Millipore®

User Guide

Cellicon™ Perfusion Solution for Process Development

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Contents

MERCK

Introduction

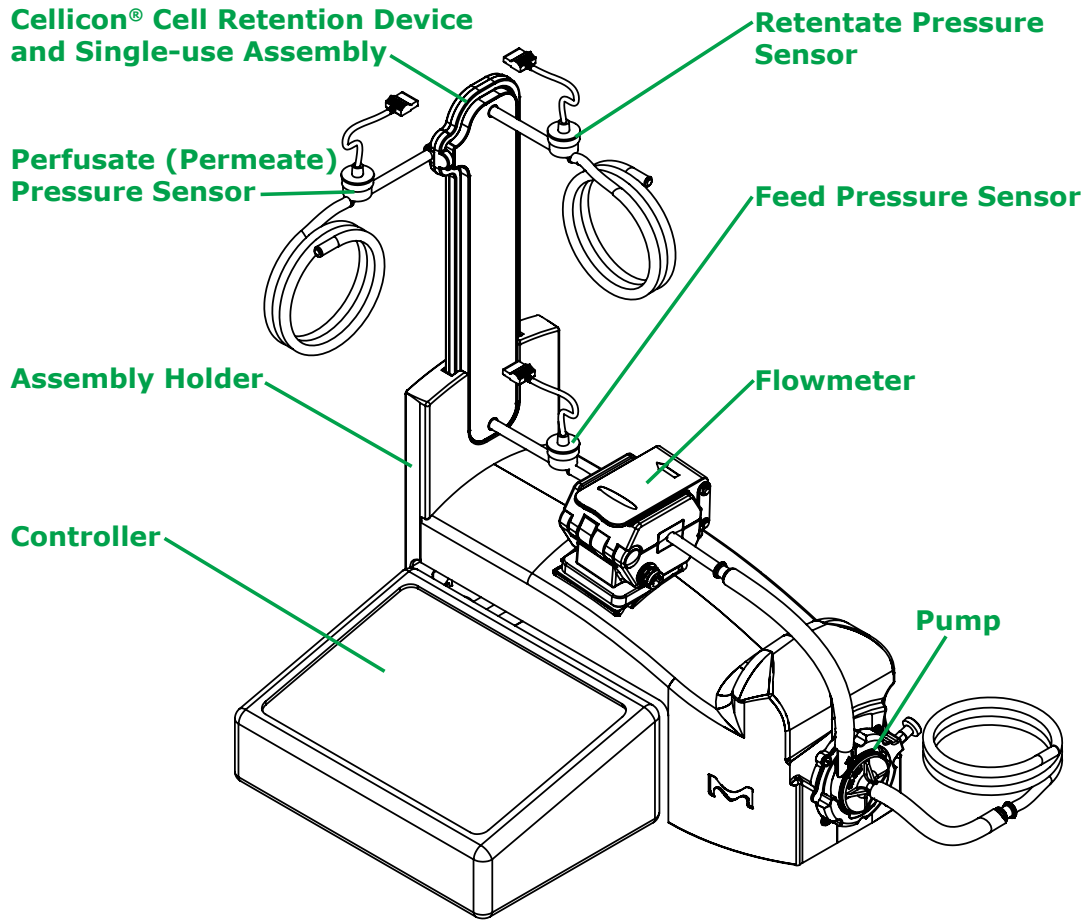
The Cellicon™ Perfusion Solution for Process Development includes scalable, single-use filters and controllers that enable seed train process intensification in upstream biomanufacturing. The filtration technology provides high throughput and membrane efficiency in a closed assembly, while maintaining a low shear environment to support cell growth.

This guide describes the installation, setup and use of the Cellicon™ Perfusion Solution for Process Development.

This system is for industrial use only and cannot be used in a residential environment.

System Components

The Cellicon™ Perfusion Solution for Process Development includes a single-use filter and assembly, a holder for the assembly, a controller, a low-shear centrifugal pump, flowmeter and feed pressure sensor.



Cellicon™ Perfusion Solution for Process Development

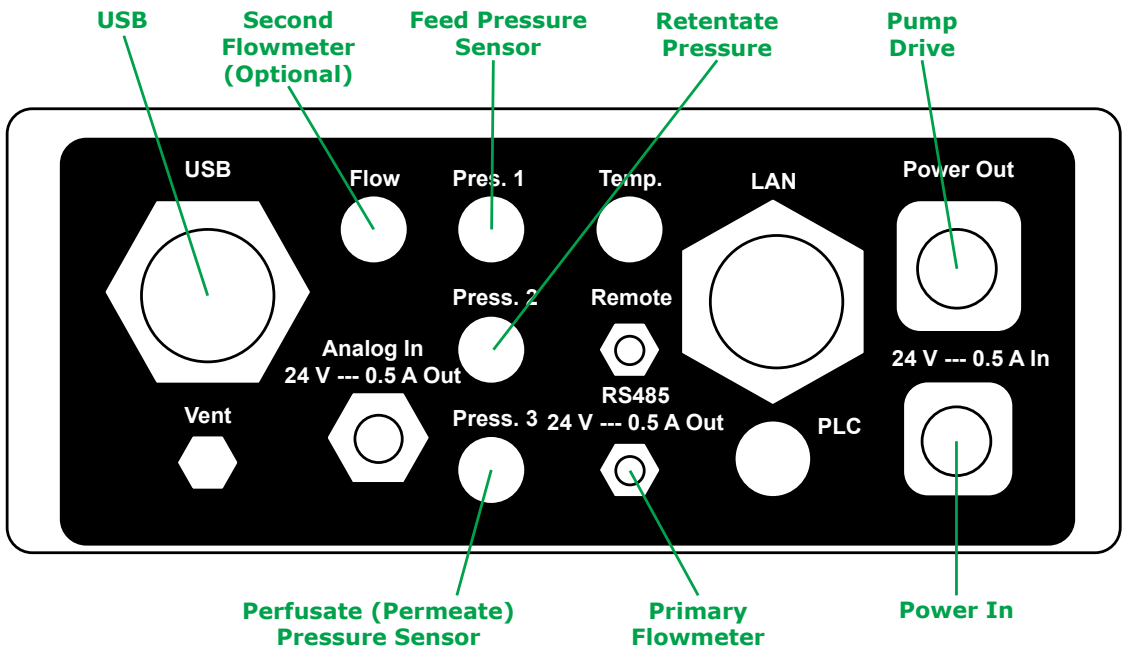
Setting Up the System

The system should be set up close to the bioreactor, with cords behind the holder. The controller screen should be easily accessible to the user.

Connecting the Component Cables

Carefully remove all the system components from the packaging.

Connect the components at the rear of the controller as shown below.



Once connected to the rear panel, connect the cables to the components as listed here:

Cable Color	Connection
Gray	Primary flowmeter
Black	Power in (24 V)

When all cables have been connected, connect the power cord to the power source.

Installing the Filter

Refer to System Components image for reference.

Prior to installation, there may be a slight kink in the feed tubing line between the filter and the pressure sensor. This is cosmetic and does not impact the function of the system. It should self-resolve by laying the tubing inside the flow meter clamp.

1. Carefully remove the filter and tubing assembly from the bags, and remove the assembly from the backing card.
2. Slide the device into the slot on top of the holder.
3. Open flowmeter clamp (located at the center of the holder) and lay tubing inside.
4. Close the flowmeter, ensure the tubing is not pinched or kinked, then clamp it.
5. Place the pump head into the pump drive by aligning the outlet barb on the pump head with green coloring on pump drive. Pull pin out and push pump head in place. Rotate pump head clockwise so barb is located over the red coloring and release pin.
6. Connect the three pressure cables from the control box to the sensors on the assembly as follows:
   Press. 1 to the feed line
   Press. 2 to the retentate line
   Press. 3 to the perfusate (permeate) line.

Connecting the Bioreactor

Use a tubing welder to connect:

- The cell retention filter feed line to the device inlet line of the bioreactor, ensuring the line is submerged, and below the sparger.
- The cell retention filter retentate line to the device outlet line of the bioreactor.

Starting the System

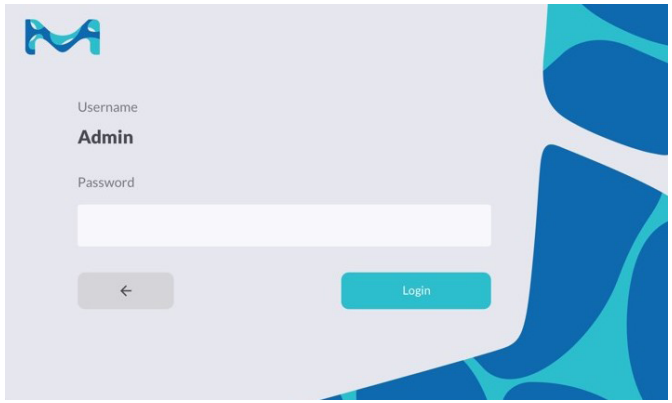
The system must be fully assembled prior to operation, as described in Setting Up the System. The filter assembly must be installed and the pump and sensors connected before starting a run.

