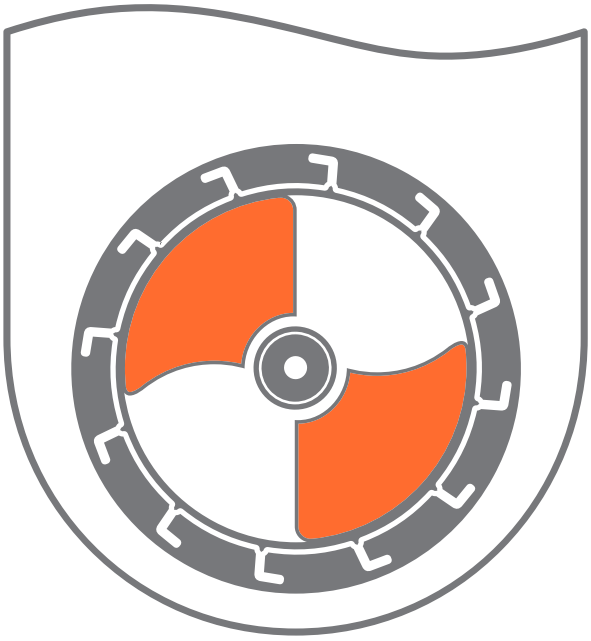
VERTICAL-WHEEL®
BIOREACTORS



PBS-MiniPRO Vertical-Wheel®

Single-Use Sensors

Single-Use Bioreactor System

User Manual

Applicable Models: IA-0.5-B-901 | IA-0.5-CM-901

PBS Biotech, Inc.

This User Manual reflects the latest product updates available at the time of publication. While every effort has been made to ensure accuracy, specifications and features may change. Please refer to the most recent product information on our website for the most up-to-date details regarding your bioreactor system.

PBS Biotech, Inc. is not responsible for printing or clerical errors.

This manual is intended as a guide to provide the user with necessary instructions on the proper use and maintenance of the PBS-MiniPRO Bioreactor System. This manual should be used in conjunction with instruction and training supplied by qualified PBS Biotech, Inc. personnel.

Any failure to follow the instructions as described, including use of materials or products not approved or recommended by PBS Biotech, Inc., could result in impaired product function, injury to the user or others, or void applicable product warranties. PBS Biotech, Inc. accepts no responsibility for liability resulting from improper use or maintenance of its products.

Utilization of PBS Biotech, Inc. products may require the user to handle and dispose of biohazardous materials. Users must fully understand and implement all regulations regarding the safe handling of biohazardous products and waste, including the policies and procedures of their facility.

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Contents

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About This Manual

This user manual shows you how to install, configure, and use the PBS-MiniPRO Bioreactor System (PBS-MiniPRO). This manual covers the Integrated Bioreactor, including the PBS Software package and the PBS-MiniPRO Bioreactor Single-Use Vessel assemblies.

Configurations are standard as of the time of publication and the software features and instructions are applicable to version 1.1.0. The “Software Release Version” can be viewed from the “System Configuration” menu.

The contents include:

- An overview of the PBS-MiniPRO’s features, components, and controls (Chapter 1 on page 11)
- A high level system description to provide an understanding of the complete PBS-MiniPRO (Chapter 2 on page 35)
- Safety considerations (Chapter 3 on page 40)
- Product specifications (Chapter 4 on page 44)
- Instructions for installing the PBS-MiniPRO and configuring users, logger settings, and alarms (Chapter 5 on page 48)
- Day-to-day use of the PBS-MiniPRO (Chapter 6 on page 62)
- A detailed description of all PBS-MiniPRO features and functions (Chapter 7 on page 133)

Note: Screenshots are illustrative of the Display Client features and are not intended to show actual or recommended settings.

For More Information

Additional documentation is located in:
`C:\Users\Public\Documents\RSView Enterprise\SE\Documentation`
For Frequently Asked Questions and more troubleshooting information, visit the PBS Biotech website at www.pbsbiotech.com, then navigate to Resources → FAQ.
For specific questions, email app.eng@pbsbiotech.com.

PBS-MiniPRO at a Glance

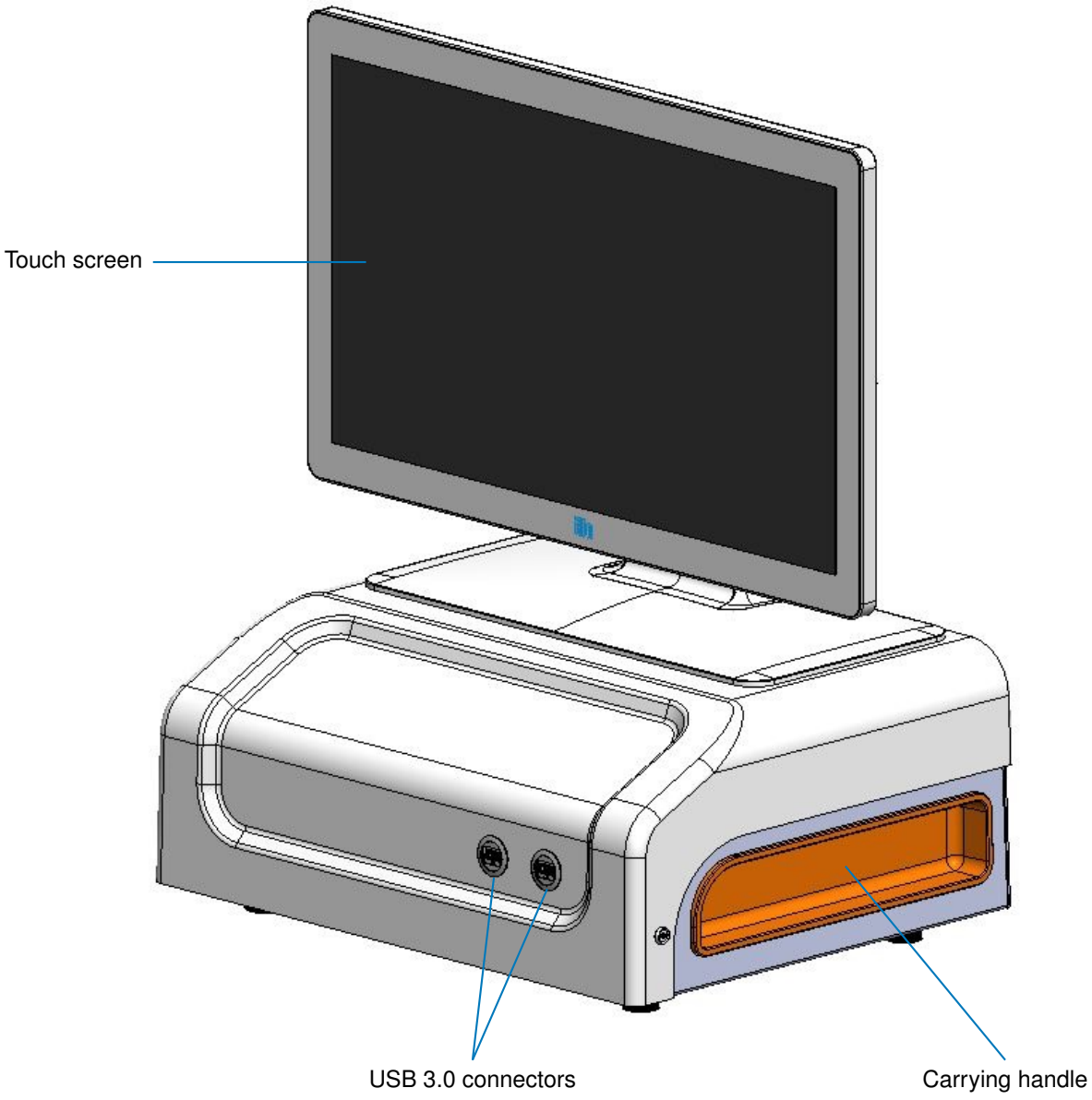
1

Use the illustrations in this chapter to become familiar with the basic features, components, and controls of the PBS-MiniPRO.

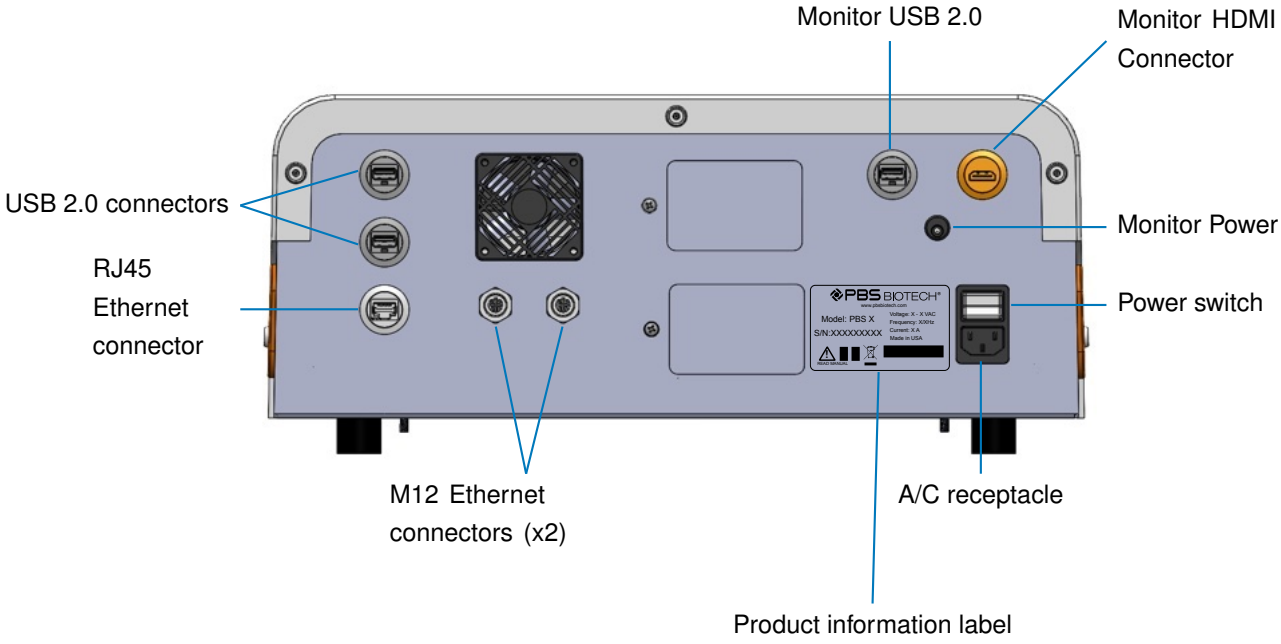
Note: Some components may be slightly different from the illustrations here, depending on the configuration you purchased.

Definitions

PV = Process Variable or Process Value
SP = Setpoint
UI = User Interface
LPM = Liters Per Minute
mLPM = Milliliters Per Minute
mL/min = Milliliters Per Minute
SCCM = Standard Cubic Centimeter per Minute
RPM = Revolutions Per Minute
CW = Clockwise
CCW = Counterclockwise
EU = Engineering Units
EM = Equipment Module
EP = Equipment Phase
CO_2 = Carbon Dioxide
N_2 = Nitrogen
O_2 = Oxygen
CCA = Clean Compressed Air when referring to an MFC
IPA = Isopropyl Alcohol
EtOH = Ethanol
MFC = Mass Flow Controller
PLC = Programmable Logic Controller
HMI = Human Machine Interface
SUS = Single Use Sensor
CV = Control Variable, or the controller output
BSC = Biosafety Cabinet
I/O or IO = Input/Output, referring to hardware


Touch screen
USB 3.0 connectors
Carrying handle

Touch screen Responds to bare fingers, latex/nitrile gloves, or a capacitive stylus.
USB 3.0 connectors Allow connection of USB devices which will benefit from the higher data transfer speeds that USB 3.0 connectors provide, such as memory sticks.
Carrying handle Allows for convenience in moving the Module.


Monitor USB 2.0
Monitor HDMI Connector
USB 2.0 connectors
Monitor Power
RJ45 Ethernet connector
Power switch
M12 Ethernet connectors (x2)
A/C receptacle
Product information label

M12 Ethernet connectors (x2)
Used to directly connect the Control Module to 2 Base Modules. Connections to additional Base Modules are made through those Base Modules. Each Control Module can be connected to up to six (6) Base Modules.

RJ45 Ethernet connector
Used to connect the Control Module to a high-speed Ethernet network. It is intended for connecting to an office network/IT network. The Control Module also has built-in Wi-Fi capability.

USB 2.0 connectors
Allow connection of USB devices which are appropriate for the lower data transfer speeds that USB 2.0 connectors provide, such as a keyboard and mouse.

NOTICE Avoid using keyboards with a power button, to prevent accidentally turning the Control Module's HMI computer off.

Monitor USB 2.0
The Touch screen monitor connects here for touch screen function.

Monitor HDMI Connector
The Touch screen monitor connects here for video output.

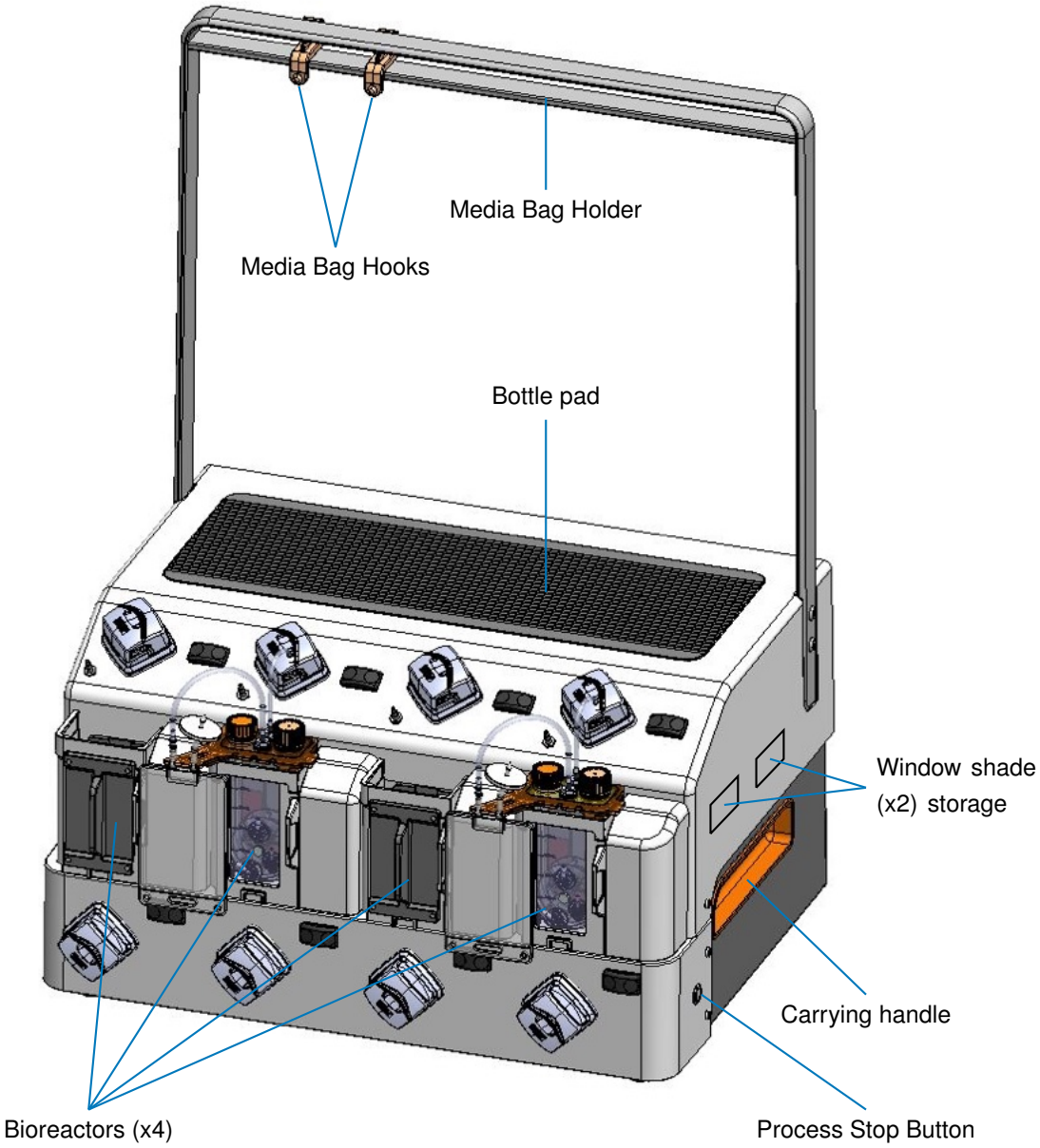
Monitor Power
The Touch screen monitor connects here for power.

Power switch
Used to power on the Module.

A/C receptacle
Connects to a grounded outlet through a desired power cord to start up the Module.

Product information label
Displays the Module's serial and model numbers, as well as safety information.

Each Control Module can be connected to up to six (6) Base Modules.



Media Bag Holder
An optional accessory available for purchase separately - it assists in hanging media bags as applicable. The weight limit for each Media Bag Hook is 9 kg (20 lbs). Do not hang anything but bags containing liquid off the Media Bag Holder. Only use the Media Bag Hooks provided by PBS Biotech to hang the bags.

Bottle pad
Stores reagent or media addition bottles during a run.

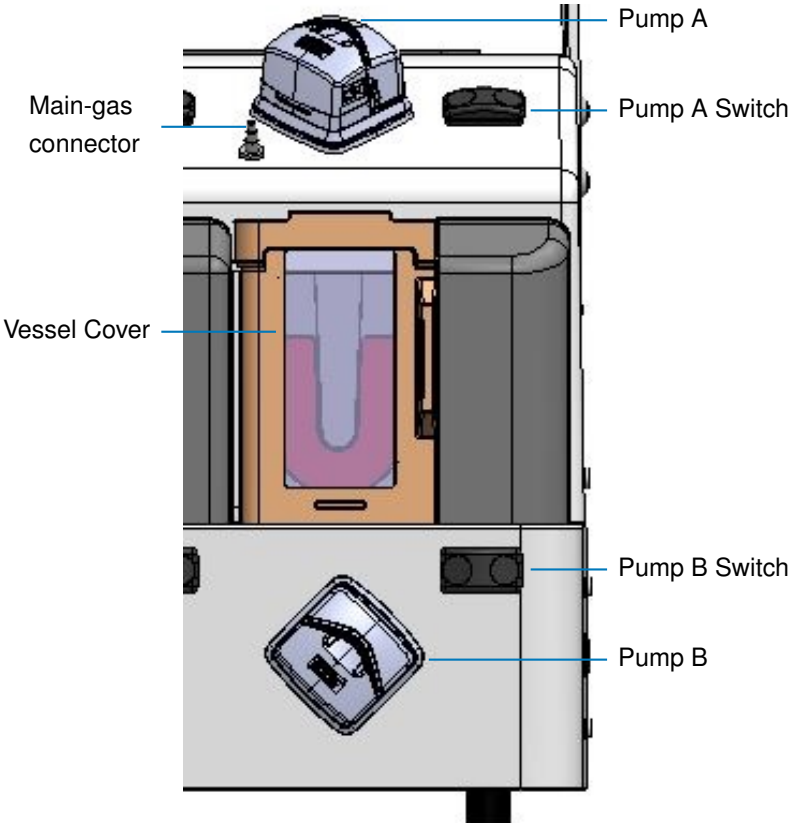
Window shade (x2) storage
Stores 2 Window shades by adhering to magnets in the Base Module's side above the handles when not in use. The other 2 Window shades are stored on the magnets above the handle on the other side of the Base Module.

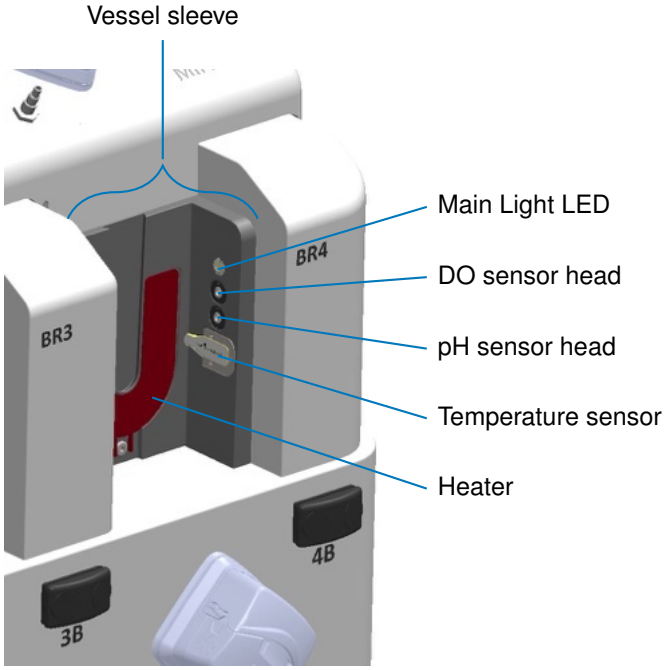
Carrying handle
Allows for convenience in moving the Module.

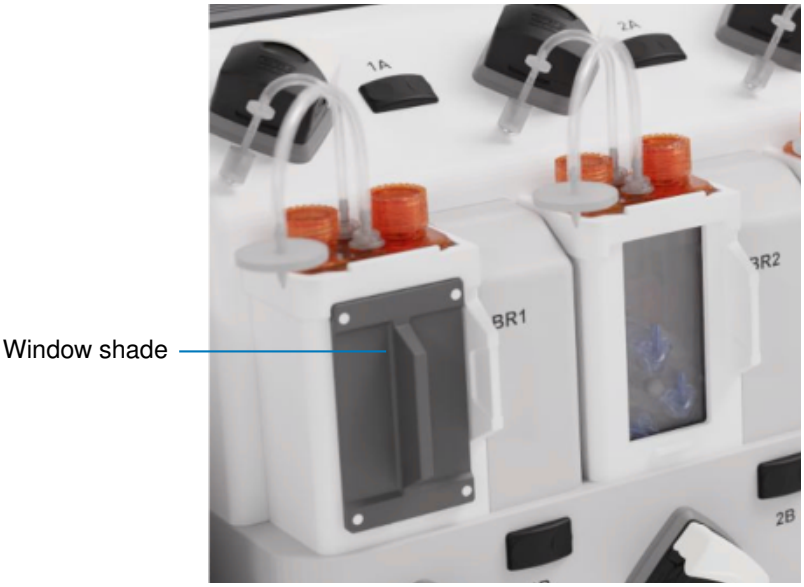
Process Stop Button
Pressing the system Process Stop Button stops all the pumps, agitators, and heaters, and closes all MFCs and valves.

NOTICE Pressing this button does **NOT** remove power to these devices, and instead it is a software stop that safely stops/closes devices across all 4 Bioreactors of the Base Module.

Bioreactors (x4)
Each Vessel gets its own Bioreactor (see next section for details). Each Base Module supports four (4) Bioreactors. To differentiate between which Bioreactor is being referred to, the convention MxRy is used, where M means Base Module, x is a number 1-6 for the Base Module, R means Bioreactor, and y is a number 1-4 for which Bioreactor on the specified Base Module. For example, M5R3 would refer to Base Module number 5, Bioreactor number 3.


Pump A
Main-gas connector
Pump A Switch
Vessel Cover
Pump B Switch
Pump B


Vessel sleeve
Main Light LED
DO sensor head
pH sensor head
Temperature sensor
Heater
BR4
BR3
4B
3B


Window shade
1A
2A
BR1
BR2
2B

Main-gas connector
Connects the vessel's Main-gas line to supplies of Air, CO2, N_2, and O_2, which are attached to the Bioreactor via the gas connection panel (see "PBS-MiniPRO Base Module - Rear" on page 20).

Vessel Cover
This attaches to the Vessel sleeve to keep the vessel insulated. Running temperature control without the Vessel Cover installed may have a significant impact on temperature control stability. Note that it is not single-use, and therefore should not be disposed of. Keep it connected to the Vessel sleeve when not in use. If it gets lost or damaged, the replacement part number is IA-0.5-BA-901.

Window shade
Attaches to the Vessel Cover to protect light-sensitive media in the vessel. The image depicts the Vessel Cover on the left with the Window shade installed, and the Vessel Cover on the right without a Window shade installed. Removable and stored by adhering to magnets in the Base Module's sides above the handles when not in use.

Pumps
Used with the vessel's tubing to add and remove liquid from the vessel during a run.

Pump Switches
These pump switches are momentary rocker switches that can operate the pump in either direction. Hold to the left for counter clockwise, and hold to the right for clockwise. The pumps can also be turned on and off in software.

Vessel sleeve
Holds the vessel so its components for DO, pH, Temperature, and Agitation align correctly. Needs to be used with the Vessel Cover, to insulate the vessel. When used with the Window shade, it keeps the vessel dark to protect light-sensitive media or other components.

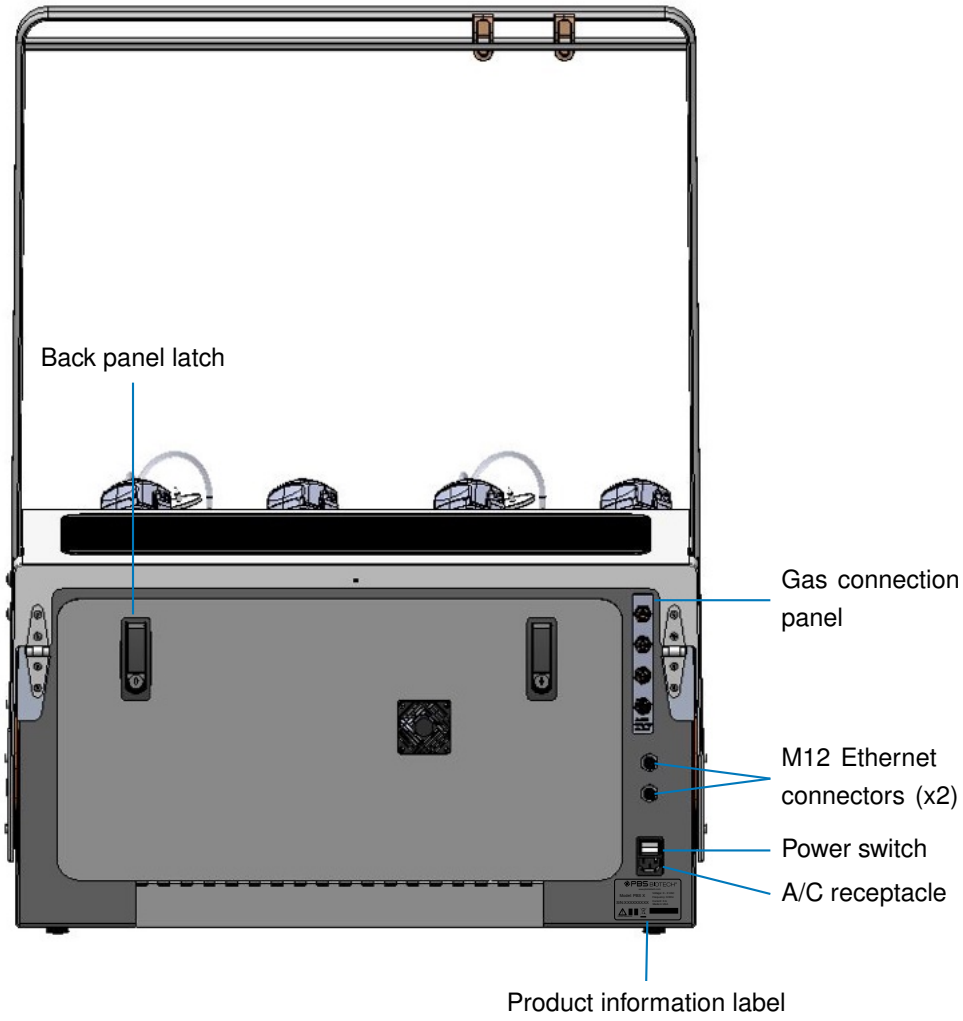
Main Light LED
The PBS-MiniPRO has a white LED light to illuminate the contents of the vessel. It can be turned on and off through the software. This light source does not impact the DO sensor spot or pH sensor spot and therefore the DO or pH PVs, but other sources of light might and therefore operators should use caution when using other light sources.

