar has a section cut out to allow a smaller contact surface with the tubing. This reduces the overall displacement of the tubing to allow smaller flow rates to be achieved. See *Post Inoculation Flow rate Settings* section more information.

The CellMax Duo system is normally operated inside a humidified, temperature-controlled CO₂ incubator. The pH of the recirculating media is regulated via diffusion of CO₂ through the walls of the silicone tubing. For most cells, a CO₂ setting of 5-10% provides a media pH that is optimum for cell growth.

The CellMax Duo can also be used with CO₂-independent media. This allows the researcher to support the growth of most cell lines using a standard 37°C incubator or warm room.

ECU Operation

The ECU is connected to the DUO Base by the flat motor cable, and controls the speed of the pump motor. The ECU is placed outside the incubator for easy visualization of the control panel during operation. The ECU contains a magnetic plate on the back of the enclosure to attach to the wall of the incubator. The ECU is activated by moving the rocker switch on the front of the ECU to the ON position. The RED LED on the rocker switch ensures that the ECU is on. The variable speed dial on the front of the ECU can adjust the flow rate.

Front Panel Features

1. Rocker On/Off Switch

- The LED will turn RED when switched to the ON position and the pump has power.

2. Variable Speed Dial

- This dial is used to adjust the pump motor speed. The higher the pump setting, the faster the pump speed, and subsequently, the flow of media to the bioreactors.

Power Supply Features

1. AC Input Transformer

- The power cord connects here to plug unit into the power outlet. If the cord supplied with the unit does not fit the electrical outlet in your country, please contact our Customer Service department for assistance.

2. Cat5 Tray Cable

- Outlet for the motor cable connection between the ECU and the Duo Base.

Cell Culture Recommendations

Aseptic Technique

- Correct, sterile technique will ensure a long and productive life for your hollow fiber bioreactor module. Shortcuts, suspect media, and careless cell culture technique will result in contaminated hollow fiber bioreactors.
- Practice safe cell culture. After the initial setup of the CellMax Hollow Fiber Bioreactor, the only connections that should be opened are the reservoir bottle cap to flow path connections when replacing the bottle cap with a fresh bottle of media.
- ALWAYS disinfect the Clave® fitting and reservoir cap-to-bottle connections with a sterile alcohol pad prior to opening. All operations must be performed in a laminar flow hood.
- A good hollow fiber bioreactor cell culture practice is to cover the reservoir cap-to-bottle junction with a protective strip of parafilm.
- Protect your hollow fiber bioreactor system from user-borne contamination. Wear powder-free gloves and a lab coat at all times.
- Use the laminar flow hood properly. Keep it clean and uncluttered. Avoid rapid movements, room air currents, and working directly over the samples.
- Keep the CO₂ incubator clean. Always wipe up spilled media. Disinfect the inside of the incubator on a regular basis with an algae inhibitor such as Spectrum Clear Bath. Use sterile water treated with Clear Bath in the humidity pan.
- Liquid cell culture growth media and supplements are typically ready-for-use. If additions of antibiotics, serum, or other supplements are to be added to the growth media, the sterility of the supplemented media is only as good as the aseptic technique of the researcher. If supplemented growth media is used, it MUST be tested prior to use. Incubate the bottle of supplemented media, or an aliquot of the supplemented media, at 37°C for 1 – 2 days prior to using it for hollow fiber bioreactor cell culture to verify the sterility of the media.
- The hollow fiber bioreactor should be precultured for 2 – 4 days prior to inoculation of cells to check for leaks, contamination, and to perform a final equilibrium of the system. Use at least 100mL of the same media that will be used for culture. Change the media in the reservoir bottle and in the extra-capillary space (ECS) at least once before cell inoculation.
- Always allow bottles of growth media, serum, or supplements to warm up to room temperature prior to opening. The reduced pressure inside a cold bottle will draw in droplets of liquid and contaminants upon opening.

- When in doubt, use a new pipette, syringe, or bottle of media. It is more cost-effective to discard a bottle of suspect media or a pipette that may have touched the surface of the hood than it is to risk contamination.
- Always use a blunt needle to draw media or cells into a syringe. Droplets of media and/or cells on the outer surface of the Luer connection will foster contamination.
- If there is media on the Luer fittings, carefully wipe it from the Luer fitting with a sterile alcohol pad.
- The CellMax and AC transformer can be wiped down with a mild disinfectant. DO NOT spray with alcohol or submerge in water for cleaning or decontaminating.

Cell Lines

- Test each new cell line to ensure that the cells are free from Mycoplasma contamination. Infected cells can exhibit normal morphology, yet suffer severe losses in antibody production levels, secreted growth factors, or doubling time.
- Some cells may exhibit clonal variation in growth characteristics or secretion levels. If applicable, test clonally isolated cells for growth rate and secretion levels prior to hollow fiber bioreactor culture.
- Use rapidly dividing cells with at least 90% viability. Split the cells no more than 1 – 2 days prior to inoculation. Feed the cells with fresh media the night before inoculating them into hollow fiber bioreactors.
- Inoculate cells into the hollow fiber bioreactor immediately after harvesting. Do not store the cells on ice for extended periods of time prior to inoculation.
- If possible, use “conditioned” media when inoculating cells in the ECS. For fastidious or slow growing cells, increasing the serum concentration in the ECS to 20 or 30% will increase viability and facilitate growth.

Startup Supplies

Below is a list of items that are most often needed by new users of the CellMax Duo. This Startup Kit should enable the researcher to immediately begin hollow fiber bioreactor cell culture. Not included in this list are pipettes, media, and media supplements.

- ✓ 10 cc Luer-Lok Syringes *for harvesting cells and ECS media feeding*
- ✓ 30 cc Luer-Lok Syringes *for harvesting cells and ECS media feeding*
- ✓ Alcohol Prep Pads *for disinfecting luer fitting and cap/bottle seals*
- ✓ 15 gauge blunt needles *for media and cell transfer into syringes*
- ✓ Autoclave bags (10x15in) *for cap/bottle sterilization*
- ✓ Sterilization Wraps or Aluminum Foil *for covering caps and luers for sterilization*
- ✓ Male Luer-Loks *sterile, replacement luer caps*
- ✓ Bottles, 500ml and 125ml *for use as media reservoirs*

The list below summarizes additional materials or kits that will be needed for hollow fiber bioreactor cell culture.

- ✓ Glucose and L-Lactate Analyzer
 - or-
- ✓ Blood Glucose Analyzer
 - or-
- ✓ Lactate Test Reagents
- ✓ Lactate Standards
 - or-
- ✓ Blood Glucose Kits

CellMax Duo System – System Setup

1. Wipe down the Duo Base with alcohol or a mild disinfectant to remove dust particles on the system. Do not directly spray the Duo Base with 100% alcohol.
2. Connect the 8 foot base cable on the Duo Base to the ECU.
3. Connect the main power cord to the ECU Power Supply and plug into a wall outlet.
4. Turn the ECU on. The RED LED on the ECU will turn on to indicate the unit is functioning.
5. At each pump station on the Duo Base, lift the spring-loaded door pin, swing the pump door open.
6. Push against the pump bars at the one station with your fingers to check that the pins are pumping (moving in and out).
7. The Duo Base can now be placed in a humidified CO₂ incubator.
8. The incubator door should readily close with the motor cable coming between the door and the door seal. Use the magnetic backing on the ECU to attach it the incubator face. The cable is also long enough to permit the ECU to sit on top of a tall incubator or on an adjacent counter.
9. **WARNING: Do not store the DUO Base in the incubator for extended periods of time with the power to the ECU turned off!**

CellMax Duo System – Module Set-up

Prior to inoculating cells into the hollow fiber bioreactor, the researcher must first perform the following procedures:

- ✓ Sterilize the reservoir cap/bottle assembly.
- ✓ Connect the sterile reservoir cap/bottle assembly to the hollow fiber bioreactor
- ✓ Connect the hollow fiber bioreactor module to the flow path, if necessary
- ✓ Preculture the system with complete growth media

NOTE: Successful, contamination-free hollow fiber bioreactor cultures are directly correlated to correct aseptic technique, spotlessly clean incubators and the proper use of laminar flow hoods.