Press the power button on the left side of the controller to turn it on. The button should light up blue when the controller is on.

Powering up the system launches the perfusion control software. The initial screen is the username selection screen.

After selecting the username, the password screen will appear.

Touch the white box on the screen to open the keyboard. Enter the password and touch Login to log into the system.

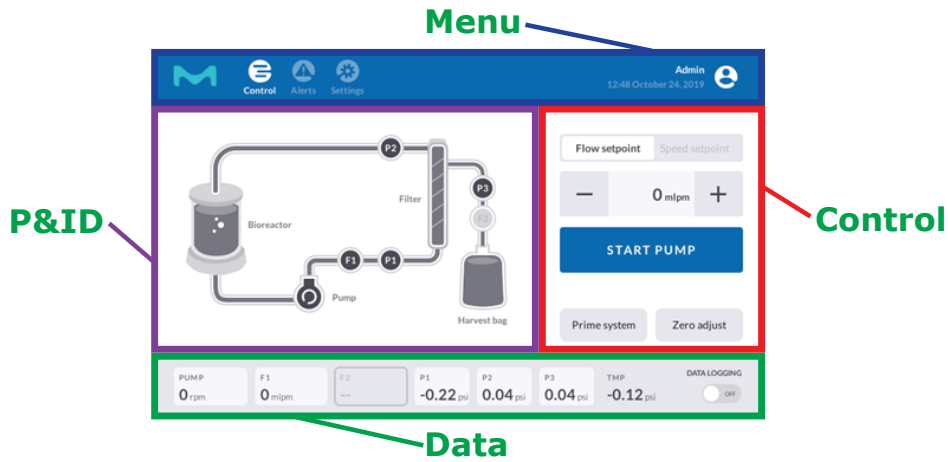


User Interface

The main screen of the system has four regions:

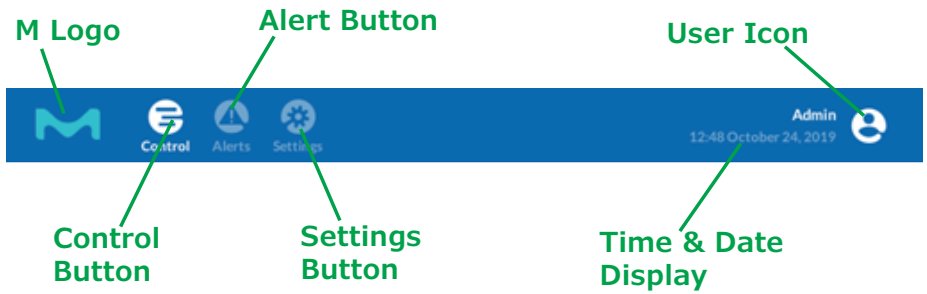
- Menu
- Piping and instrumentation diagram (P&ID)
- Control
- Status

The menu region and the status region are always visible. The P&ID and control regions are only visible when Control is selected on the menu region.



Main Menu Screen

Menu



The menu region is always visible and has five main elements.

M Logo

Selecting the M logo starts a countdown of three seconds, then generates a screenshot that is captured and saved to the USB drive with the files name LCO_Screenshotxx.png, where xx represents a consecutive number.

Control Button

Selecting the Control button returns the system to the main screen.

Alerts Button

Selecting the Alerts button displays a log of alarms and notifications. The log may also be accessed when an active alert is selected on the main screen. Each alarm or notification will display the time/date of activation, the acknowledgement status, a description of the alert, and tips for troubleshooting. See Alert Types section for explanation of the alerts page.

Settings Button

Selecting the Settings button opens the settings screen.

User Icon and Time/Date Display

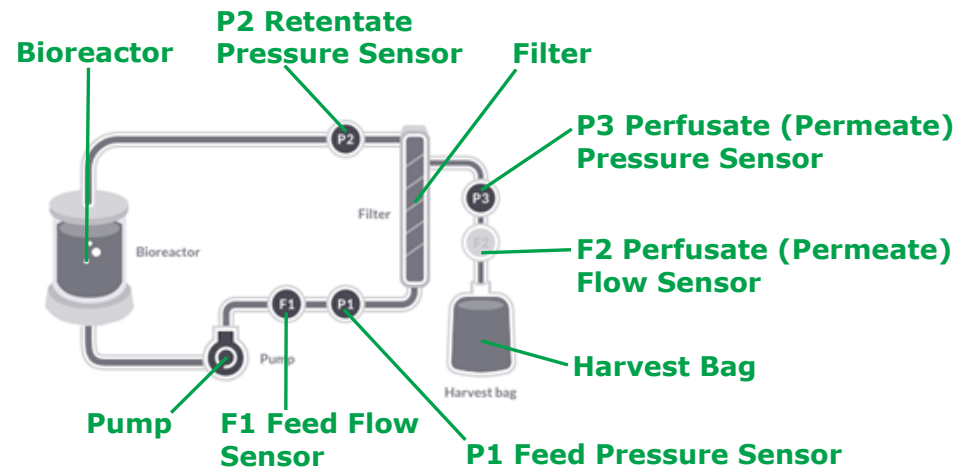
Selecting the user icon allows the user to lock the screen or switch users. Lock screen will cause the controller to go into screensaver mode.

Switch user allows the active user account to be changed.

The date and time settings can be changed in the Settings menu.

P&ID

The P&ID screen displays the components of the system connected to the bioreactor and the Cellicon™ Perfusion Solution for Process Development.



Component status is indicated by color:

Item	Color	Meaning
Component	light grey	Disconnected from the system
	dark grey	Connected to the system
Fluid Path	dark gray	Pump OFF
	light blue	Pump ON

The animation indicates the direction of flow and acts as the pump state indicator, showing that the pump is on. The perfusate (permeate) line will only turn light blue if flowmeter 2 is connected and measuring an active flow rate.

Tapping the icons listed in the following table displays the sensor output:

Icon	Display
Pump	Speed in RPM
F1	Feed flow rate in mL/min
P1	Feed pressure in selected pressure units
P2	Retentate pressure in selected pressure units
P3	Perfusate (permeate) pressure in selected pressure units
F2	Perfusate (permeate) flow rate in mL/min

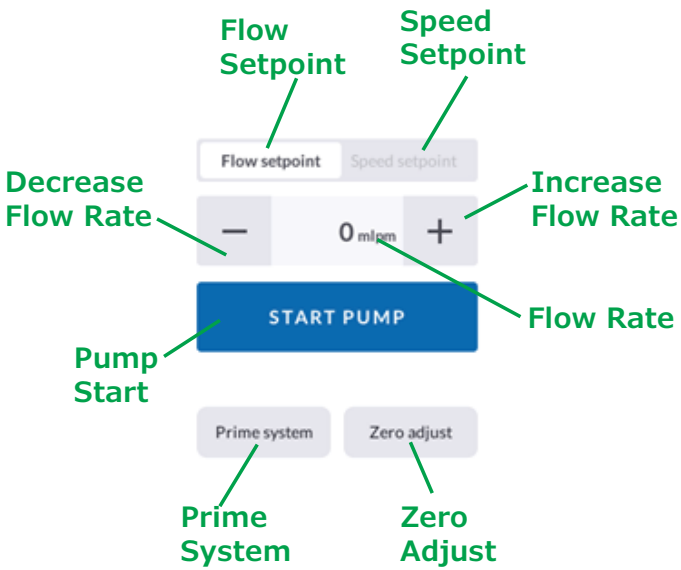
The icons change color to indicate the alert state of each component:

Color	Alert Type
Red	1
Yellow	2
Blue	3

See Table of Alerts for details on the alert classifications.

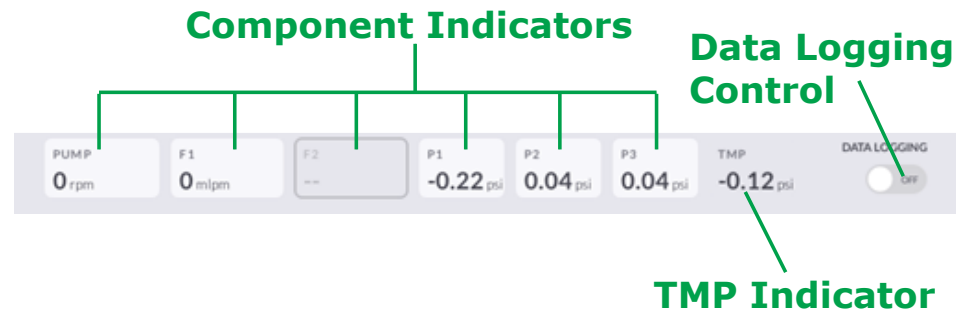
Control

Change the pump state, setpoint, zero-adjust and access guidance for priming from this screen.



Status

The current values, state of the system components and data logging switch are displayed here.



The TMP is a calculation based on P1, P2 and P3. It will only display a value when all three pressure sensors are connected.