DO sensor head
Works with the DO sensor spot preinstalled on the vessel to read the DO value of cell culture medium in the vessel.

pH sensor head
Works with the pH sensor spot preinstalled on the vessel to read the value of the pH of cell culture medium in the vessel.

Temperature sensor
Rests against the right side of the vessel to provide accurate temperature readings.

Heater
The heater contacts the back of the vessel to heat the contents.


Back panel latch
Gas connection panel
M12 Ethernet connectors (x2)
Power switch
A/C receptacle
Product information label

Power switch
Used to power on the Module.

Back panel latch
Secures the Base Module's back cover and can be locked/unlocked with a supplied key.

M12 Ethernet connectors (x2)
Used to directly connect the Control Module to 2 Base Modules. Connections to additional Base Modules are made through those Base Modules. Each Control Module can be connected to up to six (6) Base Modules.

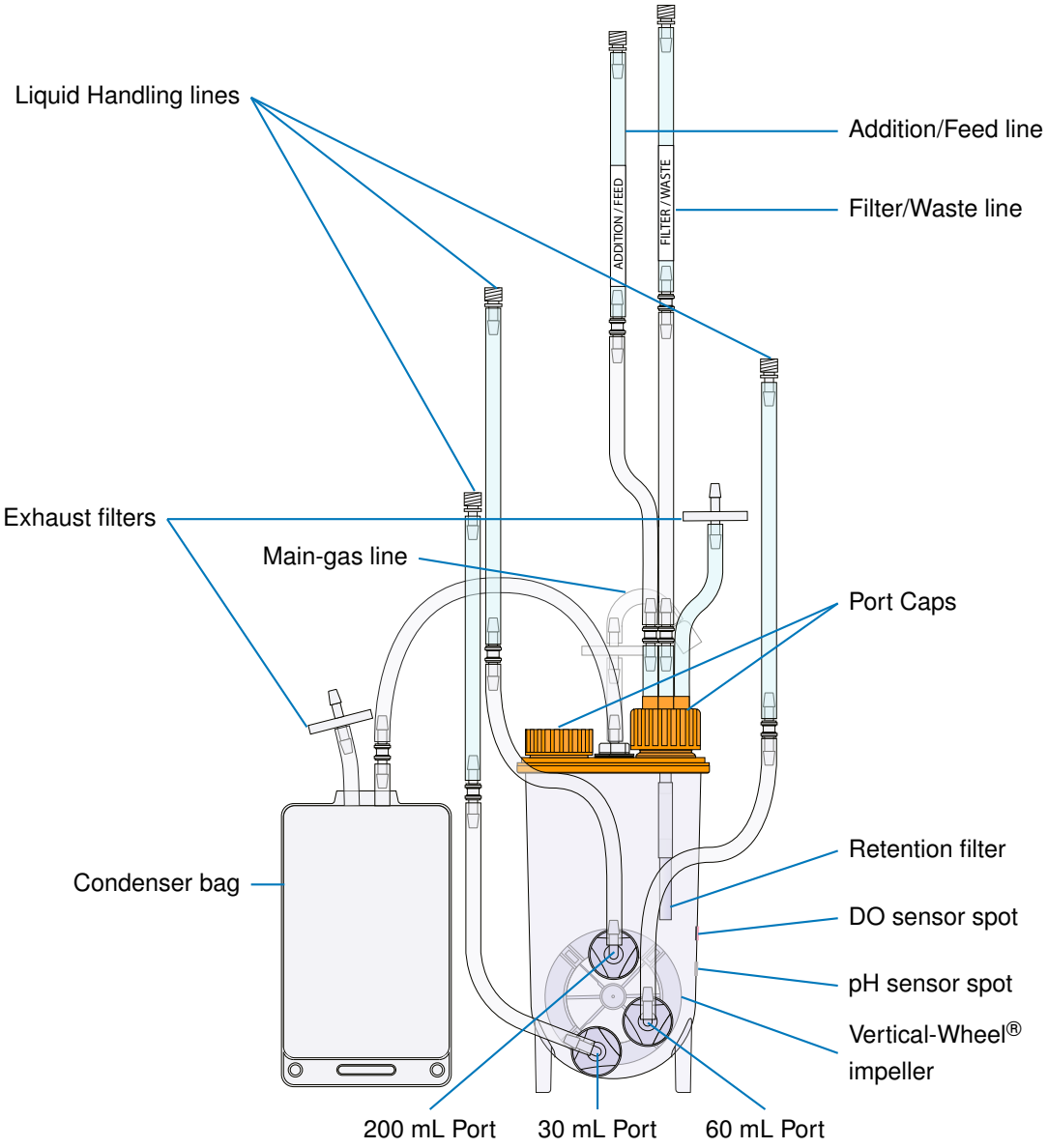
Product information label
Displays the Module's serial and model numbers, as well as safety information.

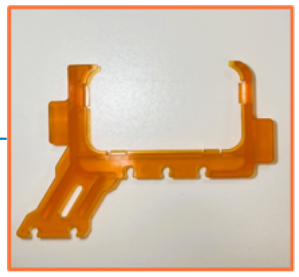
A/C receptacle
Connects to a grounded outlet through a desired power cord to start up the Module.

Gas connection panel
Connects the external N_2, Air, CO_2, and O_2 supplies to the Base Module (for specifics, see "Utility Requirements" on page 48).

NOTICE The gas connectors on the back of the Base Module are push-to-connect connectors. Disconnecting the tubing requires pushing in the orange or gray connector, then pulling out the tubing. Improper removal of tubing can break the retaining clip and impact the holding capability/seal when tubing is reinstalled.

This drawing of the vessel is illustrative of general features and is not intended to represent any particular PBS product with 100% accuracy.





Liquid Handling lines
Used to add medium, cells, and other additions, and to draw a sample or remove spent medium during medium exchanges at the specified volume. The drawing shows the corresponding volume for each port.
Note: Which ports are accessible will depend on the model of vessel purchased.

Addition/Feed line
Liquid added through this line drops from the port cap through the headspace and into the vessel. Therefore it can only be used for additions, and not for removing liquid from the vessel.

Filter/Waste line
Liquid removed through this line is filtered by the Retention filter, and its primary intended use is for removing waste media via perfusion.

Port Caps
The shallow cap on the left is intended for use in a biosafety cabinet. Its large opening can accommodate serological pipettes. The taller cap on the right is intended to be used for additions, and/or perfusion medium exchange.

Retention filter
This filters the waste medium removed through the Filter/Waste line when removed at low flow rates (such as during perfusion), leaving large particles in the vessel. The default filter's pore size is 60 µm.

DO sensor spot
Comes pre-installed and sterilized in the vessel. The spot works in conjunction with the DO sensor head to read the DO value of cell culture medium in the vessel. Shining a flashlight onto the DO sensor spot may affect the DO PV.

pH sensor spot
Comes pre-installed and sterilized in the vessel. The spot works in conjunction with the pH sensor head to read the value of the pH of cell culture medium in the vessel. Shining a flashlight onto the pH sensor spot may affect the pH PV.

Vertical-Wheel® impeller
Coupled to a driving motor by a set of magnets around its circumference. It has vanes for multidirectional mixing.

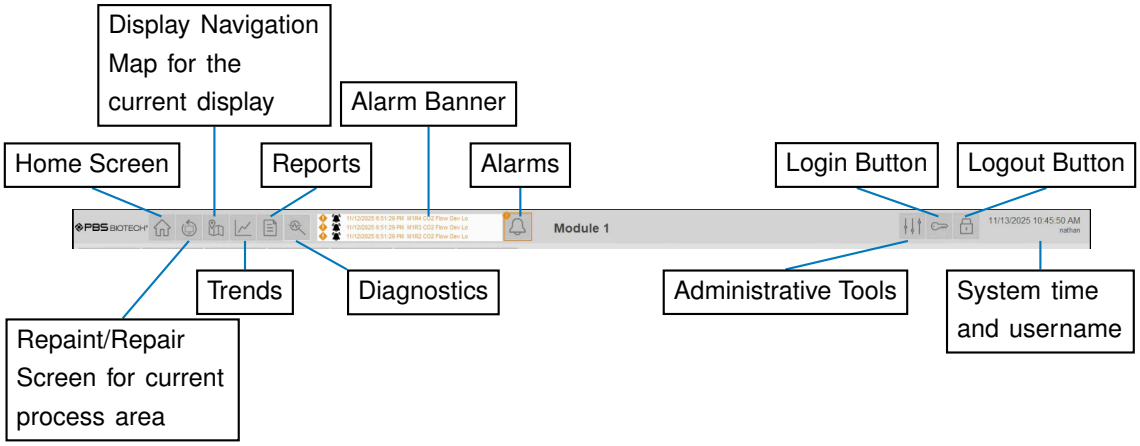
Main-gas line
Connects to the Bioreactor's Main-gas connector, which connects to external gas sources via the gas connection panel (see "PBS-MiniPRO Control Module - Rear" on page 14). Air, CO_2, N_2, and O_2 flow through this line to the overlay to control dissolved oxygen and pH.

Exhaust filters
Filter the exhaust in order to maintain sterility of the vessel contents.

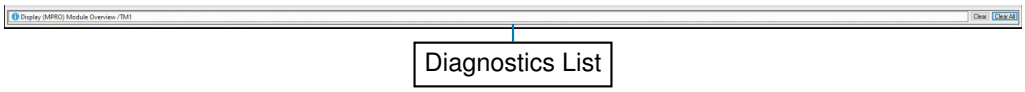
Condenser bag
Catches droplets entrained in the exhaust, preventing them from clogging the downstream exhaust filter.

Tubing guide
Each vessel comes with a Tubing guide. The operator installs it on the vessel, as part of installing the vessel in the sleeve. It holds the exhaust tubing and Liquid Handling lines, and helps prevent them from becoming kinked or tangled.

This header bar is always at the top of the software UI.



This footer bar is always at the bottom of the software UI.



Home Screen
Clicking this button brings the operator to the Base Module Overview of the active Base Module (see “PBS-MiniPRO Software - Base Module Overview” on page 30).

Repaint/Repair Screen for current process area
Refreshes the information being shown.

Display Navigation Map for the current display
Brings the operator to a display map, allowing them to navigate to any Bioreactor or Base Module.

Trends
Brings the operator to an empty TrendPro template.

Reports
Brings the operator to SQL reporting services. Also includes system-wide audit and diagnostic logs.

Diagnostics
Brings the operator to a menu for troubleshooting.

Alarm Banner
Shows the 3 most recent alarms or system-wide events. For an explanation of what the colors mean, see “PBS-MiniPRO Software - Icons” on page 26.

Alarms
Brings the operator to the alarm history/alarm explorer menu.

Administrative Tools
Brings the operator to the system configuration display.

Login Button
Click this button to log in to the software.

Logout Button
Click this button to log out of the software.

System time and username
This shows the date and time, and the name of the user currently logged in.

Diagnostics List
This includes information such as user actions, warnings, and errors. For user actions, this shows the names of the tags which were changed. Tag names follow the MxRy convention (see “PBS-MiniPRO Base Module - Front Overview” on page 16).

The Display Client uses several icons to indicate actions and statuses. The following table lists the icons and their definitions.

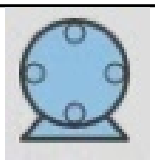
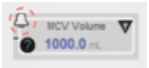
Illustration	Description	Meaning
	Current Device State: Gray	Component is off/disabled.
	Current Device State: White	Component is on/enabled.
	Current Device State: Blue	Component is dynamic/changing state.
	Flashing red outline accompanied by red circle with double exclamation marks	Discrete alarm. Input failure.
	Flashing orange outline accompanied by orange diamond with exclamation mark	LOLO or HIHI Deviation or Limit Alarm threshold exceeded and alarm has not been acknowledged. If not flashing, it means the alarm has been acknowledged.
	Flashing yellow outline accompanied by yellow triangle with exclamation mark	LO or HI Deviation or Limit Alarm threshold exceeded and alarm has not been acknowledged. If not flashing, it means the alarm has been acknowledged.
	White flashing outline accompanied by white alarm bell	Alarm condition has been cleared but not acknowledged. Acknowledgement is required.

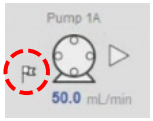
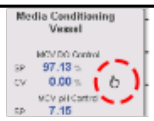
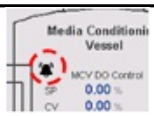
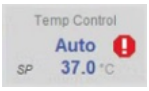
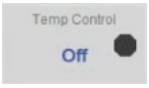
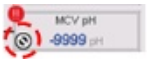
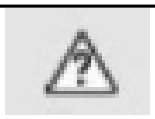
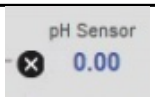
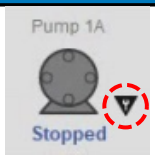
Illustration	Description	Meaning
	White flag	Device is not in its default mode (‘program’ mode is default for the MFCs, ‘operator’ mode is default for all other devices). For example, the Pump default mode is ‘operator’ but when performing a calibration, it will be in ‘program’ mode so a white flag will be displayed.
	Pointing hand	Device is in Manual mode.
	Remote	Device is in Auto mode.
	Black alarm bell	Alarm condition is active.
	Red octagon with white exclamation point	Device is interlocked and is supposed to be on. Check interlock faceplate for further detail.
	Black octagon	Device is interlocked and is supposed to be off. Check interlock faceplate for further detail.
	White circle with diagonal line through it	Device is disabled. Signal Failure.
	Triangle with question mark	PV uncertain - bad reading, before putting in Sensor Error mode.
	Black circle with X	Discrete alarm. Signal failure.
	Black circle with question mark	Bypass is enabled and a value is simulated (only available in admin).

Illustration	Description	Meaning
	Upside down triangle with wrench	Bypass Enabled for maintenance (only available in admin).

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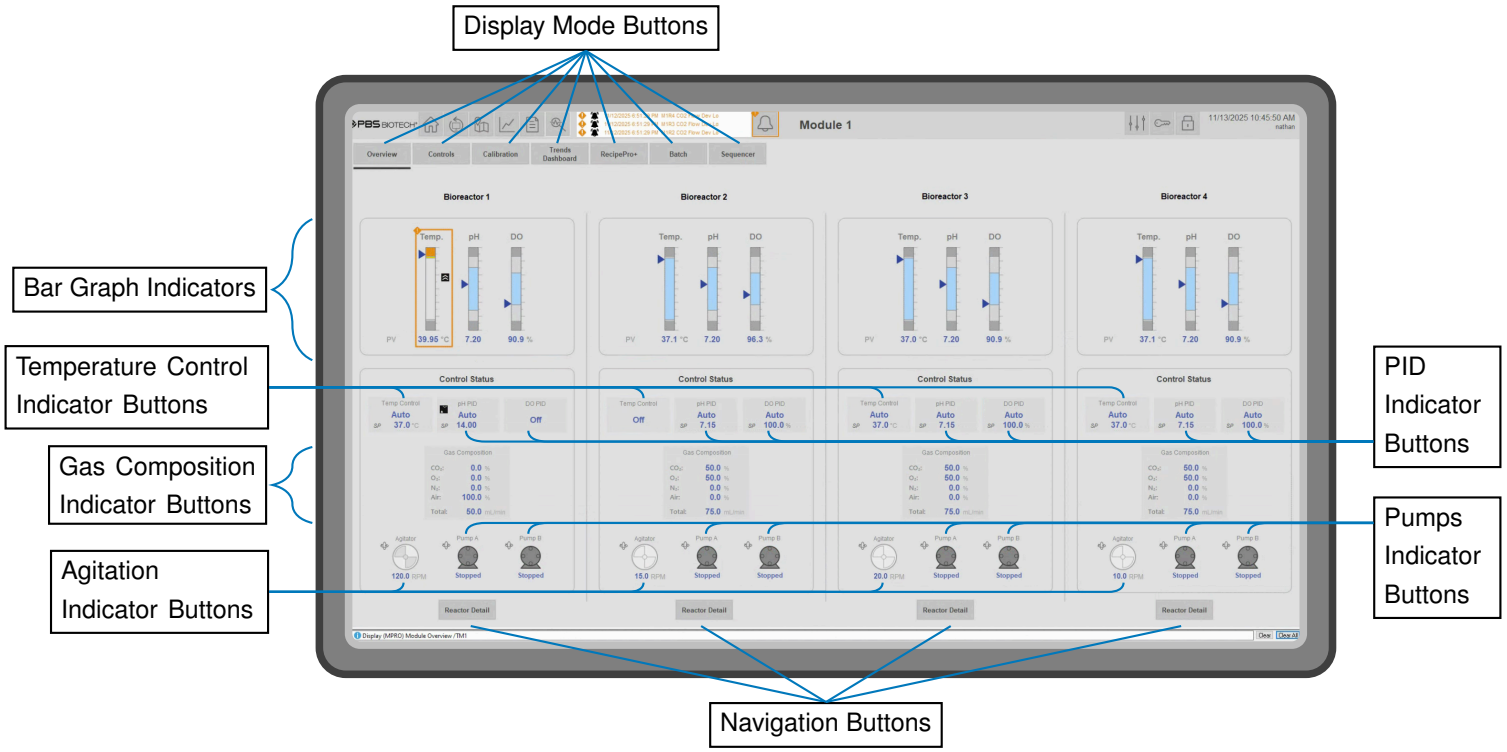
The Display Client opens automatically when the PBS-MiniPRO is powered on. It contains all control panels, configurations, and other features for standard operation and monitoring of all connected bioreactor systems. For more information, see “Display Client” on page 133. This menu shows all Bioreactors for a particular Base Module. Besides being the default menu which opens on power up and after logging out, you can also navigate to it by clicking on one of the Base Modules in the System Overview Menu, or from the Navigation Map.



Display Mode Buttons
Clicking one of these buttons changes the display mode.

- Overview - this menu.
- Controls - a menu where the operator can see and enter the control information for all 4 Bioreactors, for Agitation, Temperature, DO, pH, and Gas Control.
- Calibration- a menu where the operator can enter the manufacturer's calibration information for the DO and pH sensor spots for all 4 Bioreactors, or perform one-point calibrations on the pH sensor and high-point calibrations on the DO sensor.
- Trends Dashboard - shows graphs of the Temperature, pH, DO, and Gas Flow PVs for all 4 Bioreactors.
- RecipePro+ - Launches the RecipePro+ feature; see "RecipePro+" on page 114.
- Batch - Launches the Batch feature; see "Entering Batch ID" on page 94.
- Sequencer - Launches the Sequencer feature; see "Sequencer" on page 118.

Bar Graph Indicators
For each Bioreactor, this shows the Process Value of Temperature, pH, and DO. For an explanation of what the colors mean, see "PBS-MiniPRO Software - Icons" on page 26. Clicking one opens the corresponding faceplate for that sensor and Bioreactor, allowing the user to see trends, see active alarms, and configure alarms and other settings.

Temperature Control Indicator Buttons
This shows the Mode and Auto Setpoint for Temperature, along with the Control Variable for Manual mode; the Temperature PID Indicator Button shows the Control Variable in Auto Mode. Clicking it opens the corresponding Temperature Control Equipment Module faceplate for the Bioreactor, allowing the user to set mode and setpoint.

PID Indicator Buttons
These show the Mode and Auto Setpoint for DO and pH, along with the Control Variables when in Manual mode; the Gas Composition Indicator Button shows the Control Variables for them in Auto mode. Clicking one opens the corresponding faceplate for that PID controller and Bioreactor. All the PID faceplates allow the user to see trends, see active alarms, and configure alarms and other settings. Use the Gas Composition Indicator Button to set modes and setpoints.

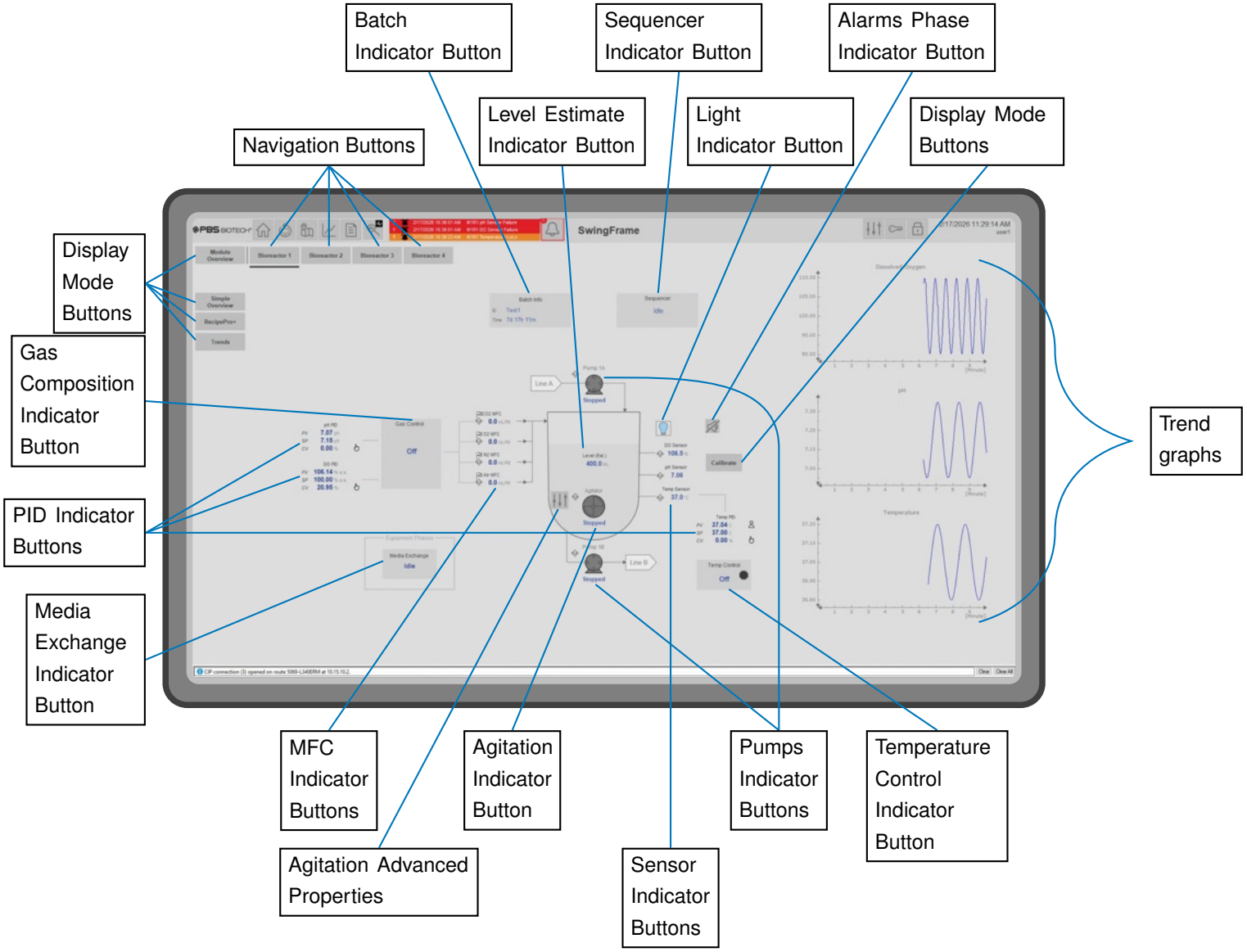
Gas Composition Indicator Buttons
This shows the total gas flow, as well as the current composition - what gases are making up what percents of the total gas flow. Clicking it opens the Gas Control faceplate for that Bioreactor, allowing the user to set mode and setpoint for pH, DO, and Gas Totalizer. It also allows the user to access the 'Settings,' 'Devices,' and 'Advanced' menus.

Pumps Indicator Buttons
This shows the current status for both pumps; whether each is on or not, and if on then what the current flow rate is in mL/min. Clicking one opens the Pump Control faceplate for that Bioreactor, allowing the user to configure and control the pumps.

Agitation Indicator Buttons
This shows the current Agitation status; whether it is on or not, and what the current RPM value is. Clicking it opens the Agitation faceplate for that Bioreactor, allowing the user to set mode and setpoint, see trends, see active alarms, and configure alarms and other settings.

Navigation Buttons
Clicking one of these brings the operator to the Bioreactor Overview for the Bioreactor.

This is a detailed view of a particular Bioreactor.



Navigation Buttons
Clicking one of these brings the operator to the Bioreactor Overview for the Bioreactor.

Batch Indicator Button
Displays the current Batch information. Clicking it launches the Batch feature; see “Entering Batch ID” on page 94 for more information.

Level Estimate Indicator Button
This shows the calculated Level Estimate. Clicking it opens the Level Estimate faceplate for the Bioreactor, allowing the user to reset or manually update the Level Estimate, see trends, see active alarms, and configure alarms and other settings.

Sequencer Indicator Button
Displays the current Sequencer information. Clicking it launches the Sequencer feature; see “Sequencer” on page 118 for more information.

Light Indicator Button
Each Bioreactor has a light in the top right side of the sleeve. Clicking this button turns the light on and off.

Alarms Phase Indicator Button
For extended conditions (such as while a Bioreactor is not being used for a cell culture run), groups of alarms can be suppressed so the software ignores the alarms which would otherwise be triggered. Clicking this button allows the operator to set the Alarms Phase and configure them. For more information, see “Alarms Phase” on page 165.

Display Mode Buttons
Clicking one of these buttons changes the display mode.

- Calibrate- a menu where the operator can enter the manufacturer’s calibration information for the DO and pH sensor spots for the Bioreactor, or perform one-point calibrations on the pH sensor and high-point calibrations on the DO sensor.
- Module Overview - Clicking this button brings the operator to the Base Module Overview of the active Base Module (see “PBS-MiniPRO Software - Base Module Overview” on page 30).
- Simple Overview - shows a simplified version of this menu.
- RecipePro+ - Launches the RecipePro+ feature; see “RecipePro+” on page 114.
- Trends - Opens the TrendPro interface, set to show the DO, pH, Temperature, and Alarms graphs for this Bioreactor. Alarms are graphed by showing a bell icon where an alarm event occurred.

Trend graphs
This shows the DO, pH, and Temperature PVs.

Gas Composition Indicator Button
This shows the total gas flow, as well as the current composition - what gases are making up what percents of the total gas flow. Clicking it opens the Gas Control faceplate for that Bioreactor, allowing the user to set mode and setpoint for pH, DO, and Gas Totalizer. It also allows the user to access the ‘Settings,’ ‘Devices,’ and ‘Advanced’ menus.

PID Indicator Buttons
These show the Mode and Auto Setpoint for Temperature, DO, and pH.

- For Temperature, it shows the Control Variable for both Auto and Manual mode.
- For DO and pH, it shows the Control Variables when in Manual mode; the Gas Composition Indicator Button shows the Control Variables for them in Auto mode.

Clicking one opens the corresponding faceplate for that PID controller and Bioreactor. All the PID faceplates allow the user to see trends, see active alarms, and configure alarms and other settings.

- For Temperature, use the Temperature Control Indicator Button to set mode and setpoint.
- For DO and pH, use the Gas Composition Indicator Button to set modes and setpoints.

Media Exchange Indicator Button
Displays the current Media Exchange information. Clicking it launches the Media Exchange feature; see “Exchanging Medium” on page 108 for more information.

MFC Indicator Buttons
These show the gas flow for each MFC. Clicking one opens the corresponding faceplate for that MFC and Bioreactor, allowing the user to see trends, see active alarms, and configure alarms and other settings.

Agitation Advanced Properties
Clicking this brings the user to the Agitation EM Settings faceplate for the Bioreactor, allowing the user to configure advanced settings.

Agitation Indicator Button
This shows the current Agitation status; whether it is on or not, and what the current RPM value is. Clicking it opens the Agitation faceplate for that Bioreactor, allowing the user to set mode and setpoint, see trends, see active alarms, and configure alarms and other settings.

