CellCube® Culture System

User's Manual
Rev. V1.02

CORNING

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Shown on front cover: CellCube® System (Corning Cat. No. 3143)

PLEASE COMPLETE THIS DOCUMENT WITH THE CELLCUBE® SYSTEM INFORMATION TO REGISTER YOUR CELLCUBE EQUIPMENT FOR WARRANTY. THIS FORM MAY BE FAXED TO 978.442.2492.

CellCube System User's Manual Rev. V1.02

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Thank you for your interest in CellCube System Applications. If you have any questions, please contact Corning®'s Technical Service department at 1.800.492.1110 within the United States. Outside the United States, please call +1.978.442.2200.

CellCube® System Information Document

PLEASE FILL IN AND MAINTAIN THIS RECORD for warranty.

Receipt Date:	(mo/day/yr) ______________
Digital Controller	Serial No. ______________
	Lot No. ______________
Circulation Pump	Serial No. ______________
	Lot No. ______________
Media Pump	Serial No. ______________
	Lot No. ______________
Oxygenator	Serial No. ______________
	Lot No. ______________
Oxygen Probe Flow Cell	Serial No. ______________
	Lot No. ______________
Flow Cell No. 2 (If applicable)	Serial No. ______________
Oxygen Probe	Serial No.. ______________
	Lot No. ______________
Oxygen Probe	Serial No. ______________
	Lot No. ______________
pH Probe	Serial No. ______________
	Lot No. ______________
Cart (4 X 100-STACK)	Serial No. ______________

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Before You Start

UNPACKING THE CELLCUBE® SYSTEM

1. Using a sharp tool carefully cut the tape on the large outer box to open.
2. Remove all inner boxes and open each.
3. Remove components from each inner box and place on a stable bench top.
4. **Important**! Save all packaging materials.

CHECK INVENTORY

Each system component box should include the following:

- Digital Controller (Corning Cat. No. 3220)
  - Digital Controller
  - Digital Controller power cable with power supply
  - Bag with different cables
- Media Pump (Corning Cat. No. 3221)
  - Media Pump
  - Power cable
  - Pump/Controller interface cable
- Single Circulation Pump (Corning Cat. No. 3222)
  - Circulation Pump
  - Power cable
  - Pump/Controller interface cable
- Dual Circulation Pump (Corning Cat. No. 3223)
  - Dual Circulation Pump
  - Power cable
  - Pump/Controller interface cable

6L Oxygenator Complete (Corning Cat. No. 3101)

- Oxygenator glass 6L
- pH Probe
- pH Probe cable
- 12 mm Oxygen probe
- 12 mm Oxygen probe cable
- Level sensor probe
- Level sensor cable
- 100 mm Head plate
- 45 mm One port sidearm assembly
- 45 mm Two port sidearm assembly
- 12 mm Oxygen probe replacement kit
- 25 mm Oxygen probe replacement kit
- Secondary O_2 probe flow cell assembly
- 25mm Oxygen probe
- 25mm Oxygen probe flow cell
- 25mm Oxygen probe cable

36L Oxygenator Complete (Corning Cat. No. 3188)

- Oxygenator glass 36L
- pH Probe
- pH Probe cable
- 25 mm Oxygen probe (2x)
- Oxygen probe flow cell (2x)
- Oxygen probe cable (2x)
- Level sensor probe
- Level sensor cable
- 100 mm Head plate
- 45 mm One port sidearm assembly
- 45 mm Two port sidearm assembly
- 25 mm Oxygen probe replacement kit

Setup Kit for the 6L Oxygenator System (Corning Cat. No. 3135)

- Single Circulation Pump cassette
- Media Pump tubing set
- Various tubing connectors
- Silicone tubing

Setup Kit for the 36L Oxygenator System (Corning Cat. No. 3115)

- Media extension set 10'
- Collection Set 25'
- Pressure relief valve assay
- Various tubing and connectors

Dual Circulation Pump Cassette (Corning Cat. No. 3187)

Quad Incubator Cart (Corning Cat. No. 9234)

Introduction

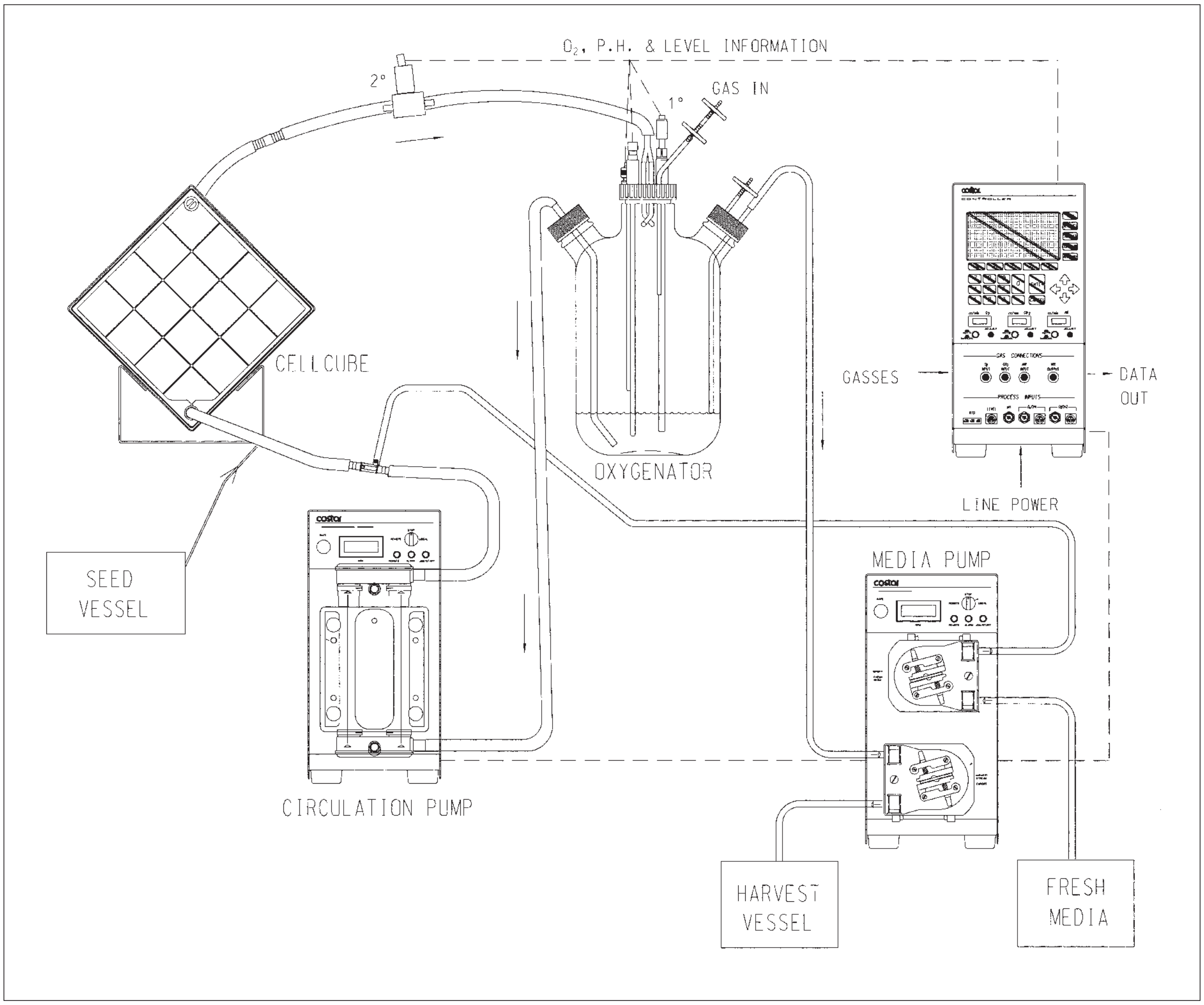


Figure 1. Overview of the CellCube System Circulation Loop

GENERAL CELLCUBE® SYSTEM OVERVIEW

The culture media within the system is removed from the Oxygenator by the Circulation Pump, and is then pumped into and distributed throughout the CellCube Module. The media flows from the outlet of the CellCube Module, back to the Oxygenator, where the media is evenly distributed down the inside surface of the glass reservoir. The media is continuously refreshed by the gas mixture supplied to the headspace of the Oxygenator by the System Controller. The fluid flow and gas exchange within the Oxygenator is carefully controlled to help eliminate turbulence, foaming and to prevent protein degradation.

Figure 2.
Corning® Standard
CellCube System
(Corning Cat. No. 3143)

CELLCUBE® SYSTEM COMPONENTS

The Standard CellCube System is comprised of the five basic components shown in Figure 2 from left to right:

- Controller
- Circulation Pump
- Oxygenator
- Media Pump
- CellCube Culture Module

The Digital Controller (Figure 3) is a fully-integrated, multichannel instrument which monitors, controls and records cell culture parameters including pH, dissolved oxygen, gas flow, and pump rates. All functions are accessible through a menu driven user interface on the faceplate. It also allows monitoring and control of the system from the user's PC. The software is fully compatible with WIN 95, 98, ME, NT4 2000 and XP operating systems. In addition, it is strongly suggested that a spreadsheet such as Excel, QuatroPro or Lotus is also installed to facilitate analysis of the data collected and downloaded from the controller through the course of a run.

The Circulation Pump is a variable-rate pump that utilizes a removable, disposable elastomeric cassette that can be autoclaved one time. The Circulation Pump provides a continuous supply of gas conditioned media and nutrients to the CellCube Module. There are two Corning Circulation Pumps: a Single Circulation Pump (Figure 4) and a Dual Circulation Pump (Figure 5).

The Oxygenator is designed to provide a constant supply of gas conditioned media to the CellCube Culture module and operates using a thin film gas exchange process. There are two sizes of Corning® Oxygenators: a 6L Oxygenator (Figure 6, page 3) and a 36L Oxygenator (Figure 7, page 3).

Figure 3. Digital Controller

Figure 4. Single Circulation Pump

Figure 5. Dual Circulation Pump

The Media Pump (Figure 8) is a variable speed, dual head peristaltic pump for the continuous infusion and removal of medium. It utilizes tubing sets of different diameters to span the 3 to 354 liters per day (LPD) perfusion range of the system. The output pump operates at or 1.2 times the rate of the input pump to continually remove spent medium from the Oxygenator and to maintain the liquid level of the Oxygenator.

The CellCube® Culture Module (Figure 9) provides a large, stable, styrenic tissue culture treated surface area for the immobilization and growth of attachment dependent cells. The CellCube module provides an environment which closely simulates in vivo conditions, and reliably distributes nutrients and oxygen, across all cells within the modules with low differential gradients.

Figure 6. 6L Oxygenator
(Corning Cat. No. 9983)

Figure 7. 36L Oxygenator
(Corning Cat. No. 9979)

Figure 8. Media Pump
(Corning Cat. No.3221)

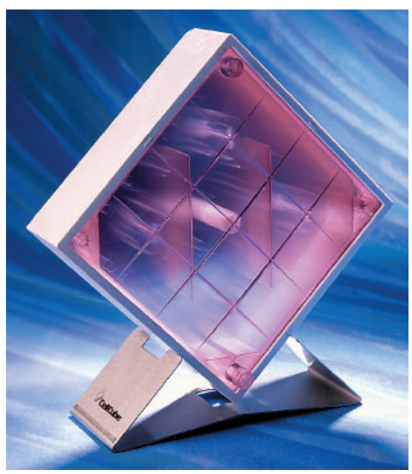


Figure 9. CellCube Culture Module 25 Layer
(Corning Cat. No.3101)

CellCube® Culture Module

The CellCube Module is an integrally encapsulated, sterile, single-use device, and is 100% pressure-hold tested before shipment (Figures 10 and 11). It is comprised of a series of parallel culture plates joined to create thin, sealed, laminar flow spaces between adjacent plates. Each culture plate receives proprietary tissue culture treatment prior to assembly to ensure dense, uniform cell growth.

The encapsulation and end plates are made from polycarbonate for strength and long term reliability. The outlet port is located on the upper rear of the CellCube module, diagonally opposite the inlet port. The outlet port interconnects all the parallel laminar flow spaces of the module. Each port (inlet and outlet) is supplied with a 25 cm (10") length of ½" ID x ¾" OD silicone tubing that terminates in a sterile wrap (only for Cat. No. 3201, 3202, 3264). Even distribution of medium to each of the channels between the plates is provided by the Flow Control Orifices (Figure 12) through a 25 cm (10") length ½" ID x ¾" OD silicon tubing located at the lower front of the module.

Figure 10. CellCube Culture Module 25-Layer (Corning Cat. No.3101)

The CellCube module is available in three basic sizes (Table 1). The 25-Layer module is comprised of 25 culture plates, while the 100-Layer module (Figure 13) is made up of four 25-Layer modules in parallel. External clip arrangements on all four corners of the 100-Layer modules ensure that the assembly runs intact and provides a robust leak-proof environment for operationng. All individual CellCube modules are separately leak tested prior to assembly and then further tested after combined assembly of the 100-Layer module. Each lot is tested on surface treatment and sterility, what is gamma radiated.The surface treatment and sterility of each lot is tested after gamma irradiation. Dye injection studies showing typical fluid flow distribution are shown on page 5 (Figures 14-18).

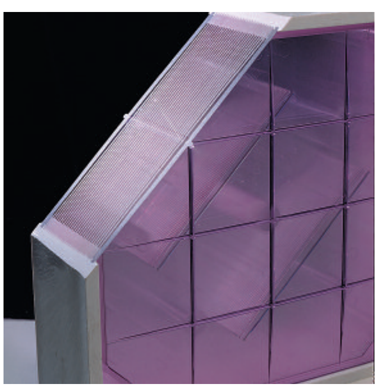


Figure 11. CellCube Culture Module with corner removed to display culture plates.

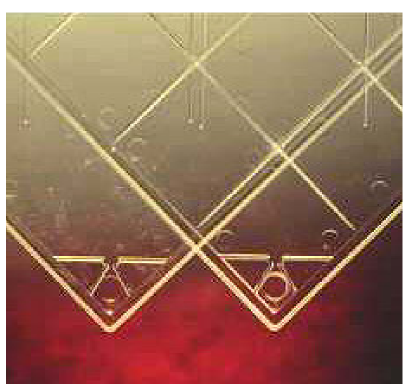


Figure 12. Flow Control Orifices

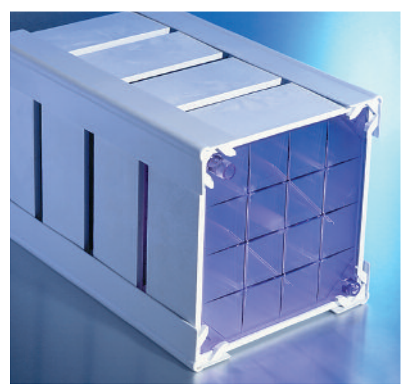


Figure 13. CellCube Culture Module 100-Layer (Corning Cat. No. 3264)

Table 1. Different CellCube® Culture Modules

Cat. No.	Description	Surface Area	Surface Area Equal to Roller Bottle*	Surface Area Equal to Flask**	CellCube Seeding Volume	Qty/Cs
3200	CellCube Modules (10)	8,500 cm^2	10	114	0.6 L	2
3201	CellCube Modules (25)	21,250 cm^2	25	284	1.5 L	1
3264	CellCube Modules (100)	85,000 cm^2	100	1,134	6.0 L	1

*Roller bottle from 850 cm^2 roller bottles (Cat. No. 3907)
**Flask 75 cm^2 flasks (Cat. No. 430198)

Figure 14-18. CellCube Culture Module Flow Progression

Figure 14.

Figure 15.

Figure 16.

Figure 17.

Figure 18.

CellCube® Oxygenator

The CellCube Oxygenator is a thin film-diffusion device (Figures 19 and 20). The entire oxygenator assembly is autoclavable and incorporates a 100 mm headplate assembly, including an autoclavable gel-filled pH probe, a stainless steel level sensor probe, and various ports for media withdrawal and a gas exchange inlet tube. A polarographic oxygen probe is also located in the 100 mm headplate assembly on the 6L Oxygenator.

The Oxygenator is designed to take advantage of the natural tendency of fluid to spread evenly across a large hydrophilic surface. Fluid is delivered to the neck of the Oxygenator, where it spreads out and flows down the interior wall of the glass reservoir. The media forms a thin film that facilitates rapid exchange of gases between the gas and media interface. This process minimizes the generation of foaming typically seen in directly sparged systems and eliminates the need for pluronic or other anti-foaming agents. The falling thin film of media is constantly maintained by an adjustable rate Circulation Pump. This provides a large surface area-to-volume ratio for efficient diffusive exchange between the circulating media and the gas mixture in the Oxygenator.

The media level is maintained by the continuous withdrawal of media through a stainless steel side-arm of the oxygenator. The pH and dissolved oxygen levels are monitored by probes that are in constant contact with the media. An adjusted gas mixture is continuously added to the Oxygenator headspace under the direction of the Digital Controller system. This provides excellent diffusion of CO_2 into and out of the media for pH control and addition of oxygen to the media necessary for maintaining the large cell density typically held by a CellCube Module. The standard 6L Oxygenator (Corning Cat. No. 3101) is designed to maintain a media volume of approximately 1.2 to 2.0 liters that is sufficient to operate the 10, 25, and 100-Layer CellCube modules.

Figure 19. 6L Oxygenator

Figure 20. 36L Oxygenator

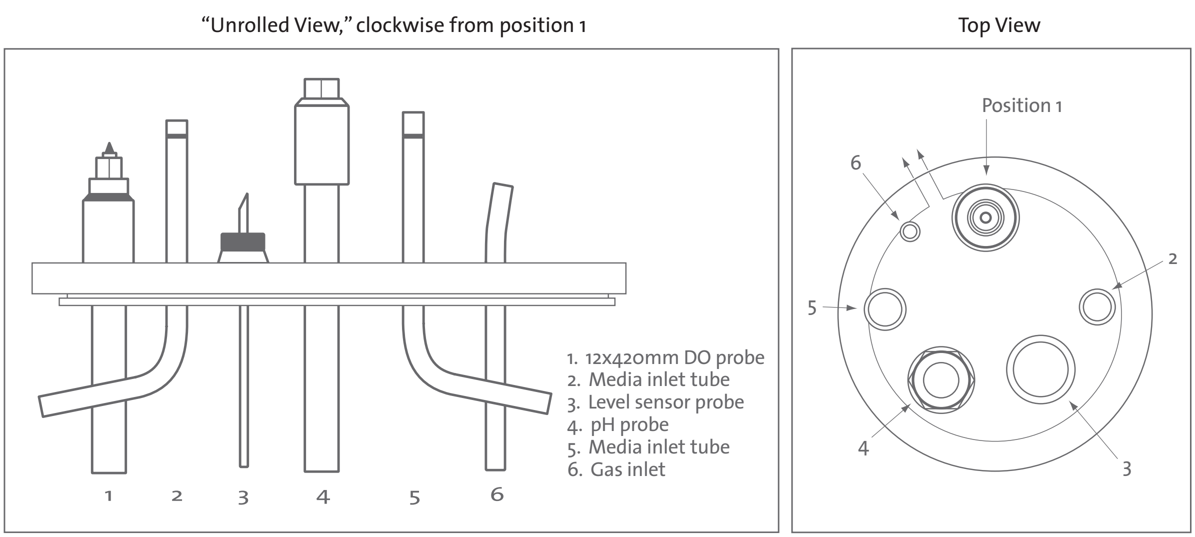


Figure 21. Schematic of a 6L Oxygenator Headplate

The 36L Oxygenator (Corning Cat. No. 3188) is designed to maintain a media volume of approximately 9.0 to 10.0 liters for operating two to four 100-Layer CellCube® Modules.

An important component in the headplate is the level sensor. In the unlikely event that the fluid level rises above a predetermined/fixed volume, contact of the media with the level sensor activates an alarm circuit which interrupts power to the Media Pump, thereby eliminating the possibility of system overflow.

Note: This level sensing function is extremely important. Do not disable this feature.

GLASS RESERVOIR CLEANING

There are a variety of cleaning methods utilized for glassware. This section discusses some of the methods used successfully to clean the glassware used in the CellCube System. The use of a laboratory glassware washer, with standard protocols, is usually all that is required to clean the reservoir.

1. Use a detergent such as 7-x® or equivalent. Avoid detergents commonly used for chemistry applications such as Alconox®.
2. Rinse as soon as practical after use. Do not allow detergents to dry on any components during washing.
3. Store items to be cleaned in a cleanser or in water with a disinfectant.
4. Thoroughly rinse all items in tap water with a final rinse of distilled or deionized water as required.

Note: Be careful when handling glassware. The Oxygenator should be decontaminated before cleaning. Avoid heat sterilization before cleaning.

A properly cleaned reservoir will promote the formation of a wide uniform liquid film when a stream of water is projected onto the surface. In the case of build up of organic material over time it may be necessary to use sodium hydroxide as outlined below to ensure the flow of a uniform fluid film.

1. Fill the reservoir to the base of the neck with a 10 mM solution of sodium hydroxide (400 mg/L), and autoclave for 45 minutes at 121°C and 1 psi, slow exhaust. Alternatively, some people prefer to use a 1Normal sodium hydroxide soak at room temperature overnight as a cleaning method.
2. Remove the glass reservoir from the sodium hydroxide solution and rinse it well with deionized water. Confirm that the O-ring contact sites are free of the cleaning solution. (Note: Organic materials may be reduced to ash by cooking the glass at 450°C for at least 3 hours. The seal rings on the glass side arms should be removed and stored prior to use of this technique.)
3. Place the clean, etched glass reservoir on a lint-free absorbent surface.
4. Do not touch the interior of the glass reservoir.

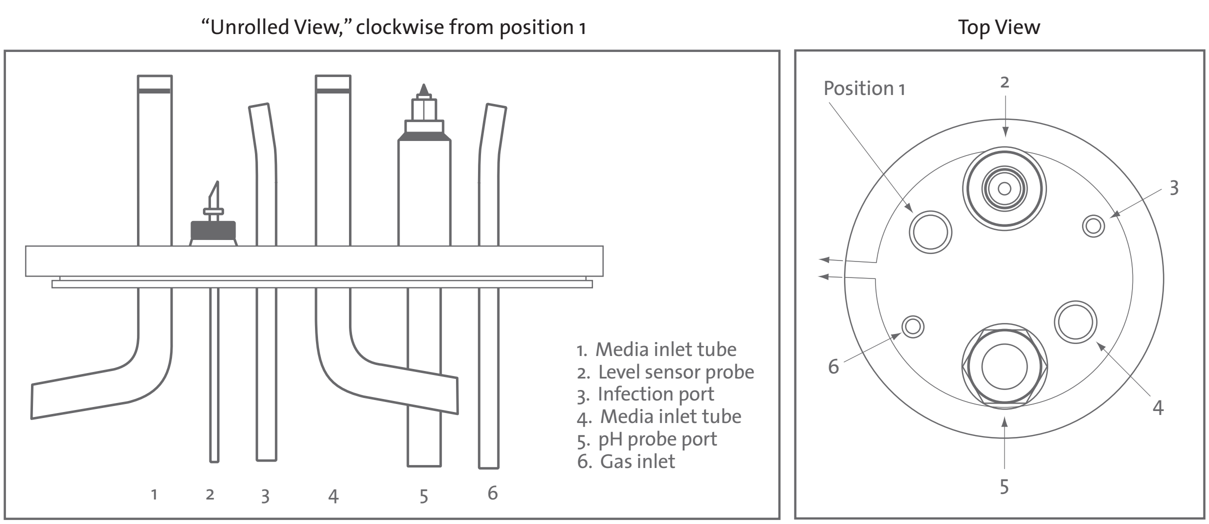


Figure 22. Schematic of a 36L Oxygenator Headplate

CellCube® Digital Controller

UNPACKING AND SETUP

Note: The CellCube Controller (Figure 23) utilizes a variety of different connectors for power and signal inputs. Each connector is different to help prevent improper connections. It is recommended that you become familiar with each connector and its purpose prior to beginning the initial testing of the controller. It is important that you never force a connector into one of the inputs.