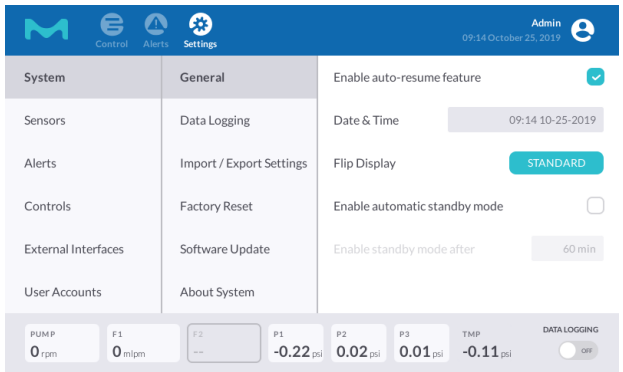
The data logging feature requires a USB drive. See Data Logging for details about how to use data logging.

Each box within the status bar change color to indicate the status of each component:

Color	Component Status
Grey	Not connected
White	Connected and ready to use (except pump, which will display a type 1 alert until single-use pump head is loaded)
Blue	Connected with a Type 3 alert
Yellow	Connected with a Type 2 alert
Red	Connected with a Type 1 alert

Settings

Selecting an element in the settings menu will open the screen for that element, and will be indicated by a darker grey background. The elements include System, Sensors, Alerts, Controls, External Interfaces and User Accounts. Refer to the example below:

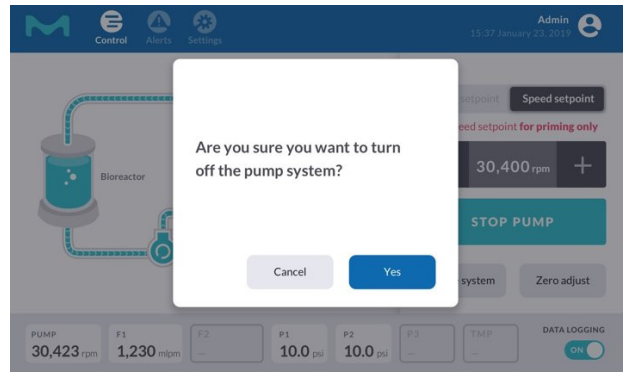


System Screen Selected

Running the Pump

Start/Stop

Start and stop the pump from the control region of the main screen. If the pump is off, the button will read START PUMP. If the pump is on, the button will read STOP PUMP. Stopping the pump will cause a confimation screen to appear.

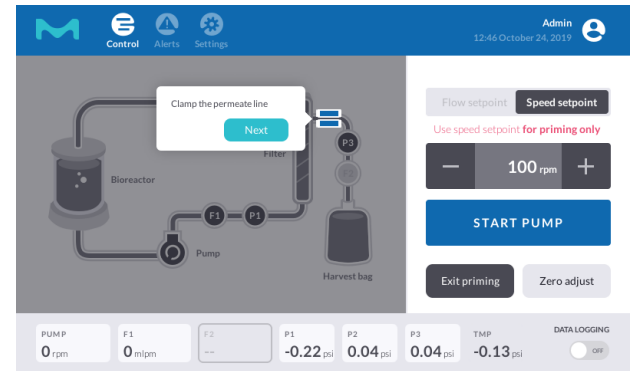


Prime System

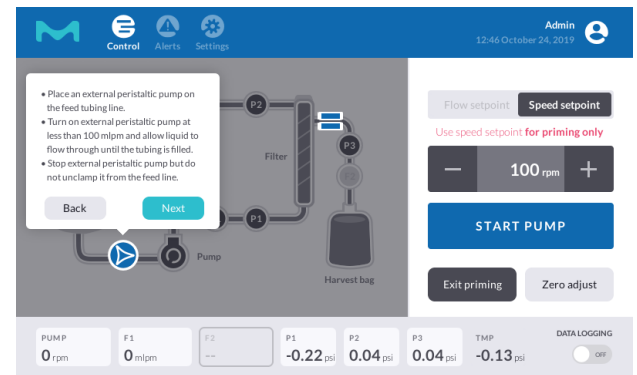
The pump needs to be primed to reduce damage to cells because the design is not positive displacement.

Prime system is accessed from the control region of the main screen. Fill the flow path with liquid prior to starting the prime sequence.

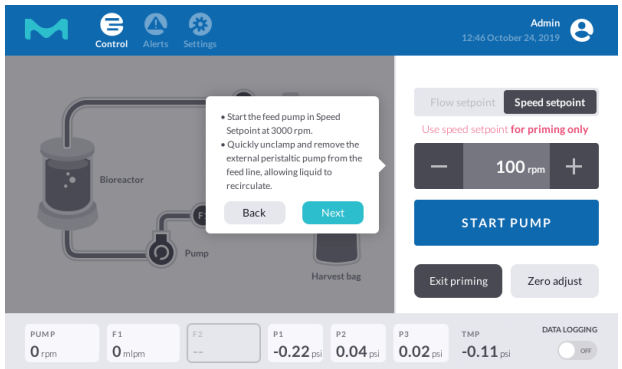
The system will switch into speed setpoint mode if Prime system is selected when the pump is off. If Prime system is selected when the pump is running, the priming guidance will start with the pump in the current setpoint mode.



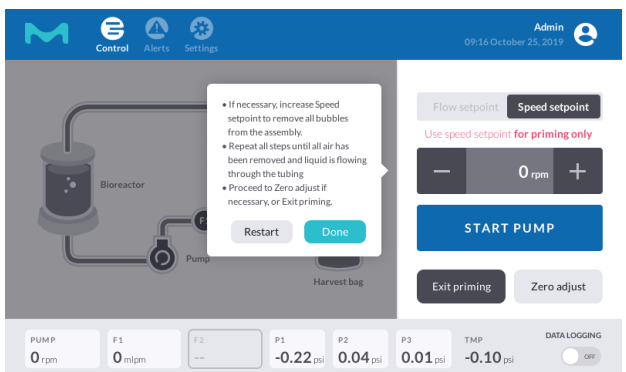
Prime System Guidance Step 1



Prime System Guidance Step 2



Prime System Guidance Step 3



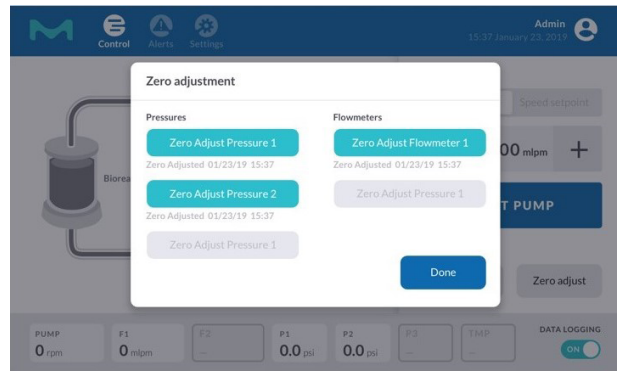
Prime System Guidance Step 4

The Next button will progress to the next step in the manual priming procedure. The Back button will revisit the previous step. When all the steps are complete, select Done. Select Exit priming to return to the main screen at any point.

When Done or Exit priming is selected, the system returns to flow setpoint mode.

Zero Adjust

Select Zero adjust from the control region of the main screen. A popup containing options for zero adjustment will appear.



If connected, the following sensors are available for zero adjustment:

- Pressure 1
- Pressure 2
- Pressure 3
- Flowmeter 1
- Flowmeter 2

Select an icon to initiate a zero adjustment of that sensor. Adjustment may take a few minutes to complete.

When the adjustment is complete, the button will return to its original state. The time and date of the last zero adjustment for each sensor will be displayed below the button. If an adjustment fails, the button will turn red and read Zero adjustment failed MM/DD/YY HH:MM.

To initiate a zero adjustment for flowmeter 1, the pump must be stopped. A pop-up will appear to confirm that the pump is stopped. Flowmeter 2 does not require that the pump is stopped.

All sensors should be zero adjusted prior to starting a run. Only one sensor can be zero adjusted at a time.

Control Modes

The pump can be run in either Flow setpoint or Speed setpoint. Choose the mode using the toggle switch at the top of the control region of the main screen.

When switching from flow control mode to speed control mode while the pump is running, the controller will default to the current pump speed (RPM). Adjust the speed using the +/- icons or enter a new speed in the field.

When switching from speed control mode to flow control mode while the pump is running, the controller will default to the last flow setpoint (mlpm) entered by the user. Adjust the setpoint using the +/- icons or enter a new flow target in the field.

Flow Setpoint

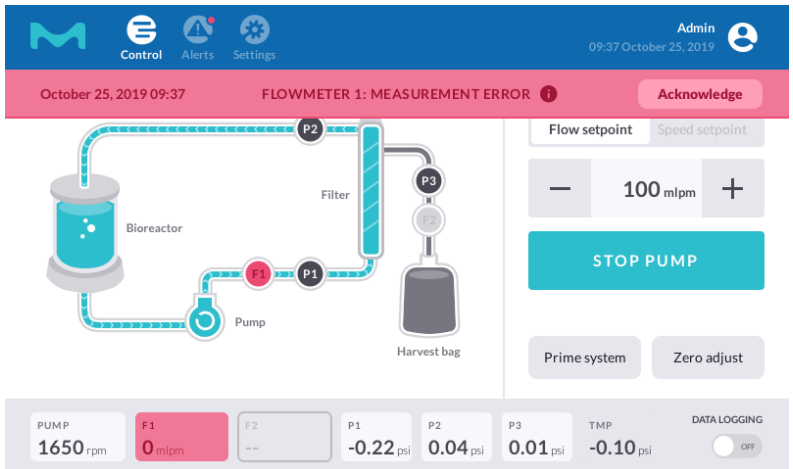
Select Flow setpoint when a perfusion run is in progress. Enter the desired flow rate and the system will maintain the flow rate by adjusting the pump speed (RPM). Speed adjustments are based on feedback from Flowmeter 1.