Sensor Indicator Buttons
For each Bioreactor, this shows the Process Value of Temperature, pH, and DO. For an explanation of what the colors mean, see “PBS-MiniPRO Software - Icons” on page 26. Clicking one opens the corresponding faceplate for that sensor and Bioreactor, allowing the user to see trends, see active alarms, and configure alarms and other settings.

Pumps Indicator Buttons
This shows the current status for both pumps; whether each is on or not, and if on then what the current flow rate is in mL/min. Clicking one opens the Pump Control faceplate for that Bioreactor, allowing the user to configure and control the pumps.

Temperature Control Indicator Button
This shows the Mode and Auto Setpoint for Temperature, along with the Control Variable for Manual mode; the Temperature PID Indicator Button shows the Control Variable in Auto Mode. Clicking it opens the corresponding Temperature Control Equipment Module faceplate for the Bioreactor, allowing the user to set mode and setpoint.

System Description

2

This chapter gives an overview of the PBS-MiniPRO Bioreactor System. It describes the high-level components and functionality of the PBS-MiniPRO and explains the principles of basic operation.

System Description

The PBS-MiniPRO Vertical-Wheel® Bioreactor System (PBS-MiniPRO) is a single-use bioreactor system intended primarily for the culture of mammalian cells and the production of cell-derived biologicals. It consists of a non-disposable PBS-MiniPRO Control Module, between 1 and 6 non-disposable PBS-MiniPRO Base Modules, and at least one Vertical-Wheel® Bioreactor Single-Use Vessel Assembly (vessel). Each PBS-MiniPRO Base Module can accommodate up to 4 vessels. The PBS-MiniPRO Control Module, Base Module(s), and vessel(s) are designed to interface closely with each other and to function as an integrated system.

This PBS-MiniPRO Vertical-Wheel® Bioreactor System provides all of the necessary process measurement and control features to ensure necessary conditions for the successful cultivation of cells. The PBS-MiniPRO Control Module consists of: an industrial controller, a touchscreen interface, a keyboard, and a mouse. The PBS-MiniPRO Base Module consists of the following for each vessel: an interface for the vessel; a four-gas module; a vessel heater; a vessel temperature sensor, DO and pH sensor heads for single-use sensors; and 2 pumps for sampling, adding medium and additional fluids, removing spent medium, and harvesting. It is able to control all critical cell culture parameters, such as agitation, temperature, DO, and pH.

The Vertical-Wheel® Bioreactor Single-Use Vessel is a uniquely shaped rectangular vessel with a round bottom incorporating the Vertical-Wheel® impeller, which has side paddles, vanes, and a hub. The vessel's shape is designed to work with the vertical impeller to offer excellent mixing and homogeneous particle suspension with very low shear stress using minimal power input.

Principles of Operation

Agitation

The PBS-MiniPRO falls into the category of stirred bioreactors. The biggest difference between PBS Biotech's Vertical-Wheel® Bioreactors and traditional stirred bioreactors, whether single-use or reusable, lies in the unique vessel

and impeller geometry, described above. The Vertical-Wheel® impeller is driven by a magnetically-coupled external motor.

Heating

The PBS-MiniPRO has a permanently-mounted temperature sensor positioned on the right side of the sleeve which senses the temperature of the vessel contents. The PBS-MiniPRO also has a permanently-mounted electric heater in the back of the sleeve, which contacts the back surface of the vessel.

Dissolved Oxygen

The dissolved oxygen is monitored by a single-use DO sensor. Single-use sensors are intended to be calibrated with the PBS-MiniPRO after the vessel has been filled with medium and equilibrated. The PBS-MiniPRO controls the DO by using a split-range PID controller. To decrease DO levels, the software increases the percent composition of N_2 flowing out of the Main-gas connector, through the Main-gas line, and into the overlay. To increase DO levels, the software increases the percent composition of O_2 flowing out of the Main-gas connector, through the Main-gas line, and into the overlay.

pH

The culture pH is monitored by a single-use pH sensor. Single-use sensors are intended to be calibrated with the PBS-MiniPRO after the vessel has been filled with medium and equilibrated. To decrease the pH, the software increases the percent composition of CO_2 flowing out of the Main-gas connector, through the Main-gas line, and into the overlay. There is no direct control for increasing pH; it is only done directly through CO_2 stripping.

Level

The software uses the calibrated pumps to totalize how much liquid has been added to and removed from the vessel, and reports that sum as the estimated volume of liquid the vessel contains.

Exhaust Filter and Condenser Bag

As sterile gas flows into the vessel, it must also leave to prevent the vessel from over-pressurizing. Each vessel has an exhaust tubing line for this purpose. The exhaust tubing line has an in-line filter to maintain the sterility of the vessel contents. To prevent clogging of the exhaust filter, each vessel is equipped with a condenser bag on the exhaust tubing to catch entrained medium droplets.

Overview of PBS Software Functionality and Architecture

Functionality

The PBS Software that is an integral part of your PBS-MiniPRO is multifunctional. Its capabilities can be grouped in the following categories:

- Sensing and Control
- Data Acquisition and Reporting
- Limit, Deviation, and Failure Alarms, and Diagnostic Alerts
- Task Automation
- Remote Monitoring and Control
- Administration

Sensing and Control

The PBS-MiniPRO has the ability to monitor and control temperature, dissolved oxygen, and pH in the vessel. It can also control the agitation, as well as calculate/report the estimated volume of the vessel contents. The three main control loops (temperature, DO, and pH) each have three user-selectable modes: Automatic, Manual, and Off. The main gas controller, which must be on for the DO and pH controls to function, only has a Manual and Off mode. The agitation controller also only has a Manual and Off mode. In Automatic mode, the control loops implement PID feedback control with a setpoint determined by the user. In Manual mode, the control loops implement an open loop scheme where the user directly selects controller output. In Off mode, the controller's output is set to zero.

Also falling under the scope of Sensing and Control are interlocks, sensor error detection, and sensor error modes. The purpose of the interlocks is to prevent the creation of unsafe conditions or conditions that would hinder the growth of cells. The purpose of the sensor error detection and mode features is to minimize the problems that could arise due to sensor failure.

Data Acquisition and Reporting

The PBS Software has the ability to collect and report multiple types of data. Data types include Process Data, Diagnostics (including Audit Events), and Alarms. All these data types are stored to a database on the HMI computer's hard drive (see "Architecture" on page 39), and can be exported as .csv (comma-separated value) files, .txt (tab-separated value) files, or as screenshots of the generated graphs. Process data includes potentially thousands of tags. For each Process data variable, the user can select

whether or not to log them, as well as how frequently to log them. The other data types always get logged to the database.

Limit, Deviation, and Failure Alarms

To assist you in monitoring the performance of the PBS-MiniPRO, a comprehensive set of parameters is continuously monitored. If any parameter falls outside of a pre-defined range, an alarm event will be generated and communicated to you. Limit alarms and Deviation alarms monitor your process variables, while Failure alarms monitor the PBS-MiniPRO's sensors and other hardware.

Each alarm can be individually configured to be enabled, temporarily shelved, suppressed, or disabled. The sensitivity of the failure alarms can be configured by the user. In addition, the limit alarm limits are entirely selectable by the user according to their particular process conditions.

Task Automation

The RecipePro+ feature allows the user to set the values of multiple tags, for multiple Bioreactors, all at once. The recipes are programmed using the RecipePro+ Editor. Once saved, the recipes are available to be run. Recipes can be used for a variety of tasks, such as setting all the controller modes and setpoints at once.

The Sequencer feature allows the user to automatically run sequences of instructions on the PBS-MiniPRO. The sequences are programmed using the Sequence Editor. Once saved, the sequences are available to be run. Sequences can also be used for a variety of tasks, such as changing a setpoint at some time in the future when no user will be present.

Remote Monitoring and Control

The software allows for remotely monitoring and controlling the PBS-MiniPRO via Ethernet Industrial Protocol.

Administration

In addition to all of the above, the software allows the user to perform additional direct control functions, such as turning pumps on and off. It also offers the user administrative capabilities to add user accounts, configure user permissions, and configure settings.

Architecture

The Vertical-Wheel® Bioreactor control system is a hybrid consisting of an industrial automation controller (the PLC) paired with a human machine interface computer (HMI).

The PLC is in charge of all sensing and control functions, including interlocks, sensor error detection, and running sequences. If the HMI were to fail (from a software crash or hardware failure), the control loops, interlocks, and sequences would continue as normal and maintain current operating conditions.

The PLC provides all data points to the HMI. The HMI is in charge of the logic that captures the data points to be recorded, and the database engine and the database are also on the HMI. If the HMI were to fail, data logging would stop, and would resume when the database engine resumed operation.

Finally, the PLC is in charge of detecting limit, deviation, and failure alarms, and the HMI communicates those alarms to the user. Alarm notifications would cease if the HMI were not to run.

Safety

3

Review the following safety information before installing the system.

	The PBS-MiniPRO is intended for bench-top use. Ensure that the selected work surface has sufficient strength to safely carry the weight of the equipment plus any accessories and process materials.
	Customer-provided safety-straps may be used to reduce the risk of tipping or damage in the event of earthquake. Do not drill into the equipment or use screws to attach straps. Industrial-strength self-adhesive straps may be affixed to the metal sides of the system.
	If any PBS Biotech equipment is used with accessories not provided or recommended by PBS Biotech or used in a manner not specified by PBS Biotech, the protection provided by the equipment may be impaired.
	The power cords are the main electrical disconnects for the equipment. To remove power from the equipment, either use the Power switch, or unplug the power cord. Do not position the equipment in such a way that it is difficult to access the Power switch or unplug the power cords. Do not apply tension on the power cords, to avoid sudden electrical disconnects.
	To provide continued protection against risk of electric shock, the equipment must be connected to a properly grounded outlet. Only use power cords provided by PBS Biotech. Do not use an adapter.
	The back panels of the equipment must only be removed by a trained technician. High voltage circuits are accessible inside and there is a danger of lethal electric shock.
	Use caution when working near peristaltic pumps. Keep fingers, jewelry, loose clothing, etc. free of the rotating pumps to prevent injury.
	The PBS-MiniPRO has hot surfaces, as indicated by hot surface warning signs. Do not touch hot surfaces.

	Vertical-Wheel® Bioreactors are not designed for pressurized operation. Always allow the bioreactor vessel to vent. Never clamp the vessel outlet lines. This could result in dangerous pressure build-up in the vessel.
	Only use vessels manufactured by PBS Biotech for the specific model of your bioreactor system.
	Pumps may restart automatically if the power is restored after an interruption.
	When using external pumps to fill a vessel installed in the bioreactor, use precautions to ensure that the vessel will not overfill, which could cause dangerous pressure build-up.
	Biological substances, such as viruses, cells, and sera, have the potential to transmit infectious diseases. If biohazardous materials are used with this equipment, follow all applicable local, state/provincial, and/or national regulations, including identification of samples with the biohazard symbol. Wear appropriate protective eyewear, clothing, and gloves.
	If the equipment has been used in a biohazardous environment, it must be decontaminated according to all applicable local, state/provincial, and/or national regulations prior to any shipment, or disposal.
	Customers are to follow local regulatory guidelines for proper recycling and disposal of PBS products.

Inspections and Preventative Maintenance

Inspections

This section describes the inspections that the user should perform on the PBS-MiniPRO Bioreactor system to verify safety mechanisms are functional. For instructions on inspecting a Vertical-Wheel® Bioreactor vessel before use, see “Install Vessel in PBS-MiniPRO” on page 66.

Media Bag Holder

An optional accessory available for purchase separately - it assists in hanging media bags as applicable. The weight limit for each Media Bag Hook is 9 kg (20 lbs). Do not hang anything but bags containing liquid off the Media Bag Holder. Only use the Media Bag Hooks provided by PBS Biotech to hang the

bags.

Safety-Related Settings

Confirm that all interlock-related settings match those listed in the Configuration Document, VV-04367, or that the values have been confirmed with PBS Biotech Technical Support. Do not attempt to verify the functionality of any interlocks - that should only be performed by a representative of PBS Biotech.

Preventative Maintenance

To keep your PBS-MiniPRO properly maintained, clean and decontaminate it after each run (see below). For other maintenance on the PBS-MiniPRO, contact PBS Biotech Technical Support.

Cleaning and Decontamination

To clean and decontaminate the PBS-MiniPRO, use 70% IPA or EtOH. Apply the cleaning solution to a clean, soft cloth - do not spray or apply cleaning solution directly onto the PBS-MiniPRO. Wipe down all surfaces of the PBS-MiniPRO, including inside the vessel sleeves. Be very gentle when cleaning the temperature sensors. Contact the manufacturers of other equipment in use, such as a keyboard or Uninterruptible Power Supply (UPS), for cleaning and decontamination instructions.

NOTICE Do not use abrasive materials on the PBS-MiniPRO. It is the user's responsibility to avoid use of decontamination or cleaning agents that could cause a hazard as a result of a reaction with parts of the equipment or material contained in it. Contact PBS Biotech Technical Support if there is any doubt about the compatibility of decontamination or cleaning agents.

This protocol is appropriate to clean and decontaminate equipment in contact with materials assigned to Biosafety Level 1. In case of operation in a higher Biosafety Level facility, please contact PBS Biotech Technical Support.

Lifting and Handling

The PBS-MiniPRO Control Module weighs approximately 11 kg (23 lbs). The PBS-MiniPRO Base Module weighs approximately 54 kg (119 lbs) without the Media Bag Holder, 61 kg (134 lbs) with it. The Touch screen is not affixed to the Control Module, so do not attempt to move them together. To prevent injury or damage to the Base Module, it should only be lifted by at least two individuals from the pallet onto the bench. Spotters should be present in front of and behind the Base Module to make sure it does not tip over during lifting. Do not move the Base Module by its Media Bag Holder as this will cause damage to the Base Module. Proper lifting technique of bending at the knees and lifting with the legs should be used when moving any of the equipment.

Product Specifications

4

Note: These specifications are for the standard PBS-MiniPRO configuration as of publication. Individual bioreactor systems may differ.

PBS-MiniPRO Specifications		
General	Size	**Control Module** Width: 46 cm (18 inches) Depth: 36 cm (14 inches) Height: 20 cm (8 inches) without the Touch screen, 59 cm (23 inches) with it **Base Module** Width: 78 cm (30.5 inches) Depth: 63 cm (24.5 inches) Height: 44 cm (17 inches) without the Media Bag Holder, 105 cm (41.5 inches) with it
	Weight	**Control Module**: 11 kg (23 lbs) **Base Module**: 54 kg (119 lbs) without the Media Bag Holder, 61 kg (134 lbs) with it (both weights are without Vessel(s))
	Minimum Space Requirements	**Control Module** (This is for having the Touch screen on top and a keyboard and mouse in front) Width: 61 cm (24 inches) Depth: 79 cm (31 inches) Height: 60 cm (23.5 inches) **Base Module** Width: 80 cm (31.5 inches) Depth: 91 cm (36 inches) Height: 74 cm (29 inches) without the Media Bag Holder, 107 cm (42 inches) with it

PBS-MiniPRO Specifications		
General (continued)	Electrical	**Control Module** 2.1/1.1 A (max), 110-120/200-240 Vac, 50/60 Hz Overvoltage Category II **Base Module** 2.7/1.4 A (max), 110-120/200-240 Vac, 50/60 Hz Overvoltage Category II
	Environmental Rating	Indoor use, Ambient Temperature: 16 - 32 °C (61 - 90 °F) Humidity: 10 - 80% RH Altitude: 2,000 m (6,500 ft) max Pollution Degree: 2
Bioreactor Geometry	Rated Working Volume	500 mL
	Minimum Working Volume	300 mL (top of wheel)
	Impeller Type	Vertical-Wheel® mixing technology
Controls	Control Interface	22″ touch screen. Network connectivity capability.
	Control Hardware/Software	Rockwell Automation PLC
	Data Communication	Built-in data historian (Microsoft SQL Server Express, remote monitoring and control via Ethernet Industrial Protocol
	Data Connection Ports	2x USB 3.0, 3x USB 2.0 1x RJ45 Ethernet Wi-Fi

PBS-MiniPRO Specifications		
Agitation	Agitation Mechanism	Brushless DC motor drive, Magnetic coupling to vessel impeller
	Agitation Control Range (Accuracy)	5 – 100 RPM (± 10% of SP)
Gassing	Gassing Mode	Headspace overlay
	Gas Control	4 mass flow controllers (for Air, N_2, O_2, CO_2 gases) Manual control of total gas flow rate Individual gas outputs as determined by Dissolved Oxygen and pH controls
	Gas Flow Rate Range	25 – 100 mL/min for Air, N_2, O_2, and CO_2
Temperature	Temperature Control Range (Accuracy)	5 °C above ambient to 40 °C (± 0.5 °C)
	Temperature Sensor Type	Class A Platinum RTD
Dissolved Oxygen	DO Control	Split-range PID Auto or Manual control of N_2 and O_2 flow
	DO Sensor Type	Single-Use PreSens® optical
pH	pH Control	PID Auto or Manual control of CO_2 gas flow
	pH Sensor Type	Single-Use PreSens® optical
Pumps	Pump A	Watson Marlow 114DV Series Bidirectional, Variable-Speed, 400 RPM max. continuous speed
	Pump B	Watson Marlow 114DV Series Bidirectional, Variable-Speed, 400 RPM max. continuous speed
Single-Use Vessel	Vessel Construction	Injection-molded polycarbonate

PBS-MiniPRO Specifications		
Single-Use Vessel (continued)	Impeller Construction	Injection-molded polycarbonate
	Product Contact Materials	All product contact materials meet requirements for USP Class VI Testing for Plastics <88> and/or ISO 10993
	Gamma Radiation Absorbed Dose	25 – 40 kGy
	Liquid Handling lines	Platinum-cured silicone/C-Flex® with female luer fitting and cap
	Exhaust Line	Platinum-cured silicone tubing with condenser bag and 0.2-micron exhaust filter
	Main-Gas Line	Platinum-cured silicone tubing with 0.2-micron filter
Safety and Regulatory	Markings (housing)	Planned completion date of Q2 2026

Installing the PBS-MiniPRO

5

This chapter gives detailed instructions on how to install the PBS-MiniPRO.

Integrated Bioreactor

Minimum Space Requirements

Before you begin, see “Minimum Space Requirements” on page 44 and confirm that your available bench space meets or exceeds the minimum space requirements listed.

Utility Requirements

General Electrical Requirements

- Outlets must be properly grounded.
- The power cords must be provided by PBS Biotech, Inc.

For other electrical requirements, see “Electrical” on page 45, and the Safety information in Chapter 3 starting on page 40.

General Gas Requirements

- The gases supplied must be clean, dry, particulate-free, and oil-free to prevent MFC damage from contaminated gases.
- All gases must be connected to their corresponding gas connector inlets on the gas connection panel unless instructed otherwise by PBS Biotech Technical Support.

Gas Tubing Outer Diameter

Depending on the bioreactor’s configuration, it will require one of the following tubing sizes for all gases:

- 1/4 inch OD tubing
- 6 mm OD tubing

Gas Tubing Material

The following materials (or equivalent) are appropriate for the gas tubing:

- Polyethylene
- Polyurethane

NOTICE The gas connectors on the back of the Base Module are push-to-connect connectors. Disconnecting the tubing requires pushing in the orange or gray connector, then pulling out the tubing. Improper removal of tubing can break the retaining clip and impact the holding capability/seal when tubing is reinstalled.

Gas Supply Pressures

Note: To ensure proper operation, gases must be regulated near the bioreactor system to a consistent pressure within the ranges specified below. Fluctuating inlet gas pressure may cause MFC flow rates to become unstable.

Gas	Imperial	Metric
Air, CO_2, O_2, N_2	30 ± 10 psig	205 ± 70 kPa

Unit Placement

To prevent bodily injury and/or damage to the product(s), see “Lifting and Handling” on page 43 and follow the safety instructions.

The units should be placed on a tabletop or benchtop where the appropriate utilities have been prepared.

Once the units are in place, the Touch screen’s HDMI, USB, and power cables may be connected to the Control Module, the Control Module may be connected to the Base Module(s) via its M12 Ethernet connectors (x2), and the utilities may be connected.

Powering On the PBS-MiniPRO

Install the appropriate power cords on the PBS-MiniPRO Control Module and Base Module(s). It is recommended to plug them into Uninterruptible Power Supplies (UPS), to allow control to be maintained in the event of a power failure. Grounded outlets are required. Flip the power switches to the ‘On’ position. The Control Module and Base Module(s) will power on, and the Display Client will automatically load once the system has finished booting.

Configuring Users and Groups

The Display Client requires a user to log in before making any changes. This section includes information about user accounts.

The PBS-MiniPRO comes with the following default user accounts for you to start with:

Username: Operator
Password: 12345
Group: Operators
Intended Use: Users in this group have permission to perform day-to-day operations, such as process calibrations, changing modes and setpoints, operating pumps, running recipes and sequences, generating reports, and acknowledging alarms.

Username: Maintenance
Password: 12345
Group: Maintenance
Intended Use: Maintenance is an intermediate level of access for technicians responsible for maintaining the equipment. Users in this group have permission to bypass devices and perform maintenance functions.

Username: Engineer
Password: 12345
Group: Engineering
Intended Use: Users in this group have permission to change settings including PID tuning, configure system parameters, configure advanced features, and configure alarms as they see fit for different cell culture processes.

The user1 account is also in the Engineering group and uses password 12345.

The PBS-MiniPRO also comes with a user account “pbstech,” which will be used by PBS Biotech Technical Support if they need to log in to your bioreactor system. This is the only account in the “OEM” user group. Do not delete or change this account or user group, and do not add other users to the user group.

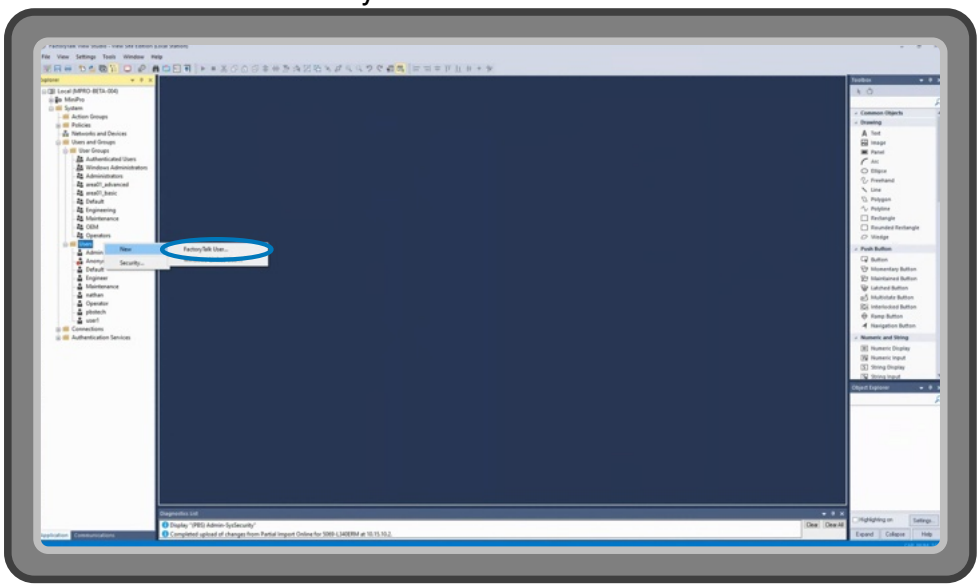
It is recommended to configure the user accounts based on your security requirements. This may include replacing the generic accounts or using them as is. The generic accounts are provided for commissioning, early system testing, and low security environments.

To manage ‘Policies’ and ‘Users and Groups,’ use the FactoryTalk View Studio application installed on the PBS-MiniPRO, under ‘System.’ See documentation

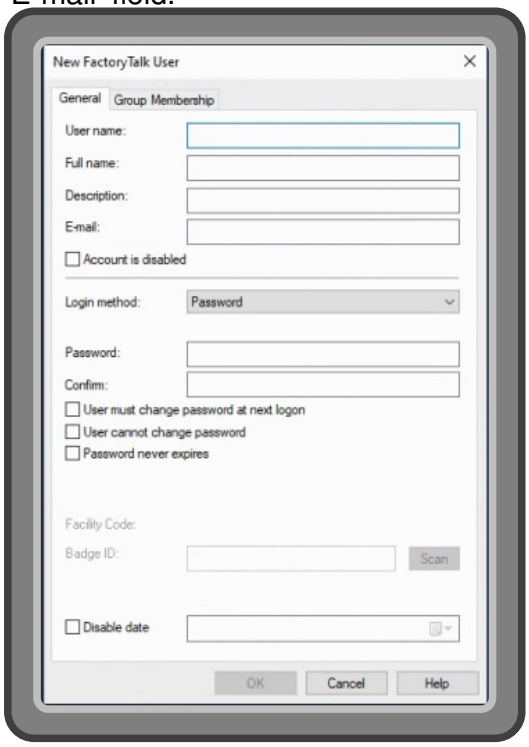
such as the “FactoryTalk View Site Edition User’s Guide” for further instruction.

Creating a New User

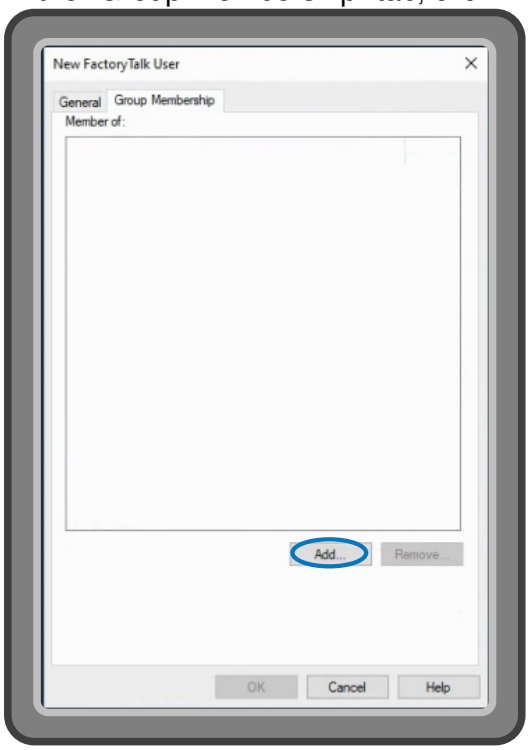
1. Open the FactoryTalk View Studio application installed on the PBS-MiniPRO.
2. Under Local → System → Users and Groups, right-click “Users” and under “New” select “FactoryTalk User...”



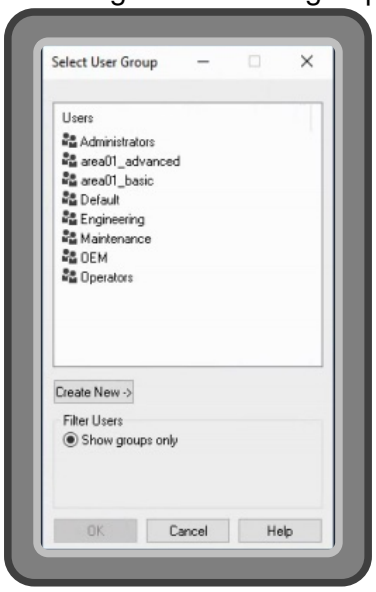
3. In the “General” tab, configure the account and login options as desired. Note that the PBS software does not use the information entered in the ‘E-mail’ field.



4. In the “Group Membership” tab, click “Add...”



5. Select the desired user group from the list, and click “OK.” For an explanation of how Permissions work with the user groups, see “User Group Permissions” on page 167. For which permissions/security tags are assigned to which groups, see Appendix 4 on page 185.



Note: Along with being added to the desired user group, new accounts also need to be added to the “area01_basic” and “area01_advanced” groups to allow the user to access controls in the UI. Additionally, users should not be added to the “Default” group as that group is read-only.

6. Click “OK” from the “New FactoryTalk User” window to save the new account, which should appear under the “Users” folder.