The CellCube Controller is packaged with the following components:

1. Controller
2. Multinational power supply
3. Support equipment kit
    - a) U.S. power cord
    - b) Universal power cord
    - c) Serial port terminator
    - d) Connector 12 pos.
    - e) Connector 8 pos.
    - f) Level sensor cable
    - g) Level sensor adapter
    - h) DB25 M/F serial cable
    - i) Temperature sensor

Figure 23. Digital Controller (Corning Cat. No.3220)

The controller is equipped with a universal power supply that automatically adjusts to any AC power source between 90 and 250 VAC.

1. Place the controller on a clean, dry surface.
2. Assure that the power selection knob on the back of the controller is in the OFF position.
3. Insert the power connector from the power supply into the socket located at the rear of the controller.
4. Insert the female end of the power cord into the power supply and the male end into the power receptacle.
5. Activate the power switch, located above the power cord (back rear panel). The controller will begin an initialization routine and after 10 to 20 seconds, you will hear a beep and the startup screen with the time and date will be displayed.

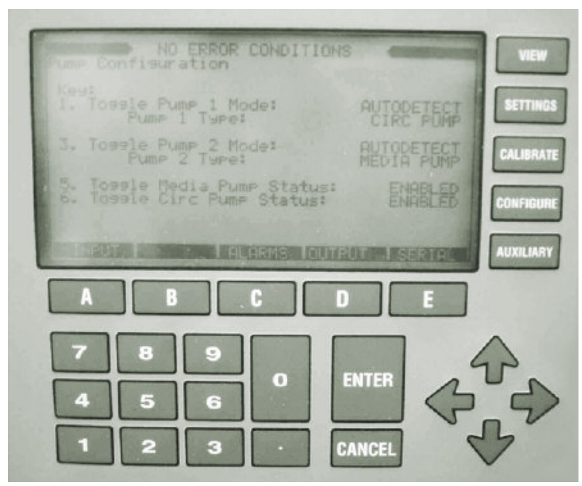


Figure 24. CellCube® Digital Controller Faceplate (3220)

SYSTEM CONTROLS

Working With Menus

As shown above (Figure 24), the Digital Controller, incorporates a menu-driven faceplate. Each function for the controller may be addressed by selecting the proper menu screen. Main menu screens are accessed through the vertical row of buttons to the right of the screen labeled View, Settings, Calibrate, Configure and Auxiliary. Sub-Menus to the Main Menu screens are selected by choosing the appropriate function buttons labeled A through E located below the screen. The Digital Controller, when delivered, is configured with factory default settings when used with probes that have characteristics similar to a "standard" probe shipped with the unit.

It is also possible to control the Digital Controller on a PC using the Corning® PC Host Software.

Entering Data

Below the Sub-Menu buttons is a numeric keypad for entering data into the Digital Controller and arrows for x-y cursor control.

Auxiliary Gas Flow Control

Figure 25. Gas Flow Control

The Mass Flow Controllers (MFC) used to manage the input gasses; O_2, CO_2, and air, have a variable, independent, manual control feature (Figure 25). Each MFC includes a LCD readout showing the flow of gas in cc/minute, a selector switch to choose between automatic and manual gas flow control, and a dial for manual gas flow adjustment.

Gas Connections

O_2, CO_2 and air are delivered to the MFC through a male luer fitting (Figure 25). The gas inlet pressure should be regulated to 5 PSI (0.34 bar). The blended gasses are delivered to the Mix Output fitting for delivery to the Oxygenator.

Process Inputs

Figure 26. Process Connectors

The front mounted process input fittings provide connections for a three wire RTD probe for temperature monitoring, Level sensor, pH and two temperature compensating polarographic dissolved oxygen (DO) probes (Figure 26).

Connecting Probes to the Controller

The Controller collects its information through the input cables from each of the probes and pumps. The Controller utilizes a variety of connectors with different pin styles and numbers. The varied styles help assure that the proper probe is connected to the correct input. It is important that you never force a connector into one of the inputs as most of the connectors are "keyed" so that they will only fit in one orientation. The Controller contains very sensitive amplifiers, for pH and DO measurement. It is beneficial whenever working with electronic instruments to be grounded prior to touching an input connection.

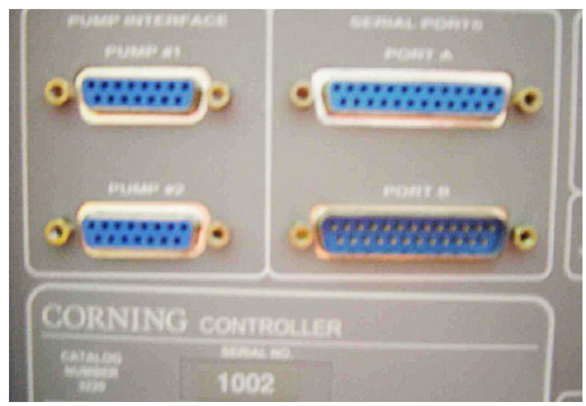


Figure 27. Smart ports of the controller

The rear of the Controller contains two pump interface ports. These ports are "smart ports," (Figure 27) that are capable of recognizing any of the Corning Media or Circulation Pumps (Corning Cat No.3221, 3222, or 3223). The data cables must be connected to the Controller for remote control of the Media Pump and to record pump data.

Corning® Pumps

BACKGROUND INFORMATION

The Controller is capable of recognizing any of the three pumps that are designed to integrate with the controller. The following pumps will be automatically recognized when connected to the controller with the pump communications cable: Media Pump (Corning Cat. No. 3221), Single Circulation Pump (Corning Cat. No. 3222), and Dual Circulation Pump (Corning Cat. No. 3223). The controller is equipped with a calibration routine to convert the pump speed into typical engineering units.

The three pumps are driven with the same servo-type brushless DC motor to provide accurate, low maintenance pump operation. Although the calibration procedure is the same for each pump, the volume collected will depend on the ID of the tubing positioned in the pump head. The calibration of the pumps is accomplished by an automatic sequence that measures the volumes collected during two-minute intervals at two different pump speeds. Typical volumes collected during a 24-hour period of operation of the standard CellCube® System are shown in Table 2. The amounts collected will depend on the size of the CellCube System and the system volume exchanges that are desired per 24-hour period.

Table 2. Volume Collected During 24-Hour Test Period

			Module Size: System Volume (l):	25-Layer 3	50-Layer 4.5	100-Layer 7.5
Tubing I.D.	RPM	Flow Rate (ml/min)	L/day	Vol Exchanges/day at Flow Rate		
1/16" Tubing:	10	4.3	6.2	2.1	1.4	0.8
	20	8.6	12.4	4.1	2.8	1.7
	30	12.9	18.6	6.2	4.1	2.5
	40	17.2	24.8	8.3	5.5	3.3
	50	21.5	31.0	10.3	6.9	4.1
1/8" Tubing:	10	17.9	25.8	8.6	5.7	3.4
	20	37.2	53.6	17.9	11.9	7.1
	30	55.8	80.4	26.8	17.9	10.7
	40	74.4	107.1	35.7	23.8	14.3
	50	93.0	133.9	44.6	29.8	17.9
3/16" Tubing:	10	40.5	58.3	19.4	13.0	7.8
	20	81.0	116.6	38.9	25.9	15.5
	30	121.5	175.0	58.3	38.9	23.3
	40	162.0	233.3	77.8	51.8	31.1
	50	202.5	291.6	97.2	64.8	38.9

CellCube® Media Pump

The Media Pump (Corning Cat. No. 3221) is designed for continuous operation at 37°C. The pump utilizes an autoclavable, disposable tubing set (Corning Cat. No. 3120). The tubing set is designed for continuous duty at the full rated speed of the Media Pump. It is generally recommended that the tubing be replaced after approximately 2,000 hours of use, or if noticeable scoring or deep gauges are observed on the tubing. To facilitate extended use of the tubing, it is advantageous to move the tubing back and forth daily to minimize extended wear of the tubing at the same place on the tube.

UNPACKING

The Media Pump is packaged with the following items:

- Power cord
- Remote control cable (15 pin D-Sub type connector – male x female)
- Data sheet – Remote control interface and alarm connections
- Two female pin connectors for auxiliary analog external alarm connections

The pump is equipped with an internal universal power supply that automatically adjusts to any AC power source between 90 and 250 VAC.

1. Place the pump on a clean dry surface.
2. Assure that the power selection knob on the back of the pump is in the OFF position.
3. Assure that the power selection knob on the front of the pump is in the STOP position.
4. Insert the power cord into the socket located at the rear of the pump.
5. Assure that the safety shield is in position covering the pump mechanism. To activate the pump the safety screw on the cover needs to be tightened with a screwdriver by turning it approximately 90° in a clockwise direction. For safety reasons the pump will not activate until this sequence has been completed.
6. Insert the plug into the wall connector. Insert end of the remote control cable into the back of the Media Pump and the other end into one of the pump interface ports at the back of the controller (Corning Cat. No. 3220).

Figure 28. Media pump (3221)

7. Activate the power switch located above the power cord on the rear panel. The red indicator on the front panel should light when the power selector switch is turned on. The green Jog/Start light on the front panel should light and flash. The green Remote light on the front panel will light only when the remote control interface is properly connected and the safety switch has been activated. To activate the safety switch, push on the Jog/Start button one time.
8. Turn the power switch, on the front panel, to the local setting on the pump. The pump should begin to operate at a rate determined by the “Rate” knob on the pump. The RPM rate is shown on the display.
9. Rotate the “Rate” knob counter clock-wise, until it stops. The rate should stabilize at approximately 2 RPM.
10. Rotate the “Rate” knob clock-wise, until it stops. The rate should stabilize at approximately 64 RPM.
11. Reduce the pump Rate to 2 RPM.

12. Safety check: Slowly loosen either of the screws holding the safety shield of either pump in position by turning counterclockwise 90º. When the cover becomes loose, the red alarm light will light, the pump should stop, and the green Jog/Start light will flash. The pumps will not restart until the safety cover is properly positioned, the screws tightened again and the Jog/Start switch is pressed to restart the pump.

TUBING INSTALLATION

The Media Pump incorporates two Watson Marlow® pump heads. The direction of rotation, clockwise, is the same for each pump. The lower export pump rotates at a rate of 1.2 times the speed of the upper import pump. The tubing set (Corning Cat. No. 3120) includes three different size import tubes that include luer connectors, and one tube that includes barbed hose connectors. Arrows on the face of the pump show the direction of the flow for each of the pump heads.

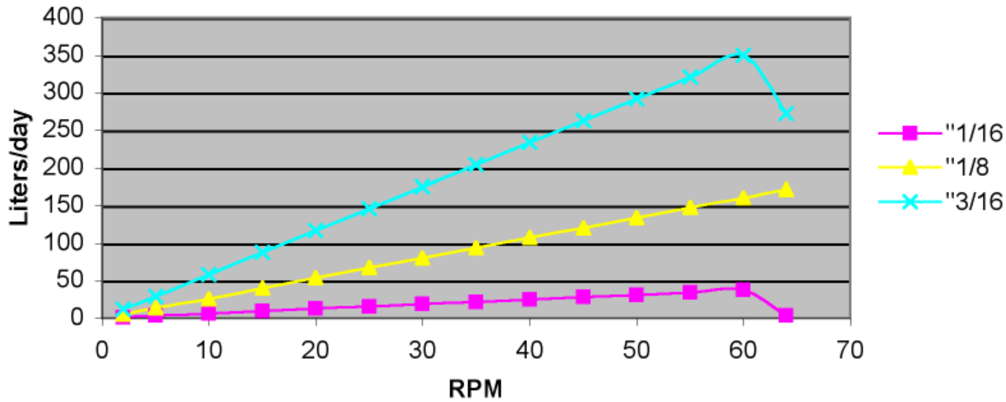
1. Turn the rate control knob counterclockwise until it stops.
2. Place the Power Selector switch in the Stop position
3. Place the rotor in the horizontal position using the Jog/Start switch.
4. Unlock and open the Safety Cover and swing out the rotor crank handle.
5. Lift up the tubing pinch clamp and take notice that the closed position is adjustable. The adjusting tab slides out away from the face of the pump. The tab should be out for the smallest tube and in for the larger tubes. The object of the pinch clamp is to stop the tubing from being drawn into the pump during operation, while not pinching so firmly that the tube is occluded.
6. Fit one end of the tubing into the pinch clamp of the pump cavity.
7. Check the desired flow of the fluid, to assure the tubing is properly placed in the pump. The tubing from the fresh media should be near the inlet of the pump and the tubing leaving the outlet of the pump should be directed towards the CellCube® system. There is a crank handle that snaps in and out of the center of the pump head that can be used to help rotate the pump head while inserting the tubing.
8. Feed the tubing between the rollers and the track, aligning it with the rotor tube guides. The tubing must lie naturally against the track without being twisted or stretched.
9. Open the outlet pinch clamp, and place the other end of the tubing in the pinch clamp and adjust the pinch clamp as detailed above.
10. Close the crank handle by snapping it back into the lock position on the pump head and shut and lock the safety cover shield, using the cover screw provided.
11. When both of the covers are tight and the safety switches are activated, the red alarm light will light and the green Jog/Start light will flash. The pump will not restart until both of the safety covers are properly positioned and the Jog/Start switch is pressed to restart the pump.

Caution: The area under the safety shield is a pinch point and extreme caution should be used while installing or removing tubing.

PUMP CALIBRATION – FLOW CHARTS

The flow charts (Figure 29, page 13) depict the typical output of the Media Pump (Corning Cat. No. 3221). The volume range that can be delivered is completely dependent on the internal diameter (I.D.) of the pump tubing used in the pump head and is independent of the size of the tubing outside of the pump head. The display indicates the rate in RPM, and it is typically linear ±0.5% FS (full scale) of the set point for the usable speed range of the pump. Mechanical variations, backpressure, tubing dimensions and actual tube location can all affect the performance of the pump. The most accurate delivery will occur when the calibration of the pump is performed prior to sterilization, in conjunction with the same size tubing connectors, fluid viscosity, suction and head pressure components and operating temperature that will be used during actual operation. It is advantageous to break in a new segment of pump tubing prior to calibration by installing it into the pump head and operating it in the pump head at a moderate speed for several hours prior to calibration.

Figure 29 on page 13 indicates the typical delivery results after calibration of the Media Pump for certain ID tubing. Manual calibrations are preformed by measuring the volume collected over a fixed time period, for a range of speeds that would cover the process. The specific details and procedures for calibrating the Media Pumps are found later in this manual. (See section on Media Pump Calibration on page 29.)



	Tubing Size		
RPM	1/16"	1/8"	3/16"
2	1.2	5.4	11.7
5	3.1	13.4	29.2
10	6.2	25.8	58.3
15	9.3	40.2	87.5
20	12.4	53.6	116.6
25	15.5	67	145.8
30	18.6	80.4	175
35	21.7	93.7	204.1
40	24.8	107.1	233.3
45	27.9	120.5	262.4
50	31	133.9	291.6
55	34.1	147.3	320.8
60	37.2	160.7	349.9
64	3.6	171.4	272.2

Figure 29. An example of a flow chart for the Media Pump

CellCube® Single Circulation Pump

Figure 30. Single Circulation pump (3222)

The Circulation Pump (Corning Cat. No.3222) is designed for continuous operation at 37°C. The pump utilizes a single use Disposable Cassette (Corning Cat. No. 3121). The cassette is designed for continuous duty at full rated speed of the Circulation Pump.

UNPACKING

The Circulation Pump is packaged with the following items:

- Power cord
- Remote control cable (15 pin D-Sub type connector – male x female)
- Data sheet - remote control interface and alarm connections
- Two pin female connectors for auxiliary analog external alarm connections

The pump is equipped with an internal universal power supply that automatically adjusts to any AC power source between 90 and 250 VAC.

1. Place the pump on a clean dry surface.
2. Assure that the power selection knob on the back of the pump is in the OFF position.
3. Assure that the power selection knob on the front of the pump is in the STOP position.
4. Insert the power cord into the socket located at the rear of the pump.
5. Assure that the safety shield is in position, covering the pump mechanism. The safety shield is held in place by four thumb screws which must be tightened sufficiently to depress the white safety switch found on the left tube guide of the pump.
6. Insert the power plug into the wall outlet. Insert one part of the remote control cable in the back of the Dual Circulation Pump and the other end in one of the pump interface ports at the back of the Controller (Corning. Cat No. 3131).
7. Activate the power switch located above the power cord on the rear panel. The red indicator on the front panel should light when the power selector switch is turned on. The green Jog/Start light on the front panel should light and flash. The green Remote light on the front panel will light only when the remote control interface is properly connected and the safety switch has been activated by attaching the safety shield. To activate the safety switch, push on the Jog/Start button one time.
8. Turn the power switch on the front panel to the local setting on the pump. The pump should begin to operate at a rate determined by the "Rate" knob on the pump. The rate shown on the display is in RPM.
9. Rotate the "Rate" knob counter clock-wise until it stops. The rate should stabilize at approximately 4 RPM.
10. Rotate the "Rate" knob clockwise until it stops. The rate should stabilize at approximately 84 RPM.
11. Reduce the pump rate to 4 RPM.
12. Slowly loosen all four of the knobs, holding the safety shield in position. When the cover becomes loose, the red Alarm light should light, the pump should stop, and the green Jog/Start light will flash. The pump will not restart, until both the safety cover is properly positioned to depress the white safety switch on the left tube guide and the Jog/Start switch is pressed to restart the pump.

CASSETTE INSTALLATION

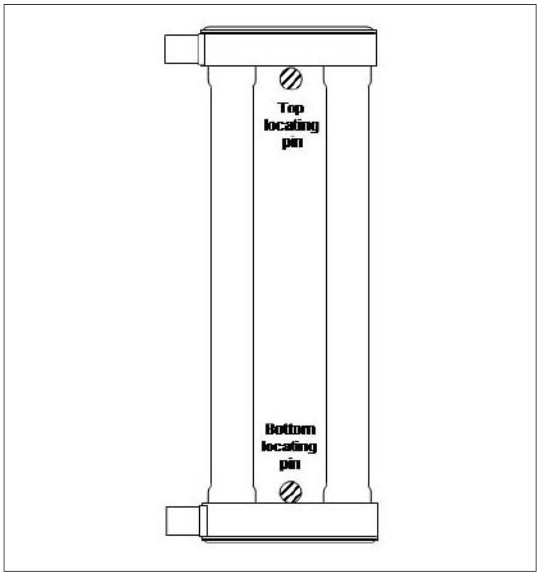


Figure 31. Drawing of the Single Circulation Pump Cassette (Corning Cat No.3121)

The Single Circulation Pump Cassette (Corning Cat. No. 3121) utilizes four stainless steel ball check valves, located in the top and bottom manifold assemblies. Each manifold is labeled in the area between the silicone tubular diaphragms. The bottom manifold is marked faintly with "bottom" and represents the inlet section of the cassette. The top manifold represents the outlet section of the cassette and is marked faintly with "top." The cassette will operate in one position only. It is important to locate the cassette in the pump with the top manifold in the "right-side up" orientation. It is a good idea to use a marking pen and write "top" and "bottom" on the front of the cassette manifolds and also indicate the direction of flow with an arrow to avoid confusion later.

Caution: The area under the safety shield is a pinch point and extreme caution should be used while installing or removing the cassette.

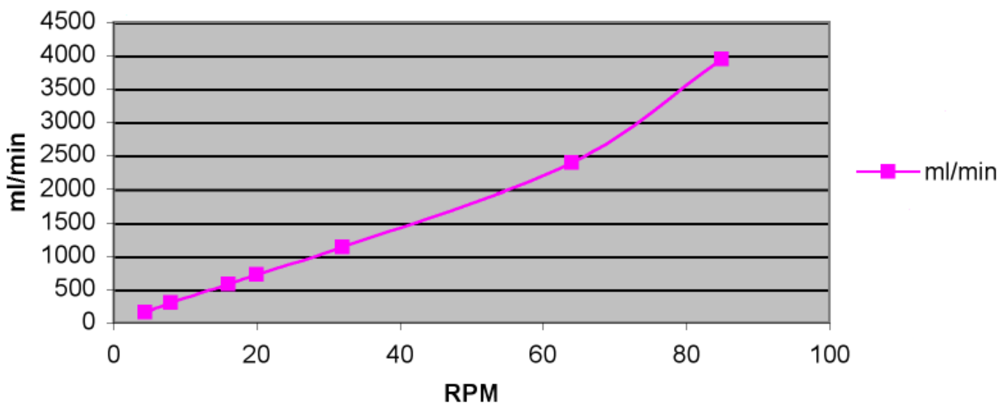
1. Place the Power Selector switch in the Stop position.
2. Using the Jog/Start switch, place one of the pistons in the full right, left position. **Note:** The Jog/Start switch needs to be continually pressed to operate the pump.
3. Remove the safety shield. The red Alarm light will light and the Jog/Start light will flash.
4. Place the cassette loosely into the pump cavity.
5. Slide the top manifold onto the top-locating pin until it settles into the notch in the pin.
6. Press down on the bottom cassette manifold, to gently stretch the cassette over the bottom-locating pin. This may take some physical exertion especially with a new cassette that is a bit stiff. Generally speaking, you will hear a sharp "snap" as the bottom manifold snaps into the notch on the bottom locating pin. Repeat with the top of the cassette if it has shifted during positioning of the lower manifold.
7. Press the Jog/Start switch intermittently to move the piston over to the other side, allowing one of the silicone tubular diaphragms to move into position. Repeat the process by pressing the Jog/Start button down until the second tubular diaphragm moves into position. When the Jog/Start button is released the piston will tend to move back toward the center point between two diaphragm tubes.
8. Check the top and bottom manifolds to assure that they are properly located in the notch of the locating pin.
9. Check the location of the tubular diaphragms. The front of the tube should be flush with the face of the tube guide.
10. Install the safety shield using the four cover screws provided.

Note: When the safety shield cover is tight and the white safety switches are activated the red alarm light will light, the pump should stop and the green Jog/Start light will flash. The pump will not restart until the safety shield is properly positioned and the Jog/Start switch is pressed.

PUMP CALIBRATION – FLOW CHARTS

The following chart (Figure 32, on page 16) depicts the typical output of the Single Circulation Pump (Corning Cat. No. 3222). The display indicates the rate in RPM (reciprocations per minute), and it is typically linear ±0.5% FS (full scale) of the set point for the usable speed range of the pump. Mechanical variations, back pressure, tubing dimensions, and actual tube location can all affect the performance of the pump. The most accurate delivery will occur when the calibration on the pump is performed prior to sterilization making sure to use the same size tubing connectors, fluid viscosity, suction and head pressure components, and operating temperature that will be used in the process. It is advantageous to "break in" a new pump tube cassette prior to calibration by installing it in the pump head and operating it in the pump head at a moderate speed for several hours prior to calibration.

Figure 32 indicates the typical delivery results after calibration of the Single Circulation Pump. Manual calibrations are performed by measuring the volume collected over a fixed time period for a range of speeds that would cover your typical process. The specific details and procedures for calibrating the Circulation Pump are found in a later portion of this manual. (See Calibrating the Circulation Pump, page 30.)



RPM	ml/min	ml/rev	L/day
4.4	156.50	35.57	225.36
8	296.08	37.01	426.36
16	577.03	36.06	830.92
20	717.61	35.88	1033.36
32	1134.17	35.44	1633.20
64	2385.00	37.27	3434.40
85	3943.33	46.39	5678.40

Figure 32. An example of a flow chart of the Single Circulation Pump (Corning cat.No.3222)

CellCube® Dual Circulation Pump

The Dual Circulation Pump (Corning Cat. No. 3223) is designed for continuous duty operation at 37°C. The pump utilizes a single use disposable cassette (Corning Cat. No. 3187). The cassette is designed for continuous duty at the full rated speed of the Dual Circulation Pump.