Speed Setpoint

Speed setpoint should not be selected when a perfusion run is in progress. When switching to speed setpoint, a confirmation popup will appear. Enter the desired RPM and the system will maintain the speed. Use speed setpoint for priming.

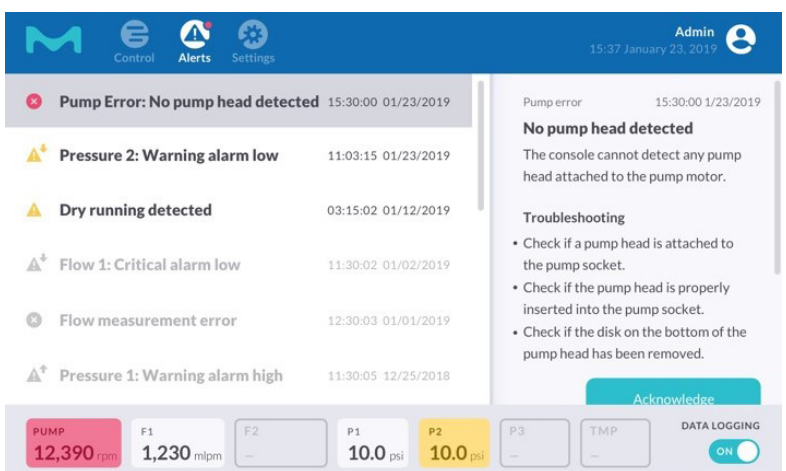
Alerts

When an alert is activated, it will appear in the alert bar on the main screen and in the alert log.



Alert Log

Select the Alerts button to access the alert log. The left side lists past alerts that were activated in the system. The right side displays the details of the alert, including acknowledgement status, suggestions to resolve the issue and when the alert was resolved.

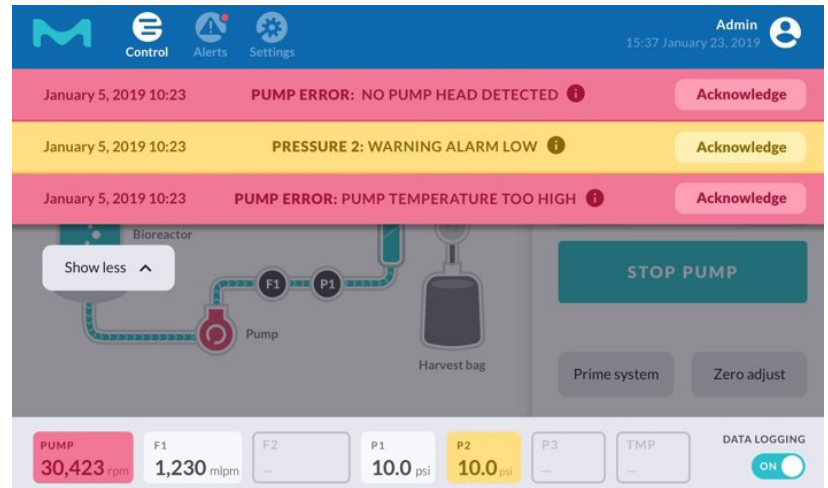


Alert Log

Alert Bar

Selecting the alert bar will display the alert log, where details about previous alerts can be viewed.

If there are multiple active alerts, expand the alert bar by selecting the down arrow on the left side of the bar. All unacknowledged active alerts will be displayed.



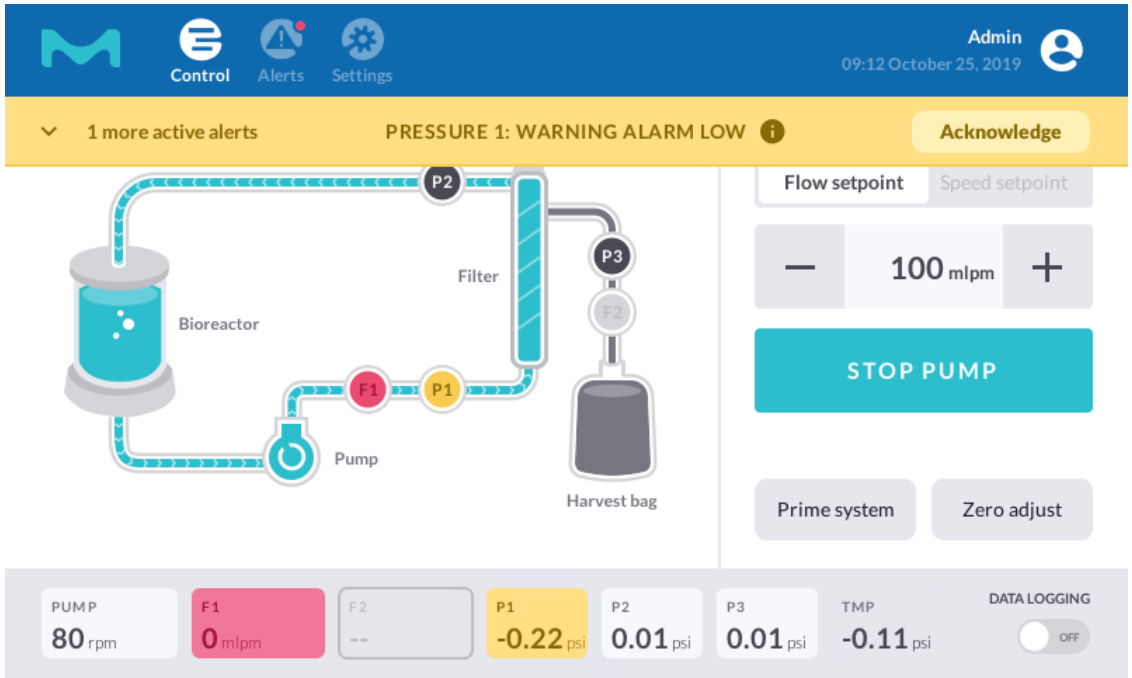
Alarm Acknowledgement

Alerts can be removed from the main screen by selecting Acknowledge from the alert bar or in the alert log. The alert will reappear on the alert bar if it remains active 10 minutes after being acknowledged.

If any alert is unacknowledged, a red dot will appear on the Alerts icon. Unacknowledged alerts will be listed in black font, and acknowledged alerts will be listed in grey font in the alerts log. If an alert is unacknowledged and unresolved, the icon will appear in the color of the alert type (red, yellow, or blue). If an alert is unacknowledged and resolved, the icon next to the listing will appear black.

Alerts Status in P&ID and Data Region

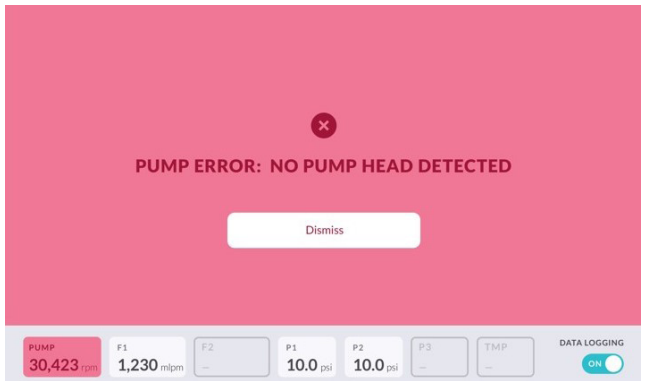
The alert status of each sensor can be viewed from the P&ID and data region of the main screen. An active alert on a sensor is indicated when the sensor appears with the alert color. The sensor color will return to dark grey once the alert is resolved.



Example of Alert on Main Menu Screen

Audible and Full-screen Alerts

Full-screen alerts can be enabled for a group of alerts under Settings > Alerts. If an alert is enabled to be audible and full-screen, the system will make a loud beep when the alert is activated. Select Dismiss to return to the main screen, then select Acknowledge on the alert bar or within the alert log. The data region can still be viewed when a full-screen alert is active.



Example of Full-screen Alert Type 1

Alert Types

Alert types indicate the severity of an alert. There are three alert types:

Type	Color	Alarm Type
1	Red	Critical
2	Yellow	Warning
3	Blue	Notification

Alerts

The list on the next page shows possible alerts.