Sterilization of the Reservoir Cap/Bottle Assembly

Two size reservoir caps are available from Spectrum Labs:

33 mm Reservoir Caps

- ✓ Insert the 33 mm reservoir cap into a clean glass media bottle. Tighten the cap one-half turn. The cap must be loose enough on the bottle to allow steam to penetrate. A 125mL glass bottle is convenient and fits easily into most autoclave bags. However, any size glass media bottle with a 33 mm neck will work.

38 mm Reservoir Caps

- ✓ Insert the 38 mm reservoir cap into a clean glass media bottle. Tighten the cap one-half turn. The cap must be loose enough on the bottle to allow steam to penetrate. A 125mL glass bottle is convenient and fits easily into most autoclave bags. However, any size glass media bottle with a 33 or 38 mm neck will work.

Note: The 38 mm Reservoir Cap fits most square plastic media bottles. These are NOT autoclavable.

1. Wrap the luer fittings on the reservoir cap assembly with autoclave paper or foil, and secure with autoclave tape for sterilization.
2. Place the reservoir cap/bottle assembly into an autoclave bag and seal with autoclave tape.
3. Steam autoclave the reservoir cap/bottle assembly at 121°C. If possible, use a sterilization program that includes a dry cycle. This will reduce the amount of liquid within the autoclave bag and the possibility of the wet, post-autoclaved bag from tearing. Wet autoclave paper is not a sterile barrier.

Note: Failure to adequately autoclave the reservoir cap/bottle assembly will result in contamination of the Hollow Fiber Bioreactor System.

4. After sterilization, immediately remove the autoclave bag containing the reservoir cap/bottle assembly from the autoclave and place it in a laminar flow hood to dry.
5. Reservoir caps are reusable. After a hollow fiber bioreactor has been used and discarded, the cap must be decontaminated and the tubing removed from the stainless steel tubes on the reservoir cap. The cap assembly should be cleaned with a dilute solution of a non-ionic, non-toxic detergent, and then immersed in distilled water for 60 minutes to remove any residual detergents or contaminants. Thoroughly rinse the cap with distilled water before reuse.

Note: Always replace the cap tubing before reuse. A Cap Tubing Kit is included with each CellMax Hollow Fiber Bioreactor for this purpose.

Reservoir Cap to Flow Path Connections

Perform all operations in the laminar flow hood.

1. To connect the inlet and outlet tubes on the sterile reservoir cap/bottle assembly to the inlet and outlet tubes on the flow path, first collect the following material in a clean uncluttered laminar flow hood:
 - ✓ Newly autoclaved reservoir cap/bottle assembly in sealed autoclave bag.
 - ✓ CellMax Hollow Fiber Bioreactor in sealed packaging
 - ✓ One bottle of complete growth media
 - ✓ Sterile 50mL conical centrifuge tubes (2 each)
 - ✓ Alcohol wipe pads (10-20 each)
 - ✓ Sterile 30 cc syringes (2 each)
 - ✓ Sterile blunt needles (2 each)
 - ✓ Scissors
2. Remove the CellMax Hollow Fiber Bioreactor from the wrapped sterile package.
3. Remove the sterile reservoir cap/bottle assembly from the autoclave bag. There is no directionality for the reservoir cap inlet and outlet tubes. Therefore, both one of the reservoir cap inlet and outlet tubes can be connected to either of the flow path inlet and outlet luer fittings.
4. Remove the autoclave paper from one of the reservoir cap luer fittings.

5. Remove the luer cap from the luer fitting of one of the two silicone flow path tubing ends and connect it to the exposed reservoir cap luer fitting.
6. Repeat steps 4 and 5 to connect the remaining flow path luer fitting to the remaining reservoir cap luer fitting.
7. Unscrew the reservoir cap, and fill the empty, sterile glass bottle with approximately 125mL of culture media.

NOTE: A minimum of 100mL of complete media should be used for preculture. Remember that the flow path and bioreactor hold approximately 35mL of media.

NOTE: The preculture media should be the same formulation that will be used for the actual cell culture. Expensive components such as cytokines can be omitted at this step. However, serum, if used, should be added to the preculture media.



Figure 1: Flow Path to Reservoir Cap Assembly

Flow Path to Bioreactor Connections

Perform all operations in the laminar flow hood.

Spectrum has introduced a new line of Bioreactors that are *preflushed, fully assembled and ready to use*. If you have ordered a previous generation of CellMax Bioreactor, where the flow path and bioreactor module are not preassembled, please connect the inlet and outlet tubes of the flow path to the inlet and outlet of the module fittings as described below.

1. Notice that the bioreactor module inlet and outlet, and the flow path silicone tubing ends, are sealed with luer caps and are held together by plastic cable ties. Carefully cut the cable ties with scissors to separate the tubing ends, taking care not to cut the silicone tubing.
2. Close the inlet and outlet tubing on the module with the slide clamps to prevent water from leaking from the bioreactor.
3. Remove the luer caps from the inlet (left) end port luer fitting of the module and from the inlet (left) flow path luer fitting. Aseptically connect these two fittings.
4. Remove the luer caps from the outlet (right) end port luer fitting of the module and from the outlet (right) flow path tubing luer fitting. Aseptically connect these two fittings.

Removal of Bioreactor Storage Solution

Perform all operations in the laminar flow hood.

As described previously, Spectrum has introduced a new line of Bioreactors that are *preflushed, fully assembled and ready to use*. If you have ordered a previous generation of the CellMax bioreactor, where storage solution is present, remove the solution as described below.

1. Aseptically remove the luer caps from the side port tubing.

NOTE: If you intend to reuse the luer caps, make certain they remain sterile.

2. Attach one sterile 20 cc syringe to each of the luer side port fittings. One syringe must have the plunger pulled out, while the other is pulled down.
3. Remove the storage water from the ECS by a simultaneous pushing in with one syringe and withdrawing the water with the second syringe.
4. Disconnect the syringe from the side port and discard.

Preculture

The hollow fiber bioreactor should be precultured at 37°C in a tissue culture incubator for at least 48 hours. The media in the ECS and the reservoir bottle should be changed at least once prior to the final media change and cell inoculation.

It is crucial to successful hollow fiber bioreactor cell culture to preculture the system for at least 2 days prior to inoculating cells into the bioreactor. Preculturing provides a sterility check for the newly assembled hollow fiber bioreactor, purges any remaining water from the membrane walls, and saturates the hollow fiber with culture media.

Filling the Bioreactor ECS with Preculture Media

The proper procedure for media transfer to the ECS is as follows:

1. Close the inlet and outlet slide clamps on the flow path to isolate the bioreactor from the flow path.

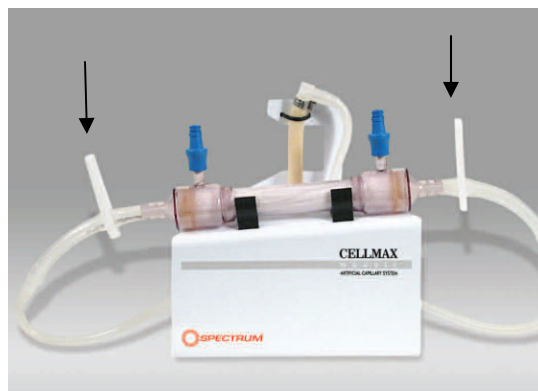


Figure 2: Close the inlet and outlet slide clamps

2. Disinfect the surface of the access ports with an alcohol swab.



Figure 3: Disinfect side access ports

3. Fill a 50 mL conical centrifuge tube with approximately 25 mL of media. One can also use a cell culture dish to hold media or cells.