Figure 33. Dual Circulation Pump (Corning Cat. No. 3223)

UNPACKING

The Dual Circulation Pump is packaged with the following items:

- Power cord
- Remote control cable (15 pin D-Sub type connector - male x female)
- Data sheet – remote control interface and alarm connections
- Two pin female connector, for auxiliary external alarm connections

The pump is equipped with a universal power supply that automatically adjusts to any AC power source between 90 and 250 VAC.

1. Place the pump on a clean dry surface.
2. Assure that the power selection knob on the back of the pump is in the OFF position.
3. Assure that the power selection knob on the front of the pump is in the STOP position.
4. Insert the power cord into the socket located at the rear of the pump.
5. Assure that the safety shields are in position, covering the pump mechanism. Each safety shield is held in place by four thumb screws which need to be tightened sufficiently to depress the white safety switch found on the left tube guide of the pump.
6. Insert the power plug into the wall outlet. Insert one part of the remote control cable in the back of the Dual Circulation Pump and the other end in one of the pump interface ports at the back of the Controller (Corning cat. No. 3220).
7. Activate the power switch located above the power cord on the rear panel. The red indicator on the front panel should light when the power selector switch is turned on. The green Jog/Start light on the front panel should light and flash. The green Remote light on the front panel will light only when the remote control interface is properly connected and the safety switch has been activated by attaching the safety shield. To activate the safety switch, push on the Jog/Start button one time.
8. Turn the power switch on the front panel to the local setting on the pump. The pump should begin to operate at a rate determined by the "Rate" knob on the pump. The rate shown on the display is RPM.
9. Rotate the "Rate" knob counter clockwise until it stops. The rate should stabilize at approximately 4 RPM.
10. Rotate the "Rate" knob clockwise until it stops. The rate should stabilize approximately 84 RPM.
11. Reduce the pump Rate to 4 RPM.
12. Slowly loosen all four of the knobs, holding the safety shield in position. When the safety shield cover becomes loose, the red alarm indicator will light, the pump should stop and the green Jog/Start light will flash. The pump will not restart, until both the safety shield is properly positioned to depress the white safety switch on the left tube guide and the Jog/Start switch is pressed to restart the pump.

CASSETTE INSTALLATION

The Dual Circulation Pump Cassette (Corning Cat. No. 3187) utilizes eight stainless steel ball check valves, located in the top valve assemblies and bottom manifold assemblies. The bottom manifold is labeled in the area between the silicone tubular diaphragms. The bottom manifold is marked faintly with “bottom” and represents the inlet section of the cassette and the pressure relief valves. The top valve assemblies represent the outlet section of the cassette. The cassette will operate in one position only. It is important to locate the cassette in the pump with the bottom manifold over the location pin near the lower portion of the arrows that indicate the direction of flow in the pump. It is a good idea to use a marking pen and write “bottom” on the front of the cassette manifolds and indicate the direction of flow with an arrow to avoid confusion in the future.

Caution: The area under the safety shield is a pinch point and extreme caution should be used while installing or removing the cassette.

There are a variety of methods used to install the cassette in the pump. The following is one method that permits the installation of the cassette with only one operator.

1. Place the Power Selector switch in the Stop position.
2. Using the Jog/Start switch, place one of the pistons in the full right, left position. **Note:** The Jog/Start switch needs to be continually pressed to operate the pump.
3. Remove both of the safety shields. The red Alarm light will light and the Jog/Start light should flash.
4. The upper tube guides contain four slotted guides to locate the outlet check valves. The tubes may be inserted into the guides by pinching the tube approximately 3 cm below the valve assembly and sliding the tube into the slot.
5. Insert the four Dual Circulation Pump tubing into each of the top tube guides.
6. Center each tube in the guide and push down on each of the valve assemblies.
7. Press down on the bottom cassette manifold to gently stretch the cassette over the bottom-locating pin. This may take some physical exertion especially with a new cassette that is a bit stiff.

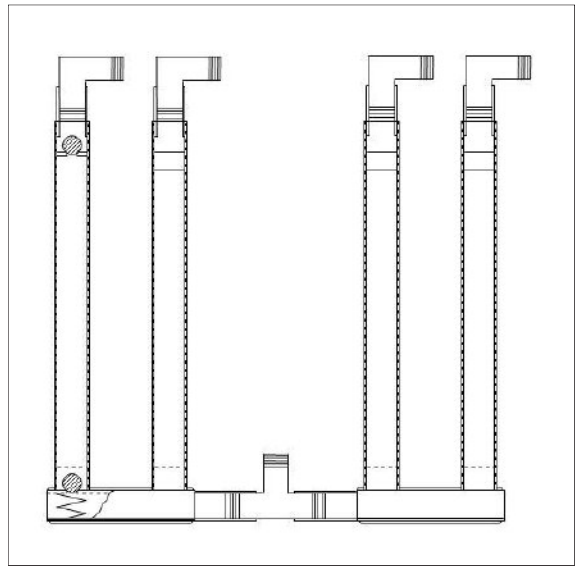


Figure 34. Drawing of the Dual Circulation Pump Cassette (Corning Cat. No. 3187)

Figure 35. Installing the Dual Circulation Pump Cassette , Top View

Figure 36. Installing the Dual Circulation Pump Cassette , Total View

8. Press down and toward the faceplate on the bottom manifold until it slides over the bottom locating pin. Generally speaking, you will hear a “snap” as the bottom manifold

snaps into the mouth on the bottom locating pins (Figure 37).

9. Slide the tube between the tube guide and the piston (the open position from step two).
10. Press the “Jog/Start” switch intermittently to move the piston over to the other side, allowing the second of the silicone tubular diaphragms to move into position. Repeat the process by pressing the Jog/Start button until all four silicone tubular diaphragms move into position. When the Jog/Start Button is released, the pistons tend to move back toward the center point between the two diaphragm tubes.
11. Check the location of the silicone tubular diaphragms. The front of the tube should be flush with the face of the tube guide.
12. Install the safety shield using the four cover screws provided for each plate. **Note:** When both covers are tight and the white safety switches are activated, the red alarm light will light, the pump should stop and the green Jog/Start light will flash. The pump will not restart, until the safety shield is properly positioned and the Jog/Start switch is pressed to restart the pump.
13. Repeat steps 1 through 12 for the opposite side of the pump.

PUMP CALIBRATION – FLOW CHARTS

The flow chart (Figure 39, page 20) depicts the typical output of the Dual Circulation Pump (Corning Cat. No. 3223). The display indicates the rate in RPM, and it is typically linear $\pm 0.5\%$ FS (full scale) of the set point for the usable speed range of the pump. Mechanical variations, back pressure, tubing dimensions and actual tube location can affect the performance of the pump. The most accurate delivery will occur when the calibration of the pump is performed prior to sterilization in conjunction with the same size tubing, connectors, fluid viscosity, suction and head pressure components and operating temperature. As with the Media Pump, it is an advantage to “break in” a new pump cassette prior to calibration by installing it in the pump head and operating it in the pump head at a moderate speed for several hours prior to calibration.

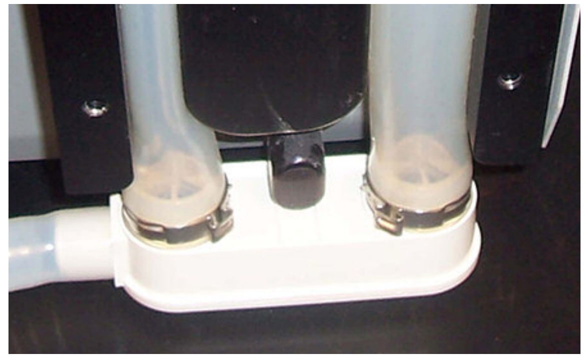
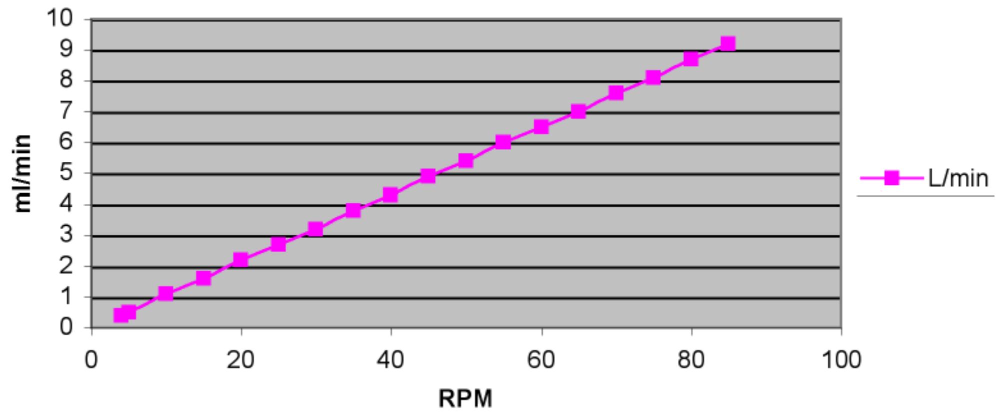


Figure 37. Installing the Dual Circulation Pump Cassette, Bottom View

Figure 38. Installing the Dual Circulation Pump Cassette , Complete

Figure 39 on the next page indicates the typical delivery results after calibration of the Dual Circulation Pump. Manual calibrations are performed by measuring the volume collected over a fixed time period, for a range of speeds that would cover the process. The specific details and procedures for calibrating the Dual Circulation Pump is found in a later portion of this manual. (See Calibrating the Circulation Pump, page 30.)



RPM	L/min	ml/rev	L/day
4	0.4	0.10	576
5	0.5	0.10	720
10	1.1	0.11	1584
15	1.6	0.11	2304
20	2.2	0.11	3168
25	2.7	0.11	3888
30	3.2	0.11	4608
35	3.8	0.11	5472
40	4.3	0.11	6192
45	4.9	0.11	7056
50	5.4	0.11	7776
55	6.0	0.11	8640
60	6.5	0.11	9360
65	7.0	0.11	10080
70	7.6	0.11	10944
75	8.1	0.11	11664
80	8.7	0.11	12528
85	9.2	0.11	13248

Figure 39. Flow chart Dual Circulation Pump (Corning Cat. No.3223)

Software

The PC Host Software is a program that is designed to run in a Windows® environment and is operational in all of the Windows operating systems from Windows 95 through Windows XP. If the PC is on a network and can be accessed via pcAnywhere 1.0*, then the program can even offer off site remote access capability.

The functions of the PC Host software include communication via serial cable and display of the LCD screen from the CellCube® Controller Unit (Corning Cat No. 3220). A graphical view of the last 24 hours, settings and calibrations of virtually all the different views can also be controlled by the Host Software.

The software is required to download the data from the controller itself in a spreadsheet compatible history file. This data is in a raw text format and can be imported in to a variety of spreadsheet programs for analysis. Transferring the capture screens from the display on the controller in a bitmap format is also an option of the Host Software.

Thirty-two controllers can be monitored with the Host Software on a PC when the controllers are serial connected to each other. Each controller can be viewed on the PC screen by selecting the corresponding controller designation number, from 0 to 31. These controller designation numbers are manually entered on the controller itself via the configuration tools which are accessible on the controller. In addition, any upgrades to the Firmware have to be done through the Host Software on the PC by transferring the new Firmware file from the PC to the Controller itself.

INSTALLING THE PC HOST SOFTWARE

Installing Version 2.00

1. Start the installation of the new PC Host Software version 2.00 by inserting “Disk 1” in the floppy drive.
2. Click “Start”, select “Run”, and then type in: “A:\Setup.exe” (substitute A for the corresponding Floppy drive letter) and hit “OK”. This will begin the Controller software installation.
3. Make sure all other applications are closed before going to the next step of the installation, then click “OK”.
4. Leave the installation directory as the default directory, “C:\Corning\Host” and press the “Installation” button to continue, inserting Disk 2 and 3 when prompted to.

When the installation has been completed, there will be a shortcut to the PC Host software program group within Program Files.

* pcAnywhere, is a registered software with Symantec Corporation. Similar software applications on the market may also act in this capacity, but have not been tested to date.

Firmware Operating System

Firmware is the working on-board program for the CellCube® Controller itself. With this software it is possible to view the last 24 hours, change settings and alarms, calibrate and identify the equipment. The firmware is responsible for the local interaction of the digital controller with all of the I/O devices necessary to operate the CellCube System including calibration set point assignments of a CellCube system gas flow control and the measurements of the different probes.

The LCD screen will show which version of Firmware is installed on the machine the moment the controller is turned on.

If upgrading the Firmware is necessary, the controller must be connected to a PC with operational Host Software. It is imperative that these up grades are performed by trained Corning® CellCube System personnel or in conjunction with guidance from a Corning CellCube System technical specialist to ensure that the Firmware and Host Software are correctly matched. After uploading on the host software, select the "control.bin" file. The Host Software will ask to confirm the update. A window will display the status of the firmware update on the PC screen. Once the firmware is updated, the controller will automatically restart itself. There is no need to restart the PC. Detailed instructions are provided from a Corning technical specialist together with the software upgrade instructions in the next section.

UPGRADING FIRMWARE

Start the Host software program. (See Figure 40.) On the menu is the option Transfer which will give you two options after clicking on it. The first option is Receive History and the second option is Update Firmware.

1. Using the left mouse button, click on Update Firmware.
2. In the middle of the screen a prompt will ask: "Are you sure you want to update the Controller Firmware?"
3. Press "Yes".
4. Put the Firmware disk in drive A.
5. Go to drives and select **driver A**:. Using the left mouse button, select the "control.bin" file on the left side of the screen and press OK.

Now the software will take over the controller and upload the firmware. After the Firmware is uploaded the Controller will reset itself automatically. You can run the menus from the controller on the PC.

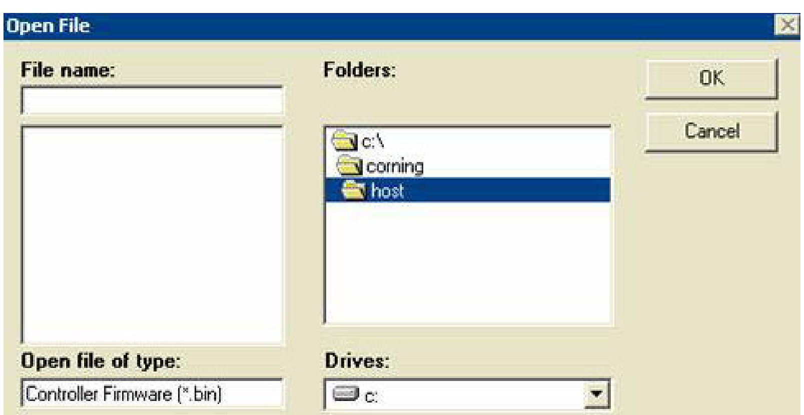


Figure 40. Starting the host software program

WORKING WITH MENUS

Start-up Screen

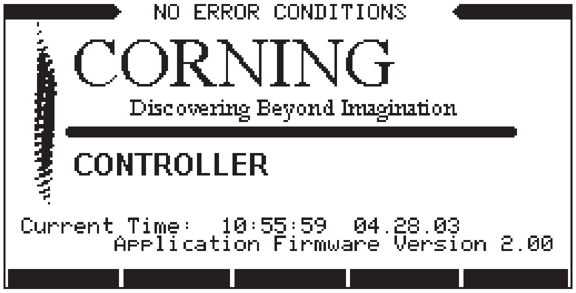


Figure 41. Start-up Screen

The start-up screen is displayed each time the controller is either powered up or a re-boot is activated. The start-up screen displays the current time, date (month/day/year), Firmware version, error conditions, and the Corning logo.

CALIBRATION MENUS

The calibration menus are self-prompting menus to assist in the process of calibrating the probes to the system controller. Sub menus are provided for the calibration of the two oxygen inputs, the pH probe, the temperature probe, and the Corning® Pumps (Circulation and Media).

The calibration menus are accessed by pressing the main selection button [Calibration], on the right side of the display (Figure 24, page 8). The first menu that appears is the Calibration Summary Screen (Figure 42).

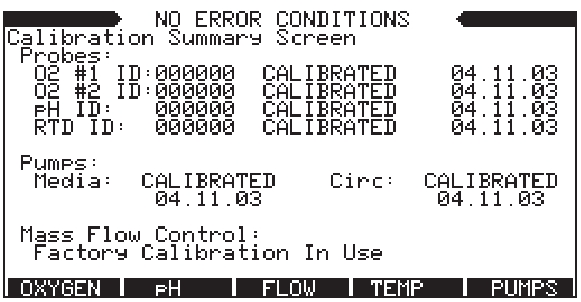


Figure 42. Calibration Summary Screen (Before ID numbers are entered)

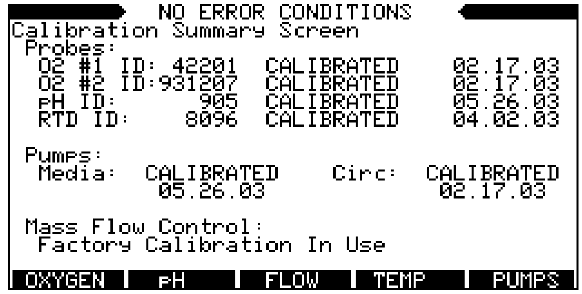


Figure 43. Calibration Summary Screen (After ID numbers are added)

The Calibration Summary Screen provides a listing of each probe's identities and calibration status. Each probe is identified by the input channel, an optional ID number (e.g., serial number), status (calibrated or uncalibrated) and the date calibrated. The calibration status of the Corning pumps and date of the calibration are also listed on this screen.

The various sub-menus are displayed at the bottom of each screen and above a corresponding function button. The functions at the bottom of the Calibration Summary Screen are: OXYGEN, pH, FLOW, TEMP and PUMPS.

After you have entered all of the ID numbers for each probe, the Calibration should appear similar to Figure 43.

Calibration of DO Probes

Maintenance of O_2 Probes

Prior to calibration, fresh electrolyte and/or a new membrane should be installed on the probe. After this procedure, the probe will need to be connected to the controller by the probe cable for a MINIMUM of 6 hours, preferably overnight. Calibration of a nonpolarized probe is a useless endeavor which will only result in faulty operation.

Changing the Membrane Cartridge –
25 mm DO Probes

The membrane cartridge in the electrode should be renewed if the electrode has been stored for several months. The manufacturer of the probes also recommends that the membrane is replaced every third autoclaving. Replacement of the electrolyte should be done prior to each autoclaving.

Further detailed information is provided by the manufacturer in the brochure accompanying each probe. To verify that the membrane cartridge is functioning properly, check the electrode zero point and calibration in room temperature air. If the membrane fails to operate flawlessly (for instance, the response is sluggish or mechanical damage is detected), it must be replaced. When replacing the membrane cartridge, strictly observe the following instructions:

Caution: The use of protective gloves and eyewear is recommended throughout the membrane cartridge refill and installation procedures. The electrode is mildly caustic and should be rinsed off of any contact with your skin. The membrane of all O_2 probes is very delicate and care must be taken to avoid damaging its integrity. Even the slightest puncture of the membrane will result in faulty readings.

1. Unscrew the cap sleeve from the electrode shaft and carefully slide it off the electrode. If the membrane cartridge remains in the end cap, dislodge it by gently pressing on the membrane end.
2. Pull the membrane cartridge from the interior of the electrode body. Discard the old membrane cartridge if it is faulty or has been autoclaved three times.
3. Rinse the anode/cathode assembly with tap water and dry it with a piece of lint free tissue paper. Inspect the glass tip to ensure that it has no hairline cracks. **Never** polish or sand it with an abrasive and avoid touching it with your fingers. Inspect the anode, or brass-colored area above the glass tip. It should have a bright finish, although an irregular or blotched finish is acceptable. If a deposit is noted use a toothbrush with soft bristles to clean the anode.
4. Check all O-rings visually for mechanical defects and replace them if necessary.
5. Completely fill the membrane cartridge with O_2 electrolyte, remove any air bubbles from the membrane cartridge by gently tapping on the side of the membrane cartridge.
6. Position the probe so that the fill groove in the electrode faces you and the probe is in a vertical position. Slowly and gently, slide the membrane onto the top of the assembly. When sliding the membrane onto the cathode, some of the electrolyte will be displaced. The electrode is mildly caustic and should be rinsed off of any contact with your skin. When the membrane is in position, rinse the outside of the membrane with clean tap water and pat dry with a clean tissue to remove any residual electrolyte.
7. Replace the cap sleeve of the probe and gently screw it on the housing. The probe is now ready to be polarized.

Changing the Membrane Cap – 12 mm DO Probes

The membrane for the 12 mm probes is an integral part of the probe. The entire tip (stainless steel sleeve and membrane) can be unscrewed from the probe. There is only a small amount of electrolyte in the probe.

1. Refer to points 3 and 4 above regarding probe inspection and cleaning.
2. The membrane cap should be filled with electrolyte to the first graduated marking line (just below the bottom of the threads) with fresh electrolyte.
3. The cap should then be carefully reinserted onto the probe.
4. While slowly turning the threaded cap onto the probe, you should look for electrolyte being displaced. It is preferable that the cap reach the O-ring without displacing the electrolyte. If electrolyte is displaced prior to reaching the O-ring, remove the cap and wipe the cathode dry with a lint free towel and reinstall.

Note: The above method assures that there is a small bubble of air inside the probe to provide room for expansion, during sterilization.

Calibration of Oxygen Channel One

A good laboratory practice is to record all of the values achieved during the calibration procedures in a notebook to ensure that a history of probe response is accumulated for the life of the probe.

Selecting the OXYGEN (Calibration) screen by pressing the **[A]** button displays, the calibration menu for oxygen channel one (Oxygen #1 Calibration). The functions that may be calibrated are displayed and are listed as items 1-4. You may also note that when the first oxygen screen is selected, that the variable function button is O2CH2 or oxygen channel two. By pressing the **[A]** button again at this point you switch and calibrate channel two.

As mentioned above, it is important, that the probes are connected to the controller and the controller is turned on for at least six hours, or the probes should be maintained in a polarized state prior to calibration. The following procedures refer to the “current value” of the probe signal during the calibration procedures. The numbers displayed are digital values of the amplifier. The amplifier is 12 bit, and the full scale is represented by 4096 digital levels. The full scale is 0 to 4095 units, with 0 units equivalent to 0 input and 4095 the maximum input. This procedure is generally performed prior to sterilization, although it can be done after sterilizing, however, the methodology for performing this step aseptically is a bit cumbersome.

1. **Press [1]** on the control pad to display the Amplifier Null screen with the current value of the probe signal (Figure 44). There are two cable connections for each of the oxygen probes. The oxygen probe signal is transmitted through the BNC connector.

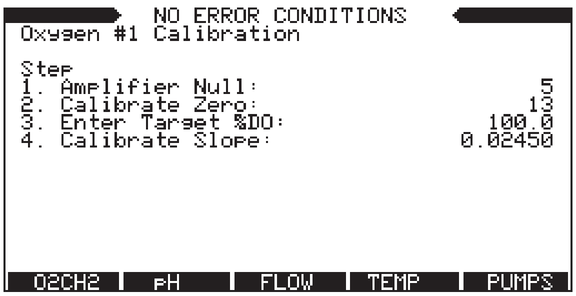


Figure 44. Calibration screen of Oxygen Channel One

The three-pin connector is connected to the thermistor that is built in to each of the oxygen probes. Disconnect the BNC connector from the controller for the amplifier null procedure (Figure 45). The value on the display should rapidly decrease to between 4 and 10 units (Figure 46). When the reading is stable, **press [ENTER]** or the **[A]** button. Reconnect the BNC connector and proceed to step two.