Table of Alerts

Alert Category	Alert Description	Full-screen	Alert Type
Pump Error	Pump not connected	Always	1
	No pump head detected		
	Pump impellor stabilization failed		
	Pump supply voltage out of range		
	Pump temperature too high		
	Pump motor type not detected		
	Internal pump error		
Flowmeter Error	Flow measurement error	Optional	1
	Flow calibration parameter error		
	Flow zero adjustment error		
	Internal flowmeter error		
	Flowmeter disconnected while in flow control mode	Always	
Flow Alarms	Flow: Critical Alarm Low	Optional	1
	Flow: Warning Alarm Low	Never	2
	Flow: Warning Alarm High	Never	2
	Flow: Critical Alarm High	Optional	1
Pressure Alarms	Pressure: Critical Alarm Low	Optional	1
	Pressure: Warning Alarm Low	Never	2
	Pressure: Warning Alarm High	Never	2
	Pressure: Critical Alarm High	Optional	1

Alert Category	Alert Description	Full-screen	Alert Type
TMP Alarms	TMP: Critical Alarm Low	Optional	1
	TMP: Warning Alarm Low	Never	2
	TMP: Warning Alarm High	Never	2
	TMP: Critical Alarm High	Optional	1
Pump Internal Alerts	Pump impellor stabilizing issue	Optional	2
	Pump supply voltage out of range		
	Internal pump communication issue		
Application Specific Alerts	Pump driver power limit reached	Optional	2
	High pump temperature		
Out of Setpoint Tolerance Alerts	Speed out of tolerance	Optional	2
	Flow out of tolerance		
Other Alerts	Data logging aborted	Optional	2
	Auto-resume has recovered previous system state	Never	
Bubble Notification	Bubble detected	Never	3

Settings

System Settings

General

Auto-resume

Allows the system to store the current system state and setpoint. If power is lost, the system recovers automatically when this feature is enabled. An alert shows the system has recovered its last working point using the auto-resume feature.

Date & Time

Adjust the values up or down. Select Done when finished adjusting the values. The date and time format is MM-DD-YYYY HH:MM.

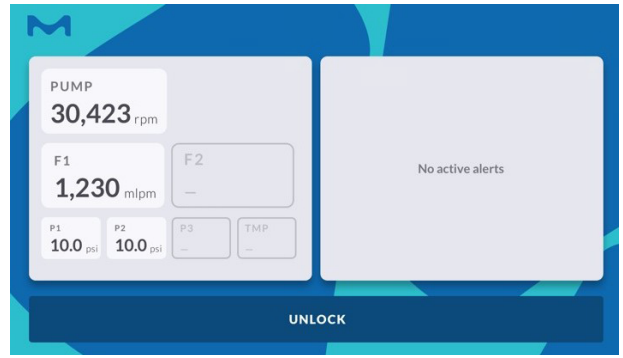
Flip Display

Change the orientation of the display to landscape, portrait or upside down.

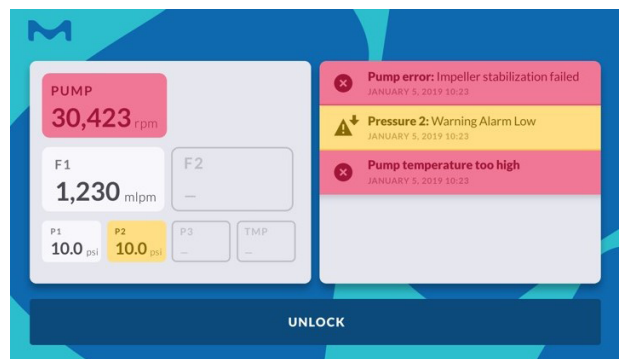
Automatic Standby Mode

Locks the screen and displays a screensaver after a defined period of inactivity (1 to 1000 minutes). Enable this feature by selecting the checkmark next to Enable automatic standby mode.

In standby mode, the screensaver shows the following information:



Standby Mode with No Active Alerts



Standby Mode with Active Alerts

To unlock the screen, select UNLOCK and enter user account credentials.

Data Logging

The beginning of the filename can be defined by adding a log filename. The ending is fixed and generated with the date followed by the time when the logfile is created.

When data logging is switched On, a prompt to enter a filename will appear. This filename will replace the text in the log filename region in the settings.

A USB drive must be inserted into the controller to start the data logging. Data will be lost if the USB drive is removed prior to ending the data logging.

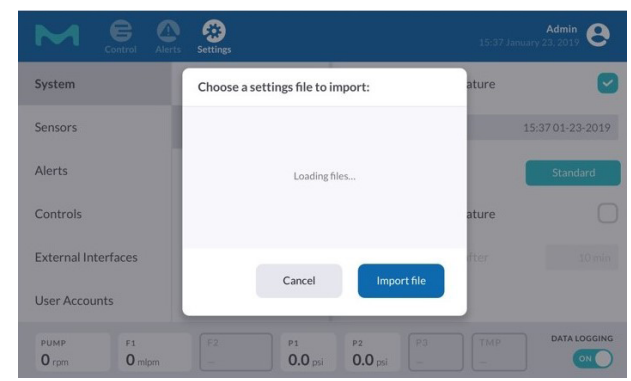
The logfile is stored as a csv-file to the subfolder/ logs on the USB drive.

The csv-file format allows a user to open the logfiles with Microsoft Excel.

The data logging settings also define log interval and the log file size.

Import/Export Settings

Settings can be imported via the USB drive. By selecting IMPORT, if any settings file is found it will be listed within the selection list on the popup window and can be loaded onto the controller. Existing controller settings will be overwritten by the settings files imported from the USB drive.



Import Settings Popup

Export Settings enables a user to store all controller settings on a USB drive and transfer them to another controller.

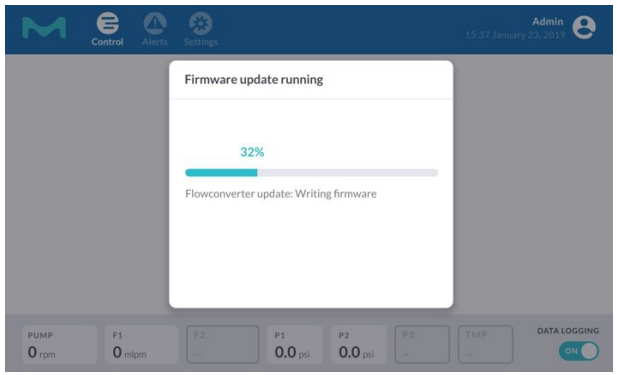
Factory Reset

By selecting RESTORE, all the settings will be reset to the factory setting values.

Note All the existing controller settings will be lost by restoring factory settings.

Software Update

Loading a software package (.levi-file) via the USB drive allows the customer to perform a software update on the controller. Select REFRESH to search the USB drive for a file, then select Update. Only one .levi file should be on the USB drive. The flow meter firmware can also be updated here.



Software/ Firmware Update Wizard

About System

This section contains information about the controller and its connected peripherals, like serial numbers, component names and software/firmware versions.

Sensors Settings

Note Only single-use pump heads should be used with this controller. The controller does not support firmware for the multi-use pump versions.

Flowmeters

Flowmeter settings contain all configuration parameters for each flowmeter. If a clamp-on sensor (LFSC-i06X) is connected, the Tubing & System Temp setting will show the calibration sets for this sensor. The user can choose from a list of tubing types and operating temperatures by selecting the box displaying the current selection. When using the Cellicon™ filter, select Advantaflex, 37⁰ C.

The low cutoff setting can be specified by the user. Every flow value below this cutoff is shown as 0 mlpm.

When a less noisy flow signal is required this signal can be smoothed by increasing the damping time. Be aware by changing this damping time the flow control performance can be influenced negatively.

The flow error ignore time defines how long a flow measurement error needs to be present until any action is taken by the system. In flow setpoint mode the defined pump behavior is triggered (Settings→Controls→Flow Control→Pump Behavior on Flow Error). In speed setpoint mode, when the ignore time has expired the flow value is set to 0 mL/min, the flowmeter shows an error.

The resolution of the flow signal can be improved by decreasing the full scale of the flowmeter. The resolution of the flow signal is always 1/10,000 of the full scale. Decreasing the full scale below 1000 mlpm affects the max flow setpoint.

Calibrate flowmeters every one to two years.

Pressure Sensors

An offset can be defined for each pressure sensor. This value is automatically adjusted when the zero adjustment is performed.

A quieter pressure signal can be achieved by increasing the damping time.

The displayed pressure unit can be either psi or mbar.

Alerts Settings

This section allows a user to configure how different types of alerts are communicated. If alerts are selected as Show, a message will appear in the alert bar area of the main screen. If alerts are selected as audible and full-screen, a full screen alarm appears with an audible beep sound. Each alert will be added to the alert log, found by selecting Alerts from the main screen.

If the controller is in standby mode when the alert occurs, the alert will appear on the screensaver. It will also be added to the backlog of alerts on the main screen.

Audible alarms can be enabled or disabled, except:

- When pump errors are configured as visible (Show pump errors), every pump error that occurs when the pump is enabled results in a full screen alert together with an audible beep sound.
- When flowmeter errors are configured as visible (Show Flowmeter errors) and the flowmeter gets disconnected while in flow control mode results in a full screen alert together with an audible beep sound.