NOTE: Always use a blunt needle to transfer media or cells from the 50 mL centrifuge tube or dish in the syringe.

4. Fill the syringe with approximately 20 mL of media from the conical centrifuge tube.
5. Aseptically place the sterile, protective cover back on the blunt needle.
6. Remove the blunt needle from the syringe and place it in a secure position.

NOTE: The blunt needle with its protective cover can be reused as long as it remains sterile. If in doubt about the sterility of the needle, always use a new blunt needle from an unbroken, sterile package.

7. Connect the syringe to the side port luer fitting, open the slide clamp and inject the growth media from the full syringe into the ECS using the empty syringe to pull the displaced air and media from the ECS.

NOTE: The new CellMax Bioreactors are assembled with a sterile access port instead of the tubing assembled with a slide clamp. Firmly connect the syringe to the access port as the silicone rubber port will apply resistance.



Figure 4: Attach syringe to side access port

8. Gently flush the media through the bioreactor 4 or 5 times to flush the ECS completely. Displace all air bubbles from the ECS into either of the two syringes. Tilt the right side of the system up if necessary to remove all of the air.

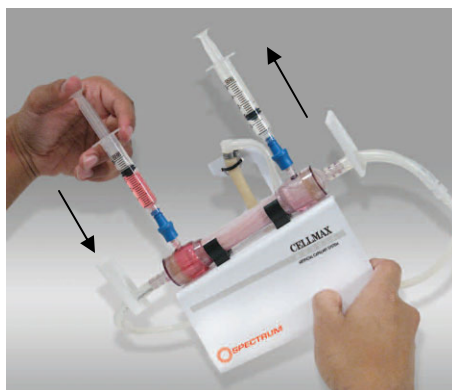


Figure 5: Tilt the system up to remove all air

9. Clamp off the side port tubes with the slide clamps for bioreactors if applicable (see Note above).

NOTE: It is permissible to leave media in one or both the syringes during the preculture procedure.

10. Release the slide clamps on the bioreactor inlet and outlet tubing.
11. Check and, if necessary, retighten all luer connections.

NOTE: Most researchers leave the syringes connected to the side ports. Doing so will not affect cell growth or interfere with removal or installation of the hollow fiber bioreactor onto the Duo. Syringes are easily replaced if their sterility becomes suspect and are easier to manipulate than the luer caps.

12. If you decide to use the luer caps, be certain that they are sterile. Remove the syringes and replace the luer caps on the side port tubing if using a previous generation CellMax Bioreactor.

Priming the Flow Path

Prime the flow path by firmly compressing and releasing the thick-walled pump station tubing with your fingers until the media has completely filled the flow path and module. Tilt the bioreactor with the outlet side up to allow air bubbles to flush out of the module. Be certain that all air bubbles have been flushed from the inlet and outlet of the hollow fiber bioreactor assembly.

Attaching the Hollow Fiber Bioreactor to the Pump Station

1. At one of the Duo pump stations, lift the spring-loaded door pin and swing the pump door open.
2. Position the pump tubing of the hollow fiber bioreactor over the pump bar at the station.
3. Close the pump door over the pump tubing until the spring-loaded door pin snaps into position.
4. Verify that all sections of the flow path tubing are free from kinks.
5. Operate the system at maximum flow rate in a 37°C incubator for at least 48 hours to verify that there are no leaks and the system is sterile. The media in the ECS and the reservoir bottle should be replaced prior to cell inoculation.
6. Air bubbles may collect on the inlet side of the module during 15 – 30 min of operation. These air bubbles will prevent perfusion of oxygen and nutrients through the upper rows of hollow fibers. If air bubbles do accumulate chase the bubbles into the reservoir by tilting the bioreactor while it is pumping so that the outlet port rises up.

Cell Inoculation

Guidelines

There important considerations to follow are:

- Use rapidly dividing cells with at least 90% viability. Split the cells no more that 1 – 2 days prior to inoculation. Feed cultures the night before harvesting.
- Minimize the time between harvesting and inoculation. Do not store the cells on ice for extended periods of time prior to inoculating them into the bioreactor.
- If possible, use conditioned media when inoculating cells into the ECS. For fastidious or slow growing cells, increasing the serum concentration of the ECS media to 20 or 30% for the first several days will greatly facilitate cell growth.

Removing Preculture Media from the ECS

1. Clamp off the silicone tubing at the inlet and outlet ports of the hollow fiber bioreactor with the slide clamps.
2. Remove the preculture media from the ECS by simultaneously pushing in with one syringe and withdrawing the preculture media with the second syringe.
3. Wipe the outside of the two side port Clave fittings with several alcohol pads.
4. Remove the syringe containing the ECS preculture media from the side port and attach the blunt needle to the syringe. Discard the preculture media into a waste container. Proceed immediately to inoculate cells in the ECS. Do not let the hollow fiber membranes dry out.

Inoculating Cells into the Bioreactor

Start with the harvest cells to be inoculated in a sterile 50 mL centrifuge tube. Harvested cells should be centrifuged and resuspended in the desired growth media (conditioned, high serum, serum-free, etc).

Inoculate cells in the ECS.

Recommendations as to the cell number and volume are contained in the product information sheets for each hollow fiber bioreactor.

NOTE: Media can be ultrafiltered through the membrane, therefore the slide clamps on the inlet and outlet end ports of the hollow fiber bioreactor MUST BE CLOSED prior to inoculating cells.

NOTE: Do not forget to release the slide clamps after inoculation.

General Protocol

1. Close the inlet and outlet tubing with the slide clamps to prevent ultrafiltration during inoculation
2. Resuspend approximately $3 - 7 \times 10^7$ cells in a volume of conditioned media 1.5 times the ECS volume of the bioreactor module.
3. Wipe the syringe-to-side port luer connection with alcohol pads and remove one of the side port syringes.
4. Connect a blunt needle to the syringe and gently load the cell suspension into the syringe.
5. Remove the blunt needle from the syringe and place it in a secure position.

NOTE: The blunt needle with its protective cover can be reused for the several steps involved in a single hollow fiber bioreactor inoculation as long as it remains sterile. If in doubt about sterility of the needle, always use a new needle from a sterile, unbroken package.

6. Flush the cell suspension gently through the module 4 or 5 times to uniformly and completely fill the ECS with cells, and to displace all air bubbles from the ECS into either of the two syringes.

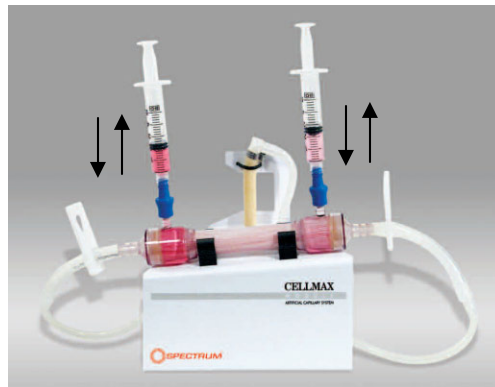


Figure 6: Slowly flush the cells back and forth

7. Open the slide clamp on the module outlet tubing. Vent the reservoir bottle by loosening the cap $\frac{1}{4}$ turn.
8. Using firm but gentle pressure on the syringes, transfer the remaining cell suspension into the ECS by forcing the excess media in the syringes through the membrane (ultrafiltration). Excess media will enter the membrane lumen and flow path.
9. Do not leave residual media and/or cells in the syringes. Cells trapped in the syringe will quickly die. The dead cells will lead to false interpretation of cell viability at the next ECS harvest.

10. Close the slide clamps on the side port tubing and tighten the cap on the reservoir bottle. Bioreactors with the Clave fitting will omit this step.