Figure 45. Controller inputs showing BNC connector disconnected

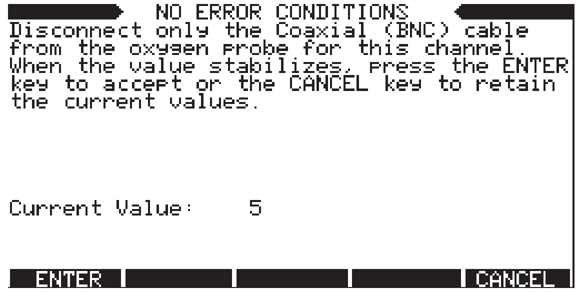


Figure 46. BNC connector disconnected screen

2. **Press [2]** to display the Calibrate Zero screen. Place the oxygen probe into an oxygen free environment. This could be a solution of sodium beta bisulfate or gas such as nitrogen that is near zero in oxygen content, prior to executing this function. The method used to reach the "zero" value will effect the time necessary to perform this test. Typically, a probe should reach a stable zero point after just a few minutes in the presence of a saturated continuous flow of nitrogen gas. The same procedure performed in the liquid phase however, may require a very long stabilization period. A graph, depicting the time required (60 minutes) to reach 3.5% DO, using a small flask and 200 cc's of water, is shown as a general indicator of the time that might be required using the liquid phase test system. The time required, may be much greater, if the volume of fluid, gas headspace, or the gas flow rate are changed.

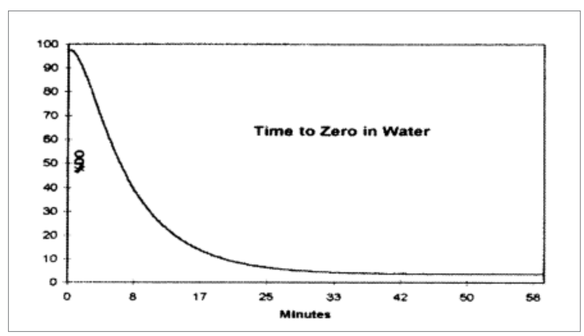


Figure 47. %DO in water during a time frame

When the probe signal has stabilized at the lowest point, **press [ENTER]** or the **[A]** button and the controller will record the value as the zero point for this probe. Typically, the value obtained in the O_2 free environment is only slightly different than the value of the amplifier null test. The difference helps the controller recognize the difference between an error condition (no electrolyte) and low oxygen (See Figure 48, page 26).

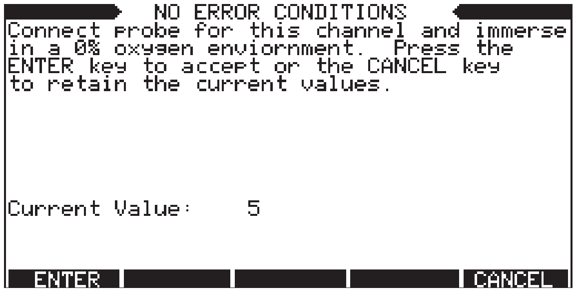


Figure 48. Calibration in an oxygen free environment

3. **Press [3]** to display the Enter Target % DO screen in order to change the target reference calibration point (Figure 49). This controller is preset at the factory to read O_2 saturated liquid as 100%. You may decide to correct this value to compensate for the effects of density altitude, depending on your location. Enter the desired value, and then **press [ENTER]**. *Unless the CellCube¤ System is used in a super saturated environment it is best to leave the value at 100%.*

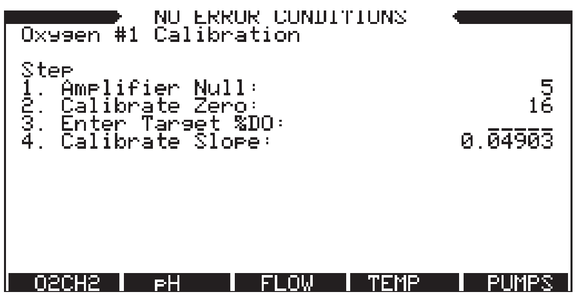


Figure 49. Enter target % DO

4. **Press [4]** to display the Calibrate Slope menu (Figure 49). Place the probe in an environment saturated with O_2 until it reaches a stable reading. This may take a few minutes. The conditions surrounding the probe should remain constant during this portion of the test. An acceptable reference is a liquid in a container open to ambient air, a cylinder of compressed air, or a small pump such as an aquarium pump. When the signal is stable, **press [ENTER]** or the **[A]** button and the procedure is complete. The procedures used to enter the ID number will be discussed in the section Configure Inputs on page 32.

Calibration of Oxygen Channel Two

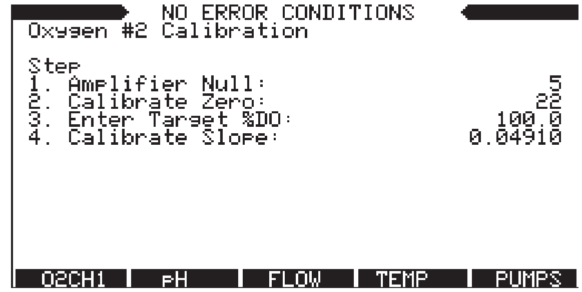


Figure 50. Calibration summary screen for Oxygen Channel Two

It is important that the oxygen probe has been connected to the controller and the controller has been turned on for at least six hours, or the probes should be maintained in a polarized state, prior to calibration. **Press the [A]** button to select Oxygen channel two.

1. **Press [1]** to display the Amplifier Null screen. There are two cable connections for each of the oxygen probes. The oxygen probe signal is transmitted through the BNC connector. The three pin connector is connected to the thermistor that is built into each of the oxygen probes. The thermistor connector should be connected to the controller and the BNC connector should be disconnected for the Amplifier Null procedure (Figure 45). When the reading is stable, **press [ENTER]** or the **[A]** button. Reconnect the BNC connector and proceed to step two.
2. **Press [2]** to display the Calibrate Zero screen. It is important that the oxygen probe is in an oxygen free environment. This could be a solution of sodium beta bisulfate or gas such as nitrogen that is near zero in oxygen content, prior to executing this function. When the probe signal has stabilized at the lowest point **press [ENTER]** or the **[A]** button and the controller will record the value as the zero point for this probe. Note that the value is only slightly different from the value of the amplifier null test. The difference helps the controller recognize the difference between an error condition (no electrolyte) and low oxygen.
3. **Press [3]** to change the target reference calibration point. We typically use room temperature air as a reference point. You may decide to correct this value to compensate for the effects of altitude, depending on your location. Enter the desired value, and then **press [ENTER]**.

4. **Press [4]** to display the calibrate slope menu. Hold the probe in an oxygen environment until it reaches a stable reading. This may take a few minutes. The conditions surrounding the probe should remain constant during this portion of the test. When the signal is stable, **press [ENTER]** or the **[A]** button and the procedure is complete. The procedures used to enter the probe's ID number will be discussed in the section Configure Inputs on page 32.

Calibration and Maintenance of pH probe

```
         NO ERROR CONDITIONS
pH Calibration

Step
1. Calibrate Stand:                2001
2. Toggle Compensation Type:  AUTOMATIC
3. Enter Fixed Compensation Value:  37.0
4. Calibrate Slope:           0.0043517
5. Drift Comp(00.00.00 00:00:00):  +0.00

 O2CH1 |        | FLOW | TEMP | PUMPS
```

Figure 51. The pH Calibration Screen

The controller is calibrated in a manner analogous to standard bench top pH meters, using a two-point calibration with pH 7.0 and a second reference. Since bioreactors using mammalian cells typically progress into the acidic range during scale up, we recommend a pH 4.0 buffer as the second standard. A pH 10 standard can be used, but the values collected will be less accurate than calibrating at pH 4.0. One major difference from benchtop pH meter calibration is temperature compensation. Since the CellCube® System is usually operated at 37°C, there are three possible compensation methods: NONE, FIXED, and AUTOMATIC. Select NONE, if you are calibrating the probe at the actual use temperature and the probe and solution are at the same temperature. Select FIXED if you are calibrating at room temperature, and the system is brought up to temperature later. Select AUTOMATIC if you have the temperature probe calibrated and connected and you are measuring the pH in a solution at the same temperature as the temperature probe.

Note: For the best results always use fresh buffer solutions (pH buffer solutions higher than 7.0 are less stable and have a shorter lifetime).

```
         NO ERROR CONDITIONS
Place electrode in a buffer solution
of 7.00pH.  When the value becomes
stable press the ENTER key to accept
or the CANCEL key to retain the current
value.


Current Value: 1889

 ENTER |      |      |      | CANCEL
```

Figure 52. The Calibration at pH 7.0

1. **Press [1]** and immerse the probe in a standard solution (e.g., pH 7.0). Stir the probe momentarily to ensure proper contact. The first calibration menu appears and the current value of the probe signal will be displayed. When the current value is stable, **press [ENTER]** or the **[A]** button. A pH of 7.00 should yield a current value of 2048, plus or minus 100 units. Values outside this limit may indicate a probe that is not clean or may have internal reference problems. Rinse the probe with D.I. (deionized) water.
2. Determine the proper compensation method to apply from the information listed above. Each time you **press [2]**, the compensation method (Toggle Compensation Type) will advance to the next type; FIXED, AUTOMATIC (preferred method), and NONE.
3. **Press [3]** for the Enter Fixed Compensation Value step. Enter the probe calibration temperature (e.g., 37°C) and then **press ENTER**.

```
         NO ERROR CONDITIONS
Place electrode in a buffer solution
of 4.00pH or 10.00pH.  When the value
becomes stable press the ENTER key to
accept or the CANCEL key to retain the
current value.


Current Value: 1890
No acceptable pH standard found
Temperature Compensation:  0.94
 ENTER |      |      |      | CANCEL
```

Figure 53. The pH Calibration Slope Screen

4. **Press [4]** to select the calibrate slope menu (Figure 52 on previous page). Place the probe, after rinsing it with D.I. (deionized) water, in a standard buffer (e.g., pH 4.0 or 10.0) and stir with the electrode to ensure proper contact. Allow the signal to stabilize, and then **press [ENTER]** or the **[A]** button. The controller will warn you if it determines that the probe response is not typical. A typical response to a change from pH 7.0 to either 4.0 or 10.0 would generate a shift of approximately 870 units, plus or minus depending on the buffer from the current value determined at pH 7.0 (e.g., 2048).

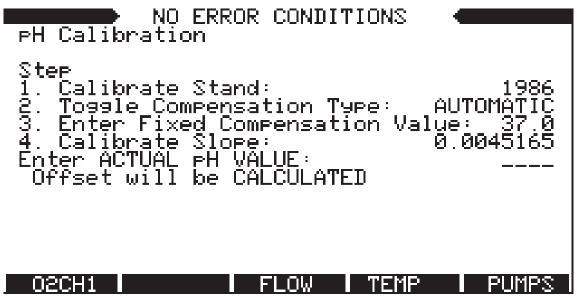


Figure 54. The pH Calibration Drift Step

During repeated autoclaving and long production runs the probe may display "drift" or movement away from the set points. This will result in a steady increase in inaccurate values. It is good practice to routinely check the pH of the medium during the course of a run using another calibrated pH meter on freshly drawn samples as close to the CellCube® System operating temperatures as possible. If drift is observed, it can be compensated for by the following procedure. Take a sample and measure it on another calibrated pH electrode. It is possible to enter this value in your run (Figure 54). During the calibration of the pH electrode, the drift will be zero and after the correction it will calculate the drift. The time and date of this correction will be monitored.

Temperature Probe Calibration

The thermistor temperature probe is very useful for tracking the actual operation temperature of the system. Although the CellCube System is typically operated at 37°C in an incubator/warm room, during long perfusion runs the medium added may be from a refrigerated environment to preserve labile components in the medium. One can use a nonsterile thermistor placed under the oxygenator to measure the circulating temperature of the media in the system. Tracking the temperature in the system will then provide valuable information for the future operation. Calibrate the temperature probe with a calibrated thermometer.

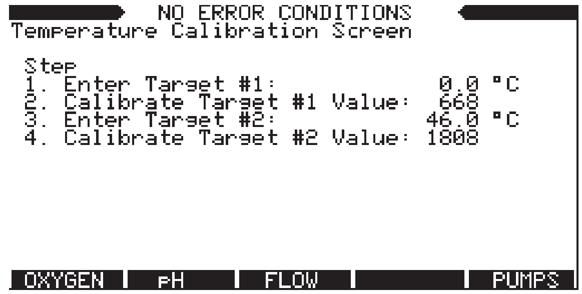


Figure 55. Temperature Calibration Screen

1. Place the thermistor in an ice bath and stir the probe momentarily to ensure proper contact. **Press [1]** to Enter Target #1. Enter the target temperature (e.g., 40°C or the temperature from the reference thermometer) then **press [ENTER]**.
2. **Press [2]** for Calibrate Target #1 Value to select the first calibration menu. The signal will stabilize as indicated by a stabile reading of the current value. **Press ENTER** to record the first standard temperature.
3. **Press [3]** to Enter Target #2. Enter the value for the second calibration point. The most accurate result will be ensured when the second calibration point is just a few degrees above operation temperature. Place the thermistor in the second temperature standard and stir the probe momentarily to ensure a proper contact.
4. **Press [4]** for Calibrate Target #2 Value to select the second calibration menu. The signal will stabilize as indicated by a stable reading of the current value. **Press ENTER** to record the second standard temperature.

Calibration of Corning® Pumps

Background Information

The CellCube Controller is capable of recognizing any of the three pumps that are designed to integrate with the controller. The following three pumps are automatically recognized when connected to the controller with the pump communications cable: Media Pump (Corning Cat. No. 3221), Circulation Pump (Corning Cat No. 3222), and the Dual Circulation Pump (Corning Cat. No.3223). The controller is equipped with a calibration routine to convert RPM (reciprocations or revolutions per minute), depending on the pump.

The three pumps are driven with the same servo-type brushless DC motor to provide accurate, low maintenance pump operation. The calibration procedure is the same for each of the pumps; however, the volume collected during the procedure will vary depending on the pump used. As with O_2 and pH probes, a two point calibration method is used. The calibration routine automatically performs a timed two-minute interval as the collection period calibration. This is repeated twice. The first with the pump operating at approximately ¾ of full speed, and the second at approximately ¼ speed. The approximate volume collected during the test period would be as shown in Table 3.

The accuracy of volumetric delivery during actual operation and reproducibility of delivery from run to run is directly proportional to the accuracy of the measurement during the calibration procedure. It is important, therefore, to accurately calibrate all pumps by weighing/measuring the output volume. The Circulation Pumps require the collection of larger volumes and will require either a large graduated cylinder or large empty containers to collect the volume of fluid during calibration. Much less volume is used during Media Pump calibration, but it is important that the same diameter of tubing is used in the pump head during calibration as will be used in the production run.

As mentioned in the section Cassette Installation on page 15, more accurate calibration is obtained when the tubing has been “broken in” by operation for a period prior to calibration.

Media Pump Calibration

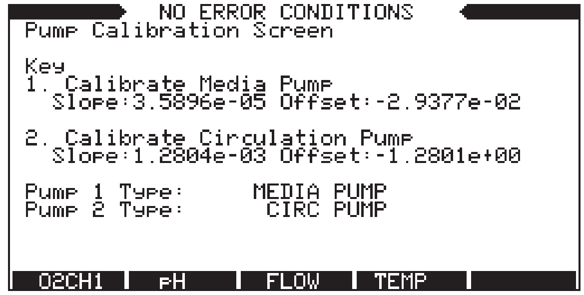


Figure 56. Pump Calibration Screen

By selecting the category Pumps from the main calibration menu, the Pump Calibration Screen is displayed (Figure 55). **Press [1]** to select the Calibrate Media Pump menu (Figure 56 on page 30). The pump should be equipped with the same ID tubing that will be used during operation. There are three choices: START, PRIME, or ABORT. The inlet section of the tube should be immersed in water and the air should be purged from the system. This can be achieved by placing the outlet tubing in the same vessel as the inlet tubing and pressing down on the PRIME button to purge all of the air in the tubing. When you release the PRIME button, the pump stops priming immediately.

Table 3. Volume Collected During a Test Period

			Module Size:	25-Layer	50-Layer	100-Layer
			System Volume (L):	3	4.5	7.5
Tubing I.D.	RPM	Flow Rate (ml/min)	L/day	Vol Exchanges/day at Flow Rate		
1/16" Tubing:	10	4.3	6.2	2.1	1.4	0.8
	20	8.6	12.4	4.1	2.8	1.7
	30	12.9	18.6	6.2	4.1	2.5
	40	17.2	24.8	8.3	5.5	3.3
	50	21.5	31.0	10.3	6.9	4.1
1/8" Tubing:	10	17.9	25.8	8.6	5.7	3.4
	20	37.2	53.6	17.9	11.9	7.1
	30	55.8	80.4	26.8	17.9	10.7
	40	74.4	107.1	35.7	23.8	14.3
	50	93.0	133.9	44.6	29.8	17.9
3/16" Tubing:	10	40.5	58.3	19.4	13.0	7.8
	20	81.0	116.6	38.9	25.9	15.5
	30	121.5	175.0	58.3	38.9	23.3
	40	162.0	233.3	77.8	51.8	31.1
	50	202.5	291.6	97.2	64.8	38.9

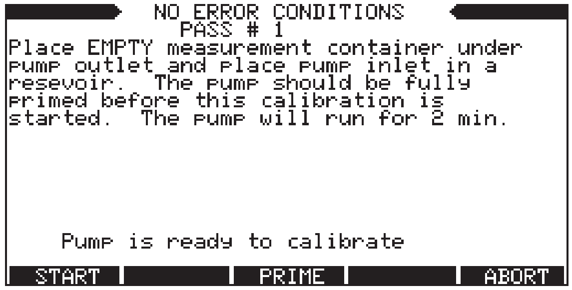


Figure 57. Calibration Pump Screen - High Speed

Place a receiving vessel of sufficient size to retain the sample under the output tube and **press START**. The calibration will begin and the pump will run for two minutes (Figure 58).

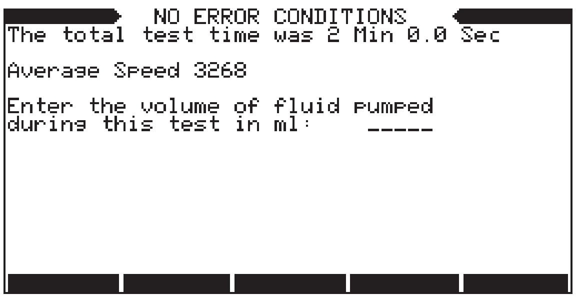


Figure 58. Calibration Media Pump - High Speed _Enter Volume

The pump will stop automatically. Measure the sample collected (volume or weight). The screen displayed in Figure 58 will prompt you for the volume collected during the first test period. Enter the volume in mL in the space as requested and **press [ENTER]**.

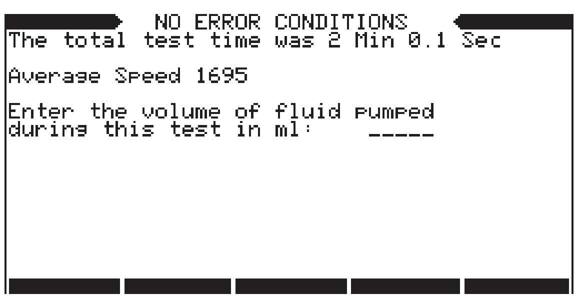


Figure 59. Calibration Media Pump Enter Volume – Low Speed

The screen displayed in Figure 59 will prompt you to prepare and to collect the second calibration sample. Repeat if necessary. Empty the receiving vessel, replace it back under the output tube and **press START**. The calibration will begin and the pump will run for two minutes.

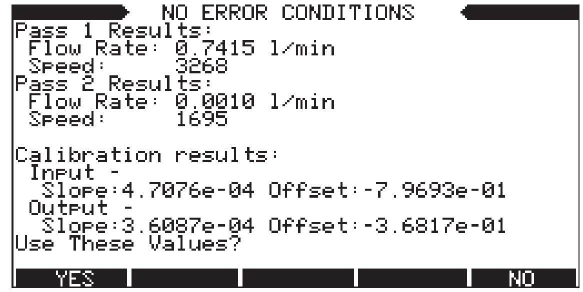


Figure 60. Summary Media Pump Calibration

The pump will automatically stop after 2 minutes. The screen displayed in Figure 59, will prompt you for the volume collected during the second test period. Enter the volume in mL in the space as requested and **press [ENTER]**.

The calibration summary screen will display the results of the two tests (Figure 60). The results may be either accepted or rejected. **Press YES** to accept the values. If results are rejected, **press NO** and the prior calibration values will be used. The Media Pump is now calibrated.

Note: It is a good laboratory practice to enter results of the calibration into a lab notebook in order to compare them to past or future results.

Warning: Each time you access the pump calibration routine, the pumps will be paused.

Calibrating the Circulation Pump

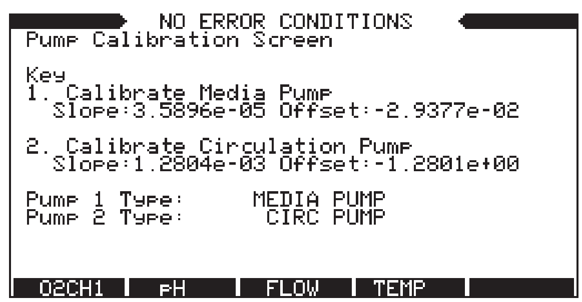


Figure 61. Pump Calibration Screen

The calibration of the Circulation Pump is a two-point calibration. This means it will be run first for two minutes at high speed followed by two minutes low speed. By selecting the category PUMPS from the main calibration menu, the Pump Calibration Screen will be displayed. **Press [2]** to select the Calibrate Circulation Pump menu. The pump should be equipped with a pump cassette similar to the one that will be used during operation. See Figure 63 for the menu displayed. There are three choices; START, PRIME, or ABORT.

The inlet section of the tube should be immersed in water and the air should be purged from the system. This can be achieved by placing the outlet tubing in the same vessel as the inlet tubing and pressing the PRIME button to remove of all the air in the tubing. When you release the prime button the pump stops priming immediately.

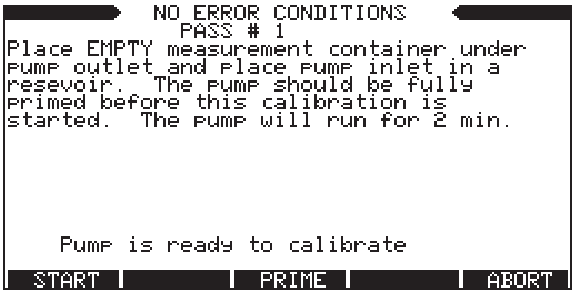


Figure 62. Calibrate Circulation Pump Screen - High speed

Once all the air is purged from the system, place a receiving vessel of sufficient size to retain the sample under the output tube and **press START**. The calibration will begin and the pump will run for two minutes (Figure 62).

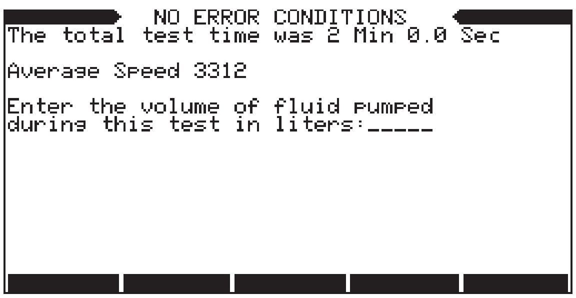


Figure 63. Calibrate Circulation Pump Screen – High Speed_ Enter Volume

The pump will stop automatically after two minutes and the sample may be measured (Figure 63). The screen displayed will prompt you for the volume collected during the first test period. Enter the volume in liters in the space as requested and **press [ENTER]**.