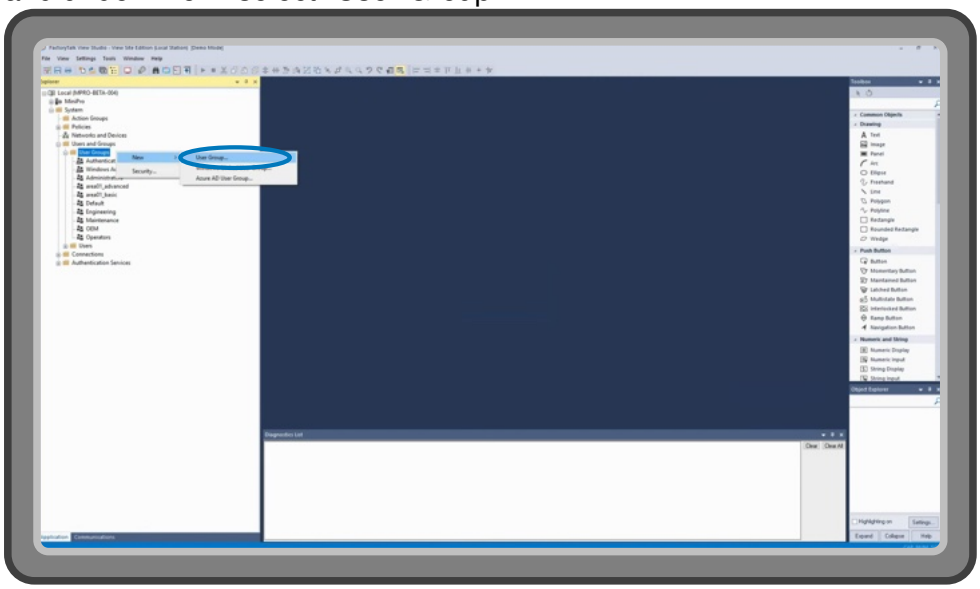
Existing user accounts can be edited by right-clicking the username under the “Users” folder and selecting “Properties...” from the menu.

Existing user accounts can be deleted by right-clicking the username under the “Users” folder and selecting “Delete.”

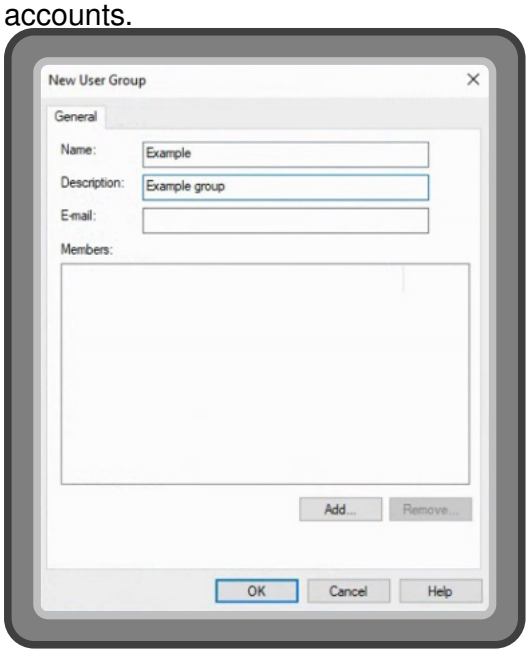
Creating a New User Group

1. Open the FactoryTalk View Studio application installed on the PBS-MiniPRO.

2. Under Local → System → Users and Groups, right-click “User Groups” and under “New” select “User Group...”

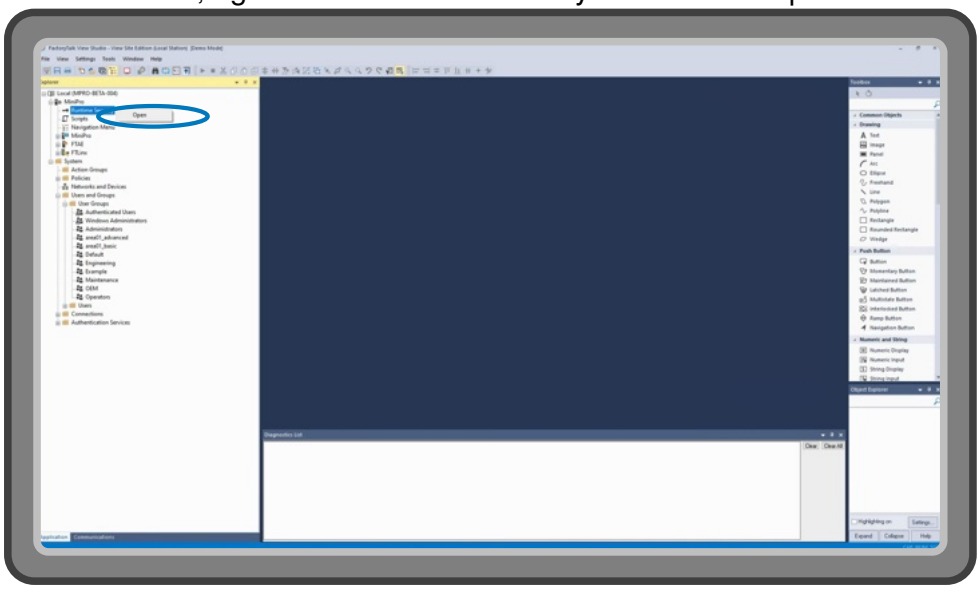


3. Configure the group Name and Description as desired. Note that the PBS software does not use the information entered in the ‘E-mail’ field. Adding members can be done here, or by configuring individual accounts.

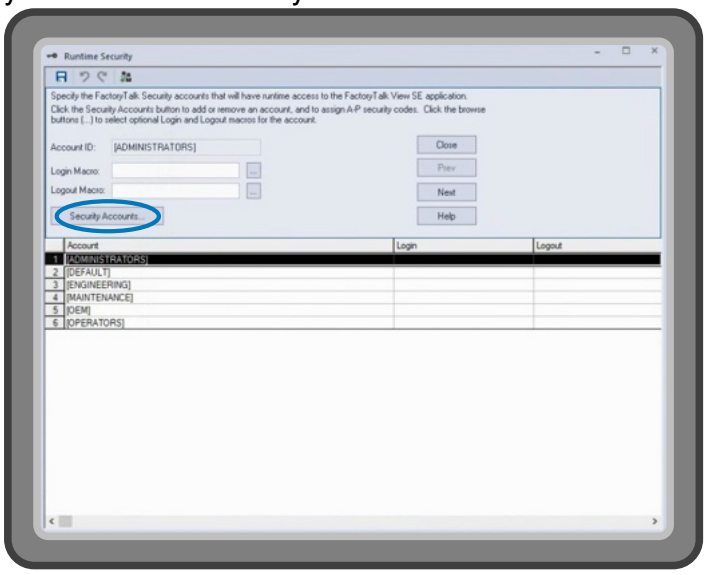


4. Click “OK.” The new group should appear under the “User Groups” folder.

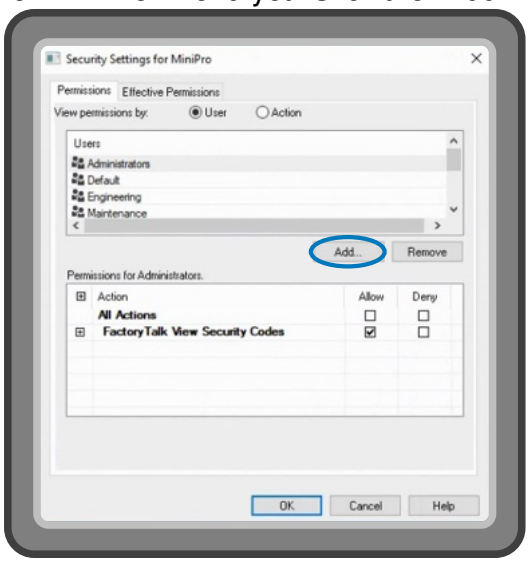
5. Under MiniPro, right-click “Runtime Security” and select “Open.”



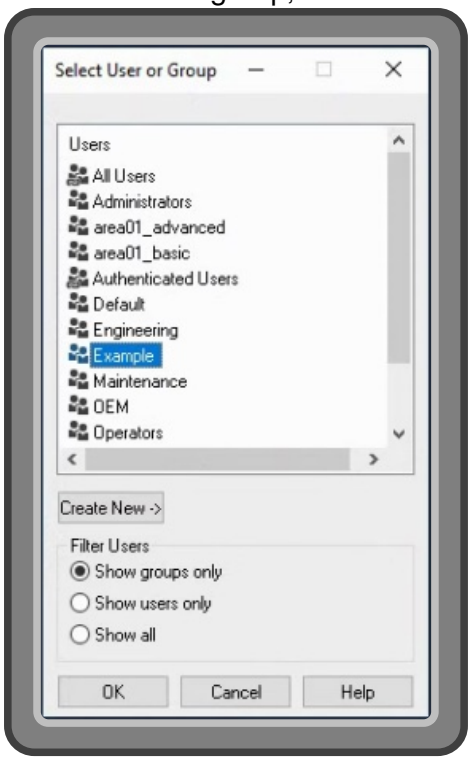
6. The new group will not show up in the list in the “Runtime Security” menu yet. Click the “Security Accounts...” button.



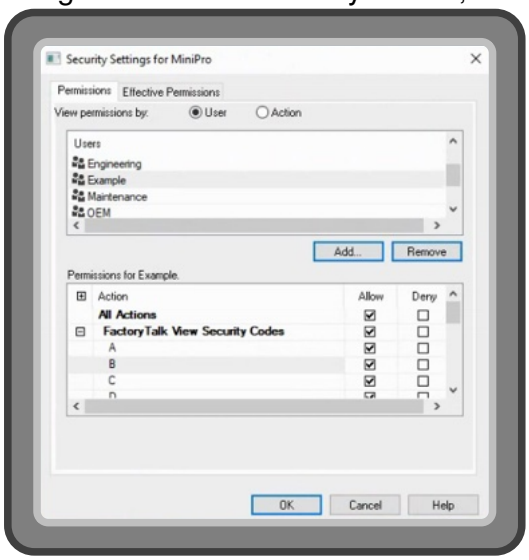
7. The new group will also not show up in the list in the “Security Settings for MiniPro” menu yet. Click the “Add...” button.



8. Select the new group, and click “Ok.”



9. In the “Security Settings for MiniPro” menu, select the desired group, and expand the ‘FactoryTalk View Security Codes.’ All the ‘Allow’ checkboxes will be checked at first. Un-check the ones for each security code you don’t want to grant to the group. For which permissions/security tags are assigned to which Security Codes, see Appendix 4 on page 185.



10. Click “OK” to close the “Security Settings for MiniPro” menu. The new group will now be listed in the “Runtime Security” menu. Close that menu to finish.

Existing group names and descriptions can be edited by right-clicking the group under the “User Groups” folder and selecting “Properties...” from the menu.

Existing group security can be modified in the “Security Settings for MiniPro” menu.

Existing groups can be deleted by right-clicking the group under the “User Groups” folder and selecting “Delete.”

Configuring Logger Settings

Before beginning a run, you should configure what data is recorded, how often, and the destination database(s).

To manage data logging, use the FactoryTalk View Studio application installed on the PBS-MiniPRO. See documentation such as the “FactoryTalk View Site Edition User’s Guide” for further instruction.

In FactoryTalk View Studio, under
Local → MiniPro → MiniPro → Data Log
expand the “Data Log Models” subfolder. The PBS-MiniPRO ships with a

default Data Log, “DefaultLog” but users can configure up to 10. Tags follow the MxRy convention (see “PBS-MiniPRO Base Module - Front Overview” on page 16).

For information about tags, see Appendix 3 on page 181.

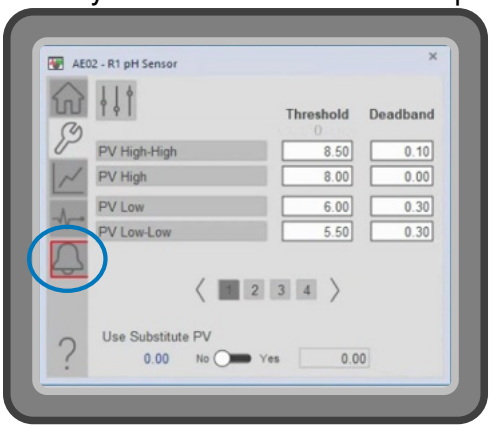
NOTICE The Standard Log Trigger is set to ‘Periodic’ at 30 second intervals, because the ‘On Change’ function only supports a percent change deadband, not absolute, and does not detect tag minimum and maximum values to properly calculate when a percent change occurs. In practice, this means that the ‘On Change’ function will log a value every time it sees a change, which is the maximum logging frequency (~every 1.5 s). HMI tags do support the minimum/maximum ranges necessary to determine change percent, but the device tags used by the software are not mapped to HMI tags, and therefore do not work with the ‘On Change’ function.

After configuring a new Data Log Model, it needs to be added to the ‘MPRO_DataLogOn’ Macro file so it is used by the software on launch.

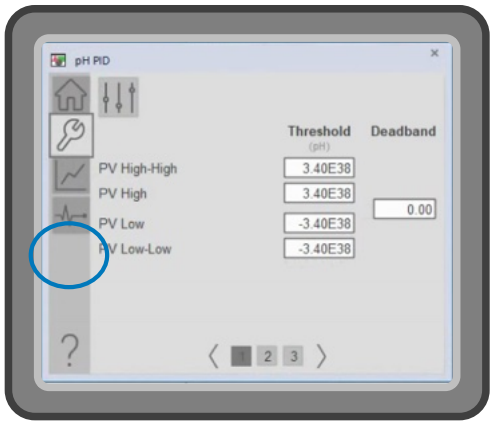
Configuring Alarm Settings

Each alarm is configured on its corresponding faceplate. This means that for one type of alarm to behave the same way on all 4 of a Base Module’s Bioreactors, the operator will have to configure all 4 of the Bioreactor-specific alarms. For information about how alarms work, see “Alarms” on page 161. For definitions of all alarms, see Appendix 2 on page 175.

The alarms corresponding to the settings in the “DO PID,” “pH PID,” and “Temperature PID” faceplates are not in use, but the settings are still visible in software. A simple way to determine if a particular faceplate’s alarms are actually in use is to see if the faceplate has an “Alarms” tab.

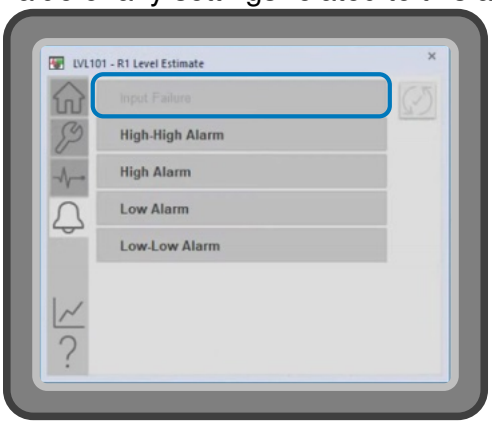


The “pH Sensor” faceplate has an “Alarms” tab, and the settings displayed in its “Maintenance” tab are applicable to Enabled alarms.



The “pH PID” faceplate has no “Alarms” tab, and the settings displayed in its “Maintenance” tab are not applicable to any Enabled alarms.

Additionally, some faceplates have settings for alarms that are in use *and* settings for alarms that are not in use. A simple way to determine if a particular alarm is actually in use is to see if the alarm is available on the faceplate’s “Alarms” tab. For example, the Level “Input Failure” alarm is grayed out in the Level Estimate’s “Alarms” tab, because this alarm is not in use. Changing the value of any settings related to this alarm will have no effect.

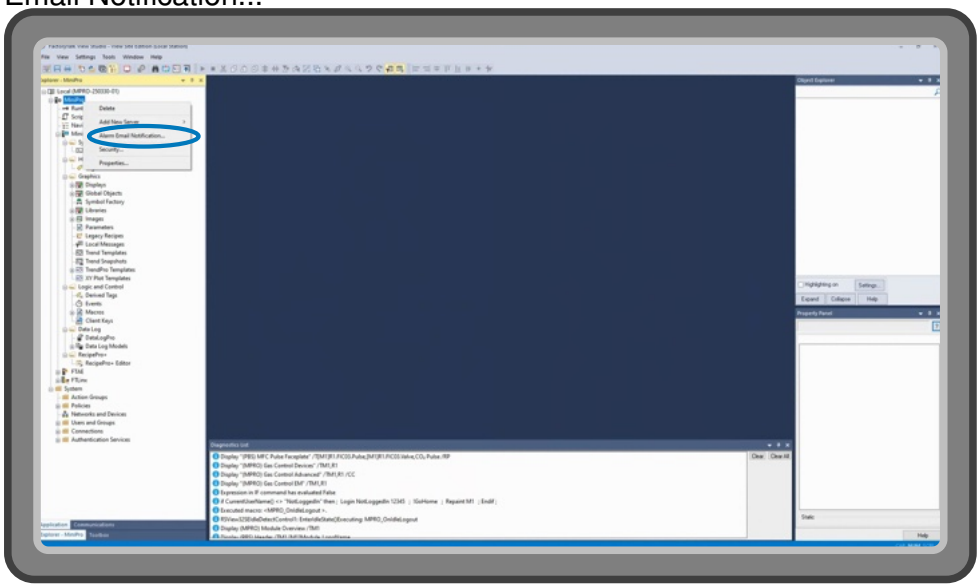


Alarms are configured within the “Maintenance” tabs of the corresponding faceplates, including the “Display Advanced Properties” menu. Clicking the alarm name opens a new window with additional configuration options. The “Alarms” tab also contains options for configuration.

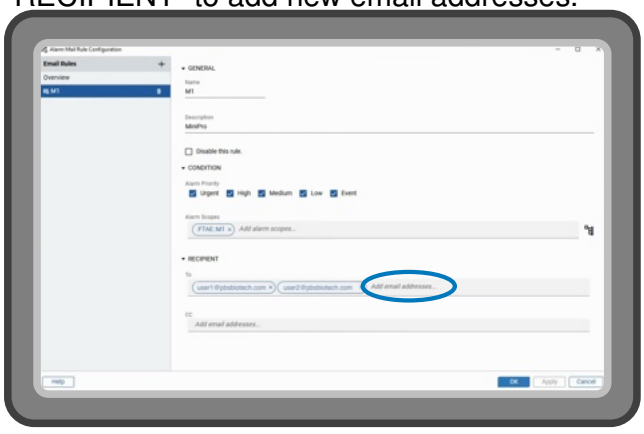
Configuring Email Function

The PBS-MiniPRO arrives with a PBS Biotech email address for sending emails. When an alarm is triggered, the email addresses on this list will receive an email.

1. Open the FactoryTalk View Studio application installed on the PBS-MiniPRO.
2. Right-click the topmost “MiniPro” item in the Explorer, and select “Alarm Email Notification...”



3. Select the “M1” Email Rule, and click “Add email addresses...” under “RECIPIENT” to add new email addresses.



4. Click “OK” when finished.

Configuring Automatic Backups

SQL Server Express is the database that FactoryTalk uses. Backups can be managed through SQL Server Management Studio, SSMS, included with the

installed software. The SQL server can also point to a remote database server, if desired. Refer to the documentation for that software for more information:
https://learn.microsoft.com/en-us/sql/sql-server/?view=sql-server-ver16

Congratulations! You have now set up your PBS-MiniPRO and configured user accounts, logger settings, and alarms. Please see Chapter 6 for more details to begin using the PBS-MiniPRO.

Using the PBS-MiniPRO

6

Before You Begin

This chapter will explain how to perform all the steps associated with a typical run, as well as tasks that a user may want to perform at any time from start to finish. Reading the preceding chapters is highly recommended before continuing.

Suggested Order of Operations

Set Up Run

1. Confirm gas source pressure matches specifications (see “Utility Requirements” on page 48)
2. Enter calibration information for vessel(s)
3. Install vessel(s) in PBS-MiniPRO Base Module(s)
4. Calibrate pumps
5. Prime addition line
6. Level adjustment to 0 mL
7. Add medium
8. Level adjustment to filled volume (if necessary)
9. Set the Bioreactor’s Alarms Phase to ‘Ramp’ mode
10. Control temperature, agitation, and main gas as for process. Control DO and pH in Manual mode.
11. Wait for equilibration
12. ‘High-point’ DO calibration
13. ‘One-point’ pH calibration
14. Control DO and pH in Auto mode
15. Take the Bioreactor’s Alarms Phase out of ‘Ramp’ mode
16. Add cells
17. Start batch

During Run

1. Take Sample
2. Perform Medium feed/exchange (if applicable)

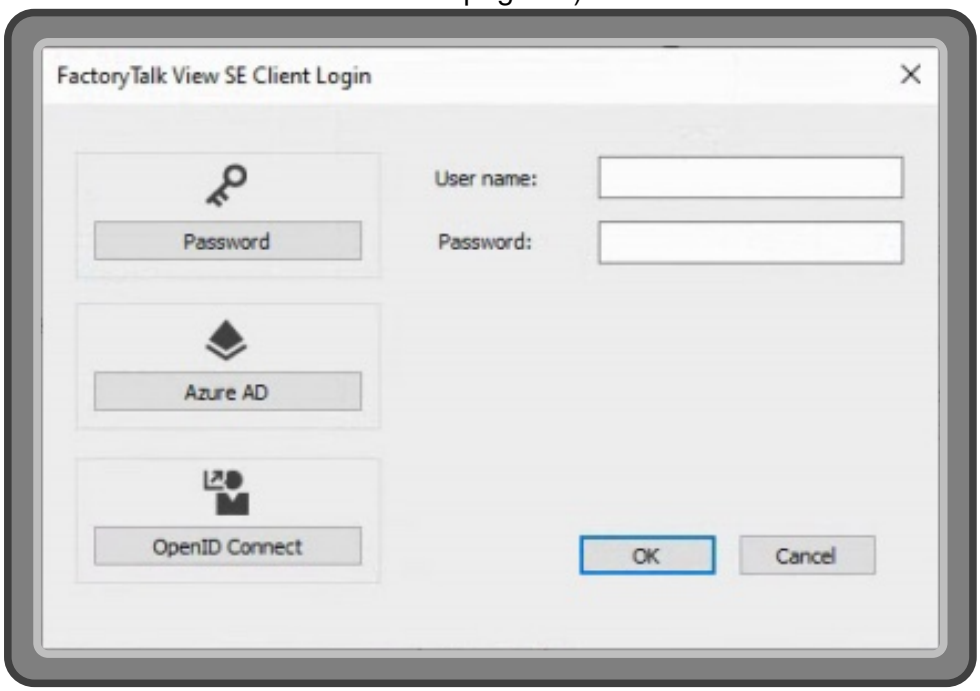
End Run

1. Set the Bioreactor's Alarms Phase to 'Standby' mode
2. Harvest
3. End batch
4. Clean/decontaminate the PBS-MiniPRO

Before Starting a Batch Run

Log In to the Display Client

1. Click the "Login" button (it has an icon of a key; see "PBS-MiniPRO Software - Header and Footer" on page 24).



2. Make the appropriate selections and enter your credentials.
3. Click "OK."

Enter Vessel Calibration Information

Each vessel comes with a single-use DO sensor spot and a single-use pH sensor spot already installed. These sensor spots have unique calibration parameters, which must be entered into the software for the pH and DO readings to be effective.

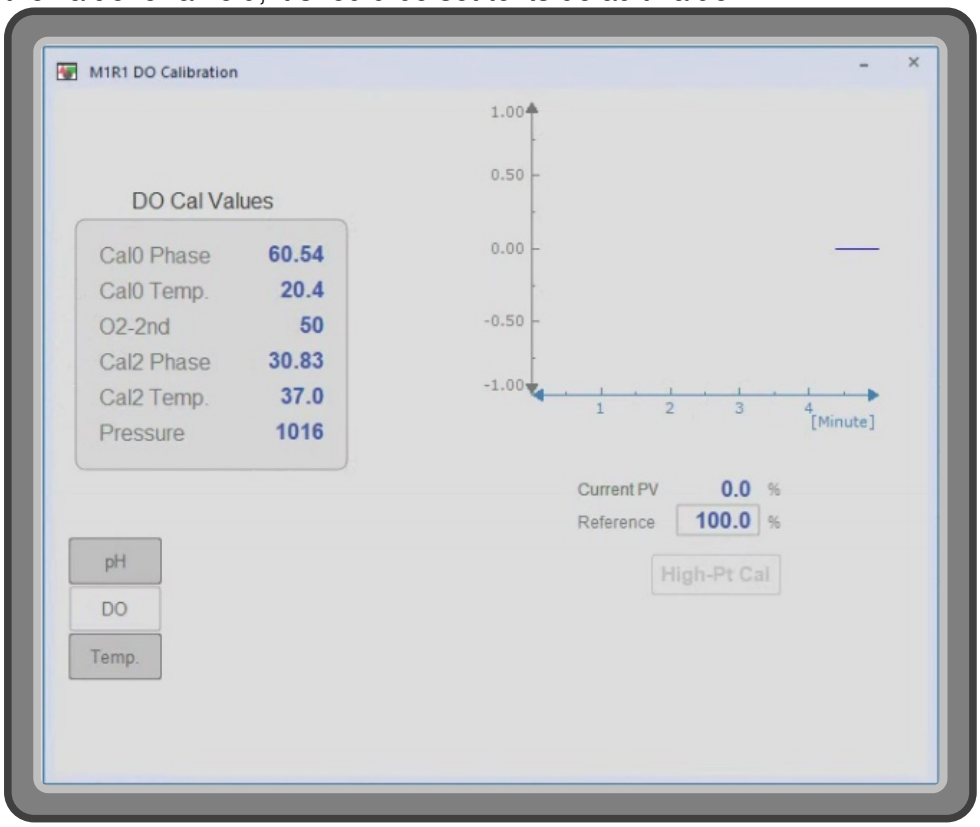
1. Retrieve the vessel-specific calibration information for the vessel you intend to install. This information is part of the Certificate of Conformance (CoC), which is included with each shipment of vessels.

2. In the software, navigate to the Bioreactor Overview menu for the Base Module you will be installing the vessel, and select Calibration (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).

   While it is possible to use the Bulk Calibration menu, accessed from the Base Module Overview (see “PBS-MiniPRO Software - Base Module Overview” on page 30) instead, this should be done with caution. If some vessel calibration values have mistakenly been applied to all vessels for a particular Base Module, the only way to undo it is to manually replace the parameters for the specific vessels that they do not apply to.

   Similarly, while it is possible to use the “Default Presens Values” recipe (see “Running/downloading recipes with an associated button” on page 117) to set the default calibration values before entering the vessel-specific values, it must be noted that running it would set *all* calibration values for *all* Bioreactors on the Base Module, including those with a loaded vessel in the middle of a cell culture run.

3. Click “DO” if the DO menu is not already selected. Enter the information in the menu. All the fields must be populated. If the CoC does not specify the value for a field, it should be set to its default value.