NOTE: Most researchers prefer to leave the syringes connected to the side port luer fittings or Clave fitting. If you decide to use this technique you must dispose of any excess cells contained in the syringe.

11. Alternatively, if you wish to use sterile luer caps to seal the side port luer fittings, remove and discard the syringes and aseptically attach new sterile luer caps to the side port luer fittings. Bioreactors with the Clave fitting will omit this step.
12. Release the slide clamps on the inlet and outlet of the module end ports.
13. Failure to release the slide clamps will prevent the flow of media to the cells. Complete loss of cell viability will occur within 30 – 60 minutes if media flow is restricted.

Post Inoculation Flow rate Settings

The CellMax Duo has been engineered to provide flow rates from 0 – 120 mL/min.

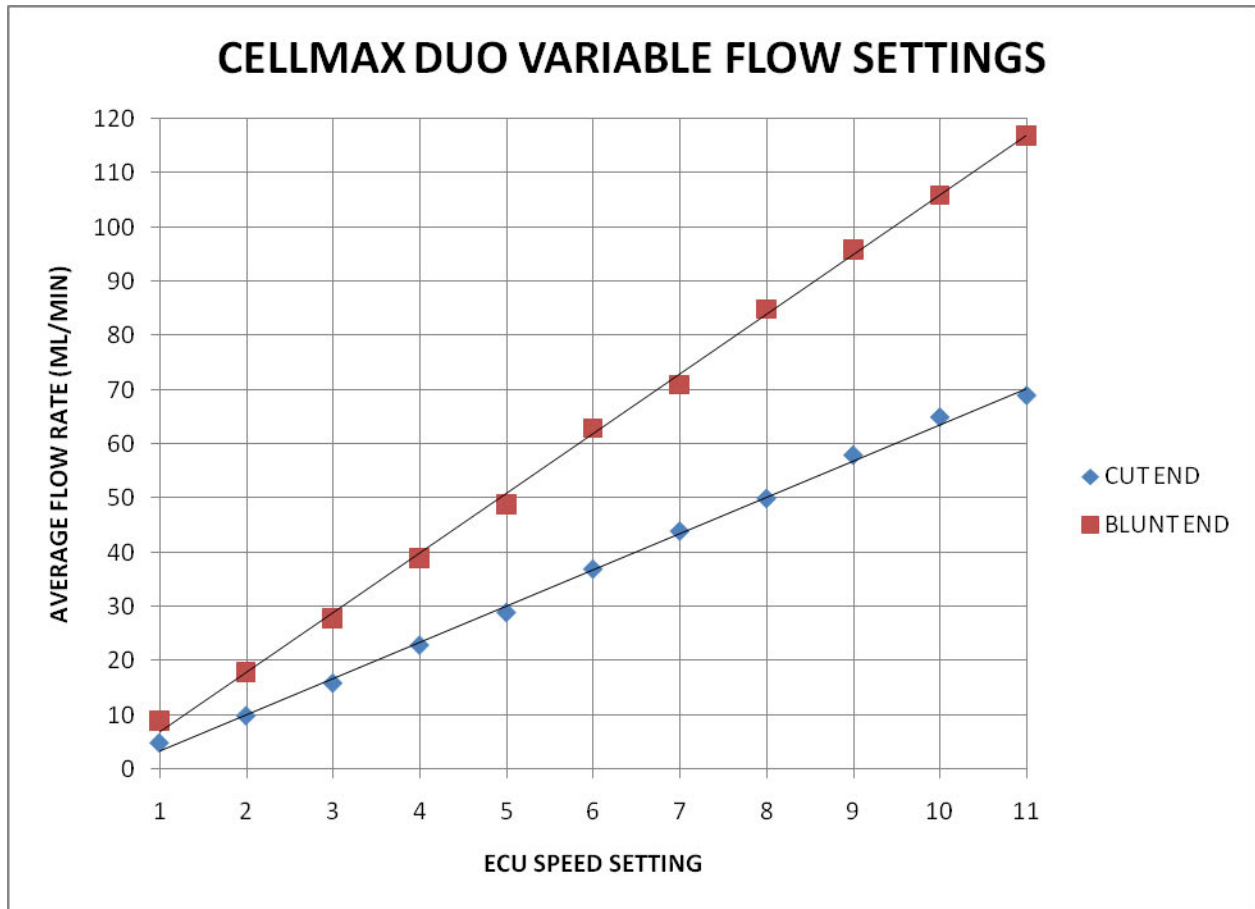
1. Incubate cultures without flow for approximately 5-10 minutes at 37°C after inoculation. This incubation period is designed to allow cells to settle on and around the network of hollow fibers.
2. Attach the bioreactor to the pump station to initiate flow. Based on your starting inoculums, set the flow rate as follows for the first 24 hours of culture:
 - 5 mL/min for less than 1×10^7 cells (ECU setting 1- Teflon Bar Pointed End)
 - 25 mL/min for $1 - 5 \times 10^7$ cells (ECU setting 4 - Teflon Bar Pointed End)
 - 50 mL/min for $5 - 10 \times 10^7$ cells (ECU setting 8 - Teflon Bar Pointed End)
3. Suggested flow rate settings after the first 24 hours based on daily calculation of lactate production rates:
 - 5 mL/min for less than 50mg/day lactate production
 - 25 mL/min for 50 – 200 mg/day lactate production
 - 50 mL/min for 200 – 2000 mg/day lactate production

Variable Flow rate

When more than one culture is being maintained at a time, it will be necessary to individually adjust the flow rate at a single pump station for a new culture.

The CellMax Duo Cell Culture system is designed to permit variable flow rates for each individual culture from 0 – 120 mL/min. Flow rates are controlled by adjusting the Pump Setting on the ECU, and by changing orientation of the Teflon Pump Bar.

The Teflon Pump bar is asymmetric. One end of the bar is blunt and the other side has been cut down to reduce the surface area in contact with the tubing. The blunt end allows maximum displacement of the tubing during each stroke. The pump curve depicts the relationship between the Teflon Bar orientation, the speed setting on the ECU and the flow rate generated. The flow rates generated in the graph are without a bioreactor in place. You may see minor variations based on the surface area of the bioreactor used.



Example:

One established culture is being maintained at DUO pump station #1 at the recommended flow rate of 50mL/min (ECU setting 5, Teflon bar blunt end). A new culture is started with an initial cell inoculum of 3×10^7 cells, and placed at pump station #2. To achieve the recommended 25mL/min flow rate (a 50% decrease) during the first 24 hours of culture, orient the Teflon bar with the pointed end facing the bioreactor tubing at pump station #2.

Daily Maintenance

Media Changes and Feeding Schedule

The rate of growth and media consumption will be different for each cell line. Therefore, feeding schedules and the rate of media replacement will vary. Depending on the cell type, the reservoir bottle can be replaced with a fresh bottle, or fresh media can be added to the conditioned media in the reservoir bottle.

To replace the media in the reservoir bottle with a fresh bottle of media:

1. Wipe the outside of the reservoir cap/bottle seam with a sterile alcohol pad. Repeat with a second alcohol pad. Sterile surfaces are required to avoid contamination.
2. Unscrew the reservoir cap and carefully lift it away from the media bottle.

NOTE: Do not touch the metal tubes of the reservoir cap to the outside of the bottle neck or to any other non-sterile surface.

3. Insert the reservoir cap tubes into a prewarmed bottle of fresh media and tighten the cap.
4. Wrap the reservoir cap/bottle interface with parafilm to prevent dust from collecting on this surface.
5. Start with a newly inoculated bioreactor with a fresh 125 mL bottle of media to maximize accumulation of conditioning factors during the first few days of culture. The total volume of growth media will be 160 mL, partitioned with 35 mL of preculture media in the flow path and 125 mL of fresh growth media in the reservoir bottle.
6. Replace the media in the 125 mL bottle once, and then shift the system to a 500 or 1000 mL bottle. With most cell lines, media usage by day 7 – 10 will reach 500 – 1000 ml per day.
7. One can usually reduce the serum concentration in the media by a step wise change at each subsequent replacement of the media; i.e., 10% to 7.5%, 7.5% to 5%, 5% to 2.5%, and so on. The lower limit of serum reduction seems to be at the 1 to 2% level. Monitor the daily glucose and/or lactate rate(s). If the rate(s) decrease significantly subsequent to a reduction in serum, increase the serum to the previous level.