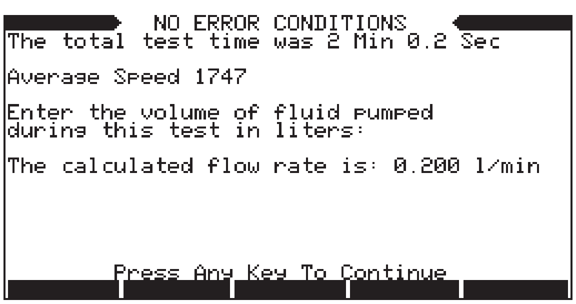


Figure 64. Calibration Pump Screen - Low speed

Figure 64 will prompt you to prepare to collect the second calibration sample. Empty the receiving vessel and replace it back under the output tube and **press START**. The calibration will begin and the pump will run for two minutes.

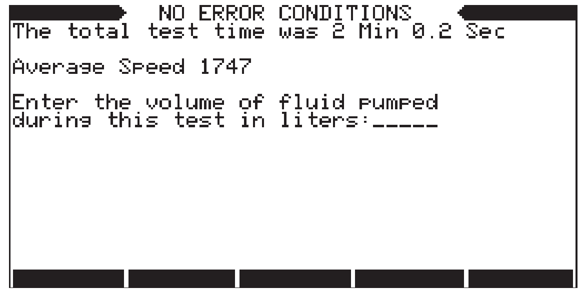


Figure 65. Calibration Circulation Pump - Low Speed_ Enter Volume

The pump will stop automatically after two minutes and the sample may be measured. The screen displayed in Figure 65, will prompt you for the volume collected during the second test period. Enter the volume in liters in the space as requested and **press [ENTER]**.

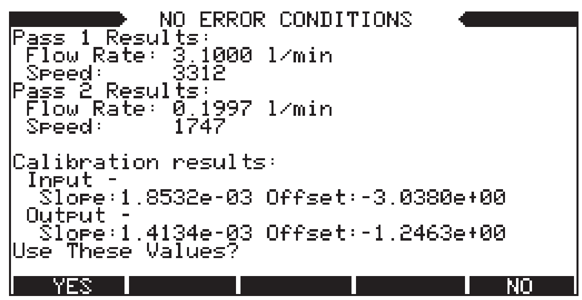


Figure 66. Summary of the Circulation Pump Calibration

The calibration summary screen will display the results of the two tests (Figure 66). The results may be either accepted or rejected. **Press YES** to accept the values. The Circulation Pump is now calibrated.

Note: It is a good laboratory practice to enter results of the calibration into a lab notebook in order to compare them to past or future results.

SYSTEM CONFIGURATION

The Configure menus are accessed by pressing the main selection button CONFIGURE on the Digital Controller Faceplate, (see Figure 24 on page 8). The first menu that appears is the Configuration Summary screen.

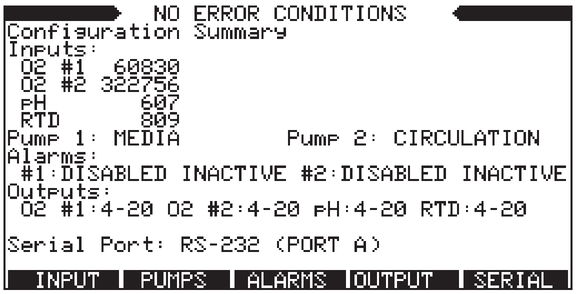


Figure 67. Configure Summary Screen

There are five selection categories under the main configure menu; INPUT, PUMPS, ALARMS, OUTPUT, and SERIAL (ports).

Configure Inputs

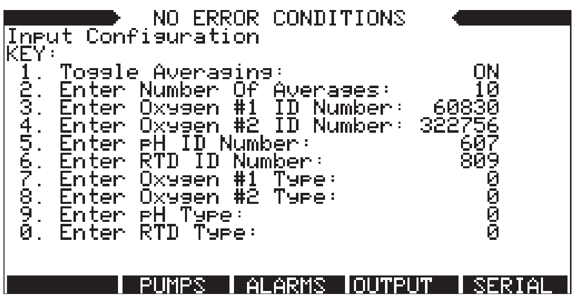


Figure 68. Configuration Input Screen

There are ten selections available in this category indicated by the numbers 1 to 0 (Figure 68).

Averaging

1. **Press [1]** to select Toggle Averaging (the averaging function) to be ON or OFF. The microprocessor continuously scans each of the input channels. The probe output signal may demonstrate some minor bounce that, when digitized, would be displayed as a rapidly changing digit representing the most sensitive level of the signal (a tenth of 80.0 % DO, etc.). Selecting the Toggle Averaging to be ON results in a displayed value, that is the average of a number of samples. Selecting the Toggle Averaging to be OFF sends the digitized value directly to the display.

Number of Averages

2. **Press [2]** to select Enter Number of Averages and input your desired number of averages. The number of averages may vary from 5 to 250 averages. The response time of the displayed value will be dampened by very high averaging numbers and that may interfere with the ability to accurately view signals that are changing rapidly. A fast capture profile would use low averaging numbers, or no averaging at all, and would be more responsive to changing conditions, perhaps excessively so.

ID Numbers

3. **Press [3] - [6]** to enter ID numbers for the probes used during a run. The ID feature is useful for batch records, probe calibration, maintenance and repairs. Many of the probes exhibit their own personal characteristics. It is important that the correct probe is connected to the same input that was used during the calibration procedure.

Type Selection

4. **Press [7] - [0]** to further identify the probes in facilities that may have probes from different vendors that perform the same function.

Pump Configuration

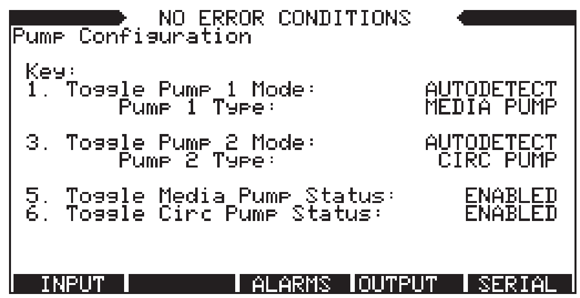


Figure 69. Pump Configuration Screen

The pump configuration section provides access menus to disable the output to the display if you are not using the Corning® pumps. The ability to receive data from non-Corning type pumps will be discussed in the Appendix on page 59. The use of the inputs for non-Corning pumps should only be attempted by skilled electronics technicians.

Alarm Configuration

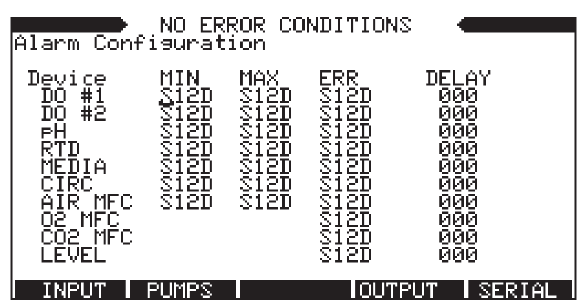


Figure 70. Alarm Configuration screen

The Alarm Configuration section provides a comprehensive array of selections that may be routed to remote switches to signal undesirable operating conditions.

Caution: Do not connect the alarms connector to your central alarm system until the system has reached a stable operating condition.

The three lower green connectors on the back of the controller are fused alarm switches. The switches may be used for a variety of functions. Switch # 1 and #2 are normally open and close on activation. In the open position (default) there is no power to the circuit. Switches #1 and #2 are addressable from the configure-alarms menu. The switches may be activated by any of the minimum, maximum or error alarm functions. When closed, power is routed through the circuit, and thus the switches may also be used to activate a buffer pump if equipped with a suitable remote switch interface. While use of a buffer pump is typically not necessary when the system is sufficiently perfused with properly buffered media, the pump could be used to deliver a basic solution as a fail-safe if the pH drops to a level below a set point at which perfusion is no longer capable of controlling the pH. The third switch is set up in the opposite orientation. Switch #3 is normally closed (i.e., always on) during normal operation and is not addressable. Therefore, if wired to an external alarm, it could be used to indicate power failure or failure of the microprocessor.

The controller provides user selectable minimum and maximum set points, alarms and error condition alarms for: DO #1, DO #2, pH, RTD, Media Pump flow rate, Circulation Pump flow rate, and the Air flow rate.

The controller provides selectable error condition alarms, for CO_2, O_2 and the level sensor.

The error conditions for the probes are as follows:

DO #1 and #2	Probe shorted
pH	Probe shorted
RTD	Probe not connected or shorted
Pumps	Interface error or not connected
Mass Flow Control	Output less than requested (loss of pressure)
Level Sense	Not connected or High Level

Status Alarms

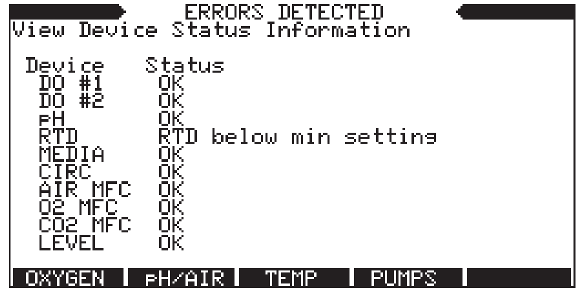


Figure 71. View Device Status Information screen

The View Device Status Information menu (Figure 71) provides a current look at all the alarm conditions showing which alarms are active, and what type of activity caused the alarm.

Alarm Categories

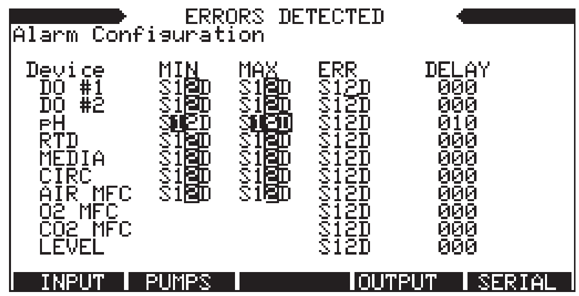


Figure 72. Alarm Configuration Screen/ Active

The alarm configuration screen displays the different categories visible with their alarm function; device, minimum, maximum, error and delay (Figure 72). By default, all alarms are set in the "off" position at the factory. This is to minimize the annoyance of loud alarm noices during initial setup of the CellCube® System when a large number of process parameters would be out of limit. Minimum, maximum and error has its own group of alarm functions, and they are labeled S12D, where S = Sound, 1 = Switch #1, 2= Switch #2, and D = Delay. Any combination of selections is acceptable. Selecting "sound" for each alarm could be annoying; each active alarm must be resolved before the aural alarm stops and that could take a few minutes. It is best to activate the second function only after a run has started and culture parameters have stabilized.

Selecting an Alarm Function

Each alarm function is selected by moving the cursor (an underscore symbol) to the desired alarm condition utilizing the left, right, up and down arrows (Figure 73). Each time one of the arrows is pressed, a “beep” (tone) is generated and the cursor moves one position in the direction of the arrow selected. Alarm functions that are not selected are displayed as a black character on a blank background. Alarm functions that have been selected are displayed as a white character on a black background. **Press [ENTER]** to either select or deselect the function.

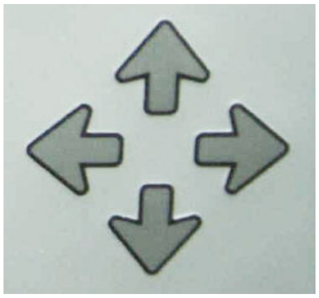


Figure 73. Arrow Cursor

Delay Function

The delay function helps prevent unnecessary activation of the connected alarm system. This is especially useful if the Controller is being operated from a remote location. Each selected alarm or set point will immediately activate the alarm switch that it is connected to whenever that limit is exceeded. One example of a condition that would set off all the selected alarms would be a power failure. When the power is restored, the oxygen probes require a certain amount of time to stabilize extending the alarm period. The pH levels may exceed the limits until the circulation has re-established the set point, which may require a number of volumetric turnovers depending on the length of time the pump was off. Delay periods should be selected based on their ability to prevent spurious alarm activation without being so long that the process is compromised.

To set a delay period, use the cursor to move to the selected Device (e.g., DO #1, DO #2) and then over to the DELAY column. Any number between 0 and 999 (seconds) is an acceptable entry. Use the keypad to enter the numbers and then **press [ENTER]** to complete that selection. You must either **press [ENTER]** or move out of the delay function area using the cursor control arrows before you are permitted to use the other main function buttons. This does not affect the control functions or actual operation.

Configure Outputs

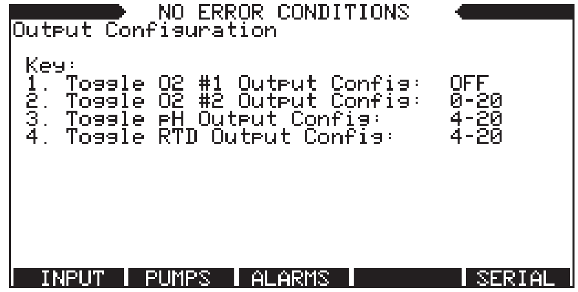


Figure 74. Output Configuration screen

In the Output Configuration screen are the four current output displays (Figure 74). Each of the outputs may be turned off or configured for either 0 to 20 milliamps (ma) or 4 to 20 ma. The output is configured by pressing the appropriate key number. Each time the key number is pressed, the output is advanced to the next level.

The current loop outputs are accessible from the rear of the controller. The outputs are isolated and provide an optional means to log data at a user selectable rate. Corning recommends using the 4 to 20 ma output. This is because using a 4 to 20 ma output will detect any wrong cables which will give a 0 ma output. Your facility or IT engineer should be able to assist in this process.

Serial Configuration

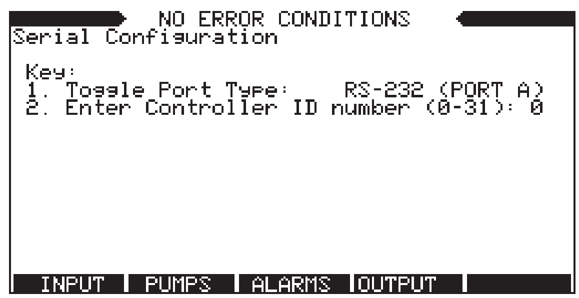


Figure 75. Serial Configuration screen

The Serial Configuration screen modifies communication between the controller and a PC. The serial port and/ or address configuration can be selected by pressing **[1]** or **[2]**.

Press [1] for Toggle Port Type to select the type of output port you are using for communication between the controller and the PC. The typical configuration for most PC’s is the RS232 setting. For system wide monitoring with long, serial cables, the RS485 is more appropriate. Your IT department should be able to assist with determining the appropriate course of action.

Press [2] to enter the controller ID. The controller ID is a number generated by the user where the acceptable range is between 0 and 31. A maximum of 32 Controllers can be daisy-chained together through the RS232 outputs to a single PC. This is done by routing the output of the 1st Controller (Part B on the back of the Controller) to the input of the second Controller (Part A), and so forth. Controller ID numbers are only changeable on the controller itself.

THE SETTINGS FUNCTIONS

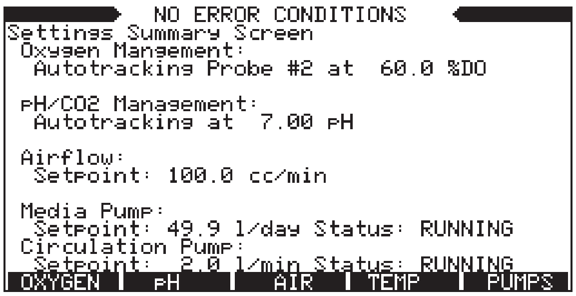


Figure 76. Settings Summary Screen

The settings function provides access to the menus where the set points and alarm values (minimum and maximum) are entered into the controller. The Settings Summary Screen displays the currently active set points and the probe selection (Figure 76). The default values of the Controller are: Oxygen is set to 80.0% and the controller tracks DO Probe #2; pH/CO_2 Management is set for Autotracking at 7.00 pH; and the Airflow Setpoint is defaulted to 200 cc/min. The pumps in this example are connected and running at their respective flow rates.

Note: The values that are expressed above are for reference only. Actual values will no doubt be developed depending on the cell lines, media, and products expressed. Those parameters as developed empirically are:

There are five sub menus: OXYGEN, pH, AIR, TEMP, and PUMPS.

Oxygen Settings

The oxygen set point and alarm values are entered here. There are nine different options displayed in the oxygen settings menu (Figure 77).

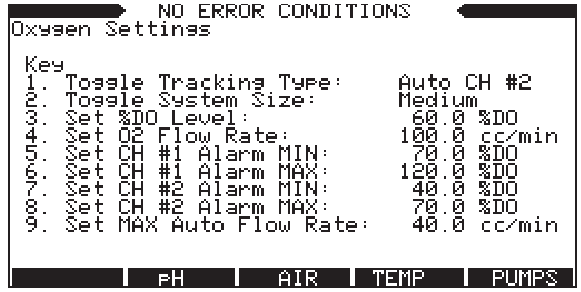


Figure 77. Oxygen Settings Screen

Press [1] to change the control method. (The control of the oxygen in the system may be controlled by O_2 Probe #1, O_2 Probe #2 or manually. There are three selections, Auto CH #1 auto tracking, Auto CH #2 auto tracking, and Manual, that can be toggled respectively. Each time that you **press [1]** the selection is advanced one position. The default setting is Auto CH #2 auto tracking.

Press [2] to select the system size and choose one of three values; Small, Medium, or Large. The size selection loads a set of algorithms that were developed to match the response of the system to the appropriate oxygenator vessel. A standard system that has the 6L Oxygenator (Corning Cat. No. 3101) with one 100-Layer CellCube® Module (Corning Cat No. 3201 or Corning Cat No. 3264) would be set to Medium. The Large setting would incorporate the 36L Oxygenator (Corning Cat. No. 3188) which can handle four modules (Corning Cat. No. 3264). The default setting is set to Medium.

Press [3] to set the %DO level which is used to enter the desired set point for the probe selected in the control method. A set point of 80 for Channel 1 and 60 for Channel 2 are the default settings. Typical starting set points for operations are 60 on Channel 1 and 40 on Channel 2.

Press [4] to set the O_2 Flow Rate. This is the flow that will be provided when the manual mode is selected as the control strategy in option #1 above.

Press [5] to set the CH #1 Alarm Min. This is the set point that will trigger a low alarm condition on channel l. A typical value here would be 10% less than the desired 0_2 set point.

Press [6] to set the CH #1 Alarm Max. This is the set point that will trigger a maximum alarm condition on channel 1. A typical value here would be 20 to 40% higher than the desired O_2 set point. The Max set point should be increased proportionally as the O_2 set point is increased to minimize overshoot that typically occurs as the O_2 set point increases.

Press [7] to set the CH #2 Alarm Min. This is the set point that will trigger a low alarm condition on channel 2. A typical value here would be 10% less than the desired O_2 set point.

Press [8] to set the CH #2 Alarm Max. This is the set point that will trigger a maximum alarm condition on channel 2. A typical value here would be 10% less than the set point.

Press [9] to set the MAX Auto Flow Rate which is used to limit the oxygen flow rate. A maximum limit may be advantageous when utilizing a fast capture profile (such as indicating low averaging numbers) for the PID controller. A typical value here would be a value equal to the air setting. Correctly adjusting the maximum flow will help prevent overshoot (exceeding the set point), especially if higher O_2 set points are desired.

pH Settings

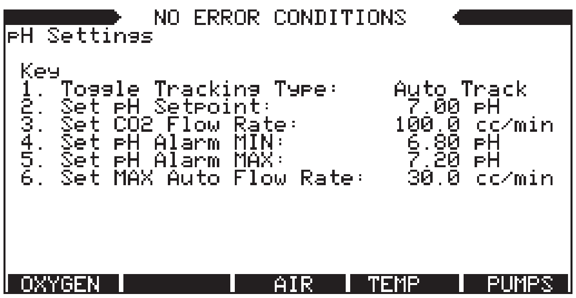


Figure 78. The pH Settings screen

It is assumed that sodium bicarbonate buffered media will typically be used as the primary means of maintaining pH in the system.

Primary pH control is therefore provided through the introduction of CO_2 into the gas inlet stream. The pH control may be either Automatic or Manual. Manual control provides a method to blend a known percentage of gasses to control pH in case the probe fails. A typical value of 5 to 10% CO_2 to the total gas flow will provide adequate control.

Press [1] to toggle between either Automatic or Manual control. Automatic control is recommended.

Press [2] you are able to input the set point, and complete the selection by pressing **[ENTER]**. A typical value would be pH 7.10 to 7.2.

Press [3] to select the CO_2 flow rate that would be provided if the manual mode option in #1 above is selected.

Press [4] to enter a minimum pH alarm value; **press [ENTER]** to complete the selection. A typical value is -0.50 pH lower then the set point.

Press [5] to enter a maximum pH alarm value; press 3 to complete the selection. A typical value is + 0.50 pH higher then the set point.

Press [6] to limit the maximum automatic CO_2 flow rate. Enter the value and **press [ENTER]** to complete the selection. A maximum limit may be advantageous when utilizing a fast capture profile for the PID controller. A typical value here would be 5 to 20% of the total gas flow (see Air Flow Settings below). Correctly adjusting the maximum flow will help prevent overshoot (exceeding the set point), especially as the cell culture process and CO_2 begins to build in the system as the result of metabolic activity of the cell population.

Air Flow Settings

The air flow settings are used to control the carrier gas flow of air into the oxygenator and the alarm settings. Although it is possible to choose another carrier gas such as nitrogen, air is the typical carrier gas into which CO_2 and oxygen are blended. It is important to maintain a continuous flow of a gas that is not being controlled. The carrier gas dilutes out the controlled gasses, providing smooth capture and control of the desired set points.

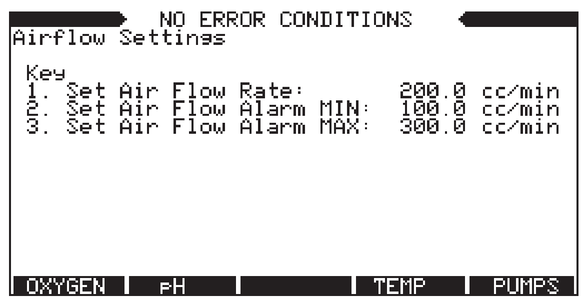


Figure 79. Airflow Settings screen

Press [1] to enter the desired Air Flow Rate and complete the selection by pressing the **[ENTER]** key. A typical value used here would be 200 cc/min for the 6L oxygenator and 400 cc/min for the 36L oxygenator. Avoid excessively high values which can displace other desirable control gasses such as oxygen or CO_2, which are less soluble in the combined carrier gas stream.

Press [2] for the Set Air Flow Alarm MIN in order to enter the desired Air Flow rate for the minimum alarm set point and complete the selection by pressing **[ENTER]**. A typical value used here would be 100 cc/min less then the Set Air Flow Rate.

Press [3] for the Set Air Flow Alarm MAX in order to enter the desired air flow rate for the maximum alarm set point and complete the selection by pressing **[ENTER]**. A typical value used here would be a 100 cc/min more then the Set Air Flow Rate.

Temperature Settings

The alarm temperature settings should be set within a range that will not generate numerous false alarms. (Tests have shown that the temperature probe placed under the oxygenator and insulated from the surface under the oxygenator very closely simulates a probe immersed in the media.) When properly calibrated against a reference it can provide valuable process control data, especially when perfusing at high flow rates with refrigerated medium.