General Alerts

Application specific alerts, data logging aborted alerts, and out-of-setpoint tolerance alerts are alert type 2 and can be found on the Table of Alerts.

Bubble alerts are alert type 3 and can be found on the Table of Alerts.

Pump Alerts

Pump errors are alert type 1 alerts and will stop the system. Pump errors can be found on the Table of Alerts.

Pump internal alerts are alert type 2 alerts and can be found on the Table of Alerts.

Flowmeter Alerts

When alerts for Flowmeter 1 are activated, the system will take the selected pump behavior on flow error (Settings→ Controls → Flow Control → Pump Behavior on Flow Error). Flow alerts can be found in the Table of Alerts.

Flow alarms can be configured to provide an alert when the actual value deviates outside a specified range.

Flowmeter Alarm	Alarm Trigger	Type
Critical Low Level	Flow falls below the critical low level	1
Warning Low Level	Flow falls below the warning low level	2
Critical High Level	Flow rises above the critical low level	1
Warning High Level	Flow rises above the warning low level	2

Refer to Table of Alerts for more information.

Critical alarms (type 1 alert) are shown in red color, and warning alarms (type 2 alert) are shown in yellow color. Neither critical nor warning alarms will shut down the pump.

The Show flow alarms selection activates all the flow alarms for that sensor. The Critical flow alarms are audible and full screen selection enables only the critical alarms to be audible and full-screen.

Pressure and TMP Alerts

Pressure and TMP alarms can be configured to provide an alert when the actual value deviates outside a specified range. These alarm levels define when a pressure or TMP alarm notification goes active.

Pressure & TMP Alarm	Alarm Trigger	Type
Critical Low Level	Pressure or TMP falls below critical low level	1
Warning Low Level	Pressure or TMP falls below the warning low level	2
Critical High Level	Pressure or TMP rises above the critical low level	1
Warning High Level	Pressure or TMP rises above the warning low level	2

Refer to Table of Alerts for more information.

Critical alarms are shown in red color (type 1 alert). Warning alarms are shown in yellow color (type 2 alert). Neither critical nor warning alarms will shut down the pump.

The Show Pressure/TMP alarms selection activates all the pressure alarms for that sensor. The Critical Pressure/TMP alarms are audible and full screen selection enables only the critical alarms to be audible and full-screen.

Controls Settings

Flow Control

The Flow Control Settings define the parameters and take effect when the system is running in flow setpoint mode. Flow setpoint mode is activated by switching the toggle on the main screen to show Flow Setpoint highlighted by a white box.

P and I parameters can be adapted to optimize the flow control behavior.

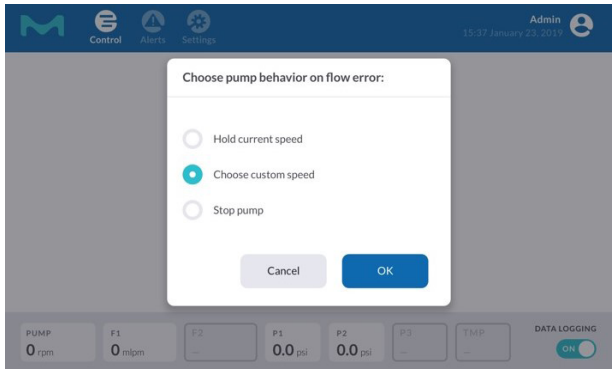
The minimum and maximum speed define the range of pump RPM that the system will allow. These settings only take effect when running in flow setpoint mode. The setpoint increment step defines which amount the flow setpoint is increased/decreased when selecting + or - on the main screen.

The setpoint tolerance parameter defines the band around the setpoint which the actual flow must reach.

Out of tolerance alert will be activated when the actual flow does not reach the setpoint tolerance band within the time defined in out of tolerance alert delay. Activate the alert to be notified when an out of tolerance situation is present (Settings → Alerts → General → Out-of-Setpoint Tolerance Alerts).

A flowmeter error can be defined when the number of seconds exceeds the setpoint in Flow Error Ignore Time (Settings->Sensors->Flowmeter). The controller takes one of the following actions, which can be defined under Pump Behavior on Flow Error:

- Hold Current Speed: The pump continuously holds the same speed.
- Choose Custom Speed: The pump defaults to the defined speed.
- Stop Pump: The pump stops.



Choose Pump Behavior on Flow Error

Speed Control

These define the parameters which take effect when the system is running in speed setpoint mode. Speed setpoint mode is activated by moving the toggle on the main screen to show Speed Setpoint highlighted by a dark grey box.

The minimum and maximum speed define the range of pump RPM that the system will allow. These settings take effect while running in speed setpoint mode. The pump speed ramp defines the rate of change when a new setpoint is selected.

The out of tolerance alert will be activated when the actual speed does not reach the setpoint within the time defined in Out of tolerance alert delay. The out of tolerance notification can be activated under Settings→ Alerts → General → Out-of-Setpoint Tolerance Alerts.

External Interfaces Settings

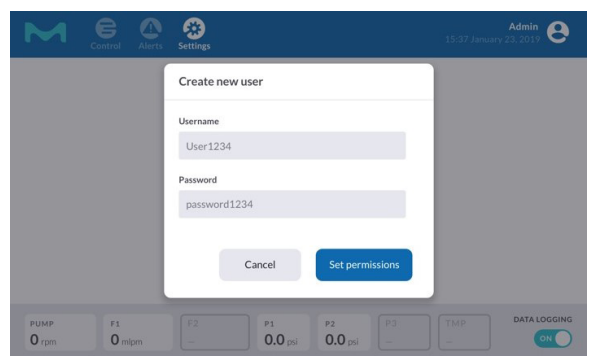
- LAN Accessibility
- Remote Control by PLC
- PLC Analog Input
- PLC Digital Input
- PLC Analog Output

User Accounts Settings

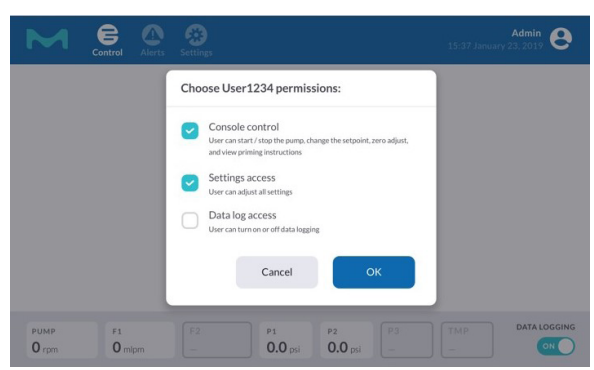
The administrator can generate, delete and edit user accounts, including password protection capabilities and rescrict access permissions.

The administrator can define up to six user accounts. After selecting the + Add new user button, enter a username and password, then press Set permissions. The following permissions can be activated or deactivated:

- Controller control – User can start/stop the pump, change the setpoint, zero adjust, and view priming instructions.
- Settings access – User can adjust all settings.
- Data log access – User can turn on/off data logging.



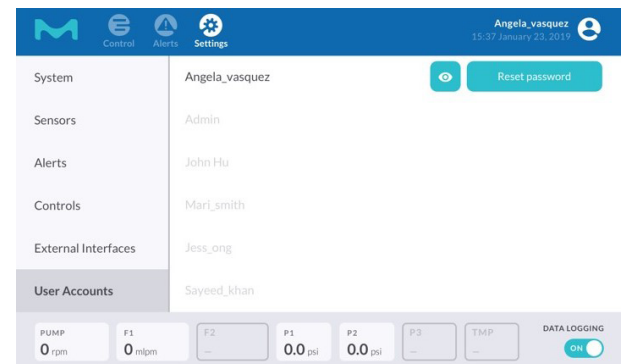
Create a New User



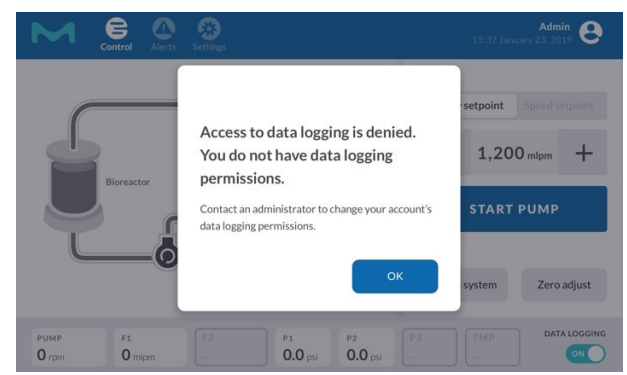
Set Permissions for New User

The active user can be changed by selecting the user account icon in the top right region of the screen and selecting Switch user. When the screen is locked, any user account can be selected to access the controller.

Only the administrator can change permissions for each user, but users can change their password when signed into their account.