Media Utilization

It is essential that the levels of glucose and/or lactate be measured, and that the rates of glucose consumption and/or lactate production must be calculated daily.

General Comments

Monitor the daily rates of glucose and/or lactate production. This measurement will provide you with a window in the metabolic state of the cells and an accurate measurement of the rate of cell expansion. Knowing the mg/day of glucose and/or lactate produced will allow you to predict the amount of media that the CellMax system will use.

Healthy populations of cells in a hollow fiber bioreactor culture usually display equimolar depletion of glucose with concomitant production of lactate. The media should be replaced when the glucose concentration is approximately 50% of the starting glucose concentration. Please refer to the Glucose Consumption Rate section of this manual.

- For RPMI-1640 with 2.0g/L of glucose, this means changing the reservoir bottle when the glucose concentration is 1.00 to 1.50 g/L. This will correspond to a lactate concentration of 1.00 to 0.50 g/L.
- For DMEM with high (4.5 g/L) glucose, the media should be changed when the glucose concentration reaches 2.0 to 1.5 g/L (lactate levels of 1.50 to 2.00 g/L).

Low Molecular Weight Hollow Fiber Bioreactors

These bioreactors require daily maintenance and monitoring the lactate production rate due to the low molecular weight cutoff (MWCO) properties of the hollow fiber. Potential cytostatic or cytotoxic secreted products can accumulate to high levels within the ECS. Most serum proteins will not diffuse in the ECS. Therefore the ECS in the low molecular weight modules should be fed on a regular basis.

Early in the culture (days 1 -5), when the cells are adapting and beginning to fill the ECS, no special feeding is needed. Once the glucose and/or lactate rates reach 100 – 300 mg per day level, a 3 – 4 day cycle of harvesting is needed. When the system reaches glucose and/or lactate rates of 1000 – 1500 mg/day, daily ECS harvest are strongly recommended to maintain optimum viability and secretion rates.

High Molecular Weight Hollow Fiber Bioreactors

These bioreactors require less maintenance than the low molecular weight hollow fiber bioreactors. The researcher should measure the daily rate of lactate production as a physiological monitor on the state of the cells. The media should be replaced when the glucose concentration reaches approximately 50% of the glucose concentration of fresh media.

- For RPMI-1640 with 2.0 g/L of glucose, this means changing the reservoir bottle when the glucose concentration is 1.0 to 1.5 g/L. This will correspond to a lactate concentration of 1.0 to 0.5 g/L.

- For DMEM with high (4.5 g/L) glucose, the media should be changed when the glucose concentration reaches 2.0 to 1.5 g/L (lactate levels of 2.5 to 3.0 g/L).

Harvesting from the ECS

The techniques and precautions used to harvest the cells from the ECS are identical to those described for inoculating cells into the bioreactor. Detailed instructions for harvesting cells or secreted products are provided in the CellMax Application Guide.

When harvesting cells and/or secreted products from the ECS one has to decide whether to collect the sample in a small volume where total recovery may be low, or in larger volumes where higher recovery is achieved with concomitant dilution of product.

For example: ECS harvest of IgG from a 1700cm² Cellulosic Module:

1 st wash	10 mL	1.45 mg/mL	14.5 mg (56%)
2 nd wash	12 mL	0.65 mg/mL	7.8 mg (30%)
3 rd wash	12 mL	0.30 mg/mL	3.6 mg (14%)

Therefore, there will be a compromise between recovery of the desired product or cells in a concentrated form and efficiency of recovery. Each application must be tested by the individual researcher. Procedures for both Low Volume and High Volume harvests are discussed below.

Regardless of the harvesting method chosen, a post-harvest wash must be performed to remove excess cells from the ECS.

Low Volume ECS Harvest

Many researchers prefer to minimize the volume of harvested antibody by collecting only the ECS media by syringe displacement. An alternative harvest technique is to flush the ECS with several 10-20 mL washes of media or buffer. Each wash can then be quantified for recovery.

In the 1700cm Cellulosic module, approximately 60-75% of the cells and/or secreted product will be contained in the first 11 – 15 mL ECS harvest. A second 15 -20 mL ECS wash will contain another 5 -20% of the cells and/or secreted product.

Cell viability may be lower than expected (75 – 80%). In a mature hollow fiber bioreactor, the cells that are more easily flushed out of the ECS during harvesting may be of much lower viability than those that are more firmly attached to the hollow fiber membrane. Daily harvests of a mature bioreactor (lactate rates of 750 – 2000 mg/day) results in a higher viability of harvested cells and increased production of secreted products.

Always use a blunt needle to draw media or cells into the syringe from a conical centrifuge tube or to dispense harvested media or cells from the syringe. Droplets of media or cells on the syringe or side port connection are certain to foster contamination.

If you spill media or if media leaks from the syringe or bioreactor onto a luer fitting, carefully wipe it from the luer fitting with a sterile alcohol pad.

Always clamp the end ports during the harvest of cells to avoid minor loss of media during the harvesting. In high molecular weight cut off hollow fiber bioreactors, the media and certain small molecules may pass easily be pushed through the membrane with the syringes attached to the side ports.

Cell/Secreted Product Harvest

1. Gently withdraw the cell suspension from the ECS into the closed syringe while simultaneously pushing air into the ECS with the other syringe.

Avoid air bubbles and rapid or forceful media displacement through the ECS.

2. Close off the side port tubing on the syringe containing the cell suspension with the slide clamp. Bioreactors with the Clave fitting will omit this step.
3. Disinfect the syringe to side port luer connection of the syringe containing the cell suspension with several alcohol pads.
4. Remove the cell suspension syringe from the side port luer and attach a sterile blunt needle. Store the protective cover in a safe place within the hood where it will remain sterile. Microfuge racks are a good place to store the cover to the blunt needle.
5. Transfer the cell suspension into a 50 mL conical centrifuge tube using gentle syringe pressure.

Post-harvest ECS Wash

The low volume ECS harvest must be followed by at least one 20 – 30 mL ECS wash to remove excess cells. Cells on the hollow fiber membranes grow rapidly. Failure to perform an adequate post-harvest ECS wash at each harvest will eventually result in an occluded bioreactor.

1. Fill a 50 mL conical centrifuge with 25 – 50 mL of growth media.
2. Withdraw about 20 – 30 mL of media into the (now empty) syringe.
3. Aseptically cover the blunt needle with the protective cover and remove it from the syringe.
4. Store it in a position where it will maintain sterility.
5. Reconnect the syringe to the side port. Use caution to avoid contaminating the syringe, needle, or end port luer fitting.
6. Open the slide clamp and fill the ECS with fresh media.

7. Use firm, but gentle syringe pressure to wash the ECS with media back and forth into the syringes.

Avoid air bubbles and rapid or forceful media displacement through the ECS.

8. Close of the side port tubing on the cell suspension syringe with the slide clamp.
9. Disinfect the syringe-to-side port luer connection of the syringe containing the cell suspension with several alcohol pads.
10. Remove the cell suspension syringe from the side port luer and attach a sterile blunt needle. Store the protective cover in a safe place within the hood where it will remain sterile.
11. Transfer the cell suspension into a 50 mL conical centrifuge tube using gentle syringe pressure.
12. Do not leave residual media and/or cells in the syringes. Cells trapped in the syringe will quickly die. The dead cells will lead to false interpretation of cell viability at the next ECS harvest. After the final ECS wash, collect all of the media and/or cells into one syringe. Aseptically remove it and discard the cells.
13. Attach the syringe to the side port tubing and seal off the side port tubing with the slide clamps.
- 14. Open the Inlet and Outlet Flow Path Slide Clamps.**
15. Return the CellMax bioreactor to the incubator and reconnect the power cable to the pump system.