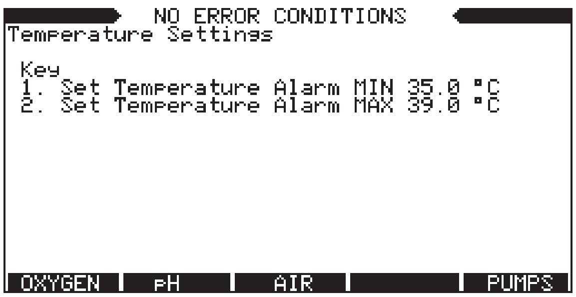


Figure 80. Temperature Settings screen

Press [1] and the desired temperature for the minimum alarm set point can be entered and the selection will be completed by pressing **[ENTER]**. A typical value used here would be two degrees below the working temperature (35°C).

Press [2] and the desired temperature for the maximum alarm set point can be entered and by pressing **[ENTER]** the selection will be completed. A typical value used here would be two degrees higher than the working temperature (39°C).

Pump Settings Screen

The pump setting screen has nine options (Figure 81). Selections 1-4 are for regulating the Media Pump and selections 5-9 are to control the Circulation Pump. Be sure to calibrate all pumps prior to adjusting the settings.

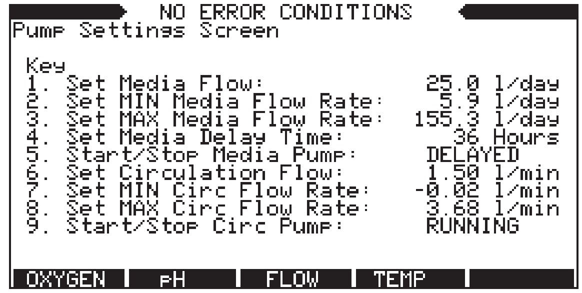


Figure 81. Pump Settings Screen

Press [1] for Set Media Flow in order to select the Media Pump flow rate. Input the desired value and then press **[ENTER]** to complete the selection.

Press [2] for Set MIN Media Flow Rate in order to select the Media Pump flow rate for the minimum flow alarm. Input the desired value and then **press [ENTER]** to complete the selection.

Press [3] for Set MAX Media Flow Rate in order to select the Media Pump flow rate for the maximum flow alarm. Input the value and then **press [ENTER]** to complete the selection

Press [4] for Set Media Delay Time in order to set the delayed start time of the Media Pump in hours. This is especially useful once process parameters have been established and the need to introduce fresh nutrients is not immediately necessary.

Press [5] for Start/Stop Media Pump in order to start or stop the Media Pump.

Press [6] for Set Circulation Flow in order to set the Circulation Pump flow rate. Input the values and **press [ENTER]** to complete the selection.

Press [7] for Set MIN Circ Flow Rate in order to select the Circulation Pump flow rate for the minimum flow alarm. Input the value and then **press [ENTER]** to complete the selection.

Press [8] for Set MAX Circ Flow Rate in order to select the Circulation Pump flow rate for the minimum flow alarm. Input the value and then **press [ENTER]** to complete the selection.

Press [9] for Start/Stop Circ Pump in order to start or stop the Circulation Pump.

VIEW MENUS

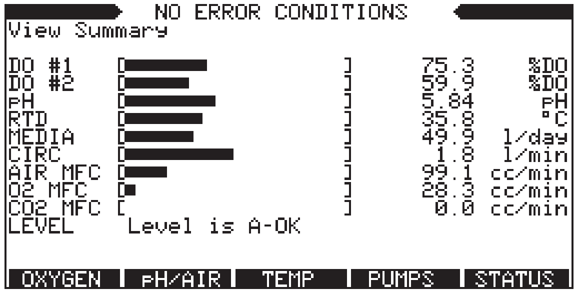


Figure 82. View Summary Screen

The menus inside **[VIEW]** provide rapid access to all the summary menus that display the operating conditions of the system for the last 24 hours. The View Summary screen provides a current display of the most critical operating parameters. There are five sub menus: OXYGEN, pH/AIR, TEMP, PUMPS and STATUS.

Oxygen

Oxygen First View (%DO#1)

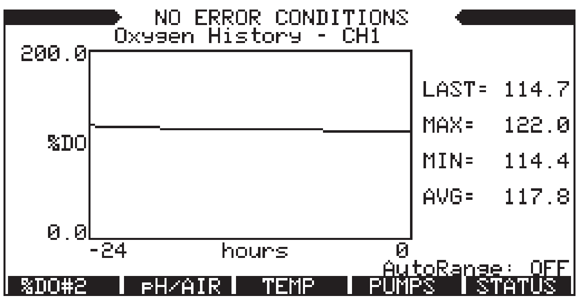


Figure 83. View Oxygen History Channel 1 Screen

Figure 83 is a typical Oxygen History chart for the last 24 hours. Note that the graph history is labeled CH1 and the Y-axis scale is 0 to 200% DO (maximum setting in this case). The minimum, maximum, average and last % DO values for the time period shown on the graph are also displayed. When a run is longer than a 24-hour period, a more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key that will auto scale to the minimum and maximum measured value for the last 24 hours. The bottom left portion of the display shows that the next possible selection is %DO#2.

Oxygen Second View (%DO#2)

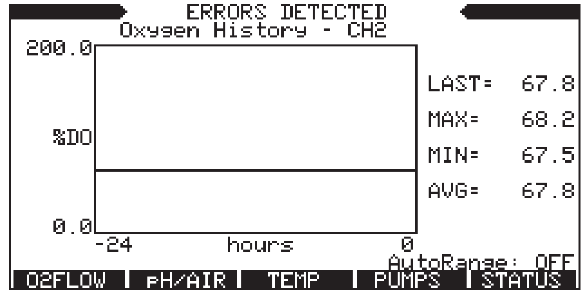


Figure 84. View Oxygen History Channel 2 Screen

Figure 84 is a typical Oxygen History chart for the last 24 hours. Note that the graph history is labeled CH2 and the Y-axis scale is 0 to 200% DO (maximum setting in this case). The minimum, maximum, average and last % DO values for the time period shown on the graph are also displayed.

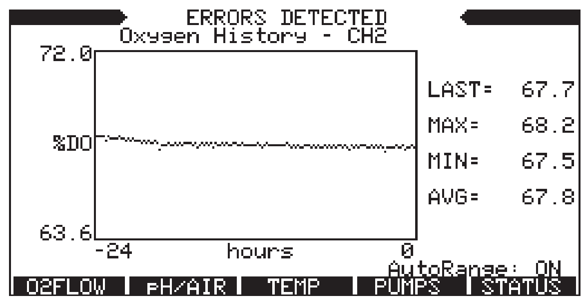


Figure 85. View Oxygen History Channel 2 Sensitive Screen

When a run is longer than a 24 hour period, a more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key which will change the scale to the minimum and maximum measured value for the last 24 hours. A typical oxygen history chart with the sensitive scale is displayed in Figure 85. The bottom left portion of the display shows that the next possible selection is O2FLOW.

Oxygen Flow History

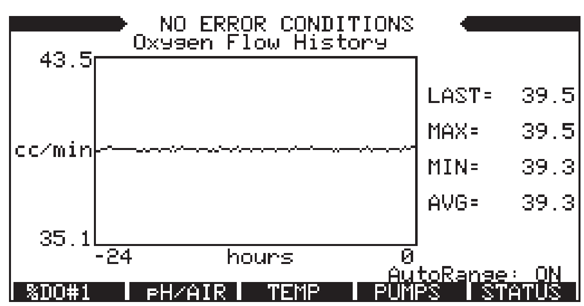


Figure 86. View Oxygen Flow History Sensitive Screen

The third oxygen view displayed is the Oxygen (gas) Flow History. A typical Oxygen Flow History chart (in auto scale mode) will have the graph history labeled Oxygen Flow History and the Y-axis scale will be 0 to 500 cc/min (500 cc is the maximum Air Flow setting in this case). The minimum, maximum, average and last oxygen air flow values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key (Figure 86). The bottom left portion of the display shows that the next possible selection is %DO#1.

pH/Air

pH History

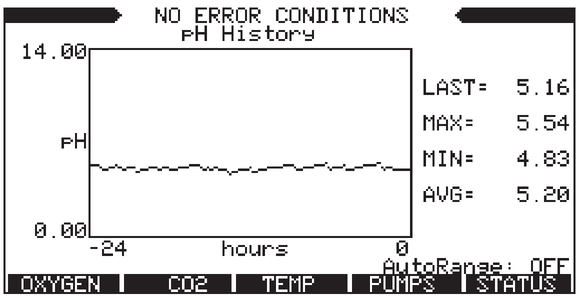


Figure 87. View pH History Screen

A typical pH History chart is displayed in Figure 87. Note that the graph history is labeled pH History and the Y-axis scale is 0 to 14 pH. The minimum, maximum, average and last pH values for the time period shown on the graph are also displayed.

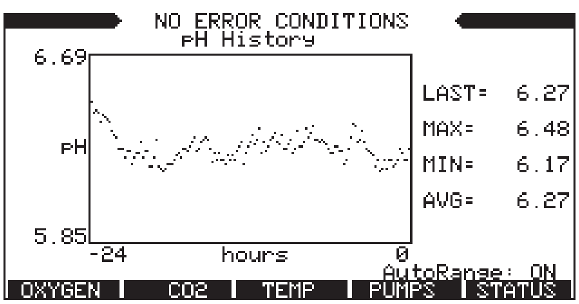


Figure 88. View pH History Screen Auto Scale

A more sensitively scale Y-axis can be viewed by pressing the **[ENTER]** key. A typical auto pH History scale chart is displayed in Figure 88. The bottom left portion of the display, shows that the next possible selection is CO_2.

CO_2 Flow History

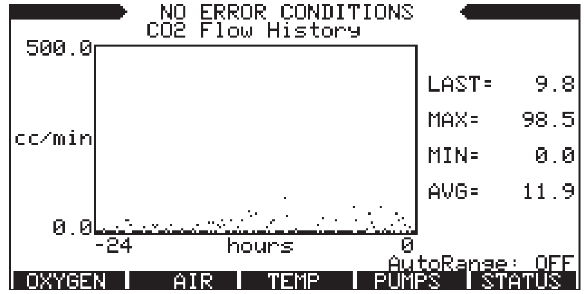


Figure 89. View CO_2 Flow History Screen

A typical CO_2 Flow History chart is displayed in Figure 89. Note that the graph history is labeled CO_2 Flow History and the Y-axis scale is 0 to 500 cc/min. The minimum, maximum, average and last CO_2 Flow values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key. The bottom left portion of the display shows that the next possible selection is AIR.

Air Flow History

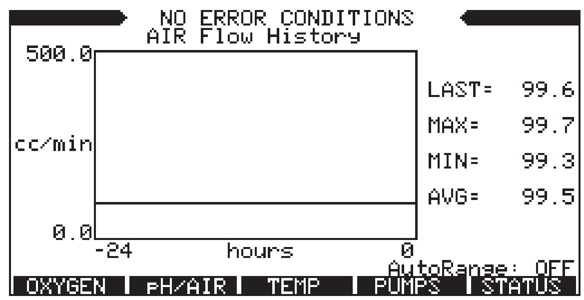


Figure 90. View Air Flow History screen

A typical air-flow history chart is shown in Figure 90. Note that the graph history is labeled Air Flow History and the Y-axis scale is 0-500 cc/min. The minimum, maximum, average and last Air Flow values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key. The bottom left portion of the display shows that the next possible selection is TEMP.

Temperature

Temperature History

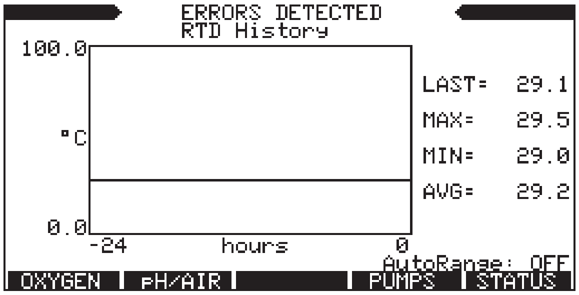


Figure 91. Temperature History

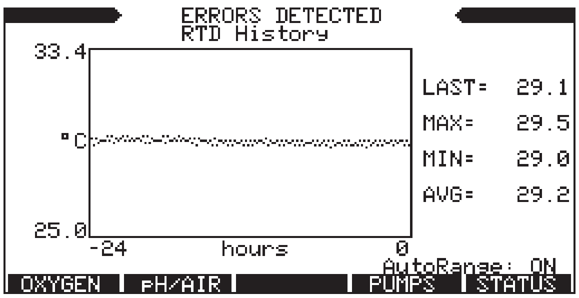


Figure 92. Temperature History (sensitive)

A typical RTD History Chart is displayed in Figure 91. Note that the graph history is labeled RTD History and the Y-axis scale is 0-1000C. The minimum, maximum, average and last RTD values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis (Figure 92) can be viewed by pressing the **[ENTER]** key.

Pumps

Media Pump History

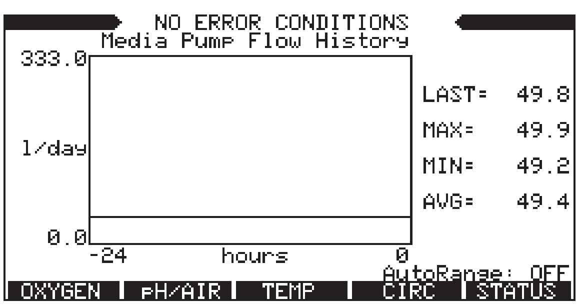


Figure 93. View Media Pump Flow History screen

A typical Media Pump Flow history chart is displayed in Figure 93. Note that the graph history is labeled Media Pump Flow History and the Y-axis scale is zero to the highest limit determined during the calibration routine. The minimum, maximum, average and last L/day values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key. The bottom left portion of the display, shows that the next possible selection is CIRC.

Circulation Pump History

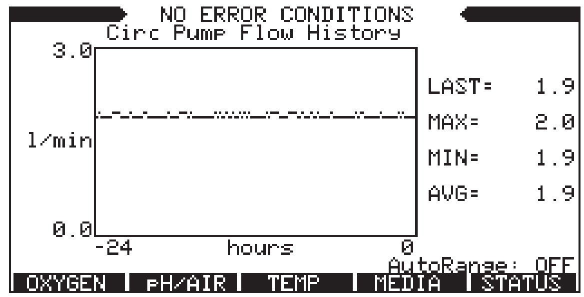


Figure 94. View Circulation Pump Flow History Screen

A typical Circulation Pump Flow History Chart is displayed in Figure 94. Note that the graph history is labeled Circ Pump Flow History and the Y-axis scale is zero to the highest limit determined during the calibration routine. The minimum, maximum, average and last L/day values for the time period shown on the graph are also displayed. A more sensitively scaled Y-axis can be viewed by pressing the **[ENTER]** key.

View Status

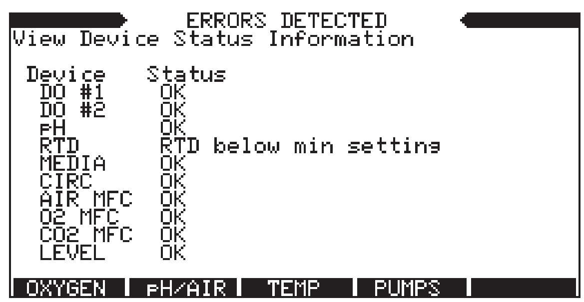


Figure 95. View Device Status Information screen

The View Device Status Information menu displays the status of all the selected alarms. A view device status menu is shown in Figure 95. When a device is operating outside its limits, an error message is displayed. The displayed menu depicts a low temperature condition. This view facilitates quick identification of any error that is detected.

AUXILIARY MENUS

Figure 96. Auxiliary Summary Screen

In the auxiliary menu you have access to start a new Batch, to save the calibration values, to change the internal clock and factory history data.

The Auxiliary Summary Screen shown in Figure 96 displays the functions that are available for BATCH, DEFAULT, TIME, and FACTORY.

Batch Menu

Figure 97. Auxiliary Batch Processing Screen

In the Batch menu you can start a Batch. By entering this START (Batch) menu, you clear the entire history of the controller. Before you do this, Corning recommends saving the current data before starting the run/batch. It will only clean the history data. The settings, calibration and configuration data will be unchanged.

Figure 98. Auxiliary Batch Start Screen

Defaults Menu

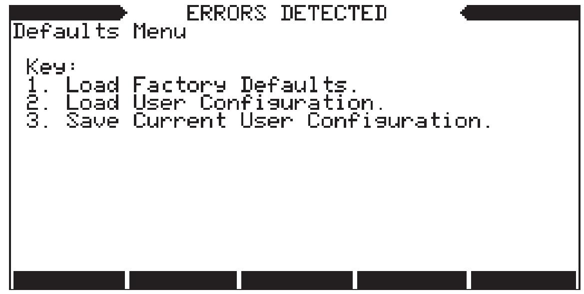


Figure 99. Auxiliary Default Screen

The Defaults Menu provides three selections: Load Factory Defaults, Load User Configuration, and Save Current User Configuration (Figure 99). Load Factory Defaults restores the values that were entered at the factory. Load User Configuration loads the values that were saved by the user (selection 3). Save Current User Configuration provides a means to save the preferred set-up parameters of the user. It is advisable to utilize this feature, after all the probes have been calibrated and the process parameters have been entered. This feature allows the user to restore the preferred calibration values to the controller if the processor resets for any reason.

Time Menu

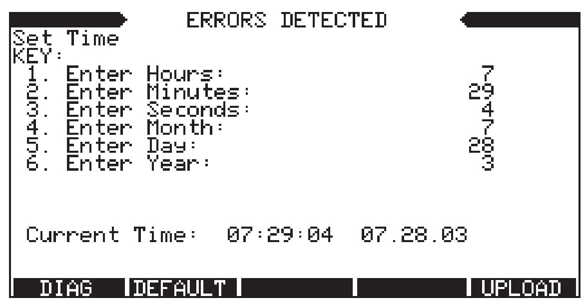


Figure 100. Auxiliary Time Screen

The Set Time menu is used to enter the current time and date into the controller. There are six selections and the values are entered using the numeric keypad.

Factory Menu

Figure 101. Auxiliary Factory Screen

Figure 102. Auxiliary Factory Settings Screen

The Auxiliary Factory Settings Screens (Figures 101 and 102) are to be accessed by Corning Technical Applications Support only. Please contact your local representative for help with these functions if necessary.

CellCube® System Setup Protocols

OVERVIEW OF CELLCUBE FLUID PATHS AND PHILOSOPHY OF OPERATION

The CellCube System typically consists of two, intersecting flow paths (Figures 103 and 104 on page 44). The primary flow path that supplies the usual nutrients, gases and controls pH is the circulation loop. It is perfectly feasible to operate the CellCube System with only this flow path in operation. This has been done in some facilities, especially once the nutritional requirements and buffering capacity of a fixed volume necessary to bring up and support a specific population of cells has been determined. Certainly, in situations where the cell themselves are the product, or where a more concentrated product is desired, this mode of operation is more practical, less expensive, and less technically challenging. Our experience, however, from both in-house and customer application input, suggest that the addition of the perfusion circuit in many cases dramatically enhances the productivity of the system, both from the man-hours for operation and productivity. Your decision to use one or both of these pathways can only be determined empirically. We typically recommend the following sequence of events in the selection process.

1. Seed and establish a viable cell population in the CellCube System.
2. Carefully monitor as many growth parameters as are feasible, such as glucose consumption, oxygen utilization, pH, lactate production, etc to create a baseline set of data from which single variable experiments can be created. While this would seem to be intuitive, we are often perplexed by investigators' reluctance to gather this information.
3. If costs of media or volume collected become a limiting factor, fed-batch strategies where only a partial volume of medium is removed and replaced from the circulation loop manually at given intervals can be both beneficial and supply a wealth of information on how to proceed.
4. Take small steps, and avoid multiple variable changes in conditions that can confound an explanation of a particular effect.
5. Lastly, especially as cell populations increase in the CellCube Modules, it is very easy for things to get "out-of-hand" in the process. Factors such as pH or available nutrients can change very rapidly when populations exceed 1×10^{11} cells in a confined area. Try and stay "ahead of the process" whenever possible. As you expect, as you grow in your expertise, you should become more adept at noticing a condition and dealing with its appropriately.

BUILDING UP CELLCUBE FLUID PATHS

The underlying flexibility of the CellCube system allows a wide variety of configurations and environments to be adopted for cell culture. While the basic pathways of fluid movements are essentially the same, minor variations may be necessary due to decisions on where the equipment will be positioned (warm room versus roll-in incubator), whether the harvest stream is heat-labile and must be refrigerated in an adjacent area or removed from the culture area, or the upstream feed stream have temperature or light constrains. We have provided you a few variations that we have compiled from a variety of sources to assist you in planning an optimal flow path. These schemes can be found in the files found on the CD/video tapes provided in your installation package. The schemes shown are for a standard CellCube system using a 100-Layer CellCube Module. Table 4 summarizes the volumes that would be appropriate for the smaller CellCube modules.

It is best to consider the circulation loop initially.

Note: It is advised to not save cost on tubing and connectors when you do not have a lot of experience with scale-up processes. However, when "thinking through" your process flow, try to minimize redundancy in tubing, especially in the assembly of the circulation loop and perfusion circuit.