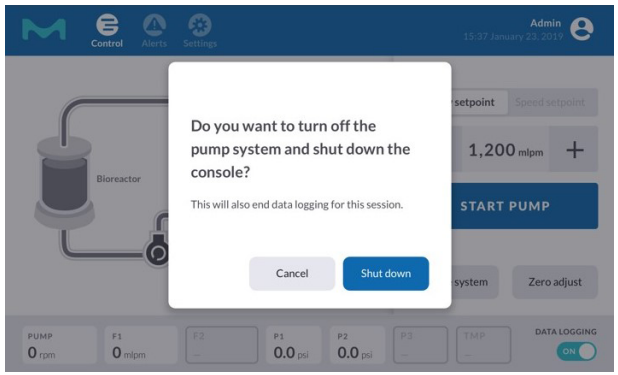
User Accounts Settings from a Non-administrator Account



Access Denied Due to User Permissions

Shutdown

When the power button is pressed a confirmation pop-up will appear. After confirmation, the controller software initiates the shutdown.



Shutdown Confirmation

When the main power button is pressed for more than 4 seconds, the hardware shutdown is triggered, the pump turns OFF and the controller immediately shuts down.

Planning for a Run

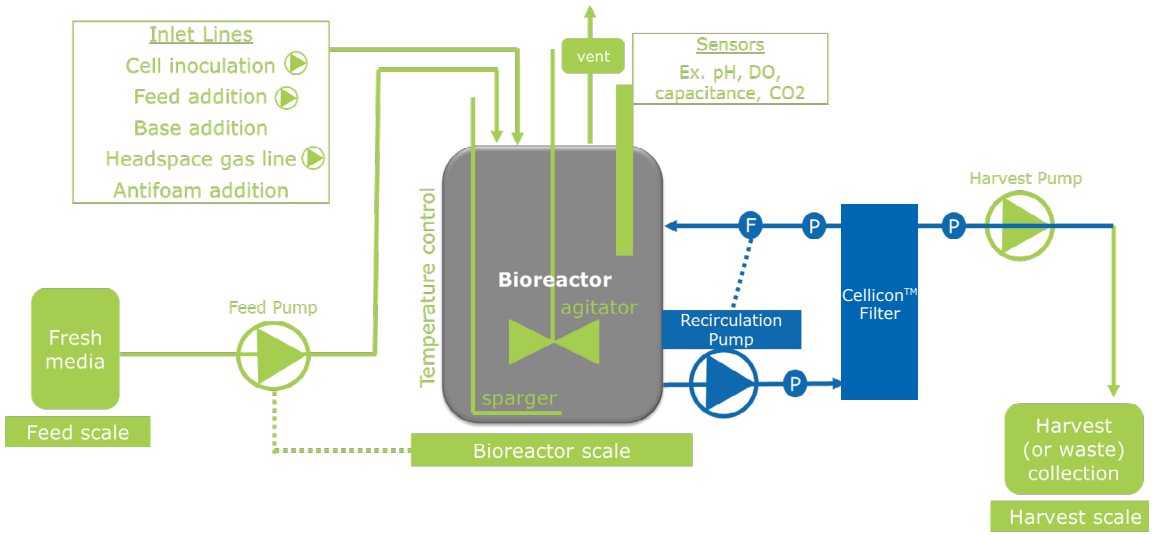
If possible, the Cellicon™ Perfusion filter should be attached to the bioreactor and the recirculation pump should be turned on during the media hold phase, before cells have been added to the reactor. This will eliminate the possible introduction of cell shear when the system is primed (see Priming).

Preparation

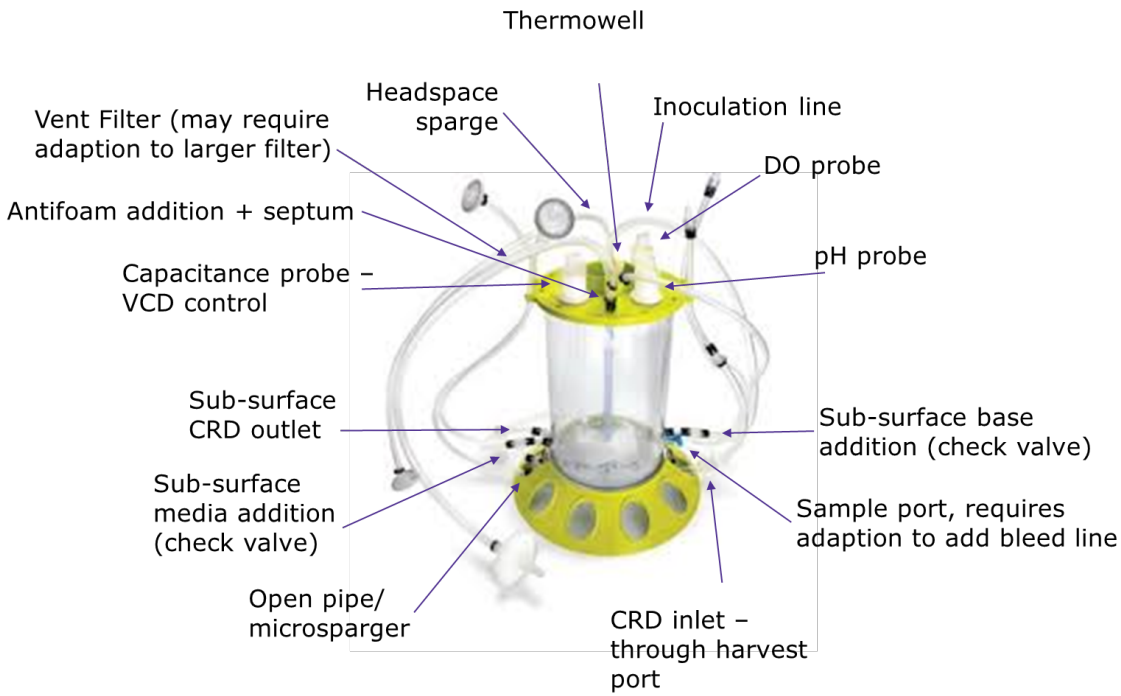
Equipment Needs

The following user supplied items may be required to execute a perfusion process:

- Assembled and sterilized bioreactor, up to 3L working volume
- Control system for the bioreactor
- Welder for ⅛ in. ID x ¼ in. OD weldable (Advantaflex) tubing to attach the filter
- Peristaltic pump for the perfusate (permeate)
- Peristaltic pump for priming
- Method to control the liquid level in the bioreactor to maintain a constant volume
- Power outlet for plugging in the cell retention system (100-240V, 1.7A max, 50/60hz)
- Sterile collection vessels to contain the fresh media and collect the spent media during the run.
- USB drive for data collection



P&ID for N-1 Perfusion Process.



Mobius 3L Bioreactor

Preparing the Bioreactor

Connect the filter to the bioreactor by welding Advantaflex tubing on the assembly.

To optimize process efficiency, reduce the holdup of cell culture volume in the recirculation loop outside of the bioreactor by shortening the tubing length between the bioreactor and the filter assembly. Tubing ID cannot be smaller than ⅛-inch at any point in the recirculation loop. If using a diptube, ensure that the ID of the diptube is between ⅛-inch and ⅜-inch.

Place the inlet to the cell retention filter below the liquid level of the bioreactor, so it will not pull in any bubbles that have been released from the sparger.

Note Any air or gas in the pump head will negatively affect pump performance and decrease the recirculation flow rate.

Mobius™ 3L Single-use Bioreactor

When using the Mobius™ single-use 3L reactor, the inlet to the cell retention filter should be welded to the harvest line (bottom) of the bioreactor. The retentate line should be welded to one of the submerged ports that does not contain a check value (see image X). This is the ideal orientation for the Cellicon™ Perfusion filter because sparger will be less likely to pull bubbles in, and priming the system is easier.

Other Bioreactors

The Cellicon™ Perfusion Solution for Process Development can be used with any bioreactor, single or multi use, with a working volume of 3L or less. On single use reactors, choose the harvest line as the inlet to the filter. On most glass reactors, use a harvest tube that extends below the sparger, or add a length of tubing that extends to the bottom of the diptube below the sparger.

Operating Parameters

The bench scale N-1 perfusion filter was designed for N-1 perfusion processes targeting:

- 3-5 days of perfusion
- 2.7 L working volume
- 2 VVD
- 50 million cells/mL cell density

The performance of the filter is specific to the cell line and media combination, as well as the chosen operating parameters. The most critical factors that impact performance are perfusate (permeate) flux, which should be less than 23 L/m²/hr, cell density, and crossflow rate.

Starting a Run

Priming

The recirculation pump for the cell retention device is a magnetically coupled centrifugal pump. It enables the assembly to be single use and disposable while providing a consistent and low shear flow rate. The pump does not self-prime (will not successfully pump unless it is filled with liquid).

When the bioreactor is in media hold and the cell retention filter has been attached, a priming procedure is performed to start recirculation. Due to the harvest port location on the Mobius™ 3L, it may prime after unclamping the feed line. In this case, the tubing will fill with liquid and recirculation flow can start from the controller. If the tubing does not self-prime, a secondary peristaltic pump is required to start the flow recirculation. Step-by-step priming instructions can be found by clicking Prime system on the main menu screen.