NOTE: Failure to release the slide clamps will prevent media flow to the cells. Complete loss of cell viability will occur within 30 – 60 minutes if media flow is restricted.

High Volume ECS Harvest

- Depending on the needs of the researcher and the growth characteristics of the cell line, one can utilize several moderate volume (10 – 25 mL) washes or one large volume (50 mL) ECS wash.
- If you desire to recover secreted products in a reduced volume, it is best to first harvest the media and cells from the ECS, followed by a vigorous flush of the ECS with 50 -100 mL of media. The wash protocol described above for the Low Volume ECS Harvest should be followed, using sufficient wash cycles to recover the needed cells.
- Always use a blunt needle to draw media or cells into the syringe. Droplets of media or cells on the syringe or side port connection will be certain to foster contamination.

- Syringe size is optional. Some researchers prefer to use 30 or 60 cc syringes, others prefer to use multiple 20 cc syringes. Make certain that the syringe is sterile.
- If you spill or leak media, carefully wipe it from the luer fitting with a sterile alcohol pad.

Final Comments

Cells and cell products can be harvested using syringes attached to the side ports. To completely recover cells, it will be necessary to flush the ECS several times with fresh media. Alternatively, a portion of the cells can be left in the ECS and further expanded. Refer to the bioreactor package insert for additional information.

Loss of cells during harvesting of cell secreted products from the ECS can be prevented by attached a sterile, 0.2um – 0.5 um filter to the side port luer and slowly withdrawing fluid from the bioreactor through the filter. Alternately, harvested cells can be pelleted by centrifugation, resuspended in fresh culture media, and re-inoculated into the bioreactor.

Reference Guide

Overview of Hollow Fiber Bioreactor Cell Culture

Cell Culture has traditionally been performed by finding conditions in which cells derived from a three-dimensional tissue are adapted to grow on a two dimensional plastic surface of culture dishes or T-flasks. The microenvironment of such cells is reflected by a non-physiological “feast or famine” growth cycle. Immediately after inoculation the cells experience the conditioning phase of their growth cycle, followed by rapid cellular growth and media utilization. The last growth phase for cells cultured by such conventional technologies is a low carbon source/lactic acid-rich environment in which cellular metabolism is severely reduced. Replenishing the media restores the cells to media rich in nutrients but depleted in cell-specific secreted products, where they once more begin the process of conditioning the growth media.

In the CellMax Hollow Fiber Bioreactor, cells are inoculated into the extra-capillary space (ECS) of the module and settle on the outer surface of the membranes. The cells are subsequently nourished within this network of hollow fibers, where nutrients and oxygen in the perfusing media readily diffuse through the membrane walls to nourish the cells.

Metabolic waste products diffuse away from the cells and are diluted in the circulating media. If the molecular weight of secreted product is too large to diffuse through the membrane walls, it will accumulate at high concentration within the ECS. Conversely, if the molecular weight cutoff is larger than the secreted product, it will diffuse through the pores of the hollow fibers into the perfusing media.

Hollow fiber bioreactor cell culture, therefore, provides a three-dimensional growth environment in which there is cell-cell contact, rapid influx of oxygen and nutrients into the ECS with concomitant diffusion of metabolic waste across the membrane walls where it is diluted into the reservoir bottle. Secreted growth factors, cell-specific and often feedback-regulated within the sphere of influence of each cell, are preserved within the ECS thereby maintaining a stable environment that approaches *in vivo* physiological conditions.

In many applications, the combination of physiological concentrations of nutrients, oxygen, growth factors and physical support, with low levels of metabolic waste enable the cells to fill the entire extra-capillary space, forming nearly solid cell masses within the network of hollow fibers.

CellMax Hollow Fiber Bioreactors therefore, enables the cells to accumulate autocrine factors for optimum cell growth conditions. Consequently, cellular requirement for exogenous serum supplements is normally reduced 5 to 10 fold. Secretion of cellular products, be they natural or recombinant, can be enhanced 10 fold or more.

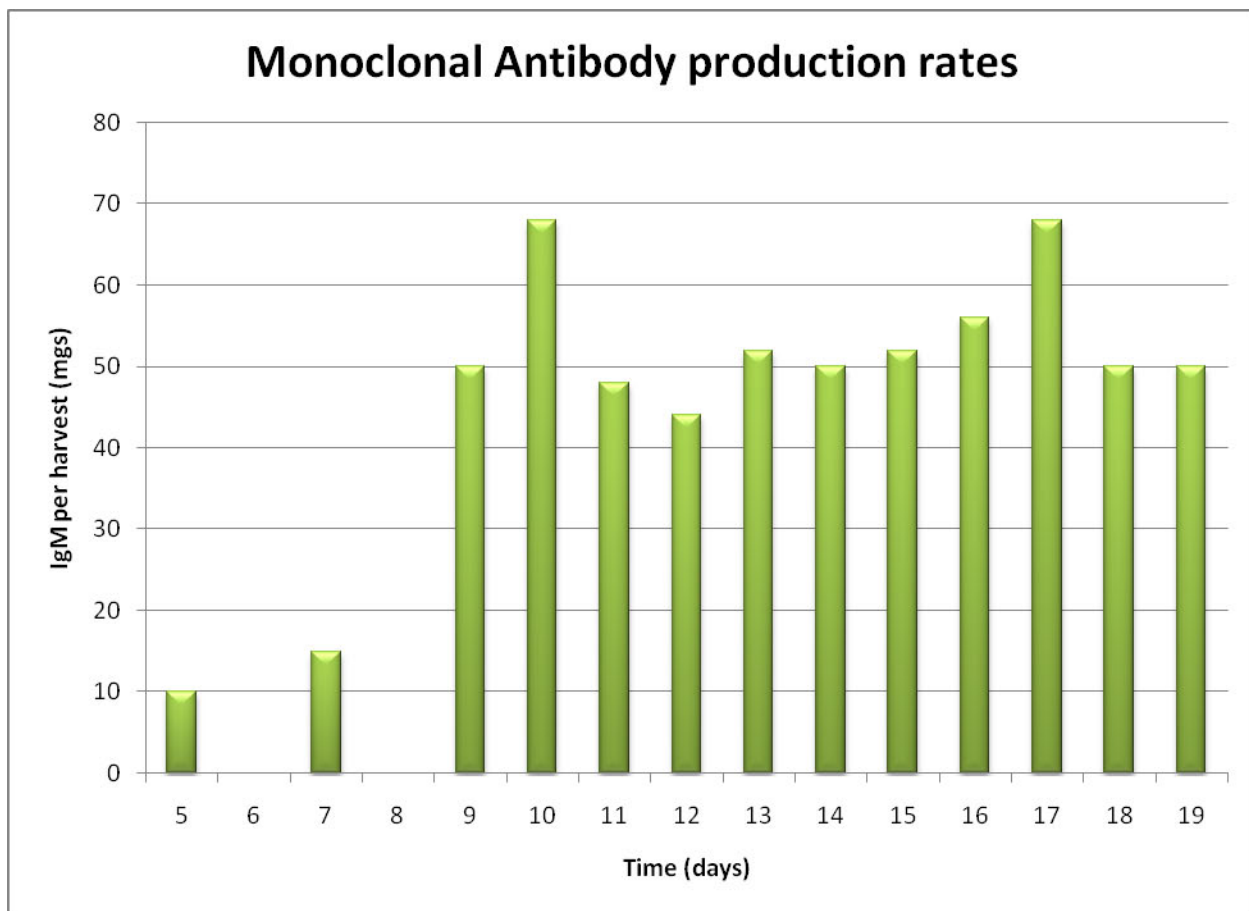
Cells grown in hollow fiber bioreactors are characterized by a reduced serum requirement, enhanced secretion of natural or recombinant proteins, and the ability to be maintained as a rapidly growing population of cells for up to several months. Daily maintenance consists of

monitoring the glucose and/or lactate concentrations in the reservoir bottle. Feeding the cells is a simple matter of changing the media bottle when the media has become depleted.