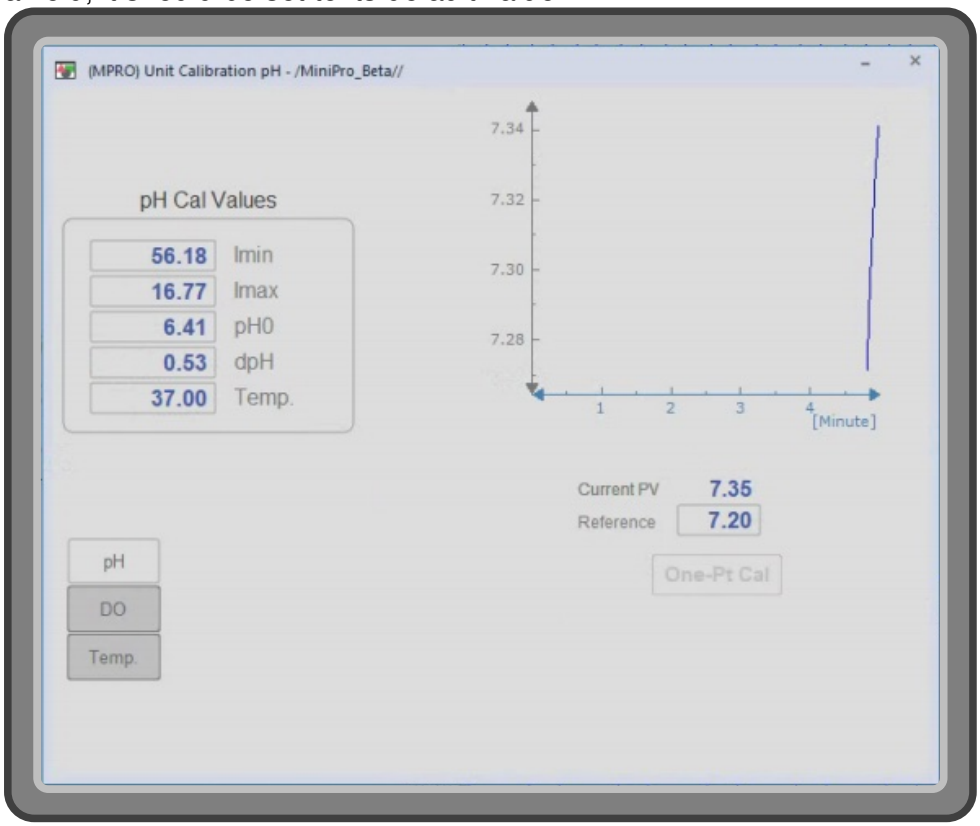


Default DO Cal Values	
Cal0 Phase	61.00
Cal0 Temp.	20.0
O2-2nd	100
Cal2 Phase	28.00
Cal2 Temp.	20.0
Pressure	1013

Default DO Sensor Constants*	
f1	0.812
m	30.26
dKSV1	3.76E-4
dKSV2	0.00E0
dPhi1	-0.07310
dPhi2	-0.00032

* Only applicable to the Bulk Calibration menu

4. Click “pH” to navigate to the pH menu. Enter the information in the menu. All the fields must be populated. If the CoC does not specify the value for a field, it should be set to its default value.



Default pH Cal Values	
Imin	60.00
Imax	20.00
pH0	7.00
dpH	0.50
Temp.	37.00

Default pH Sensor Constants*	
D_pH_Min	0.055260
D_pH_Max	0.044020
D_pH_x0	0.017440
D_pH_dx	0.002580

* Only applicable to the Bulk Calibration menu

5. Close the calibration menu.

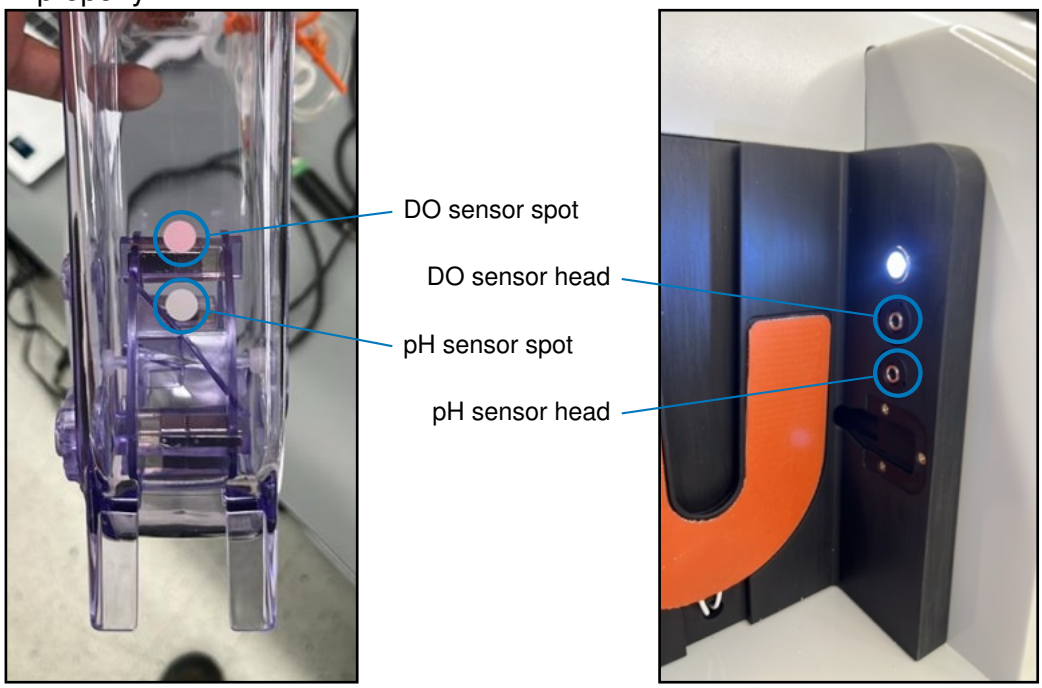
Install Vessel in PBS-MiniPRO

Note: These instructions are for the standard PBS-MiniPRO Bioreactor vessel configuration. If your vessel is different, please consult its installation protocol.

1. Connect any optional extensions in a biosafety cabinet.
   Note: Depending on which tubing sets needed to be unbundled for this

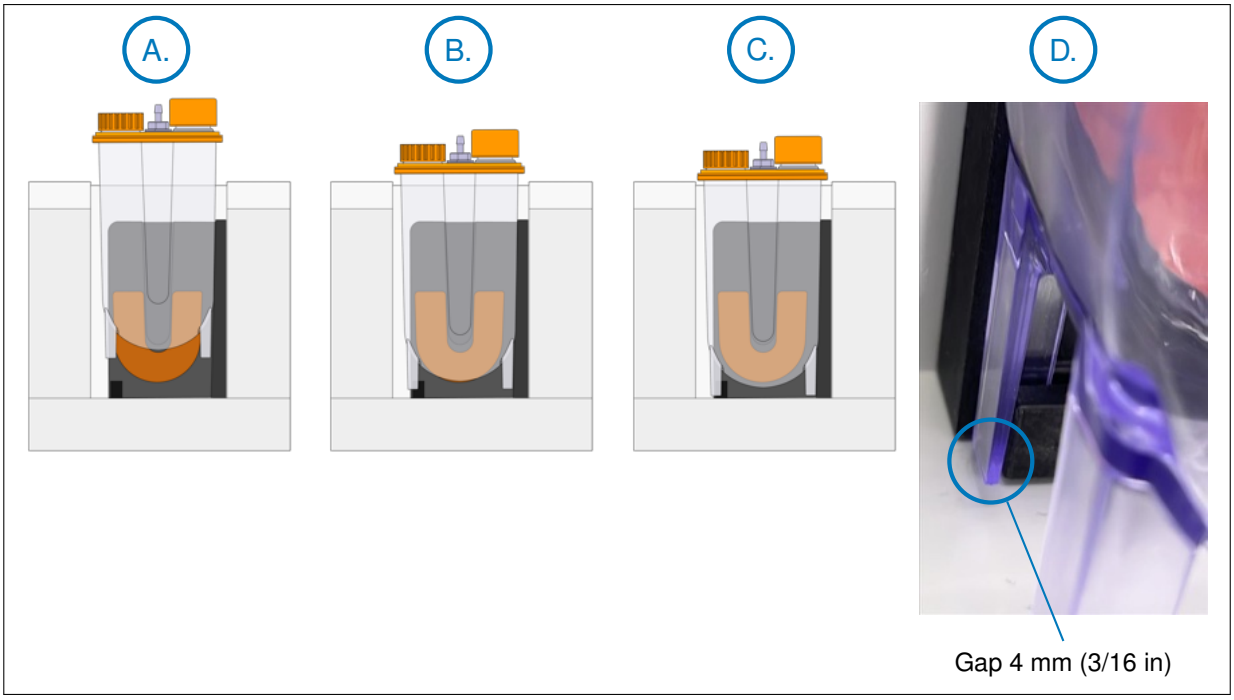
step, users may prefer to attach the Tubing guide to the vessel now, rather than in step 10 below.

2. Remove the Vessel Cover from the vessel sleeve.
3. Check that nothing is in the sleeve.
4. Note where the DO sensor spot and the pH sensor spot are on the vessel, and where the DO sensor head and the pH sensor head are in the vessel sleeve, as these will have to align for the vessel to be installed properly.

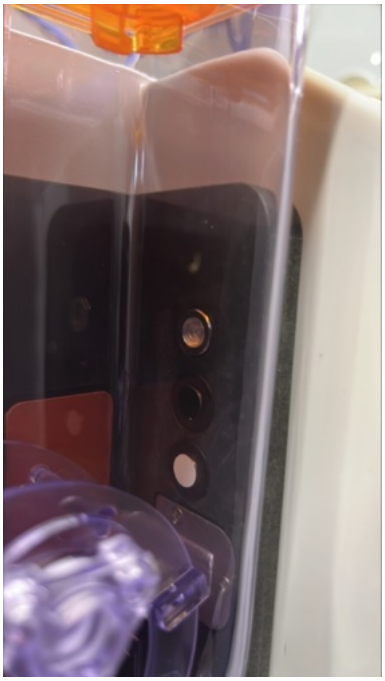


5. Hold the vessel so the front (i.e. the side with tubing coming out of it) faces towards you.

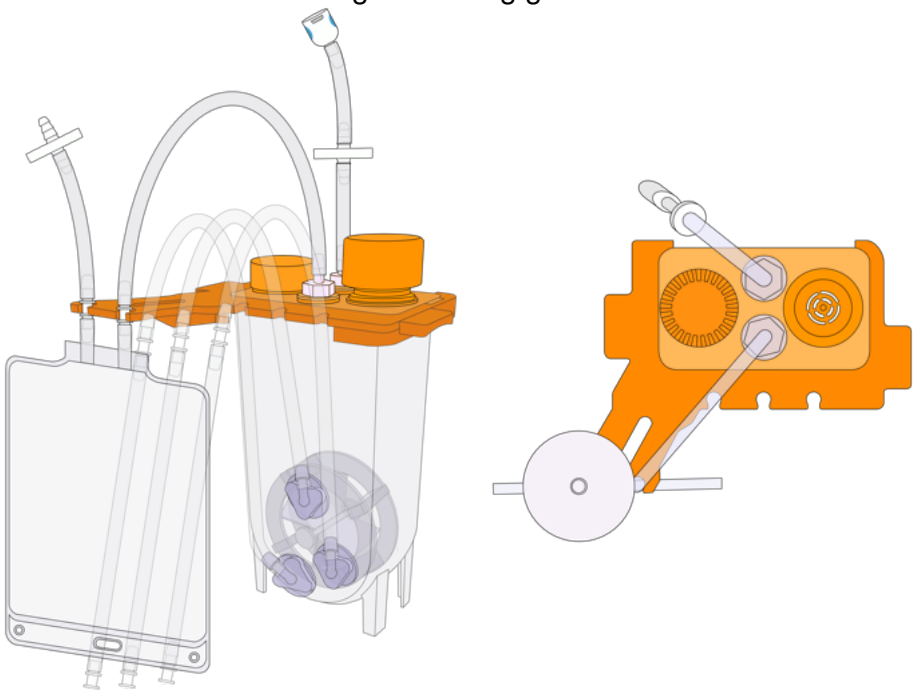
6. Match the dovetail feature on the back of the vessel with the dovetail groove in the sleeve (Diagram A.), and fully slide the vessel down the groove. The vessel will seem to stop sliding in at one point (Diagram B.) but it will be too early; keep sliding the vessel down until it is fully seated in the dovetail groove (Diagram C.), and the gap between the vessel feet and the bottom of the sleeve is no more than 4 mm (3/16 in) (Diagram D.). It is the vessel's back left foot that will slide into the notch.



7. Confirm the DO sensor spot and the pH sensor spot on the vessel are aligned with the DO sensor head and the pH sensor head in the vessel sleeve, respectively.



8. Unbundle the tubing sets.
9. Leave the tubing lines on top of the Base Module so they do not get in the way during installation.
10. Attach the Tubing guide to the vessel, and install the Condenser bag in the arm and the Liquid Handling lines in the notches, as depicted. Also refer to Document VV-04288 “PBS-MiniPRO Single-Use Vessel Insert” for more details on installing the Tubing guide.



11. Position the clamps on the Liquid Handling lines as close to their respective ports as possible to minimize dead volume between the vessel and the clamps. The clamps must be oriented with the opening to the left or right, as in either of the below images, or else they will not fit beneath the Vessel Cover. Close the clamps.


12. Attach the Vessel Cover to the vessel sleeve; it needs to slide in from the front until its tab on the upper left (Diagram A) catches in the corresponding notch on the sleeve (Diagram B), and the magnets are all engaged and holding the Vessel Cover in place (Diagram C). Attaching the Vessel Cover with a vessel already installed is depicted in Diagrams D-F.
    Keep the Vessel Cover installed while the temperature control is in operation; the controller requires the insulation provided by the Vessel Cover to function correctly.

13. Connect the Main-gas line to the Main-gas connector on the PBS-MiniPRO.

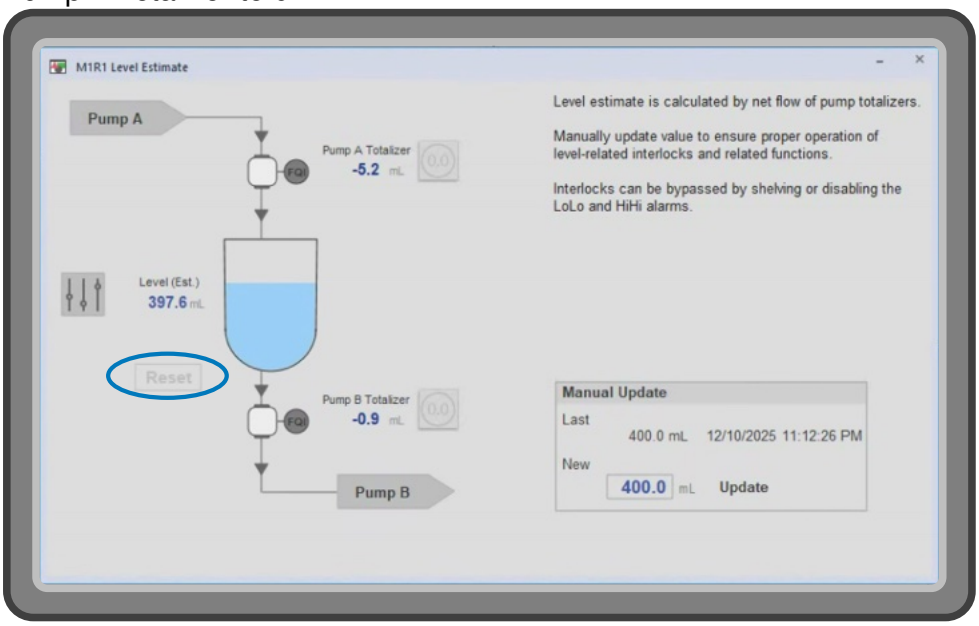
14. Route the Liquid Handling lines where they will not be in the way, either on the benchtop or the Base Module.

Level Adjustment to 0 mL

Because the Estimated Level is calculated based on the flow of the pumps (see “Estimated Level” on page 149), any pump used for addition should be primed before adjusting the Estimated Level to 0 mL (see “Using the Pumps” on page 73).

1. Navigate to the Bioreactor Overview menu for the specific Bioreactor (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).
2. Click the Level Estimate Indicator Button.

3. Click the “Reset” button. This will reset both the Pump A Totalizer and the Pump B Totalizer to 0 mL.



4. Close the Level Estimate menu.

Starting a Run

Using the Pumps

This section includes instructions for actually using the pumps. For more information about how they work, see “Pumps” on page 151.

The tubing lines on the standard vessel have a silicone section, close to the vessel, and a C-Flex® section, at the end. The C-Flex® is weldable, but not pumpable, and attempting to pump it can compromise the sterility of the vessel. Only pump the silicone tubing.

NOTICE Depending on the model of vessel being used, some of the tubing lines may not be compatible with the pumps installed on the PBS-MiniPRO and will require the use of an external pump.

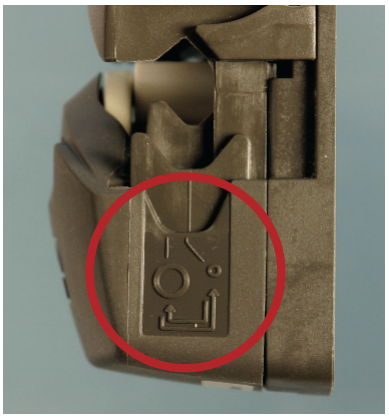
Tube holder positioning

The pumps must be adjusted for the size of tubing being used. If the outer diameter of the tubing is 1/4 in. or smaller, the “inner” position should be used. For tubing with outer diameter of 5/16 in., the “outer” position should be used.

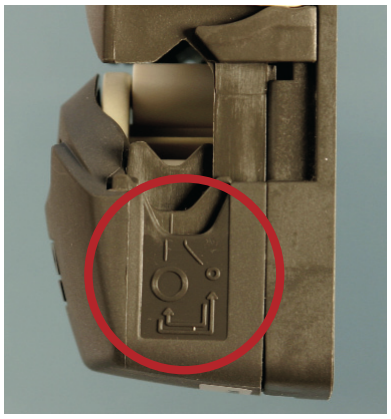
The pumphead can be adjusted to accommodate 1.6mm wall tubing in sizes from 0.5mm bore to 4.8mm bore.

Tube holder position

Tube bore size	0.5mm	0.8mm	1.6mm	2.4mm	3.2mm	4.0mm	4.8mm
Inner	✓	✓	✓	✓	✓	✗	✗
Outer	✗	✗	✗	✓	✓	✓	✓



Inner position, for small tubing



Outer position, for large tubing

With the smaller bore tubes of 0.5mm, 0.8mm and 1.6mm the inner position must be used to prevent the risk of tube slipping through the clamps and wandering across the rollers causing premature tube rupture.

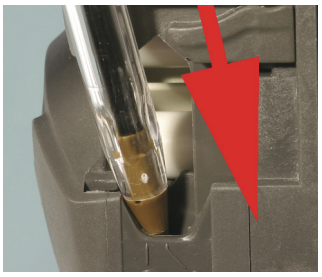
With the larger bore tubes of 4.0mm and 4.8mm the outer position must be used to prevent the flow rate being excessively reduced.

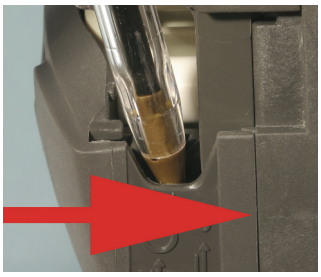
For tubing bores of 2.4mm and 3.2mm either setting may be used, as appropriate for the application. The inner setting will clamp the tube harder, reducing tube slip but has the potential to marginally reduce flow rate. The outer setting will optimise flow rate but the risk of tube slip is increased.

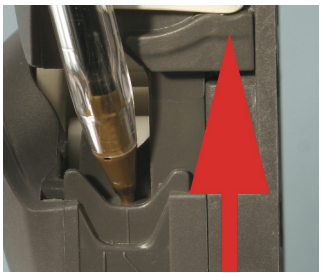
To change from the large tube to the small tube setting

Switch off the pump before changing the tube holder position. Use a pointed device such as a ball-point pen to reposition the lower tube holders **on both sides** of the pumphead.

- Lift the flip top until fully open.
- Place the pointed device pointing down into the small depression pictured here.







- Press down and slightly away from the front of the pumphead, as shown in the first picture above.
- Maintain the angled downward pressure and push away from the front of the pumphead. The jaw clicks into a new position.
- Release the pressure. The jaw rises into its correct alignment. If it does not rise, repeat the procedure, being sure to maintain downward pressure until release.
- Adjust the tube holder on the other side of the pumphead in the same way.

To change from the small tube to the large tube setting

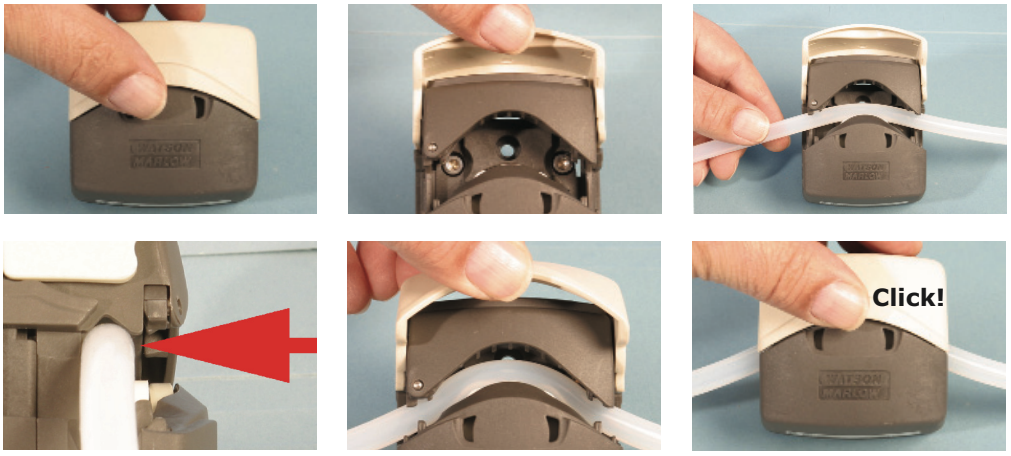
Carry out the procedure described above, but pushing towards the front of the pumphead.

Note: The pictures on the previous page show the tube holders' correct positions for small and large tubing. If a tube holder is not vertical relative to the body of the pumphead, it is wrongly positioned. Follow the instructions above to reposition it.

Tube loading

Switch off the pump before tube loading.

Check that the tube holders on both sides of the pumphead are correctly set for the size of tube you are using.



- Lift the flip top until fully open.
- Select enough tube length for the curve of the pump track. Place the tube between the rotor rollers and the track, pressed against the pumphead inner wall. The tube must not be twisted or stretched against the rollers.
- Lower the flip top until it clicks into its fully closed position. The track closes automatically and the tube is stretched correctly as it does so.

Using gravity

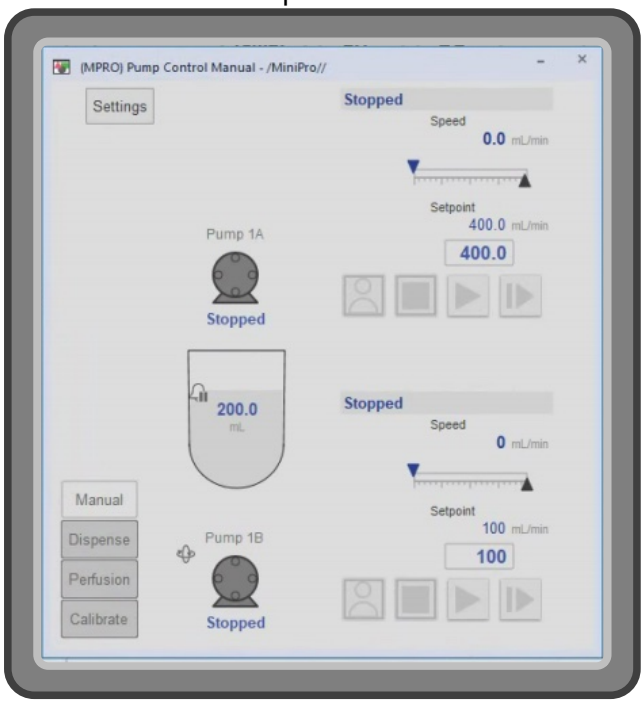
During bulk liquid transfer operations, it is possible to avoid unnecessary wear on the silicone tubing and exposing cells to unnecessary shear stress by using the pumps only to prime the tubing, and then using gravity for the rest of the liquid transfer operation.

Note: This will not update the Estimated Level, as that is calculated based on the flow of the pumps (see “Estimated Level” on page 149). The Estimated Level will have to be adjusted after this (see “Level Adjustment to Filled Volume” on page 86).

Accessing the Pumps menu

1. Log in to the Display Client.
2. Navigate to the Bioreactor Overview menu for the desired bioreactor (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).

3. Click one of the Pumps Indicator Buttons.



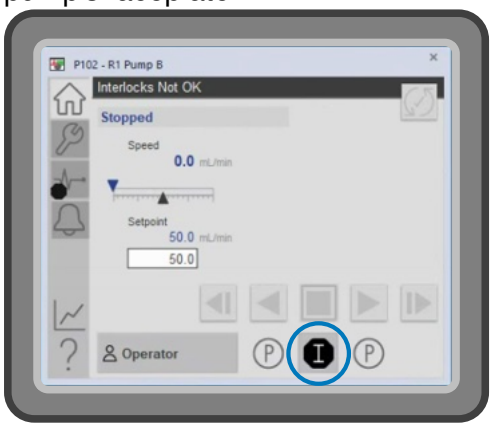
Using an interlocked pump

When a pump is interlocked, its indicator button will show a black octagon. For more information on the icons the Display Client uses, see “PBS-MiniPRO Software - Icons” on page 26.

Several conditions can result in a pump being interlocked. For a table of all interlock conditions, see “Interlocks” on page 133. For more specific information on the pump interlocks, see “Interlocks” on page 151.

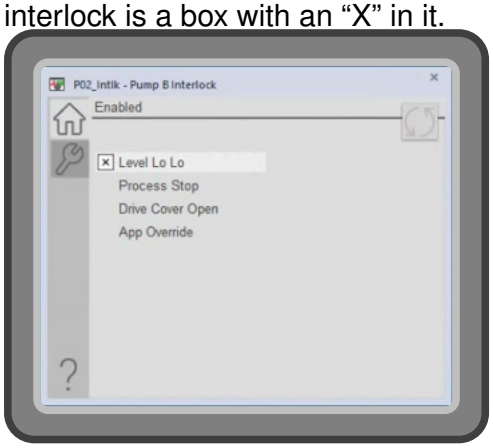
To determine the cause of a pump's interlock, check its interlock faceplate:

1. From the Pumps menu (see "Accessing the Pumps menu" on page 76), click the indicator button for the interlocked pump. This will open the pump's faceplate:



   Click the black octagon with the "I" in the center.

2. This is the pump's Interlock faceplate. Next to the condition causing the interlock is a box with an "X" in it.



   In this case, the pump is interlocked because the Estimated Level is less than the Level Lo Lo value.

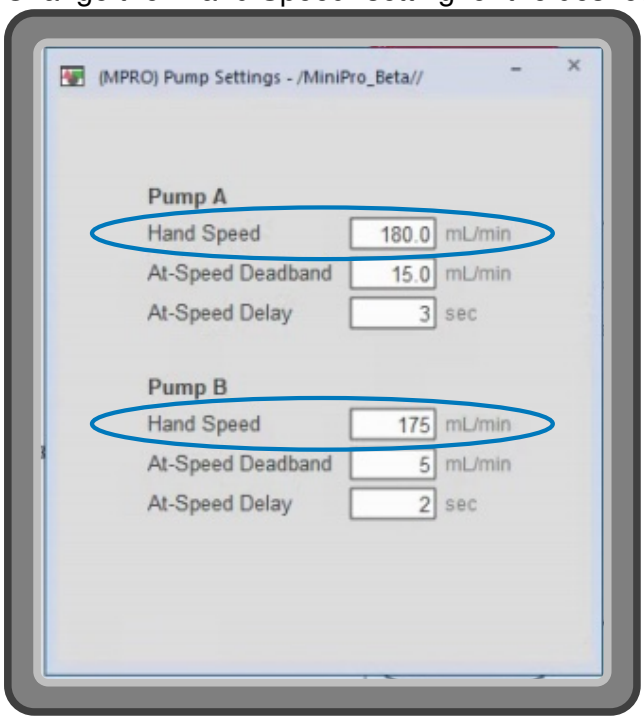
There are several ways to use Pump B if the Estimated Level is less than the Level Lo Lo value, but the simplest way is to temporarily raise the Estimated Level, perform the necessary operation (such as priming the pump or calibrating it), and then set the Estimated Level to the correct value (see "Level Adjustment to Filled Volume" on page 86). For other options, see "Interlocks" on page 151.