Priming Procedure

1. Select Prime system on the main menu screen. This will switch the controller into Speed setpoint mode and provide step-by-step guidance on how to complete the priming.
2. Ensure that the perfusate (permeate) line is clamped.
3. Take an external peristaltic pump and place the pump head on the feed line of the cell retention device loop.
4. Turn on external peristaltic pump (<100 mL/min) and allow liquid to flow until there are no bubbles left in the feed line or pump head. If necissary, adjust the angle to remove all bubbles.
5. Stop external peristaltic pump but leave it clamped so bubbles do not return to the feed line.
6. Select START PUMP on the controller with the speed setpoint at 3000 RPM. Quickly remove the external peristaltic pump from the feed line, allowing flow to recirculate through the device. Increase the RPM to clear bubbles from the loop.
7. Select Exit Priming (returns to flow setpoint mode). The flow will adjust to the setpoint that was previously entered in flow setpoint mode. Adjust the flow setpoint according to the desired recirculation rate for the run (100 mL/min).
8. Select Zero adjust from the control region of the main screen. Refer to Zero Adjust for more information.

Turning the Pump On

The system is ready to begin recirculation flow once the flow path has been primed and the sensors have been zero adjusted.

There are two ways to operate the pump between Flow setpoint and Speed setpoint, indicated by the toggle on the main screen. Ensure that Flow setpoint is selected for the perfusion run. Speed setpoint is intended to be used for priming only. See below for a description of each setpoint:

Flow setpoint	User inputs a desired flow rate and system will maintain that flow rate by adjusting pump RPM.
Speed setpoint	User inputs a desired speed in RPM and is held constant, but actual flow rate may fluctuate. For this reason, the speed setpoint is recommended only when priming the system.

In flow setpoint mode the user can define the desired crossflow rate. The bench scale filter is recommended to run at a crossflow of 100 mlpm.

Select START PUMP to begin and leave the pump on for the duration of the run.

Starting Perfusion

Once the cell retention device and system have been attached and primed, cells can be added to the bioreactor. Typically there is a delay between the inoculation and the start of perfusion. The recirculation pump can remain on during this time.

Before starting perfusion, a closed and sterile perfusate (permeate) line must be welded to a collection vessel (bag or bottle) to collect the spent media. Attach a peristaltic pump (not included with the cell retention system) to the perfusate (permeate) tubing line to pull spent media out of the cell retention device. Continuously run the perfusate (permeate) pump at a flow rate of 23 LMH or less. If the flow rate of the perfusate (permeate) pump is greater than 23 LMH it might prematurely foul the filter. The flow rates chosen (VVDs – vessel volumes per day) are specific to each process.

Run Maintenance

Data Logging

Insert a USB flash drive into the USB port in the back of the controller. The controller can record process data while a run is in progress. Data logging can be initiated by selecting the toggle on the bottom, right-hand corner of the main menu screen to On. Select the toggle again to switch back to Off and stop data logging. Removed the USB drive from the controller.

To adjust data logging settings, select Settings → System → Data Logging. Choose the name of the file saved on the USB drive, the log interval, and the log file size. Choose a log interval of 60 seconds or greater and a log file size of 65000 lines.

Insert the USB drive into a computer to view data. Open Microsoft Excel and click the Data tab on top, then download data From Text/CSV. Choose the correct file from the flash drive and follow prompts to open data in a new sheet.

Sampling the Perfusate (Permeate)

A sampling container should be used to take samples from the perfusate (permeate) of the device. The sampling container should be placed between the perfusate (permeate) pump and the perfusate (permeate) collection bag.

Follow this procedure to take a sample:

1. Locate sample collection vessel.
2. Briefly stop perfusate (permeate) pump, clamp line to collection vessel (Clamp 2) and unclamp line to sampling container (Clamp 1).
3. Start the perfusate (permeate) pump and fill sampling container until the appropriate volume is collected.
4. Stop the perfusate (permeate) pump, clamp sampling line (Clamp 1), unclamp collection vessel line (Clamp 2).
5. Restart perfusate (permeate) pump.
6. Use a syringe to collect sample from sampling container.

Changing the Filter

The Cellicon™ Perfusion filter has been designed to have a high throughput to support N-1 perfusion applications. The pressure profile of the pressure sensors and the recorded TMP can describe fouling of the membrane. If fouling occurs it will cause the perfusate (permeate) pressure (P3) to become negative, and will increase the TMP reading (TMP = [(P1 + P2) / 2] – P3). Stop the run when TMP > 5 psi.

If the run is not complete when TMP = 5 psi, replace the old filter with a new filter and continue the run. Follow these steps to replace the filter:

1. Pause the perfusate (permeate) pump.
2. Turn off the recirculation pump by selecting STOP PUMP on the main menu.
3. Use a tube sealer to seal the lines connecting to the old filter. Three seals are needed on: the feed line, the retentate line, and the perfusate (permeate) line. Ensure there is enough space on the bioreactor side to complete another weld when the new device is added.
4. Cut through the middle of all three tube seals to disconnect the old filter.

5. Locate a new filter and place it in the holder.
6. Use a tubing welder to connect the new filter to the tubing lines that were designated for feed, retentate, and permeate.
7. Unclamp the feed and retentate lines, but ensure that the perfusate (permeate) is clamped.
8. Prime the system again. If using a peristaltic pump, run at a low flow rate to avoid causing shear damage to the cells in the line.
9. Restart the recirculation pump by selecting START PUMP on the main menu. Ensure that the flow rate has reached the desired setpoint.
10. Restart the perfusate (permeate) pump.

Troubleshooting

System Troubleshooting

The following chart can be used to help identify issues related to system operation.

Symptom	Corrective Action
No power	Plug in system and press power button
System component appears light grey and does not read values	Connect cables from component to controller
	Install correct software
Component is in an alarm state, appears red or yellow	Plug in component cables
	Pump alert: Place pump head into motor
	Flow alert: Place tubing into flowmeter and prime tubing
	Adjust flow alarm ranges
	Follow instructions provided for specific alert
Assembly does not fit into holder	Follow order of operations for installation found in user guide
Pump head does not fit into motor	Pull out locking pin during installation and move barb from covering green area to covering red area
	Place pump head facing upwards
System does not allow changes	Check login credentials and user permissions
	Disable “When LAN Accessibility is enabled, console control is disabled” setting under Settings->External Interfaces->LAN Accessibility
System will not prime	Use external peristaltic pump to start flow
	Choose peristaltic pump appropriate for the tubing size
	Ensure dip-tube is open and submerged in liquid
	No pinching or kinking of tubing lines, welds are open

Symptom	Corrective Action
System leak	Check for cuts, cracks, or damage to assembly
	Ensure system pressure is less than 5 psi
	Ensure flowmeter clamp does not pinch the tubing
Unexpected or unstable pressure readings	Ensure cable connections are good
	Proper installation of P1 on Feed, P2 on retentate, and P3 on permeate
	Perform zero adjustment prior to run, after priming
	Clear air from the sensor
Unexpected or unstable flowmeter readings	Ensure cable connections are good
	Clean flowmeter and tubing with IPA
	Close clamp around tubing and ensure flowmeter clamp does not pinch the tubing
	Ensure pump is running and flow is moving
	Prime tubing in flowmeter, remove air bubbles
	Choose correct calibration set in system settings (tubing type, temperature)
	Perform zero adjustment at the correct temperature, prior to run and after priming
	Re-zero sensor
	Check flowmeter calibration against reference flowmeter
Pump unable to achieve target flow rate	Re-prime tubing to remove air bubbles
	Remove pump head from pump to clear air from inside
	Check for tubing pinch or kink
	Check for filter fouling (high P1 with low P2)
	Check dip-tube placement and ensure it does not pull air from sparger
	Check system mode, "flow control" vs "speed control"
No flow through permeate	Ensure permeate line is not clamped or pinched

Symptom	Corrective Action
Early filter fouling (High P1 or low P3)	Check that crossflow rate is at setpoint
	Check system mode, "flow control" vs "speed control"
	Ensure maximum flux is within recommended range
	Swap out filter
	Check flowmeter calibration setting is correct
	Check flowmeter calibration against reference flowmeter
System pressure increase (High P1 and P2)	Check bioreactor pressure (ex. vent filter clogging)
	Check for tubing pinch or kink on retentate line
	Check flowmeter calibration setting is correct
	Check flowmeter calibration against reference flowmeter
Cell growth issues in bioreactor	Check flow rate and pump RPM are in recommended range
	Check flowmeter calibration setting is correct
	Check for cell loss in permeate line
	Prime filter during media hold only
	Re-prime system to remove air bubbles
	Check flowmeter calibration against reference flowmeter
Cells found in permeate	Swap out filter
Unexpected or undesired alerts	Adjust alert settings
	Adjust alarm limits
	Perform factory reset
	Follow recommendations provided for each alert
Data logging is not saved	Ensure USB is inserted during run
	Ensure USB drive has adequate space
	USB is not removed until data logging is turned off and software indicates it is safe to remove

Ending the Run

Disposing of the Filter

The filter is completely single use and should be disposed of after use. Follow company procedure for waste disposal.

Cleaning the Hardware

All parts of the system should be wiped down with a cloth dampened with a 70% ethanol or isopropyl alcohol solution.

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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