The CellMax system operates in a standard CO₂ incubator where oxygen from the incubator's atmosphere diffuses through the gas permeable walls of the system's silicone tubing providing oxygenation and pH control. Equilibration of the media within the hollow fiber bioreactor modules to the temperature and gases within the incubator is rapid. If one desires to use a CO₂-independent media, the CellMax can be operated in any incubator that can be temperature regulated at 37°C.

CellMax Bioreactors can be quickly removed from the incubator and transported to the cell culture hood for sampling and harvesting. Cells and/or their secreted products are easily harvested from the extra-capillary space, with cell harvests reaching 5 x 10⁹ lymphoid cells per bioreactor.

Yields of IgG or IgM from hybridoma cultures can reach 20 to 60mg per day. The concentration of antibody contained in the ECS ranges from 1 to 5 mg/mL, concentrations that rival ascites production in mice. In addition, the ability to reduce serum to 1 to 3%, or to adapt to serum-free or protein-free media formulations, allows the researcher to harvest secreted products in a media that significantly reduces downstream processing times. Unlike ascites, predictable antibody harvests can be performed on a daily basis.



CellMax Hollow Fiber Bioreactor have been successfully used to culture a broad spectrum of cell types; primary and established, normal and transformed, adherent, and non-adherent. Each bioreactor can support up to $0.5 - 1 \times 10^9$ lymphoid cells and $2 - 5 \times 10^9$ hybridoma cells, which is the equivalent of approximately 50 to 100 T-150 flasks.

Cell Lines List

A partial list of cell lines successfully grown in CellMax Hollow Fiber Bioreactors

Tissue	Subset	Cell Line	Species	Type	Product	Hollow Fiber
Adenocarcinoma	Colon	LS174T	Human	Transformed	CEA	Polypropylene
Adenocarcinoma	Colon	WIDR	Human	Transformed	CEA	Cellulosic
Bone Marrow		Bone Marrow	Human	Normal	Cells	Polypropylene
Bone Marrow		Osteoblast	Mouse	Transformed	Cells	Cellulosic
Carcinoma	Breast	MCF7	Human	Transformed	Growth Factor	Cellulosic
Embryo		SF9	Insect	Normal	Cells	Cellulosic
Endothelial		primary	Human	Transformed	Cells	Cellulosic
Epidermoid		A431	Human	Normal	Proprietary	Cellulosic
Epithelium	Mammary	NMuMg	Mouse	Transformed	TGF- α	Cellulosic
Epithelium	Mammary	C127	Mouse	Transformed	Pro-Insulin	Cellulosic
Fibroblast	Embryo	NIH 3T3 (N-Ras)	Mouse	Normal	Growth Factor	Cellulosic
Fibroblast	Breast	primary	Human	Normal	Growth Factor	Cellulosic
Fibroblast	Fetal	primary	Human	Normal	Growth Factor	Cellulosic
Fibroblast	Skin	primary	Human	Transformed	Cells	Cellulosic
Fibrosarcoma		primary	Rat	Transformed	Hyaluronic Acid	Cellulosic
Heart		Heart	Mouse	Transformed	Cells	Cellulosic
Hybridoma		COL 12	Mouse	Transformed	IgG	Cellulosic
Hybridoma		COX	Mouse	Transformed	IgG	Polypropylene
Hybridoma		END	Mouse	Transformed	IgM	Polypropylene
Hybridoma		NOT	Human	Transformed	IgM	Polypropylene
Hybridoma		TAC 5	Mouse	Transformed	IgG	Polypropylene
Hybridoma		AA3A	Mouse	Transformed	IgG	Cellulosic
Hybridoma		OKT 1	Mouse	Transformed	IgG	Cellulosic
Leukemia		Daudi	Human	Transformed	Cells	Polypropylene
Leukemia		HL60	Human	Transformed	Cells	Polypropylene
Lymphocytes	Breast Tumor	TIL	Human	Normal	Cells	Cellulosic
Lymphocytes	Melanoma	TIL	Human	Normal	Growth Factor	Cellulosic
Lymphocytes	Ovarian Ascites	TIL	Human	Normal	Cells	Cellulosic
Macrophage		U937	Human	Transformed	Protein	Polypropylene
Muscle		L8	Rat	Transformed	Cells	Polypropylene
Myeloma		J558	Mouse	Transformed	IgA	Polypropylene
Myeloma		Transfectoma	Mouse	Transformed	Protein	Cellulosic
Myeloma		Transfectoma	Mouse	Transformed	Protein	Cellulosic
Ovary		CHO	Hamster	Transformed	Protein	Cellulosic

Selecting a Growth Media

Often, the growth characteristics of a given cell line in T-flask culture, in terms of growth rate, media usage, and protein secretion characteristics, provides one with a preview of cell growth and secreted product levels in hollow fiber bioreactor culture. Cells that exhibit fastidious growth conditions in T-Flasks will require more maintenance than those cell lines whose growth and viability are relatively unaffected by the low pH/high lactic acid conditions of infrequent feeding.

Growth of cells in hollow fiber bioreactors, in contrast to T-Flask or culture dishes methods, is characterized by the following:

Cell-specific growth factors are concentrated in the extra-capillary space while metabolic waste products, such as lactate and ammonium ion, diffuse into the lumen of the hollow fiber membranes and are removed from the extra-cellular environment. Cells are, therefore, nurtured in a microenvironment containing auto regulated concentrations of cell-specific growth factors for extended periods of time.

Most cell lines can be easily adapted from media containing 10-15% serum to media supplemented with 1 -3 % serum. Serum usage is, therefore, typically reduced five to ten fold.

With appropriate maintenance schedule, which must be determined for each cell line/growth media application, a mammalian cell line “chemostat” can be established. Within 7 – 10 days after inoculation, the bioreactor module will reach the “mature” phase growth where the cell density is sufficiently high to utilize 500 – 1000 mL of media per day with a serum requirement significantly reduced as a result of sufficient concentrations of cell-specific secreted growth factors. This mature hollow fiber bioreactor can then be harvested on a one or two day cycle. Since the cells haven’t been traumatized by non-physiological pH, oxygen, and nutrient conditions, the portion of cells remaining in the CellMax hollow fiber bioreactor module will immediately resume logarithmic growth.

General Considerations

It is easiest to perform hollow fiber bioreactor cell culture of the desired cell line in the same growth media used for flaks, culture dish, spinner, or shaker culture. Each cell line or clonal variant may display individual media-dependent metabolic variations. Changes in growth media should be followed by an analysis of growth rates and secretion levels in the new media formulation.

Media usage, measured as mg/day of glucose consumed or lactate produced, is used to monitor the metabolic activity of the cells.

If the media can be obtained in a high glucose formulation, e.g., DMEM with high (4.5g/L) glucose, more cell metabolism per bottle can be expected.

Regardless of the media and glucose formulation chosen, one must still maintain a feeding schedule that avoids extreme lactate concentrations above 2.0 g/L.

Serum-Free or Protein-Free Formulations

Most cell lines are characterized by individualized media requirements and growth properties. Consequently, no general rules exist for selection of serum-free or protein-free media. Test each new media first for the ability to support cell growth, secreted products, or antibody production.

Always adapt the cells to a new media formulation prior to inoculating the cells into the hollow fiber bioreactor.