Using the Pump Switches

The pumps can be controlled via the Pump Switches on the Base Module (see "PBS-MiniPRO Base Module - Front Overview" on page 16). Holding the

switch to the left will run the corresponding pump counter clockwise, and holding the switch to the right will run the corresponding pump clockwise. The pump will flow at the “Hand Speed” set in the software. To set it:

1. Navigate to the Pumps menu for the desired bioreactor (see “Accessing the Pumps menu” on page 76).
2. Confirm the pump is not interlocked (see “Using an interlocked pump” on page 77).
3. Click the “Settings” button.
4. Change the “Hand Speed” setting for the desired pump.



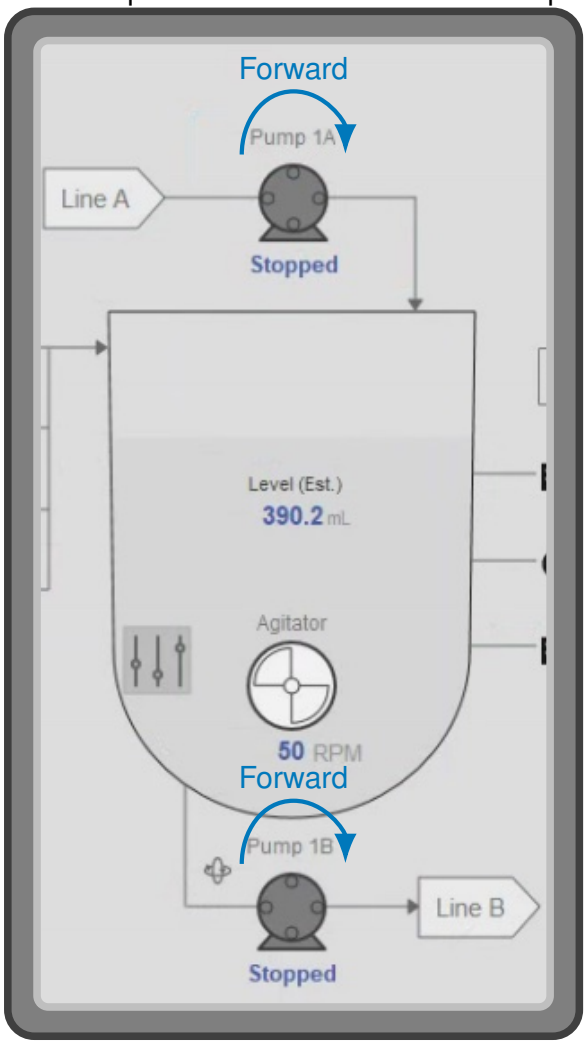
5. Close the menu.

Priming a Pump

The software cannot tell whether a pump is flowing air or liquid when it is turned on, and so for operations that depend on flowing a precise amount of liquid, the pump needs to be primed so the next time it turns on, it is flowing liquid and not air.

Note: The software's Estimated Level changes whenever either pump turns on, including during priming operations. It may be necessary to adjust the Estimated Level after priming a pump (see "Level Adjustment to Filled Volume" on page 86).

1. Install a section of silicone tubing as appropriate in the desired pump. Clockwise is considered 'Forward' by the software for both pumps, so the source liquid should be on the left of the pump.



2. Open any clamps on the tubing.
3. Confirm the pump is not interlocked (see "Using an interlocked pump" on

page 77).

4. Use the Pump Switches (see “Using the Pump Switches” on page 78) to flow liquid through the tubing from the source through the pump and to just before the destination. Try to stop the pump so the liquid does not go into the destination, but it will immediately go into the destination the next time the pump turns on.

   Note: The pump acts as a clamp, so the remainder of the tubing does not need to be clamped while the tubing is installed in a pump.

5. Adjust the Estimated Level, if necessary (see “Level Adjustment to Filled Volume” on page 86).

Note: For operations that involve removing liquid from a vessel using one of its Liquid Handling lines, priming is not necessary. This is because when the pump starts flowing away from the vessel, it will remove the volume of liquid from the vessel as it displaces the air in the tubing with liquid.

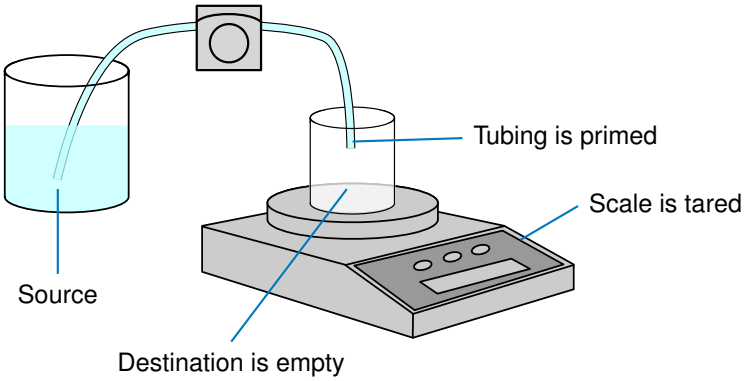
Calibrating the Pumps

Different tubing diameters and wall thicknesses will result in different volumes delivered per pump revolution. For information about the tubing sizes the pumpheads can accommodate and the tube holder position needed for different sizes, see “Tube holder positioning” on page 74. Additionally, tubing shows wear with use, and that will also affect the accuracy of the pump flow rate. It is recommended to calibrate the pumps in software before each cell culture run It may also be necessary to calibrate the pumps during a cell culture run if the operators notice large discrepancies in actual volume flowed versus what the software indicates, whereas small discrepancies can be handled by adjusting the Estimated Level (see “Level Adjustment to Filled Volume” on page 86)..

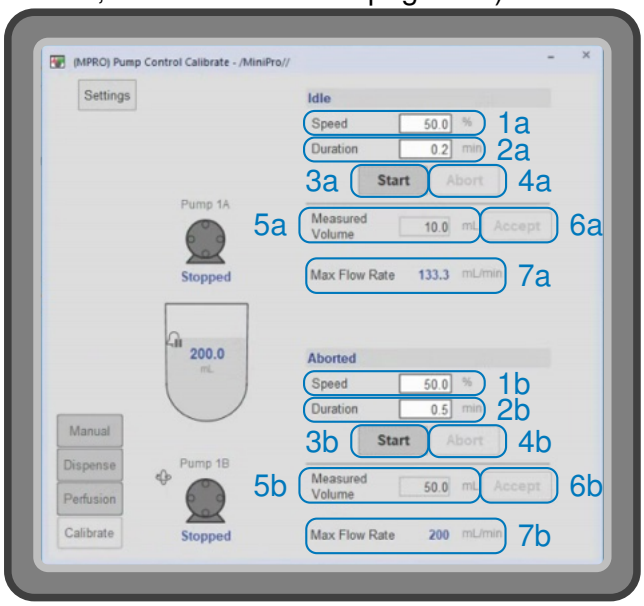
1. Prepare a source of liquid with silicone tubing and a way of measuring the volume of liquid flowed by the pump. Typically, this is a nonsterile section of tubing matching that on the vessel intended to be used for this cell culture run with the ‘source’ and ‘destination’ ends in 2 different open containers. To measure the total volume delivered, a graduated cylinder could be used as either the source or destination, or the liquid source or destination could be weighed with a scale.
2. During calibration, the pump will flow clockwise/forward. Install the tubing so the source of the liquid will enter the pump on the left side.
3. Confirm the pump is not interlocked (see “Using an interlocked pump” on page 77).

4. Prime the tubing line using the Pump Switches so it is completely filled with liquid (see “Priming a Pump” on page 80).
5. Measure the starting volume of either the source or destination.

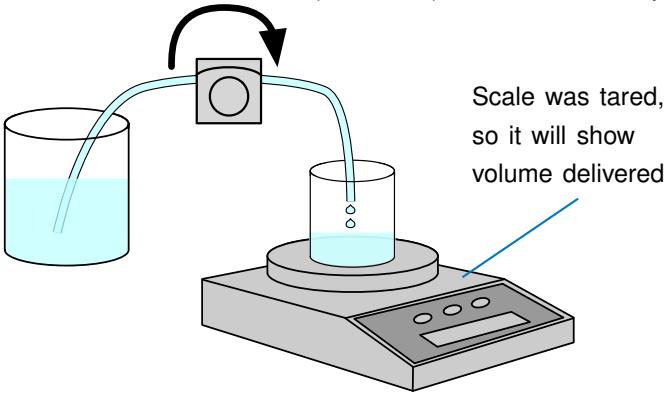
An example setup:



6. Navigate to the Pumps menu for the desired bioreactor (see “Accessing the Pumps menu” on page 76).
7. Click the “Calibrate” button.
8. The menu has controls for Pump A at the top, and Pump B at the bottom. Populate the ‘Speed’ (1a or 1b) and ‘Duration’ (2a or 2b) fields for the desired pump. It is recommended to calibrate using flow speeds and durations close to what will be used during the process. If this is unknown, calibrating to a speed of 50% for 0.2 minutes will suffice. If setting up for a perfusion process, it is recommended to calibrate to a speed of 50% for 1 minute (for instructions on using the Perfusion feature, see “Perfusion” on page 110).



9. Click the “Start” button (3a or 3b) for the desired pump.



10. After the pump has finished dispensing the liquid, measure the final volume of either the source or destination (depending on which was measured earlier), and calculate the total volume flowed.
11. Enter the volume flowed in the ‘Measured Volume’ field (5a or 5b), and click the “Accept” button (6a or 6b). The ‘Max Flow Rate’ indicator (7a or 7b) will update.
12. Repeat for the second pump.
13. Close the menu.

Note: The software’s Estimated Level changes whenever either pump turns on, including during calibration operations. It may be necessary to adjust the Estimated Level after calibrating a pump (see “Level Adjustment to Filled Volume” on page 86).

Adding Medium or Other Fluids

Note: It may be necessary to adjust the Estimated Level after adding liquid, depending on whether the tubing was properly primed before (see “Level Adjustment to Filled Volume” on page 86).

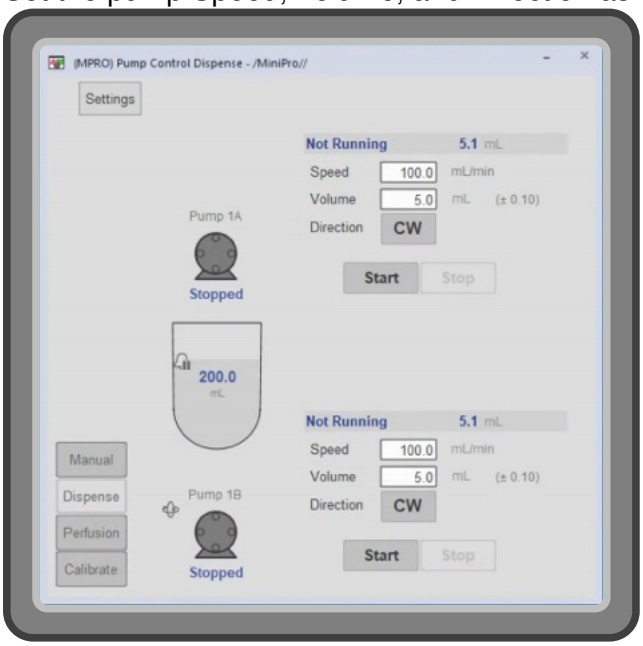
Setup

1. Navigate to the Pumps menu (see “Accessing the Pumps menu” on page 76).
2. If the desired pump is on, click the button to turn it off. If the pump is being used for Perfusion, you will have to navigate to the Perfusion menu and stop it.
3. Form a sterile connection between the desired liquid handling line and the medium bottle/bag source, by welding the tubing or using the connectors.

4. Install the silicone section of the tubing in the desired pump so its configuration is consistent with how the Estimated Level totalizer works. If using Pump A, the pump should run clockwise to add liquid to the vessel and to increase the Estimated Level, and if using Pump B, the pump should run counter clockwise to add liquid to the vessel and to increase the Estimated Level (see “Using the Pumps” on page 73 and “Estimated Level” on page 149).
5. Check that the addition line tubing clamp(s) is/are open. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while adding liquids to open the clamp and then close it after liquid addition.

Using the Dispense Menu

1. Complete the setup steps (see “Setup” on page 83).
2. Prime the tubing line using the Pump Switches (see “Priming a Pump” on page 80).
3. Click “Dispense” to go to the Dispense menu.
4. Set the pump Speed, Volume, and Direction as appropriate.



5. Click the “Start” button for the desired pump.
6. Wait for the pump to dispense the requested volume. It will stop on its own.
7. Clamp the Liquid Handling lines as close to their respective ports as possible to minimize dead volume between the vessel and the clamps. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for

instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.

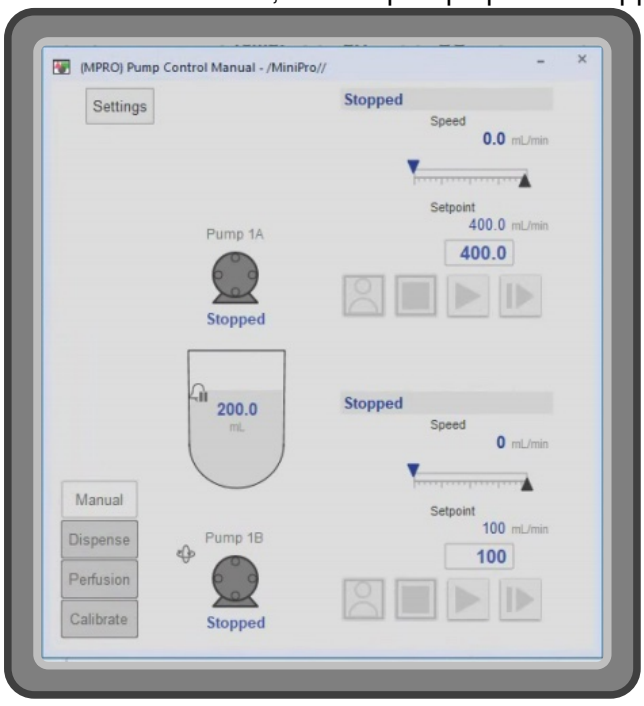
Using the Pump Switches

1. Complete the setup steps (see “Setup” on page 83).
2. Confirm the “Hand Speed” setting is appropriate (see “Using the Pump Switches” on page 78).
3. Hold the desired pump’s switch in the appropriate direction, until the desired amount of medium has been added.
4. Let go of the pump’s switch.
5. Clamp the Liquid Handling lines as close to their respective ports as possible to minimize dead volume between the vessel and the clamps. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.

Using the Manual Menu

This menu allows users to turn the pumps on clockwise at a user-set speed. Because this menu only supports running the pumps clockwise, users should only use it with Pump A when adding liquid to the vessel, and with Pump B when removing liquid from the vessel.

1. Complete the setup steps (see “Setup” on page 83).
2. In the Manual menu, set the pump speed as appropriate.



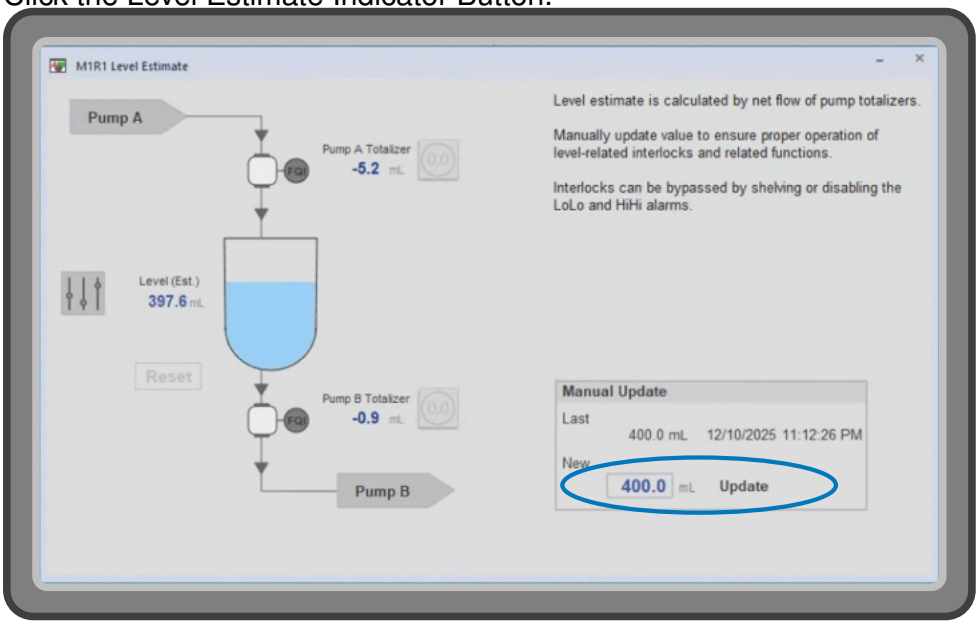
3. Click the “Start CW” button to turn the desired pump on.
4. Click the “Stop Motor” button to turn the desired pump off after adding desired amount of medium.
5. Clamp the Liquid Handling lines as close to their respective ports as possible to minimize dead volume between the vessel and the clamps. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.

Note: Alternatively, you could hold the “Jog CW” button until the desired amount of medium has been added, and then release the button when completed.

Level Adjustment to Filled Volume

Because the Estimated Level is calculated based on the flow of the pumps (see “Estimated Level” on page 149), this may have to be performed at various points during a cell culture run if the Estimated Level deviates from the actual level in the vessel.

1. Navigate to the Bioreactor Overview menu for the specific bioreactor (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).
2. Click the Level Estimate Indicator Button.



3. Enter a new value under “Manual Update” and then click the “Update” button.
4. Close the Level Estimate menu.

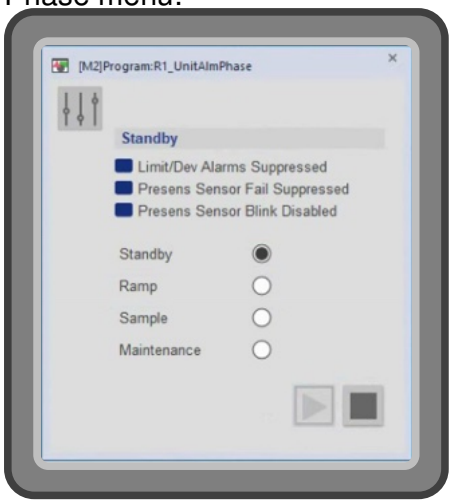
Set the Bioreactor's Alarms Phase to 'Ramp' Mode

After a vessel has been installed and medium has been added, it is important for the DO and pH sensor failure alarms to be un-suppressed, and for the DO and pH optical sensor blinking to be enabled.

Until this point, the Bioreactor's Alarms Phase should have been in 'Standby' mode. While in 'Standby' mode, the software ignores the DO, pH, and Temperature alarms which would be triggered when a cell culture run ends. This includes limit alarms caused by PVs being too low or too high, and failure alarms for the DO and pH sensors from not having a vessel installed. The software also stops the blinking for the DO and pH optical sensors. Because the failure alarms should not be ignored at this point in the process, and the DO and pH optical sensor blinking should be re-enabled, the operator should take the Bioreactor's Alarms Phase out of 'Standby' mode, and put it in 'Ramp' mode, at this time.

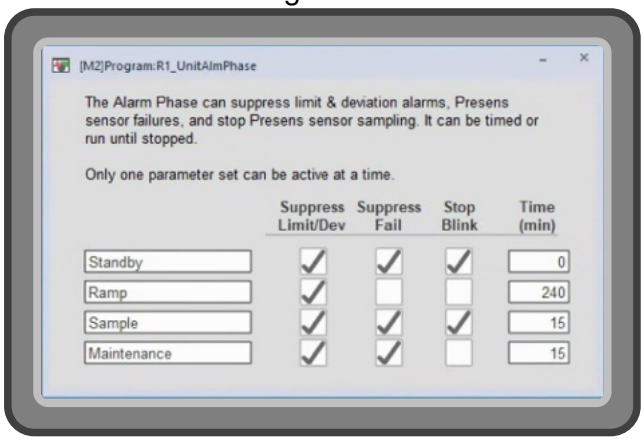
While a Bioreactor's Alarms Phase is in 'Ramp' mode, the software continues to ignore the DO, pH, and Temperature limit alarms which would be triggered while setting up for a run, from the PVs being too low or too high.

1. Navigate to the Bioreactor Overview menu for the specific Bioreactor, and click its Alarms Phase Indicator Button (see "PBS-MiniPRO Software - Bioreactor Overview" on page 32). This will bring you to its Alarms Phase menu:



2. Select the desired mode, in this case 'Ramp.' If the Alarms Phase was already in another mode, it will switch to the newly selected mode, and the "Start Phase' button will be grayed out. If the Alarms Phase was not set to any mode, then it will be necessary to click the "Start Phase' button.

3. Any of the modes can be configured so they stop after a specific amount of time has elapsed, or configured so they run until they are stopped. To configure this, click the “Display Advanced Properties” button in the top left corner to be brought to the Alarms Phase Settings menu:



4. Setting the ‘Time (min)’ to 0 means that the mode will run until it is stopped.
5. To stop a mode so none is running, click the “Stop Phase” button in the Alarms Phase menu. This will un-suppress all alarms and enable the DO and pH optical sensor blinking.

For more information about the Alarms Phase feature, see “Alarms Phase” on page 165.

Turning Controls On

After filling the vessel with medium, the controls need to be turned on, to condition the medium. This accomplishes 3 things: (1) it allows the DO and pH sensors to equilibrate, so ‘high-point’/‘one-point’ calibrations can be performed, (2) it brings the PVs to within the appropriate ranges for the cell process, and (3) it acts as a sterility hold, so operators have the opportunity to determine whether the medium has been contaminated before inoculating.

First, the agitation, temperature, and main gas controllers must be turned on. Then, the DO and pH controllers can be set to Manual mode, to achieve the desired process parameters you intend to use before inoculating. For an explanation why it is recommended to use DO and pH in Manual mode rather than Auto mode before the ‘high-point’/‘one-point’ calibrations are performed, see “Pre-Calibration Medium Conditioning Strategy” on page 154.

To control the DO in Manual mode, first remember that the DO is scaled so 100% equals atmospheric O_2 conditions. The amount of air entering the vessel and therefore saturating the medium can be displaced with either CO_2 or N_2.

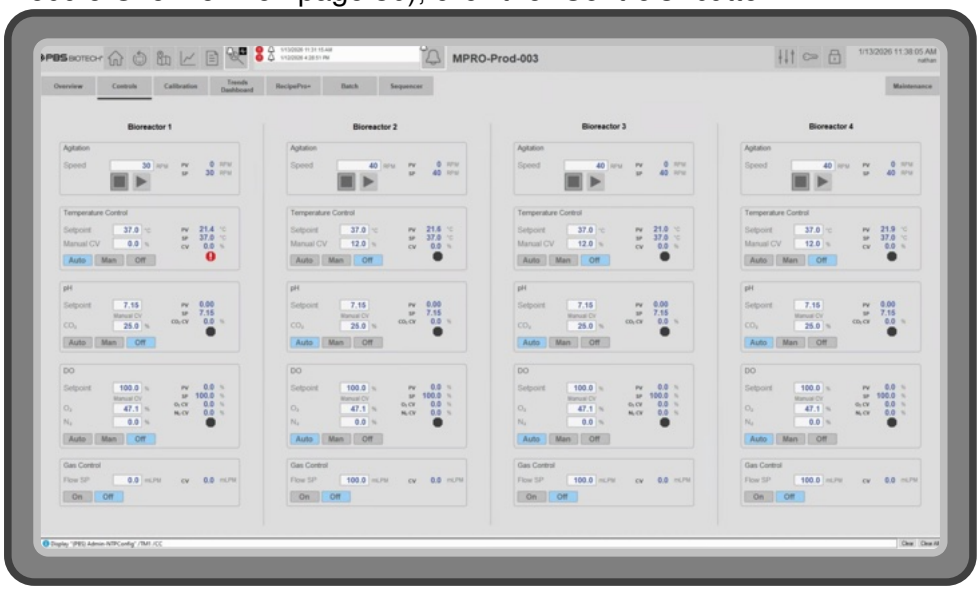
For example, to control to a DO PV of 50% with pH set to 5% CO_2, set N_2 to 45% and O_2 to 0%.

To control the pH in Manual mode, set CO_2% to the value that will provide the desired pH, using the “$NaHCO_3$, CO_2%, and pH at 37 °C” chart on page 146.

Using controls:

These instructions are for using controls from the Base Module Overview, because they are the simplest, but this can also be accomplished from the Bioreactor Overview Menu (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).

1. From the Base Module Overview (see “PBS-MiniPRO Software - Base Module Overview” on page 30), click the “Controls” button.



2. If Auto mode, change the contents of the ‘Setpoint’ field for the desired Bioreactor and controller.

Auto Mode Variables and Setpoint Units	
Agitation	N/A - only Manual mode available
Temperature	Degrees Celsius (°C)
Main Gas	N/A - only Manual mode available
Dissolved Oxygen	% Air Saturation
pH	pH units

Recommended Auto Mode Setpoints	
Agitation	N/A - only Manual mode available
Temperature	37 °C
Temperature Deadband	0 – 0.05 °C
Main Gas	N/A - only Manual mode available
Dissolved Oxygen Setpoint	25 – 100% Dissolved Oxygen
Dissolved Oxygen Deadband	0 – 2% Dissolved Oxygen
pH Setpoint	6.8 – 7.4 pH units
pH Deadband	0 – 0.05 pH units

Note: The deadbands for Temperature, Dissolved Oxygen, and pH can be changed in their associated PID faceplates, in one of the pages under Maintenance → Display Advanced Properties → Maintenance.

3. If Manual mode, change the contents of the 'Manual CV' field(s) for the desired Bioreactor and controller. For Agitation, this is the 'Speed' field, and for Gas Control this is the 'Flow SP' field.

   Note: It is recommended that Temperature is only used in Manual mode.

Manual Mode Variables and Controller Output Units	
Agitation	Vertical-Wheel® Impeller Revolutions Per Minute
Temperature	Main heater % duty
Main Gas	Total gas milliliters per minute
Dissolved Oxygen – N_2	Total gas % N_2 composition
Dissolved Oxygen – O_2	Total gas % O_2 composition
pH – CO_2	Total gas % CO_2 composition

A typical Agitation setpoint is 20 – 50 RPM.
Note: When switching from Manual mode to Auto mode, the controller output will gradually increase or decrease to transition from the user-selected output in Manual mode to the PID-calculated output.

4. Select the desired mode. The CV(s) should change to reflect the new

mode and/or setpoint.

‘High-Point’/‘One-Point’ Calibrations After Equilibration

After the medium has been conditioned and the temperature, DO, and pH have equilibrated, PBS Biotech recommends performing ‘high-point’/‘one-point’ calibrations on the DO and pH sensors. It is recommended to calibrate the DO sensor first, because calibrating the pH sensor requires taking a sample, and clearing a sample line with air can temporarily change the DO PV.

‘High-point’ DO calibration:

Note: The ‘high-point’ DO calibration is not named that because it is meant to be performed at the maximum value the sensor is capable of measuring. If the highest DO setpoint to be used for a cell culture run is 50%, then the ‘high-point’ DO calibration ideally would be performed at 50%.