For an in depth look at how to start up a CellCube system, please refer to the Power Point presentation in the CellCube system Video/CD#1. (If you do not have a set of videos or CDs, please contact your local sales representative.) This Video/CD

Table 4. Fluid Capacities for CellCube® Modules

Approximate Fluid Capacities of CellCube Systems:

Module	RB Equivalent (850 cm2)	Total Surface Area (cm2)	Approx. Module Dead Volume (ml)	Oxygenator and Circ. Loop (liters)	Total Volume (liters)
10-Layer	10	8,500	600	1.5	2.1
25-Layer	25	21,250	1500	1.5	3
50-Layer	50	42,500	3000	1.5	4.5
100-Layer	100	85,000	6000	1.5	7.5

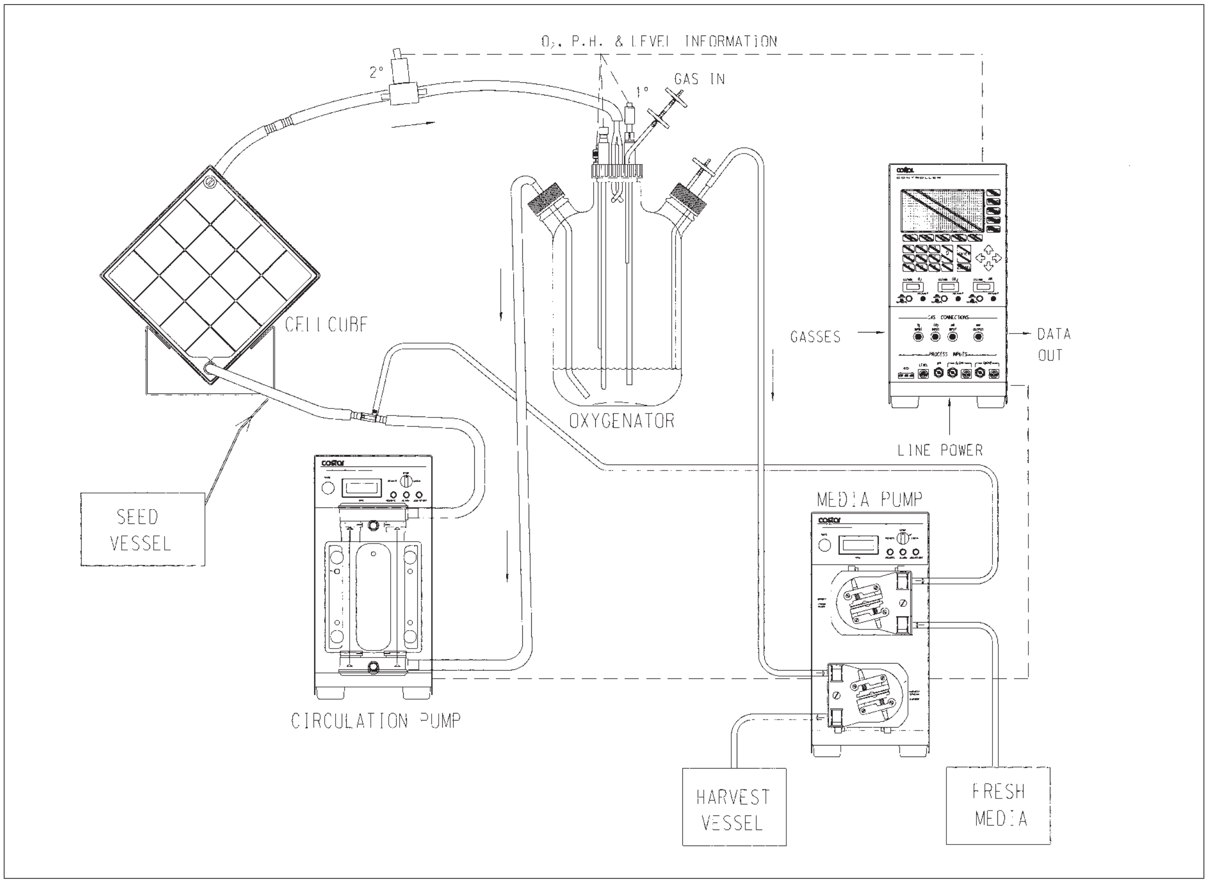


Figure 103. CellCube® System with 6L oxygenator (Corning Cat. No. 3143)

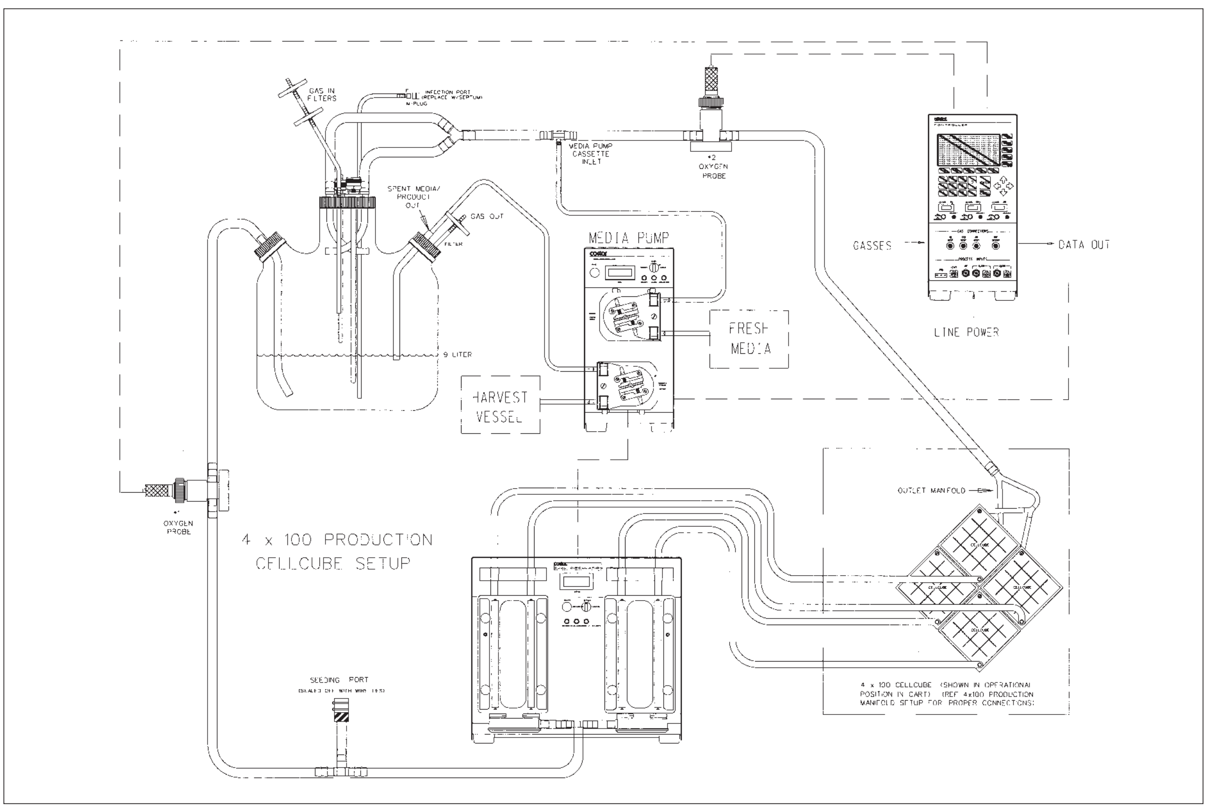


Figure 104. CellCube System with 36L oxygenator (Corning Cat. No. 3400)

contains detailed instructions on how to set up a CellCube® System for your particular application with the lengths and diameters of tubing that may be required. For instance, a CellCube system that is completely in a warm room may have different tubing requirements than a System that is set up in a roll – in incubator and utilizes media feed and harvesting bags in another location.

ASSEMBLY OF THE OXYGENATOR HEADPLATE

The assembly of both headplates is essentially the same. The exception is that the 36L Oxygenator has two 25 mm oxygen probes in line, while the 6L Oxygenator has a 12 mm oxygen probe in the headplate and one 25 mm oxygen probe in-line. Because of the liquid flow, the tubing lengths will be different for both systems.

Be sure that the glass vessel has been treated with sodium hydroxide as was described earlier (see page 7) so that proper fluid flow of the medium over the glass is maintained. The pH and Oxygen probes should be calibrated and polarized prior to assembly.

1. HEADPLATE

   Install the 100 mm headplate assembly onto the oxygenator bottle. It may be necessary to slightly tip the headplate so that the inlet tubes fit into the vessel neck. Be sure to inspect the sealing ring on the headplate so that it is correctly positioned (the concave portion should rest on the top of the glass rim), and that it is not nicked or distorted if the System is being

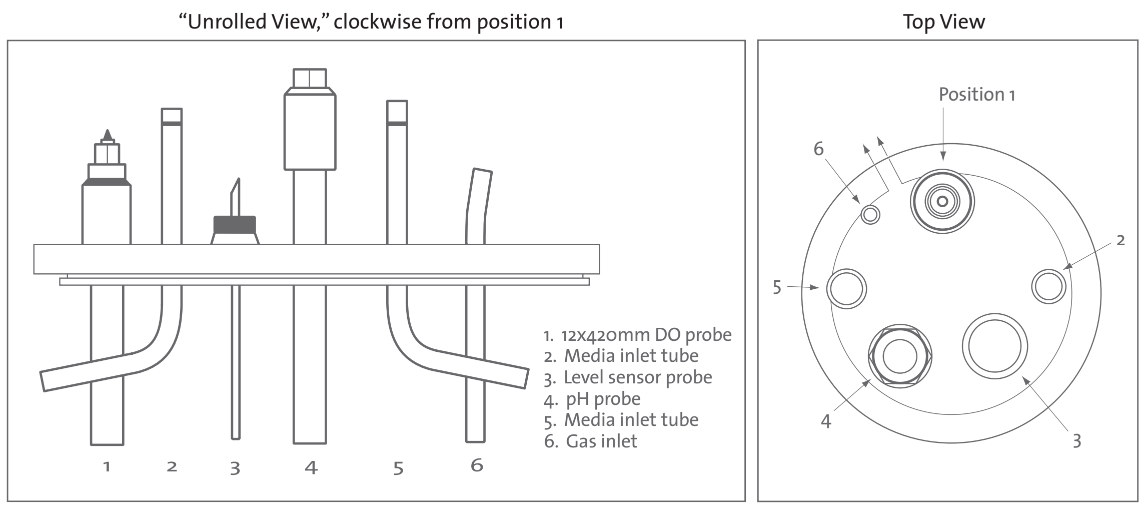


Figure 105. Drawing of a 6L Oxygenator Headplate

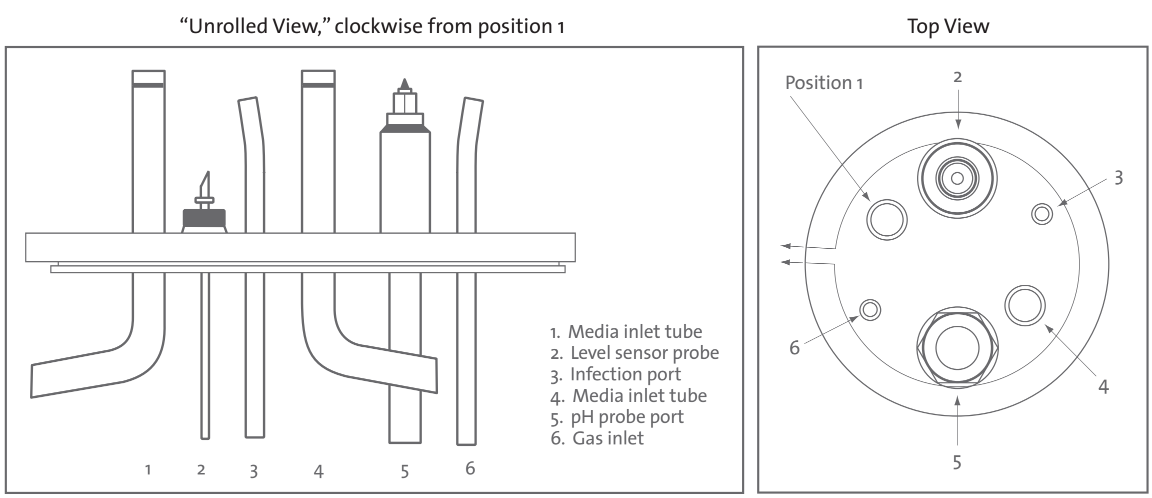


Figure 106. Drawing of a 36L Oxygenator Headplate

used after initial operation. Secure using the orange headplate securing ring.

2. Position: LEVEL SENSOR

   Insert the level sensor and cinch bushings into the appropriate opening. Finger tighten the level sensor cinch bushings.

3. Position: pH PROBE

   Wet the probe with distilled water to facilitate installation. Insert the pH probe in the pH probe fitting by gently twisting into place. The pH sensor should be threaded, installed into position, and finger tightened. To relieve stress on the pH probe during autoclaving, loosen by turning counter-clockwise a half turn.

   Refer to the subsection Calibration and Maintenance of pH probe in the Calibration Menus section (page 27).

4. Position: OXYGEN PROBE

   The 6L Oxygenator (Corning Cat. No. 3101) has one oxygen probe in the headplate and one in-line. The 36L Oxygenator has both probes in-line. Remove the DO probe membrane protection (black vinyl cap on the end of the probe). Wrap the threads on the probe with a small piece of polytetrafluorethylene (PTFE) tape. Wet the probe with distilled water to facilitate installation. Insert the probe in the probe fitting by gently twisting into place. The oxygen probe should be threaded, installed into position and finger tightened.

   Note: Do not twist the DO probe counter clockwise during insertion because the DO probe membrane is held in position with a clockwise thread.

5. Position: GAS INLET

   Prepare two 0.20 µm hydrophobic vent filters for sterilization by connecting in series utilizing a short length of 3/16" x 3/8" silicone tubing. Insert the barb end on one of the filters onto a 7 cm (2.5 in) 3/16" ID x 3/8" OD silicone tubing and place the other end of the tubing on the gas inlet of the oxygenator headplate.

6. Position: GAS VENT

   Use 3/16" ID x 3/8" OD silicone tubing for connection in order to support the weight of the filter. Cut a tubing length of 7 cm (2.5 in.) and place this on the short stainless steel tube of the combo side-arm fitting. Insert a single 0.20 µm hydrophobic vent filter for sterilization on the other end of the tubing.

Note: Be sure to place the appropriate protective cap for autoclaving over the attachment point for the electronic wiring on both the pH and oxygen probe prior to autoclaving.

BREAKDOWN OF THE OXYGENATOR HEADPLATE AFTER OPERATION

1. Decompress, remove and store the level sensor cinch bushings; remove and store the level sensor, pH and DO probes.
   a. Inspect the O-rings on the level sensor.
   b. Make sure to protect the end of the DO probe on the 6L vessel by replacing the black vinyl cap.
2. Loosen the orange headplate securing ring and remove the 100 mm headplate assembly from the glass reservoir of the oxygenator.
   a. Inspect the sealing ring on the headplate to be sure it is not damaged.
   b. Remove and store as necessary.
   c. Go to the Glass Reservoir Cleaning Protocol described in the CellCube® Oxygenator Section on page 7 if the vessel displays significant proteinaceous buildup after a lengthy operation.

ASSEMBLY OF THE CIRCULATION LOOP

1. Position: HARVESTING PORT

   Add an appropriate length of tubing (use 3/16" ID x 5/16" OD silicone tubing) for use between the long harvest tube on the stainless steel combo side-arm fitting of the oxygenator and the export pump tube set (harvest tube) (3/16" by 5/16" Pharmed or Bioprene) for the Watson-Marlow® pump head; alternate sizes may result in improper operation. Choose a pump tubing diameter size that is the same or one size larger than will be used on the import pump; i.e., if using the 1/16" tubing on the import pump, then use the 1/8" tubing on the export pump. At the other end of the harvest tube attach some silicone tubing with a female luer on the end. This can be of sufficient length to connect directly to a harvest bottle/bag, or a shorter piece that will act as a bridge between the pump and a harvest container. Place a clamp ~7 cm (2.5") before the female luer fitting. Wrap the end in autoclave paper. A male luer plug can be wrapped separately and autoclaved to plug the opening on the female luer after autoclaving. Secure all connections with a cable tie.

2. Position: MEDIA RETURN

   The connector kit (Corning Cat. No. 9267) contains a pre-assembled tubing set that includes two 1/4" x 7/16" silicone tubes on a Y-connector and terminating in a 1/4" x 1/2" male adapter. This portion of the connector kit is used between the two media inlet tubes of the oxygenator headplate and the 25 mm oxygen probe holder. Place one each of the shorter tubing pieces on the Y-connector over each of the media inlet tubes on the oxygenator headplate. Connect a piece of 1/2" ID x 3/4" OD silicone tubing, of appropriate length, between the media inlet adapter and the 25mm probe holder. Connect the same length of silicone tubing between the other side of the 25mm probe holder and the outlet tube of the CellCube® System. Install a clamp midway on this piece of tubing and terminate with a 1/2" male/male/female luer connector. Prepare for sterilization by wrapping the outlet adapter, and loosely inserting male plugs in the luer fittings. Alternatively, a bridge piece (~1 foot/30 cm length) of 1/2" ID x 3/4" OD silicone tubing can be used to connect between this 1/2" male/male/ female luer connector and its equivalent on the Single Side-arm Port during autoclaving, acting in essence as a substitute for the CellCube during the autoclave process to complete a loop. Each connector would then be removed after autoclaving and reinserted into the inlet/ outlet 1/2" ID x 3/4" OD tubing leading into the CellCube.

   Note: If the tubing length exceeds 1 meter (3 feet); add approximately 2 mL of distilled water per meter (3 feet) of tubing.

3. Position: SINGLE SIDE-ARM PORT

   Prepare the single side-arm port assembly of the oxygenator for connection to the inlet (bottom) manifold of the Circulation Pump cassette. Connect a piece of 1/2" ID x 3/4" OD silicone tubing from the single side-arm port to the inlet manifold (bottom) of the Circulation Pump cassette. Connect the outlet (top) manifold of the Circulation Pump cassette by connecting a piece of 1/2" ID x 3/4" OD silicone tubing of appropriate length with a clamp approximately 7 cm (2.5 in.) from the end of the tube, terminating with a 1/2" male/male/female luer connector that will be connected to the CellCube module. After attaching the tubing to the two female luer fittings on the 1/2" male/male/female luer connector (see below, Step 4), prepare for sterilization by wrapping the exposed connector in autoclave paper. Add approximately 5 mL of deionized water to the manifolds by holding the manifold in a horizontal position before connecting the tubing. If using a bridge piece (see Step 2 above), then insert the 1/2" male/ male/female luer connector into the other end of the bridge piece.

4. Position: CELL SEEDING PORT AND MEDIUM ADDITION PORT

   Cut an appropriate length of 1/4" ID tubing to bridge between the 1/2" male/male/female luer connector and the input pump on the Media Pump Unit. Position a clamp on the tubing approximately 7 cm (2.5") from one end. Place a male luer connector on the same end and connect it to the female luer fitting on the 1/2" male/male/female luer connector that will be closest to the CellCube inlet tube. This will become the cell seeding tubing, and its connection point will be the CELL SEEDING PORT. At the other end of this tubing attach a luer fitting to mate with an appropriate luer fitting on the Cell Seeding Reservoir. Repeat the process above with another similar length of tubing that you connect to the other female luer fitting with a male luer fitting. This will become the medium addition tubing, and its connection point will be the MEDIUM ADDITION PORT. At the other end of this tubing, insert a male luer fitting and a clamp as was done previously for the CELL SEEDING PORT. Attach this to one of the 3/16", 1/4" or 5/16" Pharmed tubings provided in the Inlet Pump Tubing Set. The selection of tubing is dependent on the amount of medium to be perfused into the CellCube System. Table 5 on page 48 will provide you with a guide to the range of volumes that can be delivered. If you are unsure of the amounts that will be used, then connect all three tubings in the Inlet Pump Tubing Set in a line of increasing size, and connect the smallest tubing to the line above. At the other end of the Inlet Pump Tubing, attach a length of 1/4" ID tubing with a male luer fitting sufficiently long to reach the Medium In reservoir or bag. Wrap the ends of the Cell Seeding Tubing, the Medium Addition Tubing, and the 1/2" male/male/female luer connector in autoclave paper. The appropriate luer plugs can be wrapped separately and autoclaved to plug the opening on the luer fittings on the tubing after autoclaving. Secure all connections, with a cable tie. If using a bridge piece (see Steps 2 and 3), then insert the 1/2" male/male/ female luer connector into the other end of the bridge piece.

5. Place the oxygenator/tubing/Circulation Pump assembly and cassette into an autoclavable container. The entire container can then be covered by the perforated autoclave bag. Check to see that all the connections have a cable tie and that the sterile wrappings will not rest in any potential condensate puddles while the assembly is cooling down. Invert or lay the Circulation Pump cassette down during the autoclave cycle in order to disable the check valves for sterilization. Detailed information on autoclaving is provided in the next section.
6. Collect and autoclave the appropriately sized vessels to seed, use for media and harvest. Vessels should be autoclavable and assembled with 0.20 µm air vents and full length dip tubes with a luer fitting on the end (this allows for a quick tubing connection). Example is shown in Figure 107.

Table 5. Fluid Handling Considerations for CellCube® System

			Module Size:	25-Layer	50-Layer	100-Layer
			System Volume (L):	3	4.5	7.5
Tubing I.D.	**RPM**	**Flow Rate (ml/min)**	**L/day**	**Vol Exchanges/day at Flow Rate**		
1/16" Tubing:	10	4.3	6.2	2.1	1.4	0.8
	20	8.6	12.4	4.1	2.8	1.7
	30	12.9	18.6	6.2	4.1	2.5
	40	17.2	24.8	8.3	5.5	3.3
	50	21.5	31.0	10.3	6.9	4.1
1/8" Tubing:	10	17.9	25.8	8.6	5.7	3.4
	20	37.2	53.6	17.9	11.9	7.1
	30	55.8	80.4	26.8	17.9	10.7
	40	74.4	107.1	35.7	23.8	14.3
	50	93.0	133.9	44.6	29.8	17.9
3/16" Tubing:	10	40.5	58.3	19.4	13.0	7.8
	20	81.0	116.6	38.9	25.9	15.5
	30	121.5	175.0	58.3	38.9	23.3
	40	162.0	233.3	77.8	51.8	31.1
	50	202.5	291.6	97.2	64.8	38.9

Figure 107. Flask with Portcap

Autoclave Preparation

The recommended autoclave cycle is between 45 and 90 minutes on slow exhaust at 121°C. It is simplest to assemble the System in the morning or early afternoon, autoclave it and then let the system cool down over night in a hood or a biological safety cabinet. The use of a plastic or metal container, sufficiently large enough to hold the oxygenator and tubing while in the autoclave is recommended. It is also a good idea to slide the entire assembly into an autoclave bag that is loosely closed. This provides additional safety after autoclaving while transporting the unit to the biohood for cooling and final assembly.

Note: The above is a conservative general recommendation and should be replaced by your validated procedure.

1. Secure wire ties around each luer and plastic connector and clip off the excess from each wire tie.
2. Securely tighten any luer-to-luer connections and loosen any clamps on the tubing to ensure free movement of steam through the fluid paths.
3. Loosely gather the longer lengths of tubing into large loops that have no constrictions and loosely secure with a large wire tie or lab tape. It is a good idea to drape the large loops over components of the oxygenator that can act as a “hanger,” such as the side-arms on the oxygenator or a probe on the headplate.
4. Ensure that any exposed ends of tubing are wrapped with autoclave paper.

STERILE SET-UP AFTER AUTOCLAVING

The following procedures require standard aseptic procedures for assembly:

1. Check all the connections to be sure that everything is still securely tightened.
2. Remove the oxygenator/tubing/Circulation Pump cassette assemblies from the autoclave and place into a biohood or a biological safety cabinet.
3. Unravel the tubing and lay it out in a hood or safety cabinet in a logical flow path orientation.
4. Place the CellCube® module, sample septum, media in, cell seeding and harvest tubing in a sterile biohood.
5. Connect the CellCube module outlet tube to the ½" male/male/female luer connector near the secondary oxygen probe and the inlet tube to the ½" male/male/female luer connector near the pump cassette. This is done by unwrapping any autoclave paper or removing the connectors at the bridge pieces and inserting them aseptically into the inlet and outlet tubes of the CellCube module.
6. If making all of the connections to Media In, Seeding Reservoir and Harvest Reservoir in the hood, complete those connections to the appropriate reservoirs before carefully moving the completed assembly out of the biohood and into the incubator, or to a cart holding the CellCube Circulatory Pump, Media Pumps and Oxygenator that will be moved into a warm room. Alternatively, if a biohood/safety cabinet is positioned next to the incubator, or if a Sterile Connect Device (SCD) is to be used to make future connections, the Circulation Loop and Oxygenator can be moved into the warm room/incubator, and connections can be done by moving the long leads to the reservoirs into the biohood/safety cabinet and making the connections in the biohood at those specific locations. A similar arrangement would occur with the use of the SCD, except that the lengths of tubing to the reservoirs could be considerably shorter. Attach the sample septum to the appropriate location.
7. If it was not done so prior to autoclaving, place quick-ties on every point of tubing connection, both to the Oxygenator and Pump Cassette, and to all luer fittings, barb fittings or quick connects that will experience any pressurization during operation.
8. Place the Circulation Pump cassette in the Circulation Pump. **Note:** Ensure the orientation of the cassette is correct.
9. Insert the Import and Export tubing segments into the appropriate pump heads on the CellCube Media Pump Unit. If using the Harvest Pump to introduce medium into the Oxygenator, be sure to orient the tubing in the pump head in a reverse orientation to be able to pump into the Oxygenator. Alternatively, an external peristaltic pump with a greater pumping action can be used for this purpose.

10. The system is ready for use when all the proper connections have been made ensuring there are no loose or open connections. It can now be transported to an environmental chamber if that has not already been done.
11. Close or open the appropriate clamps on any lines that are necessary to fill the system prior to inoculation.
12. With a reservoir of fresh medium attached, turn on the appropriate pump and begin to fill up the system with medium. Use the slowest speeds initially to ensure that there are no constrictions in the fluid paths, or that a clamp has not been accidentally left open.
13. We recommend that the system is operated at least overnight at the appropriate temperature to ensure that there are no leaks and as a sterility check prior to introducing the cells for attachment.