Determination of growth rate and secreted protein levels must always be performed after the cells have been successfully adapted to growth in that media. ELISA or radial immunodiffusion test kits are a convenient and accurate means to quantify secreted product levels during this media screening process.

Many cells will continue to secrete products for up to 48 hours after being switched to a serum-free growth media. Only a slight loss of viability occurs during this process. After harvesting and centrifugation, the cell pellet can be resuspended in complete media and re-inoculated in part or whole into the ECS.

Some cells readily adapt to a serum-free media, exhibiting rapid growth and high levels of secreted product. In these cases, one can shift a mature cells culture from the serum-free media to a protein-free version of the same media. One can then initiate a 24 to 48 hour protein-free production period analogous to the serum-free protocol described above.

CO₂- Independent Media

The CellMax Hollow Fiber Bioreactor System has been successfully employed to culture cells using CO₂-independent media. This media relies on cell-generated CO₂ and a media-derived buffering system to maintain a pH suitable for growth.

Cells must first be adapted to CO₂-independent media prior to inoculation into hollow fiber bioreactor. Moderately heavy inoculums of 5 – 8 x 10⁷ cells should be used to seed the bioreactor. This will ensure that adequate CO₂ is generated to maintain the media at the proper pH in the open system environment of the hollow fiber bioreactor cell culture.

The lactate production rate can be used to assay the metabolic activity of cells growing in CO₂-independent media. The spectrophotometric lactate diagnostic test kits produce reliable values for lactate production in CO₂-independent growth media.

Relative growth rates and production of secreted products, antibody, etc., depends on the cell line and the CO₂-independent media formulation. In general, if a cell line grows well in T-flask culture in CO₂-independent media then use of hollow fiber bioreactor cell culture in CO₂-independent media will produce the same benefits as seen in CO₂-dependent tissue culture media.

Calculation of Glucose and Lactate Rates

Background

In hollow fiber bioreactor cell culture one cannot directly observe the cells growing within the membrane bundles. One can, however, generate useful data describing cellular growth on the hollow fiber by monitoring the depletion of the media carbon source and the generation of catabolic products. Such measurements are a direct window into the physiology of the cells. Glucose consumption and the concomitant production of lactate production are both directly related to cell growth.

To determine the doubling time of cell growth, plot day of culture versus mg lactate produced and/or glucose consumed per day. The slope of this line on a semi-log graph of rates versus days of culture will provide an accurate estimate of cell doubling time. An example of this is presented below:

Glucose Consumption Rate

The equation to determine glucose consumption rate is listed below

$$\text{Glucose Consumption Rate} = (V_n * G_n + V_r * G_r - V_t * G_c) / t_c - t_p$$

V_n = liters of new media introduced at the time of the previous glucose reading

V_p = liters of unreplaced media residing in the reservoir, circuit, and bioreactor at the time of the previous glucose reading. This is exclusive of the volume of any fresh media added.

V_t = liters of total amount of media present in the reservoir, tubing, and bioreactor at the time of the current glucose reading.

G_n = glucose concentration (grams/liter) of new media.

G_p = glucose concentration (grams/liter) of media at the time of the previous glucose measurement.

G_t = glucose concentration (grams/liter) of media at the current time.

t_c = current duration of culture in fraction of days

t_p = duration of culture in fraction of days at the time of the previous glucose measurement.

Example

- CellMax Hybridoma Module 430-011
- RPMI-1640, Fetal Bovine Serum (FBS) and Penicillin/Streptomycin
- IgM-Secreting Hybridoma
- 5×10^7 cells were inoculated into the extra-capillary space (ECS) on Day 0
- Glucose Readings were performed using a Tracer II Glucose Reader.

Day of Culture	Glucose Rate (mg/24h)	Reservoir Bottle Size (mL)	Media Change (mL)	FBS in Media (%)	ECS Harvest (viable cells)
1	55	125	100	12	
2	120	125	125	12	
3	150	500	500	12	
5	230	500	NONE	12	1.2E+08
6	330	500	500	12	
7	246	500	NONE	10	
8	486	500	500	10	9.0E+07
9	540	500	500	10	
10	650	1000	1000	10	1.4E+08
13	560	1000	1000	8	1.0E+08
14	521	1000	NONE	8	
15	558	1000	1000	6	1.4E+08
16	790	1000	NONE	6	
17	854	1000	1000	4	1.5E+08
20	542	1000	1000	4	2.1E+08
21	976	1000	1000	4	
22	1123	1000	1000	2.5	1.5E+08
23	1075	1000	1000	2.5	9.0E+07
Total	765 mg/day (day 10 +)		10225	650 mL	1.2E+09 Cells

Lactic Acid Production Rate

$$\text{Lactic Acid Consumption Rate} = V_t * L_c - (V_n * L_n - V_p * L_p) / (t_c - t_p)$$

V_n = liters new media introduced at the time of the previous lactic acid measurement

V_p = liters of unreplaced media residing in the reservoir, circuit and bioreactor at the time of the previous lactic acid reading. This is exclusive of the volume of any fresh media added.

V_t = liters of total amount of media present in the reservoir, tubing, and bioreactor at the time of the current lactic acid reading.

L_n = lactic acid concentration (mg/mL) of new media, usually 0.03 g/L.

L_p = lactic acid concentration (mg/mL) of media at the time of the previous lactic acid measurement.

L_c = lactic acid concentration (mg/mL) of media at the current time.

t_c = current duration of culture in fractions of days.

t_p = duration of culture in fraction of days at the time of the previous lactic acid measurement.

Example

In the following example, the hollow fiber bioreactor culture was setup at 4 PM on day 0 with 120 mL of serum-free media. The reservoir bottle contained 85 mL of media and the flow path system contained 35 mL of media.

Lactate concentration in the reservoir bottle was measured on a daily basis.

By day 6, the cells had metabolized enough glucose to raise the lactate concentration of the media to 1.08 mg/mL. 120 mL of fresh growth media was added to the reservoir bottle bringing the total system volume (V_t) to 240 mL.

The cells in the hollow fiber bioreactor were growing rapidly at this point. As part of the daily maintenance schedule, the lactate concentration of the media in the reservoir bottle was checked the next morning (day 7). A lactate value of 0.98 mg/mL required the addition of fresh growth media. Therefore, an additional 250 mL of fresh media was added to the reservoir bottle bringing the total volume of the CellMax system to 490mL.

On day 8 the process was repeated with an additional 500 mL of media.

Day	Time	Volume	Lactate	Volume Fed	Lactate Rate
0	4:00 PM	0	0	120	---
6	10:00 AM	120	1.08	120	22.5
7	10:00 AM	240	0.94	250	86.4
8	2:00 AM	490	0.88	500	175.7

Rate of lactate production per day equals:

$$\text{LACTIC ACID RATE (mg/day)} = \{V_t * L_c - [(V_n * L_n) + (V_p * L_p)]\} / (t_c - t_p)$$

On day 6, the first calculation of the rate of lactate production (mg/d) proceeds as follows:

$$= \{(120 * 1.08) - [(120 * 0) + (0 * 0)]\} / (5.75 - 0)$$

$$= 22.5 \text{ mg/day}$$

On day 7, the rate of lactate production is

$$= \{(240 * 0.94) - [(120 * 0) + (120 * 1.08)]\} / (6.75 - 5.75)$$

$$= 96.0 \text{ mg/day}$$

Notes:

- mg/mL = g/L
- Since Serum-free media was used, the term $(V_p * L_p)$ is always zero.
- If serum is used, the media will contain a lactate concentration of approximately 160 mg/liter for 10% FBS, and 90 mg/L for 5% FBS.
- The term $(V_p * L_p)$ for the current reading is equal to the term $(V_t * L_c)$ from the previous reading if you have not removed any media in between the two readings.

Bibliography

Coming soon