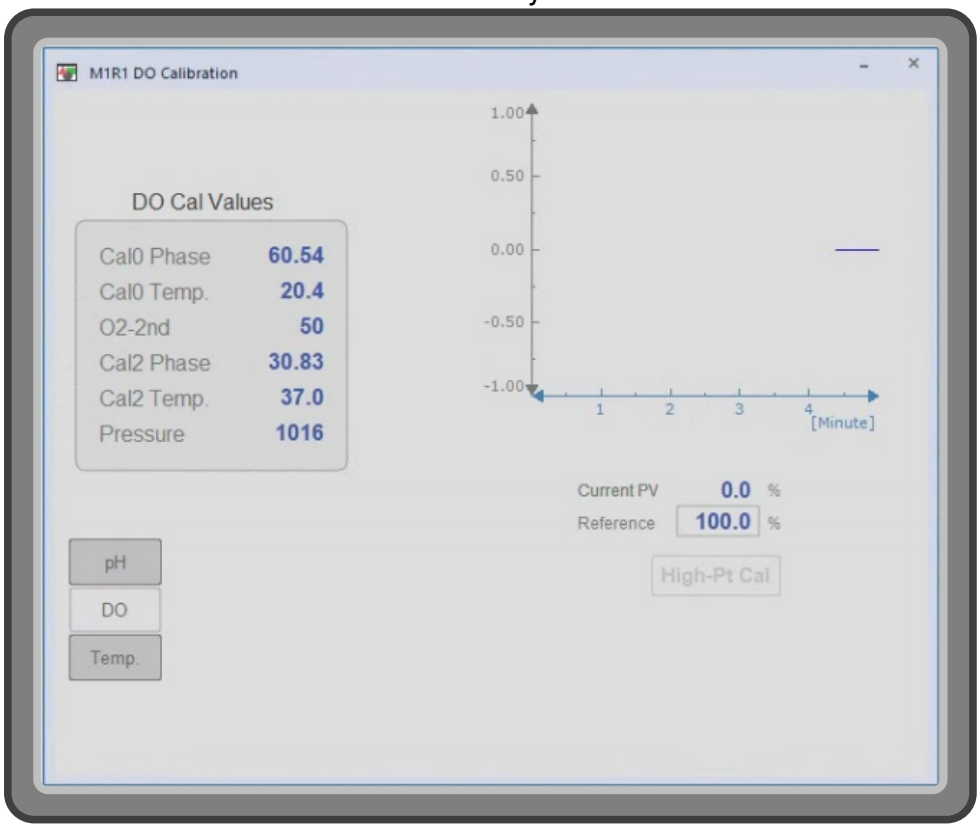
The following is recommended for DO calibrations:

- Only perform a ‘high-point’ DO calibration before inoculating with cells
- Perform the ‘high-point’ DO calibration using the headspace gas composition as the reference, rather than the measured DO of a sample
- Do not perform additional DO calibrations of any type during a cell culture run

For an explanation, see “Dissolved Oxygen” on page 156.

1. Confirm DO process value has stabilized.
   Note: If the medium is 100% air saturated, the DO PV should be between 80% and 120% before performing ‘high-point’ calibration.
2. In the software, navigate to the Bioreactor Overview menu for the specific vessel, and select Calibration (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).

3. Click “DO” if the DO menu is not already selected.



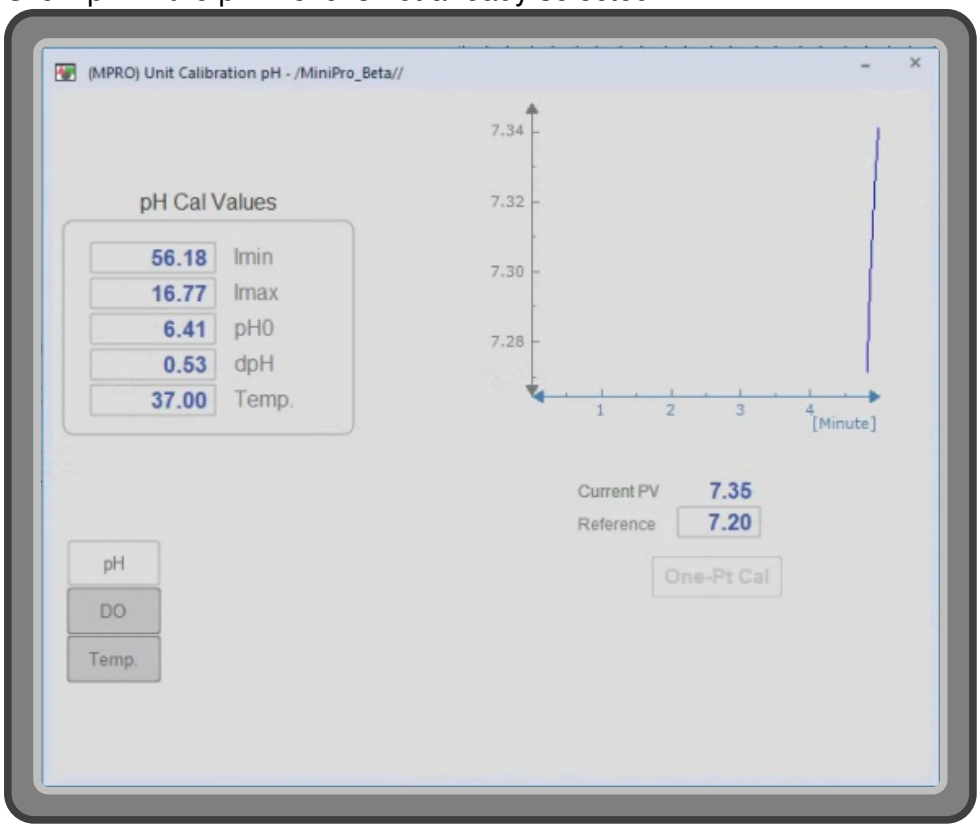
4. Enter the correct DO PV in the ‘Reference’ field. If the only gas flowing into the bioreactor was air, then the medium is 100% air saturated. Otherwise, before inoculating, the DO PV should equal 100 - CO_2% - N_2%.
5. Click the “High-Pt Cal” button.
6. Confirm the new calibration.
7. Close the menu when finished.
8. Set DO to Auto mode, if desired (see “Turning Controls On” on page 88).

‘One-point’ pH calibration:

It is recommended to do this before inoculating with cells, and regularly throughout a cell culture run to counteract the pH sensor drift.

1. Take a sample (see “Take Sample” on page 95, “Sampling” on page 157, and “Sampling for pH Measurement” on page 158). Note pH process value when taking sample.
2. Measure the pH of the sample (see “Sampling for pH Measurement” on page 158).

3. In the software, navigate to the Bioreactor Overview menu for the specific vessel, and select Calibration (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).
4. Click “pH” if the pH menu is not already selected.



5. Enter [(pH PV) – (pH PV when taking sample) + (actual pH of sample)] in the ‘Reference’ field.
6. Click the “One-Pt Cal” button.
7. Confirm the new calibration.
8. Close the menu when finished.
9. Set pH to Auto mode, if desired (see “Turning Controls On” on page 88).

Take the Bioreactor’s Alarms Phase out of ‘Ramp’ Mode

After sensors have been calibrated and the important variables are within the appropriate ranges for your cell line/process, it is important to un-suppress the limit and deviation alarms before inoculating.

The limit and deviation alarms are un-suppressed by taking the Bioreactor’s Alarms Phase out of ‘Ramp’ mode. When the Bioreactor’s Alarms Phase is in ‘Ramp’ mode, the software ignores the alarms which would be triggered while setting up for a run, such as the PVs being too low or too high before turning

on controls. Because these alarms should not be ignored during a run, the operator should take the Bioreactor's Alarms Phase out of 'Ramp' mode at this time.

1. Confirm the Limit and Deviation Alarms settings for your run. Note that if a setting is configured such that the PV is outside the appropriate range, an alarm will be generated immediately after taking the Bioreactor's Alarms Phase out of 'Ramp' mode. For more information, see "Settings" on page 131.
2. If more than the 'Time (min)' for 'Ramp' mode has elapsed, the Bioreactor's Alarm Phase will have already left 'Ramp' mode automatically. Otherwise, see "Set the Bioreactor's Alarms Phase to 'Ramp' Mode" on page 87 for instructions.
3. For how to view and acknowledge alarms, see "Alarms" on page 129.

Inoculate with Cells

After sensors have been calibrated and important variables are within the appropriate ranges for your cell line/process, it is safe to add the cells. Add cells by following the same instructions as for adding any other fluids (see "Adding Medium or Other Fluids" on page 83).

Entering Batch ID

To name a batch:

1. Navigate to the Bioreactor Overview Menu for the desired Bioreactor (see "PBS-MiniPRO Software - Bioreactor Overview" on page 32).

2. Click “Batch.”

3. If a batch is running, end it by clicking the “End” button and confirming.
4. Enter a Batch ID/name, and an optional Description if desired.
5. Click “Start.” The Record ID and Start Time fields will automatically populate. Running batch information will be visible in the Bioreactor Overview Menu.

Take Sample

For information about concerns when taking a sample, handling the sample, and measuring a sample, see “Sampling” on page 157.

The following sub-sections are not exhaustive, and there are likely many additional ways for operators to take a sample out of the bioreactor without compromising the sterility of the vessel.

Note: A sample of 5 mL or larger is recommended for cell counts.

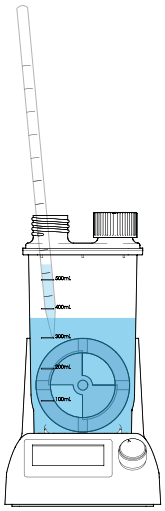
Sampling by pipette

This sampling method is considered “open system.” Sampling methods which are “closed system” use a sampling manifold (see “Sampling manifold example” on page 99).

Note: When a pump is not used to take a sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. For instructions on how to manually adjust the Estimated Level, see “Level Adjustment to Filled Volume” on page 86.

1. Install a Mini Base Unit in the biosafety cabinet for sampling.
   Note: This is not the PBS-MiniPRO Base Module, but a different PBS product.
   Note: Having a Mini Base Unit in the biosafety cabinet allows sampling to be performed under continuous agitation so an accurate sample can be taken. If a Mini Base Unit is not available to provide agitation during sampling, any resulting cell counts may not be representative of the culture condition.
2. Set the Bioreactor’s Alarm Phase in ‘Sample’ mode (see “Set the Bioreactor’s Alarms Phase to ‘Ramp’ Mode” on page 87), and turn off all controls and pumps for the vessel to be sampled.
3. Disconnect the Main-gas line from the Main-gas connector.
4. Clamp and remove tubing from any pumps on the Base Module.
5. Remove the Vessel Cover, and then the vessel.
6. Transfer the vessel to the biosafety cabinet and install it onto a Mini Base Unit.
7. Agitate at an appropriate speed to evenly suspend the vessel contents and ensure a representative sample is taken. It is generally recommended to use an RPM that is 10% faster than that used for the run condition on the PBS-MiniPRO.
8. Allow culture to mix for 10-20 seconds prior to sampling to ensure homogeneity.

9. Insert a serological pipette vertically from the left side port of the vessel until the tip is right above the side of the Vertical-Wheel® impeller (near the middle of the vessel).
   Note: The right side port cannot be used, as the inside is too narrow to accommodate most serological pipette sizes, and/or the perfusion cap is installed on that port.



10. Remove a sample and transfer to a sample container.
    Note: Do not attempt to distribute multiple samples from one serological pipette draw. Each individual sample should be fully independent. The same serological pipette can be used to take multiple samples if perfect aseptic technique is used. To reduce contamination risk, use a new serological pipette each time.
11. Reinstall the vessel (see “Install Vessel in PBS-MiniPRO” on page 66).
12. Turn on controls (see “Turning Controls On” on page 88).
13. Take the Bioreactor’s Alarm Phase out of ‘Sample’ mode (see “Set the Bioreactor’s Alarms Phase to ‘Ramp’ Mode” on page 87).
14. Because a pump was not used to take the sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. Measure the sample volume, and manually adjust the Estimated Level accordingly by following the instructions in “Level Adjustment to Filled Volume” on page 86.

Open sampling by syringe

This sampling method is considered “open system.” Sampling methods which are “closed system” use a sampling manifold (see “Sampling manifold example” on page 99).

Note: When a pump is not used to take a sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. For instructions on how to manually adjust the Estimated Level, see “Level Adjustment to Filled Volume” on page 86.

Note: Proximity to a BSC is required for this procedure.

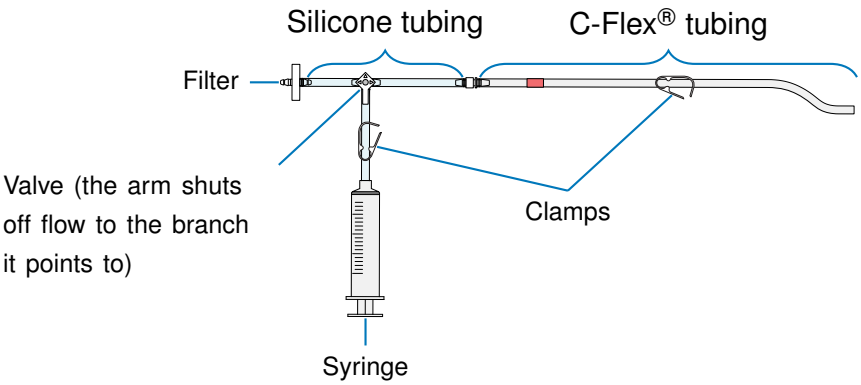
1. Position the end of one of the vessel’s tubing lines in the biosafety cabinet.
2. Open a 50 mL syringe or similar inside the biosafety cabinet.

3. Fill the syringe with 10 mL of sterile air.
4. Connect the syringe to the Luer fitting on the end of the vessel's tubing line.
5. Confirm the vessel's tubing line is not clamped. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while sampling to open the clamp and then close it after taking the sample.
6. Holding the syringe so its tip is down, pull a sample of the desired size from the vessel.
7. Arrange the syringe so the tip is pointing upwards, and push the air in the syringe back into the vessel, to clear the line.
8. When bubbles form in the vessel it means the tubing line is clear of liquid. Stop pushing air through the syringe at this point.
9. Clamp the tubing line as close to its port as possible to minimize the dead volume between the vessel and the clamp, remove the syringe, and either replace it with the Luer cap or with another sterile syringe. See step 11 of "Install Vessel in PBS-MiniPRO" on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.
10. If sampling to measure pH or DO, expel the head gas from the syringe, and cap it to make the sample more stable. Measure the pH or DO as soon as possible.
11. Because a pump was not used to take the sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. Measure the sample volume, and manually adjust the Estimated Level accordingly by following the instructions in "Level Adjustment to Filled Volume" on page 86.
12. If the Vessel Cover was previously removed to open and close any clamps, reinstall the Vessel Cover at this time.

Sampling manifold example

To take a sample while keeping the system closed, operators can sterilely weld a sampling manifold such as this one to any of the vessel's tubing lines with weldable tubing.

Sampling manifold assemblies similar to the one depicted here can be purchased from PBS, already sterilized and ready to be sterilely welded onto a vessel. Contact PBS Sales for more information.



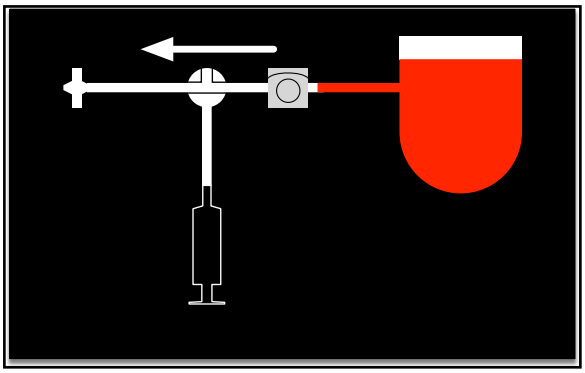
To take a sample with a sampling manifold and a pump:

Using a pump to take a sample can decrease the sampling variability between operators. It can also, however, expose the sample to more shear stress. For instructions on how to take a sample without using a pump, see "To take a sample with a sampling manifold, single-syringe pull, and gravity drain" on page 102 and "To take a sample with a sampling manifold and dual-syringe pull" on page 104.

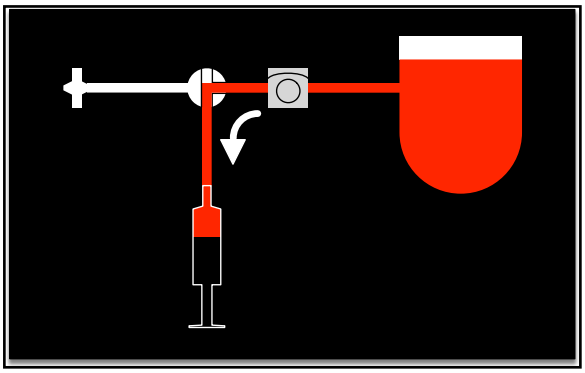
Note: These instructions are for using Pump A. If using Pump B instead, the flow directions (clockwise and counter clockwise) would need to be reversed, and the sampling manifold installed the opposite direction in Pump B.

1. Navigate to the Pumps menu (see "Accessing the Pumps menu" on page 76).
2. If Pump A is on, click the button to turn it off. If either pump is being used for Perfusion, you will have to navigate to the Perfusion menu and stop it/them.
3. Confirm the "Hand Speed" setting is appropriate (see "Using the Pump Switches" on page 78).
4. Unclamp all clamps on the sampling manifold. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while sampling to open the clamp and then close it after taking the sample.

5. Clear the sampling manifold of air, to minimize the amount of air that will end up in the syringe:
   (a) Install the silicone tubing between the valve and vessel in Pump A as in the image below.
   (b) Configure the valve to block flow to the syringe. This will push the air in the sampling manifold out of the filter.
   (c) Use the Pump A Switch to turn Pump A counter clockwise. Be prepared to execute the next step.

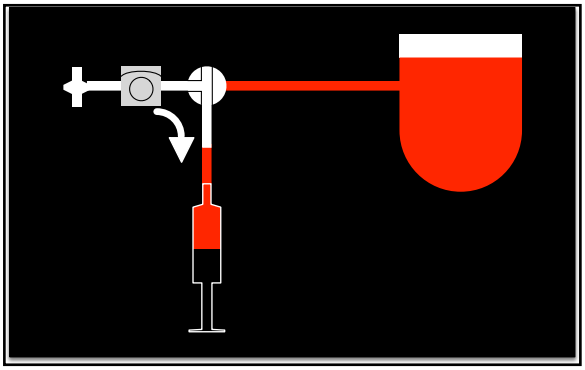


6. Bring the sample into the syringe:
   (a) Shortly before the liquid reaches Pump A (as in the image above), change the direction of the valve to block flow to the filter. This will bring the liquid from the vessel into the syringe (as in the image below). Not changing the valve direction in time risks wetting the filter, which can clog it and prevent other samples from being taken.
   (b) When there is sufficient sample in the syringe, stop Pump A.

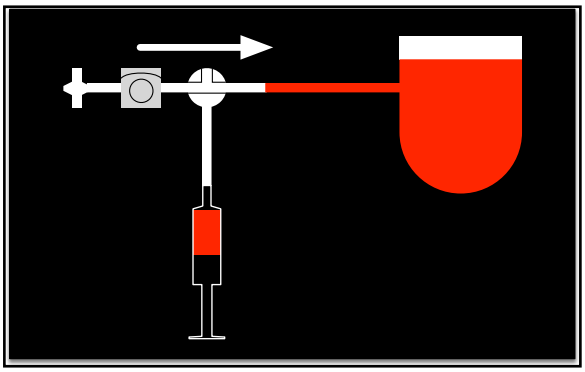


7. Clamp the line between the valve and syringe, and remove the tubing from Pump A.

8. Push the remaining liquid between the valve and syringe into the syringe:
   (a) Install the tubing between the filter and valve in Pump A as in the image below.
   (b) Configure the valve to block flow to the vessel. This will push filtered air into the syringe, clearing the line between the valve and syringe of liquid.
   (c) Unclamp the line between the valve and syringe.
   (d) Use the Pump A Switch to turn Pump A clockwise. Be prepared to execute the next step.



9. Push the remaining liquid between the valve and vessel back into the vessel:
   (a) When the line between the valve and syringe is clear of liquid, configure the valve to block flow to the syringe. This will push filtered air into the vessel, clearing the line between the valve and vessel of liquid.



10. When bubbles form in the vessel it means the line is clear of liquid. Turn off Pump A.
11. Clamp the tubing lines, and remove the syringe. When clamping the tubing line between the valve and vessel, place the clamp as close to its port as possible to minimize the dead volume between the vessel and the clamp. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the

Vessel Cover.Using proper aseptic technique, either replace the syringe with a sterile one, or weld on a new sampling manifold.

12. If sampling to measure pH or DO, expel the head gas from the syringe, and cap it to make the sample more stable. Measure the pH or DO as soon as possible.
13. Depending on how long you cleared the line with filtered air, you may have to manually adjust the Estimated Level - see “Level Adjustment to Filled Volume” on page 86.
14. If the Vessel Cover was previously removed to open and close any clamps, reinstall the Vessel Cover at this time.

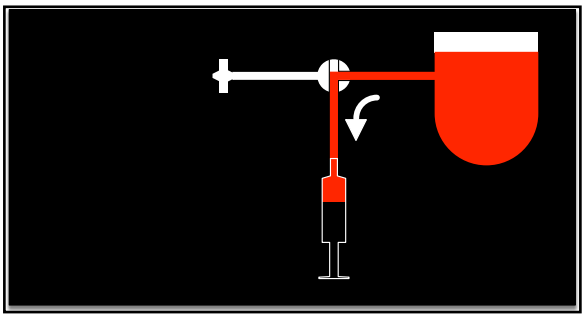
To take a sample with a sampling manifold, single-syringe pull, and gravity drain:

Taking a sample without using a pump can expose the sample to less shear stress. It can also, however, introduce more sampling variability between operators. This method also exposes the sample to much more air than the other methods using a sampling manifold, and is therefore not appropriate for measuring the pH or DO of the sample. For an alternative method of taking a sample without using a pump, see “To take a sample with a sampling manifold and dual-syringe pull” on page 104. For instructions on how to take a sample using a pump, see “To take a sample with a sampling manifold and a pump” on page 99.

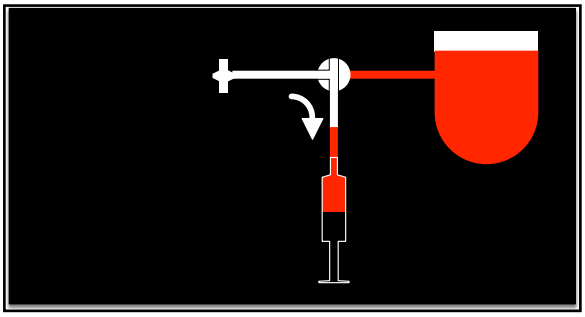
Note: When a pump is not used to take a sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. For instructions on how to manually adjust the Estimated Level, see “Level Adjustment to Filled Volume” on page 86.

1. Unclamp all clamps on the sampling manifold. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while sampling to open the clamp and then close it after taking the sample.

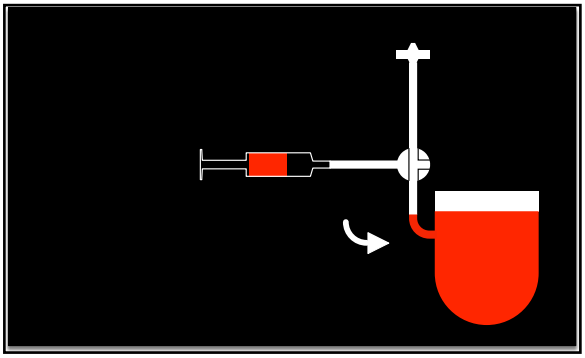
2. Pull the sample into the sample syringe:
   (a) Configure the valve to block flow to the filter. This will allow the sample syringe to pull the liquid from the vessel into the sample syringe.
   (b) Pull the sample from the vessel using the sample syringe. Note that you will first be pulling the air in the sampling manifold into the syringe.
   (c) When there is sufficient sample in the syringe, stop pulling.



3. Pull the remaining liquid between the valve and sample syringe into the sample syringe:
   (a) Configure the valve to block flow to the vessel. This will allow the sample syringe to pull filtered air into the sample syringe, clearing the line between the valve and sample syringe of liquid.
   (b) Pull air from the air transfer syringe through the filter and into the sample syringe.
   (c) Stop pulling air into the sample syringe once the line between the valve and sample syringe is clear of liquid.



4. Drain the remaining liquid between the valve and vessel back into the vessel:
   (a) Hold the filter and valve above the liquid level line of the vessel.
   (b) Configure the valve to block flow to the sample syringe. This will allow air to pass through the filter and into the vessel, clearing the sampling manifold.
   (c) Raise the sampling manifold above the liquid level line of the vessel, to allow gravity to drain as much of the liquid as possible back into the vessel.



5. Clamp the tubing lines, and remove the syringe. When clamping the tubing line between the valve and vessel, place the clamp as close to its port as possible to minimize the dead volume between the vessel and the clamp. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.Using proper aseptic technique, either replace the syringe with a sterile one, or weld on a new sampling manifold.
6. Because a pump was not used to take the sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. Measure the sample volume, and manually adjust the Estimated Level accordingly by following the instructions in “Level Adjustment to Filled Volume” on page 86.
7. If the Vessel Cover was previously removed to open and close any clamps, reinstall the Vessel Cover at this time.

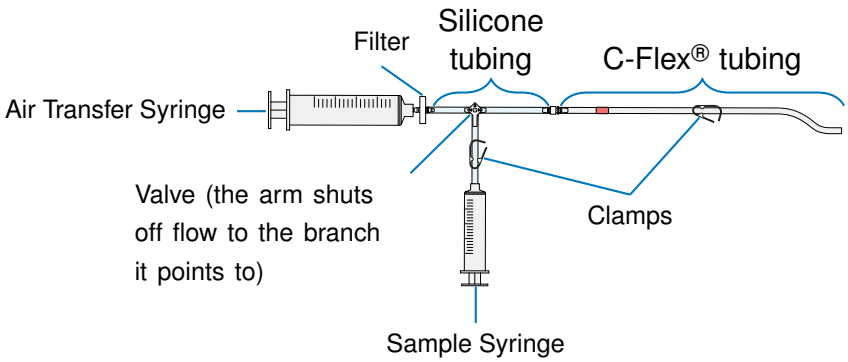
To take a sample with a sampling manifold and dual-syringe pull:

This method requires a 60 mL syringe or similar, installed on the filter on a sampling manifold. Taking a sample without using a pump can expose the sample to less shear stress. It can also, however, introduce more sampling variability between operators. For instructions on how to take a sample using a pump, see “To take a sample with a sampling manifold and a pump” on page 99. For an alternative method for taking a sample without a pump, see “To take a sample with a sampling manifold, single-syringe pull, and gravity

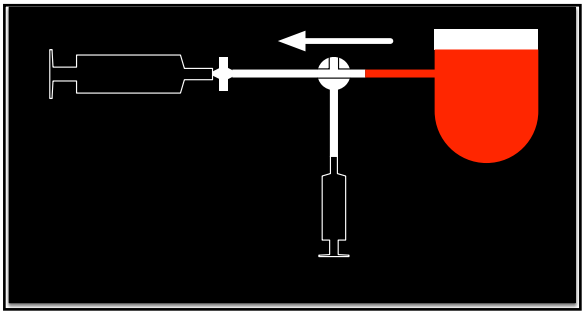
drain” on page 102.

Note: When a pump is not used to take a sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. For instructions on how to manually adjust the Estimated Level, see “Level Adjustment to Filled Volume” on page 86.

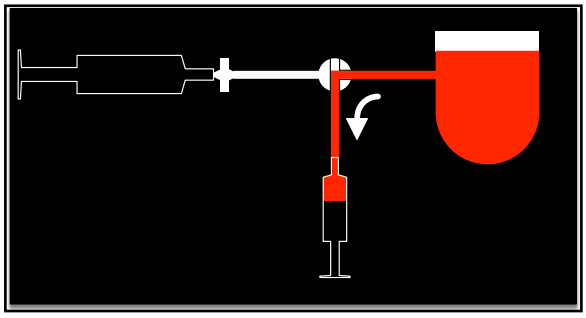
Sampling manifold assemblies similar to the one depicted here can be purchased from PBS, already sterilized and ready to be sterilely welded onto a vessel. Contact PBS Sales for more information.