Note: If a CellCube® System run is planned for the next day, place the CellCube module and media in an incubator overnight in order to bring it up to temperature for the following day's run.

CellCube® System Operation

Because of its unique design, the CellCube System requires minimal supervision during operation. During the growth phase of the culture, daily adjustments to the media infusion rate and occasional adjustments to the gas flow may be required.

GROWTH PHASE

We refer the reader to the tutorial on the CD/video tapes provided in the setup package that discusses various options for initial control strategy. This tutorial details typical scenarios observed in CellCube System operation and “how to/what to do” strategies for operation. Generally speaking, the first few days of culture are usually satisfied by the media contained within the system after the initial seeding. The length of time between the initial setup and the time when media perfusion begins depends on the initial seed density and the metabolic rates of a particular cell line. The measurement of the residual glucose concentration in the circulating media and oxygen consumption are good indicators of the status of the culture. During the process of establishing a standard operating procedure, it may be beneficial to monitor glucose concentration several times daily at a variety of perfusion flow rates to determine the most economical and productive operating parameters for the system, through the growth and production phase of culture. In most cases glucose values should not fall below 50%. If this is observed, adding fresh medium or increasing oxygen to the system may be warranted. Similarly, a drop in the O_2 value at the secondary oxygen probe is typically observed. The difference between the O_2 values in the primary probe and secondary probe (the “delta” O_2) should become more pronounced, indicating that the cell population in the CellCube is in an active metabolic state.

Remember that the cells within the system rapidly reach a higher number of cells/mL than most traditional culture systems. Many of the typically used basal media, such as MEM, are designed to support only $1x10^6$ cells/mL per day. A typical CellCube System using four 100-Layer modules (Corning Cat. No. 3264), runs with approximately 85,000 cm^2 surface area per module and contains approximately six liters of media within the module. The ultimate cell density, depending on cell line, exceeds $1x10^7$ cells/mL in the culture vessel. At confluency, 2 to 4 reactor volumes of media are typically required per day to meet the demands of the increased cell number.

A conservative approach, if perfusion is to be used, might be to begin perfusion on day two to day four with one-eighth the total system volume, then double the volume perfuse each day until day 7 or 8. Confluency would ordinarily be reached on day seven, depending on the doubling times of the cell line and the initial seeding density.

PRODUCTION PHASE

The timing and parameters of the production phase of the culture depend on the type and ultimate use of the particular cell line. Many cultures require a medium for production that is different from that used in the growth phase of the culture. The transition from growth phase to production in traditional cell culture technology generally involves various washing and rinsing steps. A wash process might require as little as 10% wash volume for roller bottles, up to as much as 4 reactor volumes for a stirred tank reactor. The media and buffer used during these procedures are not generally taken up by the cells because of the short contact time involved.

The CellCube System can employ a perfusion methodology to accomplish this goal. The benefit of perfusion is the ability to provide a gentle transition between various operating conditions as opposed to fed-batch arrangements where fresh media can shock the cells and introduce a lag phase and a delay in productivity. Traditional washing steps may be unnecessary with the perfusion system.

One object of a wash step might be to reduce or remove the serum component of the growth media. The use of a perfusion system allows the user to easily pick the rate of change and the time of completion. For example, if the media from the first day of production is usually discarded, and a perfusion rate of 3 reactor volumes per day is being used, the reduction in serum content is greater than 75% per day.

The introduction of serum-free medium one day prior to reaching confluency may help adapt the cells to the new environment while still providing some residual serum for the transition. Another benefit of perfusion is that the transition from serum containing to serum-free media is gentle.

The cells remain at the appropriate temperature and usually stay firmly attached in the CellCube® Module. This reduces lost time and the expense of wasted medium while the cells adapt to a rapid shift in the medium environment. Alternatively, the perfusion rate may be increased to a rate equal to 4 to 6 reactor volumes/day to yield a similar result, although at a potentially greater expense.

Once working parameters are established for a cell line that are reproducible, it may be possible to eliminate perfusion by providing a single layer media bag or carboy which can hold an entire supply of medium for the duration of the run. Please contact Corning Technical Service for additional information using this culture strategy.

pH and Oxygen Control

The pH high limit control is coupled to the CO_2 gas flow. This is needed mainly during the initial growth phase of the culture. The carrier gas (air) is provided by an external air pump capable of delivering up to 1.0 LPM of air through a 0.2 µm filter. As the airflow rate is increased, the CO_2 flow should be increased to maintain the same proportional mix of air and CO_2 during the initial stages of culture. Often when cell densities reach sufficient levels the CO_2 may be lowered since the cells will provide sufficient CO_2 from metabolism to maintain pH set points.

The Oxygenator affords excellent O_2 transfer and CO_2 removal. Generally, increased gas flow and increased perfusion are all that are required to maintain the culture at the desired state.

Oxygen Addition

The addition of oxygen to the headspace gases increases the transfer of oxygen to the media returning to the oxygenator. Oxygen addition may be required for some cell lines depending on the size of the culture module used, the metabolic activity of the cells cultured, and the minimum dissolved oxygen level desired. Oxygenation can be controlled in two ways. If the 1º (primary) probe is selected, then additions of O_2 will occur based on the readings at the upstream oxygenator end of the process. If the 2º (secondary) probe is selected, then additions of O_2 will occur based on the readings at the outlet of the CellCube. Either strategy is acceptable. Typical set points for oxygen control are 60% on the 1º (primary) probe, or 40% on the 2º (secondary) probe. Higher initial O_2 settings on the primary probe should be avoided because over shoot of the desired set point is more probable and may have a deleterious effect on some cell lines. The controller will then add O_2 as necessary to maintain the desired set point. The flow rate should be increased when the set point cannot be achieved.

Note: The recommendations and observations are guide lines to help you better understand the general operation of the system. Each cell line and product may have special requirements not mentioned here.

Large Scale Operation

THEORY OF LARGE SCALE CULTURE OPERATION

Large scale operation of the CellCube® System is similar to the operation of the standard CellCube system. Special care should be taken when using the 4 x 100 assembly to make sure that each phase of operation from seeding to operation to production can be accomplished with generally acceptable time limits for that particular phase of operation. An example is the seeding of a 4 x 100 CellCube System assembly. The 4 x 100 assembly requires approximately 26 liters of cell suspension to fill the modules. The connectors, connections, and associated tubing should all be capable of delivering the cell suspension to the CellCube System at the low (2 to 3 psi) pressures recommended in the setup procedure. The delivery of the suspension should be completed in a period of time that assures that the cells remain in suspension and are not settling. Air should be removed from the inlet and the outlet manifolds to ensure better distribution.

The vessels or reservoirs for media and product/ waste should also be sized to accommodate the operation of the system in its most active state (30 to 100 LPD). Flow rates of all supplies (air, CO_2, oxygen), as well as media, should be set to control the system during unsupervised periods while accommodating the most active needs of the system. Failure to control the system with either enough media or gasses, may cause the loss of pH control or the premature reduction in growth rate within the entire system.

DESCRIPTION OF THE CART

The CellCube Quad Production Cart (Figure 108) is a device designed for simplified installation, filling, seeding, operation and harvesting of up to four 100-Layer CellCube Modules (Corning Cat. No. 3264). The quad production cart is a stainless steel, autoclavable, mobile rack that holds four 100-Layer CellCube modules or 340,000 cm^2 of culture surface in a small footprint. All operational procedures may be performed in this device because the CellCube modules can be fully rotated 360 degrees. The “cage” in the center of the unit can rotate freely, facilitating the attachment process of cells in the CellCube modules. To seed the module, rotate the cage first 90º counter-clockwise (Figure 109) from the operational horizontal position (Figure 110). After attachment on the first side, the cage is turned 180º in the clockwise direction to allow the cells to attach to the second side of the CellCube module. After attachment the cage is turned back 90º counter-clockwise back to the horizontal operating position.

The quad production cart has an adjustable wheelbase and can be sized to fit in a wide selection of tracks founds within a full size standard roll-in incubation or environmental chamber during growth periods.

Figure 108. Production Cart with 4 x 3264 (Corning Cat. No. 9234)

Figure 109. Seeding Position of Cart (Corning Cat. No. 9234)

Figure 110. Operation Position of Cart (Corning Cat. No. 9234)

General Material Advisory

Below are some general guidelines for GLP/GMP environments. Corning recommends replacing all the disposables each batch including the vent filters and silicone tubing.

- All CellCube® Modules are pre-sterilized and single-use disposable items. DO NOT autoclave a CellCube module at the end of a run because it may compromise the integrity of the CellCube module.
- The Circulation Pump cassette and the dual Circulation pump cassette are one-time autoclavable single-use items. These items should be discarded after each CellCube system run.
- The pH and dissolved oxygen probes, from either Broadly James or Ingold, are supplied along with the manufacturer's recommended procedures for cleaning and maintenance. These instructions are included with each new probe and should be filed for your reference. For further explanation of this maintenance refer to the instruction video tape/CD presentation and the information in the Appendix.
- Vent filters should be replaced after four autoclave cycles by proper use. For GMP and GLP batches we recommend single-use.
- Silicone tubing should be replaced after each CellCube system run.
- All O-rings should be replaced after 10 autoclave cycles or earlier if noticeable fraying or cracking of the O-rings is observed.
- Nylon ties are considered nonfunctional after autoclaving.
- The sample septum Corning supplies is pre-sterilized and a single-use disposable. Under no circumstances should the sample septum be autoclaved since it may melt during the autoclave process.

Appendices

VENDOR LIST

Tubing

Platinum Cured Silicone

$\frac{1}{2}$" ID X $\frac{3}{4}$" OD Silicone Tubing
Durometer 50

$\frac{3}{16}$" ID X $\frac{5}{16}$" OD Silicone Tubing
Durometer 50

$\frac{3}{16}$" ID X $\frac{3}{8}$" OD Silicone Tubing
Durometer 50

PharMed

$\frac{1}{8}$" ID x $\frac{1}{4}$" OD PharMed Tubing

Norprene

For the tubing in pumps and the size will be dependent on pump head size

pH and Oxygen Probes, including membranes replacement kits are available from the following vendors:

Broadley James	Ingold Electrodes Inc.
1714 S. Lyon Street	261 Ballardvale Street
Santa Ana, CA 92705	Wilmington, MA 01887
Tel. 714.547.8061	Tel. 508.658.7615
Fax 714.547.8988	Fax 508.658.6973
www.broadleyjames.com	www.elscolab.nl.com

Polycarbonate Connectors

Qosina Corp.
150-Q Executive Drive
Edgewood, NY 11717
Tel. 631.242.3000
Fax 631.242.3230
www.qosina.com

$\frac{1}{2}$" x $\frac{1}{2}$" male/male with two female luer ports (Qosina Cat. No. 27210)

$\frac{1}{2}$" x $\frac{1}{2}$" male/male with two female luer ports (Qosina Cat. No. 27226)

$\frac{1}{2}$" x $\frac{1}{2}$" X 1/2” male/male/male 45 connector (Qosina Cat. No. 60127)

$\frac{1}{4}$" x $\frac{1}{2}$" reducer connector with female luer port (Qosina Cat. No. 27224)

$\frac{1}{4}$" x $\frac{1}{2}$" reducer connector; no female luer port (Qosina Cat. No. 27215)

Or
Alternatively from Corning — directly in the start up kit

Vent Filters

Corning Incorporated
Life Sciences
45 Nagog Park
Acton, MA 01720
Tel. 978.442.2200
Fax 978.442.2476
www.corning.com/lifesciences

50 mm, 0.22 µm vent filter; 12/pk
Cat. No. 131261 Syrfil-FN

Miscellaneous

Cole-Parmer Instrument Co.
7425 North Oak Park Avenue
Chicago, Il 60648
Tel. 800.323.4340
Tel. 847.549.7600
Fax 847.247.2929
www.coleparmer.com

	C-P Cat. No.
Nylon Cable Ties	L-06830-54
Nylon $\frac{3}{16}$" tubing connectors	6463-10
Nylon $\frac{1}{2}$" tubing connectors	6463-25
Nylon $\frac{5}{16}$" tubing clamps	L-06833-00
Quick tie tensioning tool	G-06830-00

Alternatively, the cable ties may be purchased at a local hardware stor or as part of the Corning® CellCube® System Start-up Kit.

Buffer Addition Pumps

New Digital System
Watson-Marlow, Inc.
220 Ballardvale St.
Wilmington, MA 01887
Tel. 800.282.8823
978.658.6168
Fax 978.658.0041
www.watson-marlow.com

Model # 101 U/R
#07518-10 Pump Head

Luer Tubing Fittings

Value Plastics, Inc.
3325 Timberline Rd.
Fort Collins, CO 80525
Tel. 970.267.5200
Fax 970.223.0953
www.devicelink.com

Nylon
Male luer with lock 3/16" hose barb
VP Part No. MT LL 250-1

Male luer with lock 1/8" hose barb
VP Part No. MT LL 230-1

Female luer 3/16" hose barb
VP Part No. FT LL250-1

Polyproplyene
Male luer with lock 3/16" hose barb
VP Part No. MT LL 250-6

Male luer with lock 1/8" hose barb
VP Part No. MT LL 230-6

Female luer 3/16" hose barb
VP Part No. FT LL250-6

Nylon 3/4" Tubing Clamps

McMaster-Carr
P.O. Box 4355
Chicago, Il 60680-4355
Tel. 630.833.0300
Fax 630.834.9427
www.mcmaster.com

M-C Part No. 5031 K-13

GENERAL SPECIFICATIONS

Digital Controller (Corning Cat. No. 3220) Specs

Environmental	
Temperature:	0 to 600°C
Max Humidity:	95% Noncondensing
Static Discharge:	Meets IEC 801-2
Radiated Electric Field Susceptibility:	Meets IEC 801-3
Conducted Transient Susceptibility:	Meets IEC 801-4
Power Supply	
AC Input:	100 to 240 V/250 50 to 60 Hz 1.0A (max) 45 Watt
DC Input:	13.8VDC ±10% 2.5A (max)
Inputs	
Resolution:	12 Bits
Oxygen Channels 1 & 2	
Measurement Range:	0.0 to 200.0%DO
Resolution:	up to 0.1%DO
Bias Voltage:	-0.685VDC (typ)
Input Current Range:	0 to 130nA (typ)
pH Channel	
Measurement Range:	0 to 14pH
Measurement Resolution:	0.01pH
Input Impedance:	> 1012 ohm
Input Voltage Range:	-450mV to +450mV
RTD	
Measurement Range:	0 to 1000C
Sensor Current:	1 mA
Sensor Type:	3 wire Platinum
Sensor Resistance:	100 ohm @ 0.00C
Sensor Curve:	European (alpha = .00385)

Level Sensor

Nominal Frequency:	1.1 Khz
High Level Threshold:	50K ohm (typ)

Outputs

Analog	
Resolution:	12 Bit
Span:	0-20mA or 4-20mA
Max Output Voltage:	10VDC
Max Load Impedance:	500 ohm
Alarm	
Type:	Dry Contact
Rating:	30 V/1 Amp (max)

Mass Flow Controllers/Gas Connections

Flow Range:	0 and 10 to 500 cc/min
Accuracy & Linearity:	±1% of Full Scale
Temperature Coefficient:	0.1% / 0C
Repeatability:	Within 0.2% Full Scale at any constant temperature.
Response Time:	1.2 sec
Input Pressure Range:	1 to 10 psi

Safety/Emissions

Meets Safety Requirements:	EN 61010-1 UL 3103-1 CSA C22.2
Emissions:	EN 50081-1 FCC Part 15, Class A

Serial Ports

Port A	RS-232 (DCE) & RS-485
Port B	RS-232 (DTE) & RS-485

Physical Dimensions

Height	37 cm (14.4")
Width	18 cm (7")
Depth	436 cm (14")
Weight	15.5 kg (34 lbs)
Approvals	ETL, ETL-C, CE

Host Software System Requirements (optional)

Computer:	486-33 Mhz (min.)
Operating System:	Windows 95 or more recent operating systems (required) (Not compatible with Mac operating systems)
RAM:	8 Meg (min.)
Hard Drive Space:	5 Meg (min.)
Serial Port:	16550 UART (recommended)

Circulation Pump (Corning Cat. No. 3222) Specs

Specifications	
Fuses - 2 ea.	2.0 amp 250V, 5 x 20 mm
Universal Power Supply	
Frequency	47 to 63 Hz
Auto-switching Voltage	Range
1st Stage	90 to 132 VAC
2nd Stage	180 to 264 VAC
Power Consumption (max.)	140 Watts
Normal Range	4 to 84 RPM 0.5 to 3.9 LPM
Normal Operating Temperature	10 to 40°C (50 -104°F)
Physical Dimensions	
Height	36.6 cm (14.4”)
Width	17.6 cm (6.9”)
Depth	40.0 cm (15.8”)
Weight	21 kg (46 lbs)
Approvals	ETL, ETL-C, CE

Media Pump (Corning Cat. No. 3221) Specs

Specifications	
Fuses - 2 ea.	2.0 amp 250 V, 5 x 20 mm
Universal Power Supply	
Frequency	47 to 63 Hz
Auto-switching Voltage	Range
1st Stage	90 to 132 VAC
2nd Stage	180 to 264 VAC
Power Consumption Max	40 Watts / 172 VA
Normal Range	2 to 64 RPM 3 -354 Liters/day
Normal Operating Temperature	10 to 40°C (50 -104°F)
Physical Dimensions	
Height	36.6 cm (14.4”)
Width	17.6 cm (6.9”)
Depth	40.0 cm (15.8”)
Weight	26 kg (57 lbs)
Approvals	ETL, ETL-C, CE

Dual Circulation Pump (Corning Cat. No. 3223) Specs

Specifications	
Fuses - 2 ea.	2.0 amp 250 V, 5 x 20 mm
Universal Power Supply	
Frequency	47 to 63 Hz
Auto-switching Voltage	Range
1st Stage	90 to 132 VAC
2nd Stage	180 to 264 VAC
Power Consumption Max	140 Watts
Normal Range	4 to 84 RPM
	0.4 to 9.2 LPM
Normal Operating Temperature	10 to 38°C (50 -100°F)
Physical Dimensions	
Height	36.6 cm (14.4”)
Width	42.0 cm (16.5”)
Depth	39.4 cm (15.5”)
Weight	30.5 kg (67 lbs)
Approvals	ETL, ETL-C, CE

PUMP INTERFACE

The pump interface connections provide access to the advance features of the pump, this permits computer control and monitoring, including the following:

1. Current loop input (remote speed control)
2. Current loop output (remote monitoring of speed)
3. Alarm circuit
4. Frequency output (calibration)
5. Status indicators
6. Pump Identification (binary)

Caution: A technician, with electronic circuits' experience, should make the following connections.

Explanation of Remote Interface

Pins #1 & 2: 4-20 mA Current Loop Input

Pin #1 is positive. The best results are achieved when pin #2 is grounded to pin #15 and the input current source is connected between pins #1 & #2. The pump will run at the slowest speed with a 4 mA input and at the fastest speed with a 20 mA input.

The intermediate speeds, may be calculated, with the formula:

$$RPM = MIN + ((I - 4) \times (MAX - MIN)/16),$$

where MIN and MAX are the minimum and maximum speed settings that may be achieved with the front speed pots. The formula may be simplified to:

$$RPM = 4 + [(I - 4) \times 5]$$

for the Corning Cat. No. 3222 Circulation Pump.

There is a resistance of 250 ohms, between pins #1 and #2. A 20 mA current will be developed with an applied voltage of +5 VDC to pin #1 and common to pin #2. The best results are achieved, when pin #2 is connected to ground. Digital to analog outputs (0 to 5 VDC), may be connected using this method. You may use other voltage inputs, with the addition of the proper current limiting resistor.

It is important NOT TO EXCEED 20 VDC or 20 mA on these INPUT CONNECTIONS!

The remote interface is buffered from the actual control circuit. The accidental destruction of the remote interface should not have any effect on the operation of the pump while in the local control mode. One safety feature of the pump is that the actual speed control of the pump, will default to the setting of the rate control on the front panel, if all the remote connections are not complete.

Pin #3

Pin #3 is not used at this time.

Pin #4: Remote Enable Connection

Pin #4 is the remote enable connection. When pin #4 is connected to ground (Pin# 15) the pump will run in the remote mode (see front panel) if the remote available pin is also active (see Below). Do not apply voltages above 12 V to this pin! The current sink capability must be less than 5 mA.

Pin #5: Remote Available Connection

Pin #5 is the remote available connection. This pin must be tied to ground (Pin #15) before the pump will run in the remote mode. It may be connected directly to ground or it may be connected to a watchdog circuit of the controlling device. If the connection is open, the pump will display an alarm condition, and it will run at a speed, determined by the speed pot on the front panel. Do not apply voltages above 12 V to this pin! The current sink capability must be less than 5 mA.

Pin #6: Is not used at this time.

Pin #7: Frequency Output

Pin #7 is the frequency out connection. The frequency out connection, is a current sinking output, that provides a frequency, that is directly proportional to the pump speed. The formula to calculate the speed for the Circulation Pump (Corning cat. No.3222) is

$$F_{out} = (RPM) \times (42.47)/15 \quad \text{or}$$

$$F_{out} = RPM \times 2.831333.$$

Pins #8 and 9: 4-20 mA Current Loop Output

Pins #8 and #9, are the 4 to 20 mA current loop output of the Circulation Pump (Corning Cat. No. 3222). Pin #8 is positive. These pins provide a current output that is proportional to the pump speed. The speed of the output can be determined by using the following formula:

$$I = 4 + RPM * 16/MAX$$

where MAX is Maximum speed available from the speed pot on the front panel of the pump. The formula can be simplified to:

$$I = 4 + (RPM \times 0.1904761)$$

The maximum voltage that can be produced at pin #8 is 20 VDC. The maximum load between pins #8 and #9 is 1000 ohms. The use of a 500 ohms resistor would provide a 2 to 10 volt output, that may be read or recorded directly.

DO NOT APPLY ANY VOLTAGE TO THESE PINS!!

Pin #10: Alarm

Pin # 10 is the alarm output connection. This is a sinking current output that goes active when there is an alarm condition in the pump. There are three conditions, which can activate an alarm in the pump.

The pump is in the local or remote mode and the pump mechanism has been stopped mechanically, seized, or broken.

The pump is in the local or remote mode and the interlock mechanism has been activated or is still activated and the start switch has not been pressed.

The pump is in the remote mode and the remote available signal is not active (pump runs at the local speed setting).

Pin #11: Remote Ready Indicator

Pin #11 is the remote ready indicator. This connection is a current sinking output that goes active, when the pump is in the remote mode and ready to accept input from a remote source.

Pin #12

Pin #12 is not used at this time

Pins #13 and #14: Pump ID

Pins #13 and #14 are the pump ID pins. These pins provide a binary code that defines the type of pump to a Controller (Corning Cat. No. 3220).

	Pin #14	Pin #13
Not Used	OPEN	OPEN
#3223	OPEN	GROUND
#3222	GROUND	OPEN
#3221	GROUND	GROUND

Pin #15: Ground

Pin #15 is the common ground connection for the remote interface. This is the ground reference for all of the input and output connections to and from the pump.

External Alarm Connections

The pump provides a remote alarm connection on the rear panel and a mating connector. The alarm contacts are dry contacts, rated at 30 VDC. The contacts are normally open. The contacts close under the following alarm conditions.

1. Door open
2. Improper remote connection
3. Loss of rotation (seizure) in the run state (30 second delay)

This alarm connection may be used to signal your in house alarm system, visual or aural alarm system.

Notes

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 1.800.492.1110. Customers outside the United States, please call +1.978.442.2200 or contact your local Corning sales office listed below.

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f 978.442.2476

www.corning.com/lifesciences

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f 81 3-3586 1291

Korea
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f 82 2-796-9300

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f 65 6861-2913

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f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
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f 31 (0) 20 659 76 73

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