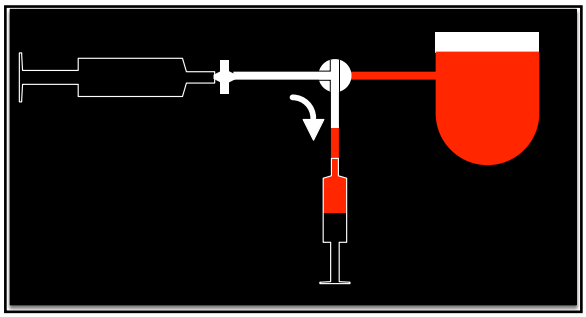
1. Install a 60 mL syringe or similar on the filter on the sampling manifold. Instructions will refer to this as the “air transfer syringe.”
2. Unclamp all clamps on the sampling manifold. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while sampling to open the clamp and then close it after taking the sample.
3. Clear the sampling manifold of air, to minimize the amount of air that will end up in the sample syringe:
   (a) Configure the valve to block flow to the sample syringe. This will allow the air transfer syringe to pull the air in the sampling manifold through the filter and into the air transfer syringe.
   (b) Using the air transfer syringe, pull air out of the sampling manifold.
   (c) Stop before the liquid in the vessel gets to the valve. Continuing to pull liquid past the valve risks wetting the filter, which can clog it and prevent other samples from being taken.



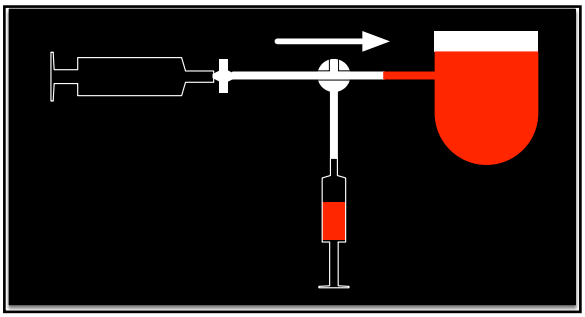
4. Pull the sample into the sample syringe:
   (a) Change the direction of the valve to block flow to the filter. This will allow the sample syringe to pull the liquid from the vessel into the sample syringe.
   (b) Pull the sample from the vessel using the sample syringe.
   (c) When there is sufficient sample in the syringe, stop pulling.



5. Push the remaining liquid between the valve and sample syringe into the sample syringe:
   (a) Configure the valve to block flow to the vessel. This will allow the air transfer syringe to push filtered air into the sample syringe, clearing the line between the valve and sample syringe of liquid.
   (b) Push air from the air transfer syringe through the filter and into the sample syringe.
   (c) Stop pushing air into the sample syringe once the line between the valve and sample syringe is clear of liquid.



6. Push the remaining liquid between the valve and vessel back into the vessel:
   (a) Configure the valve to block flow to the sample syringe. This will allow the air transfer syringe to push the air in the air transfer syringe through the filter and into the vessel, clearing the sampling manifold.
   (b) Using the air transfer syringe, push air into the sampling manifold. You will likely have to disconnect the air transfer syringe, pull more air into it, and reconnect it to the filter to completely clear the sampling manifold. Because the air is being pushed through the filter, this will not compromise sterility.
   (c) When bubbles form in the vessel it means the sampling manifold is clear of liquid. Stop pushing air through the air transfer syringe at this point.



7. Clamp the tubing lines, and remove the syringe. When clamping the tubing line between the valve and vessel, place the clamp as close to its port as possible to minimize the dead volume between the vessel and the clamp. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.Using proper aseptic technique, either replace the syringe with a sterile one, or weld on a new sampling manifold.
8. If sampling to measure pH or DO, expel the head gas from the syringe, and cap it to make the sample more stable. Measure the pH or DO as soon as possible.
9. Because a pump was not used to take the sample, the software will not automatically update the Estimated Level to reflect the new volume in the vessel. Measure the sample volume, and manually adjust the Estimated Level accordingly by following the instructions in “Level Adjustment to Filled Volume” on page 86.
10. If the Vessel Cover was previously removed to open and close any clamps, reinstall the Vessel Cover at this time.

Exchanging Medium

Setup

Follow these instructions, whether setting up for a discrete medium exchange (see “Discrete Medium Exchange” on page 109) or for perfusion (see “Perfusion” on page 110).

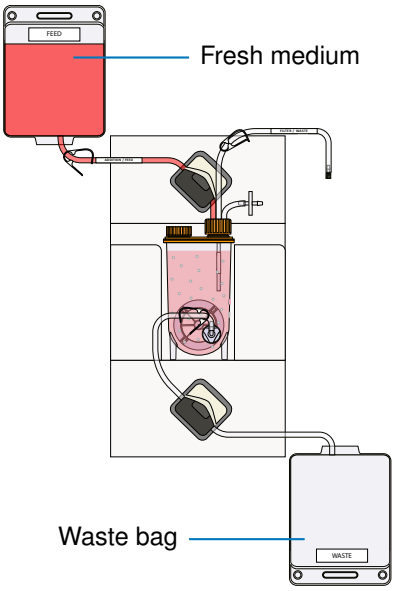
1. Navigate to the Pumps menu (see “Accessing the Pumps menu” on page 76).
2. If either pump is on, click the button to turn it/them off. If either pump is being used for Perfusion, you will have to navigate to the Perfusion menu and stop it/them.
3. For setting up removal for a discrete medium exchange, form a sterile connection between the appropriate aspiration line or dip tube line and the waste media bottle/bag destination by welding the tubing or using the sterile connectors. The volume of settled cells should be less than the aspiration level of the selected tubing line.

   For setting up removal for perfusion, form a sterile connection between the perfusion-specific vessel’s Filter/Waste line and the waste media bottle/bag destination by welding the tubing or using the sterile connectors.
4. Install the silicone section of the removal tubing in Pump B so the section leading to the waste media bottle/bag destination comes out of the right side of the pump head. When Pump B flows clockwise, the software removes the volume flowed from the Estimated Level.
5. Check that the removal line tubing clamp(s) is/are open. If there are clamps behind the Vessel Cover, you will have to remove the Vessel Cover while exchanging medium to open the clamp and then close it after exchanging medium.
6. For addition, form a sterile connection between the desired liquid handling line and the medium bottle/bag source, by welding the tubing or using the connectors. For perfusion, it is recommended to use the perfusion-specific vessel’s Addition/Feed line, but this line can also be used for discrete medium exchanges.
7. Install the silicone section of the addition tubing in Pump A so the section leading to the vessel comes out of the right side of the pump head. When Pump A flows clockwise, the software adds the volume flowed to the Estimated Level.
8. Check that the addition line tubing clamp(s) is/are open.
9. Prime Pump A to fill the line with liquid up to the port (see “Priming a Pump” on page 80).

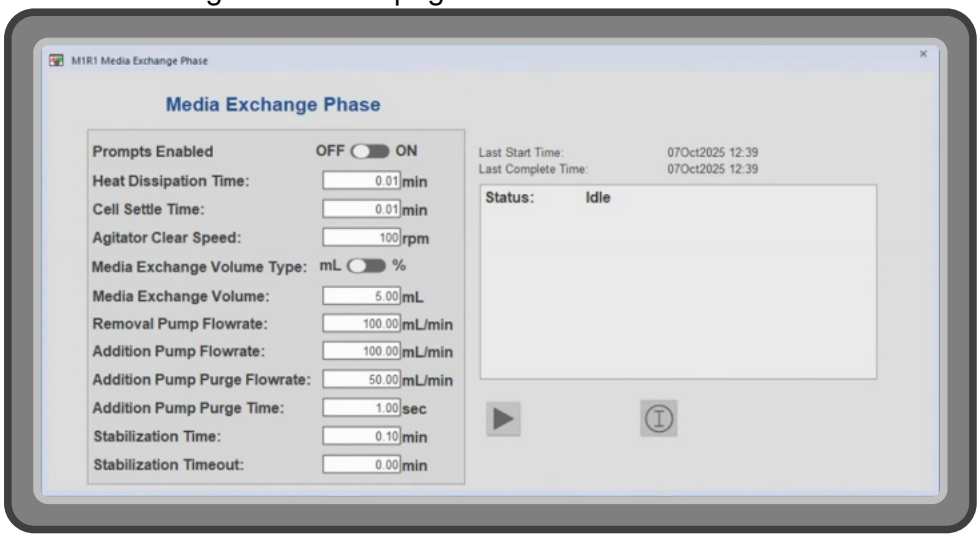
Discrete Medium Exchange

Follow these instructions to remove a specified amount of medium and then replace it. For exchanging medium via perfusion, see “Perfusion” on page 110.

1. Complete the setup steps (see “Setup” on page 108).



2. In the software, navigate to the Bioreactor Overview menu for the desired vessel, and click the Media Exchange Indicator Button under “Equipment Phases” (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).
3. Confirm the parameters in the Media Exchange Phase menu are set up correctly. For more information on how the feature works, see “Media Exchange Phase” on page 159. For definitions of these parameters, see “Media Exchange Phase” on page 173.



4. Click the “Start” button.

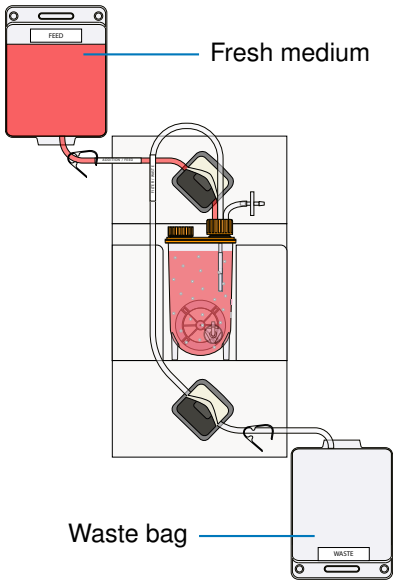
5. Wait for the software to go through the entire sequence. For details of what steps the software goes through, see “Media Exchange Phase” on page 159.
6. Clamp the Liquid Handling lines as close to their respective ports as possible to minimize dead volume between the vessel and the clamps. See step 11 of “Install Vessel in PBS-MiniPRO” on page 70 for instructions on how the clamp must be oriented so it fits beneath the Vessel Cover.
7. If the Addition Pump ‘Purge’ feature was used, it may be necessary to adjust the Estimated Level after performing the Medium Exchange (see “Level Adjustment to Filled Volume” on page 86).

Note: If performing multiple medium exchanges, reposition tubing through the pump head if it starts to wear out in order to pump with a fresh section of tubing.

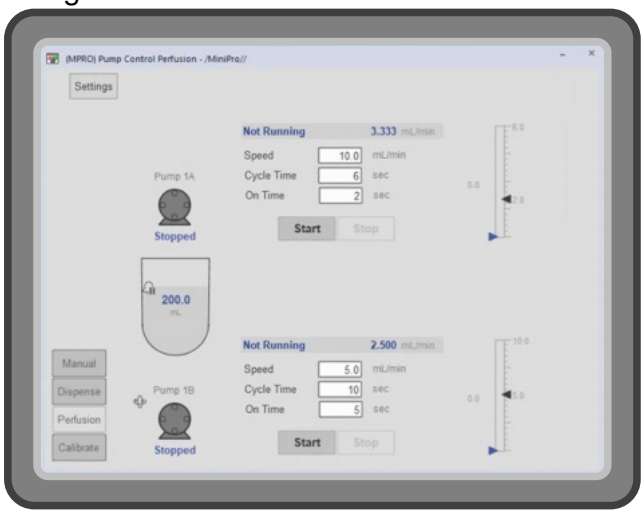
Perfusion

Follow these instructions to continuously exchange medium in the vessel by supplying fresh medium and removing spent medium at low flow rates. For instructions on performing a discrete medium exchange, see “Discrete Medium Exchange” on page 109. For more information about the Perfusion feature, see “Additional Perfusion Information” on page 152.

1. Complete the setup steps (see “Setup” on page 108).



2. Navigate to the Perfusion menu.



3. Based on the volume of liquid in the vessel and the desired Vessel Volumes per Day (VVD) to be exchanged, set the ‘Speed,’ ‘Cycle Time,’ and ‘On Time’ for each pump.

   The pumps turn on for the ‘On Time’ at the ‘Speed’ or flow rate, then turn off for the remainder of the ‘Cycle Time.’ It is generally recommended to use a ‘Speed’ of 1.0 mLPM, ‘On Time’ of 60 seconds, and to set the ‘Cycle Time’ to a value which will provide the desired VVD.

   For more information, see “Additional Perfusion Information” on page 152.

4. The menu will display the calculated net flow rates in mL/min based on the entered ‘Speed,’ ‘Cycle Time,’ and ‘On Time’ for each pump. Confirm these flow rates are indeed what is desired, and that both pumps’ net flow rates match.
5. Click the ‘Start’ buttons for both pumps.

For more information, see “Pumps” on page 151.

Harvesting a Run

Setup for Harvest

1. Set the Bioreactor’s Alarms Phase to ‘Standby’ mode (see “Set the Bioreactor’s Alarms Phase to ‘Ramp’ Mode” on page 87).
2. Set all control modes and pumps to Off.

Harvest with the 30 mL Port line

These instructions are only applicable with a vessel with a 30 mL Port line; if your vessel does not have one, see “Open System Harvest” on page 113.

1. Complete the setup steps (see “Setup for Harvest” on page 111).
2. Form a sterile connection between the 30 mL Port line and the harvest bottle/bag destination by welding the tubing or using the sterile connectors.
3. Check that the tubing clamp is open, and its branched tubing clamp is closed, if applicable.
4. Install the silicone section of the tubing in the desired pump so its configuration is consistent with how the Estimated Level totalizer works.

   If using Pump A, the tubing coming from the vessel should enter the pump on the right, and the pump should run counter clockwise to remove liquid from the vessel and to decrease the Estimated Level.

   If using Pump B, the tubing coming from the vessel should enter the pump on the left, and the pump should run clockwise to remove liquid from the vessel and to decrease the Estimated Level.

   For more information, see “Using the Pumps” on page 73 and “Estimated Level” on page 149.
5. Use the appropriate pump switch, in the appropriate direction, to remove as much of the vessel contents as possible through the 30 mL Port line.

   As the Estimated Level decreases below the Level Lo Lo value, Pump B will become interlocked (see “Using an interlocked pump” on page 77). If this is the pump being used, the simplest workaround is to raise the Estimated Level (see “Level Adjustment to Filled Volume” on page 86). For other options, see “Interlocks” on page 151.
6. Remove the Vessel Cover, and then the vessel. Tilt the vessel so the pump can continue removing the vessel contents through the 30 mL Port line. Turn off the pump when finished.

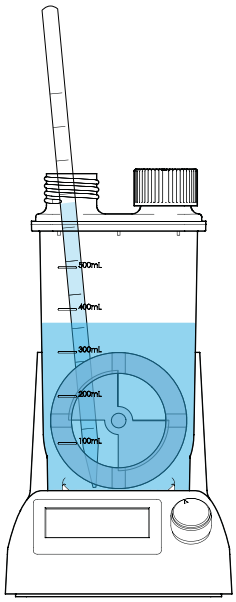
   NOTICE Be sure not to discard the Vessel Cover or Window shade - they are not single-use.
7. Turn off the light, if it is on.
8. End batch (see “Entering Batch ID” on page 94).
9. Clean/decontaminate the PBS-MiniPRO (see “Cleaning and Decontamination” on page 42).

Note: If performing multiple harvests, reposition tubing through the pump head if it starts to wear out, to pump with a fresh piece.

Open System Harvest

Not all vessels have a 30 mL Port line. Some users may also prefer to not use it. These instructions are appropriate in those situations.

1. Install a Mini Base Unit in the biosafety cabinet (see “Sampling by pipette” on page 96).
2. Complete the setup steps (see “Setup for Harvest” on page 111).
3. Turn off the light, if it is on.
4. End the batch (see “Entering Batch ID” on page 94).
5. Disconnect the Main-gas line from the Main-gas connector.
6. Clamp and remove tubing from any pumps on the Base Module.
7. Remove the Vessel Cover, and then the vessel.
   NOTICE Be sure not to discard the Vessel Cover or Window shade - they are not single-use.
8. Transfer the vessel to the biosafety cabinet and install it onto a Mini Base Unit.
9. Agitate at an appropriate speed to evenly suspend the vessel contents.
10. Allow culture to mix for 10-20 seconds prior to harvesting to ensure even suspension.
11. Insert a 25 mL serological pipette vertically from the left side port of the vessel until the tip is right above the side of the Vertical-Wheel® impeller (near the middle of the vessel).
12. Collect the desired volume of cell culture and media and transfer to an appropriately sized sterile container. Once the Vertical-Wheel® impeller is exposed to air, turn off agitation and continue to collect the remaining volume, gently pipetting up and down 1-2x if needed to resuspend cell culture. To reach the last 5-10 mL of volume, switch to a 5 mL serological pipette to reach between the Vertical-Wheel® impeller and vessel wall.



13. Clean/decontaminate the PBS-MiniPRO (see “Cleaning and Decontamination” on page 42).

Other Features

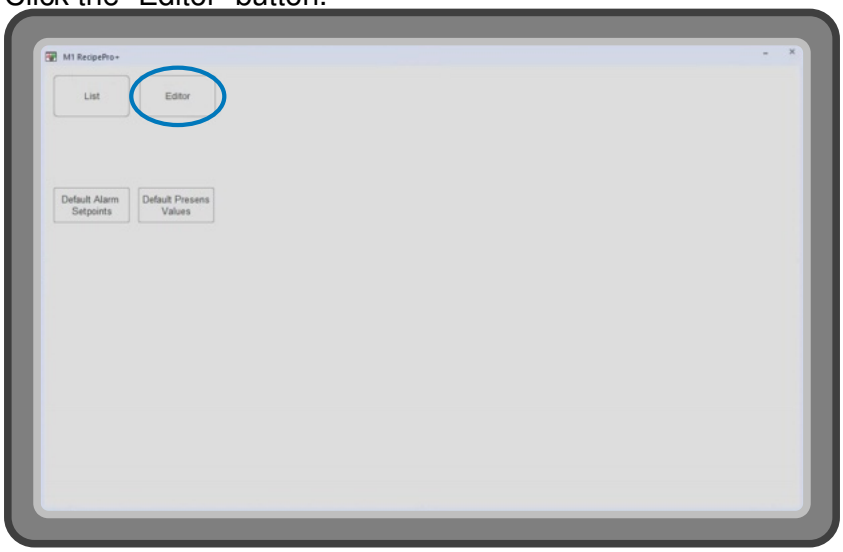
RecipePro+

Recipes in RecipePro+ are a collection of ‘Ingredients’ where each ‘Ingredient’ sets a single tag to a pre-determined value. Tags follow the MxRy convention (see “PBS-MiniPRO Base Module - Front Overview” on page 16), which means each one is for a single Bioreactor on a particular Base Module.

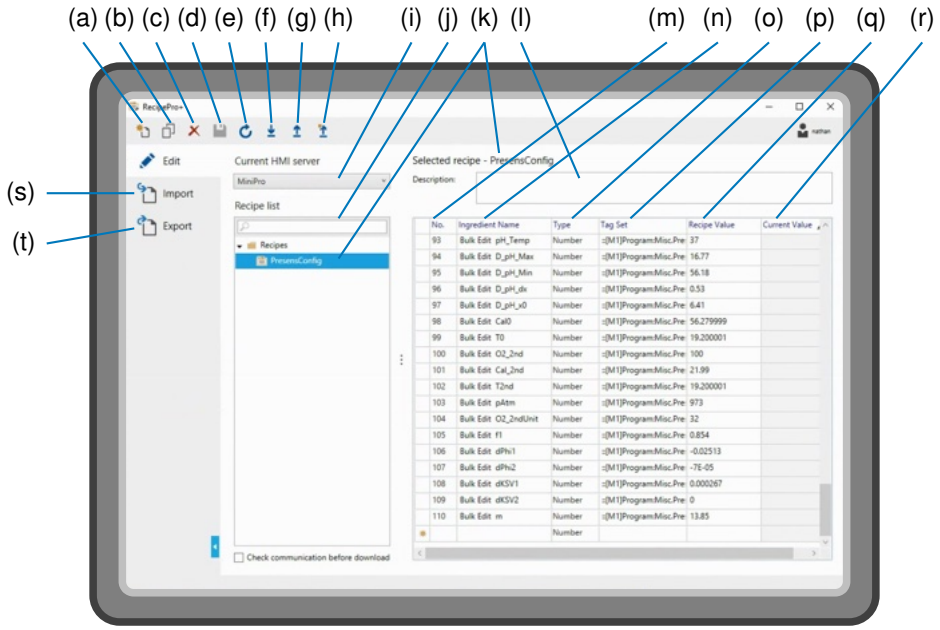
Recipes can be useful for setting all controls on a particular Bioreactor to Off, or to specific modes and setpoints for standard operations, for example.

Creating or editing recipes

1. Click the “RecipePro+” button.
2. Click the “Editor” button.



3. This is the editor. Note that none of the buttons have a confirmation or warning, so be careful when clicking them.



(a) New Recipe File

(b) Duplicate Recipe File - makes a copy of the selected recipe

(c) Delete Recipe File - deletes the selected recipe

(d) Save Recipe File

(e) Refresh Recipe Values - refreshes the “Current Value” column

(f) Download Recipe File - this runs the selected recipe

(g) Upload Recipe File - this reads the current values of all the tags listed in the selected recipe and makes those the values specified in the selected recipe file

(h) Upload and Create New Recipe File - This creates a new recipe file with the current values of each tag in the selected recipe file

(i) Current HMI server

(j) Recipe list

(k) Selected recipe

(l) Description - an optional description of the selected recipe

(m) Number - the order the step will be executed

(n) Ingredient Name - a user-set text field, to make it easier to understand the ingredient

(o) Type - either ‘Number’ or ‘String’ depending on the type of tag being set

(p) Tag Set - the name of the tag being set

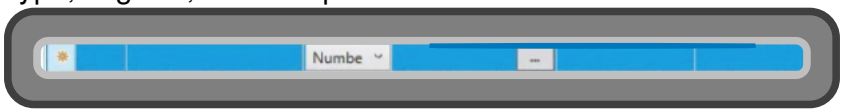
(q) Recipe Value - the value the tag is being set to

(r) Current Value - the current value of the tag

(s) Import - allows importing a recipe file

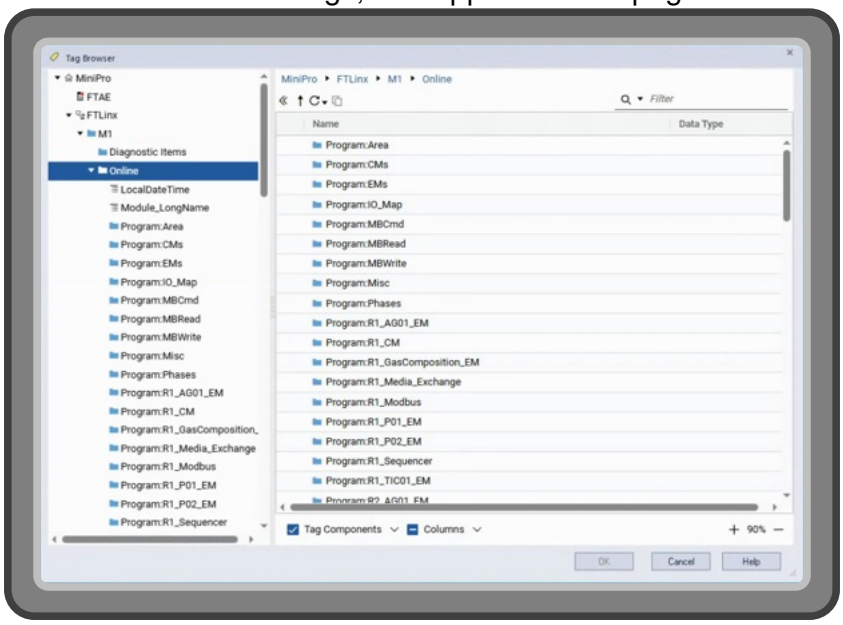
(t) Export - allows exporting a recipe file

4. Scrolling to the end of the ingredient list of the selected recipe allows the user to add a new ingredient to the end, setting the Ingredient Name, Type, Tag Set, and Recipe Value.



5. Clicking the 3 dots in the “Tag Set” field opens the Tag Browser. The filepath to use is MiniPro → FTLinx → M1 → Online, then navigate to the desired tag. After selecting the desired tag, click “Ok.”

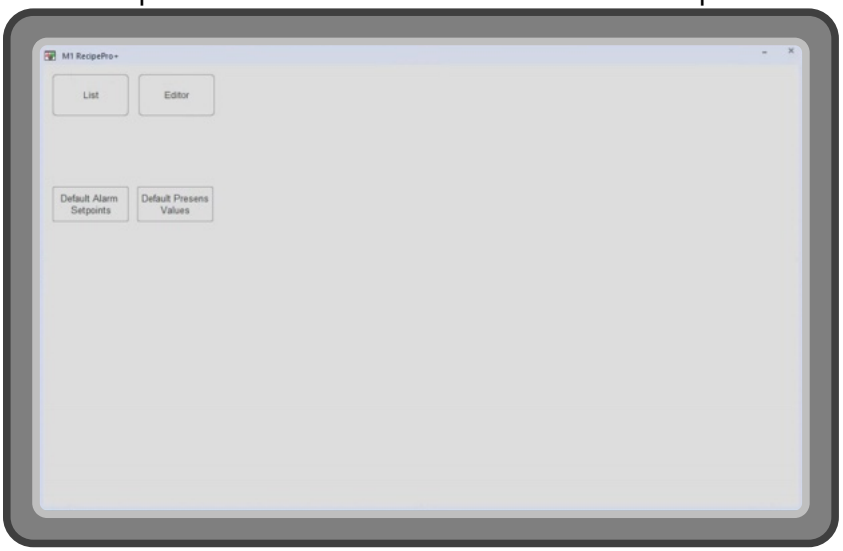
   For information about tags, see Appendix 3 on page 181.



6. Right-clicking any ingredient allows the user to delete it from the recipe.
7. Configure the recipe as desired, and save it.

Running/downloading recipes with an associated button

Some recipes have an associated button in the RecipePro+ menu:



Recipes with Associated Buttons	
Default Alarm Setpoints	This sets all the values of all alarm Thresholds and Deadbands to their default values, for all Bioreactors (for more information, see “Alarms” on page 161).
Default Presens Values	This sets the calibration values for the single-use DO and pH sensors to their default values for all Bioreactors.

1. Click the “RecipePro+” button to open the RecipePro+ menu.
2. Click the button corresponding with the recipe you wish to run.

Running/downloading recipes without an associated button

1. Navigate to the RecipePro+ ‘Editor’ menu.

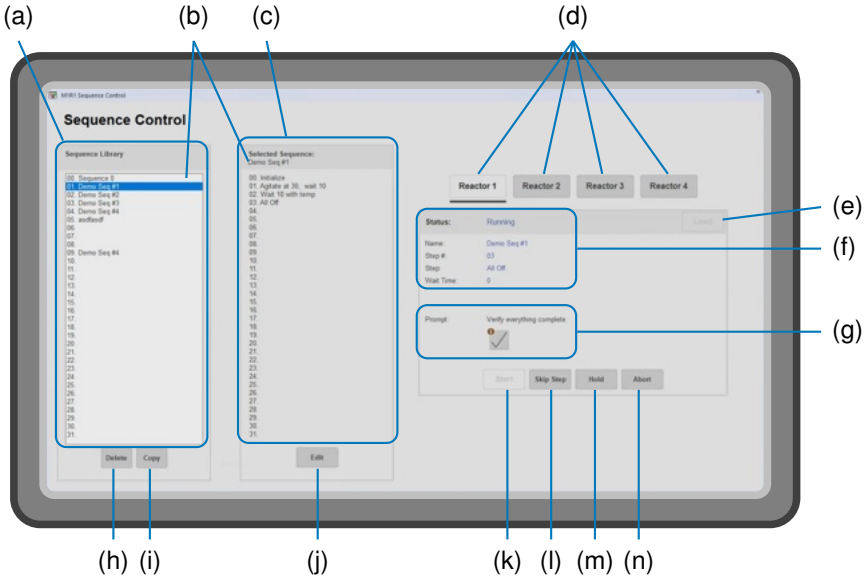
   Note: The RecipePro+ ‘List’ menu also offers users the same “Download Recipe File” and “Upload Recipe File” functions as in the ‘Editor’ menu.

2. Select the desired recipe.
3. Click the “Download Recipe File” button.

Sequencer

Creating or editing sequences

1. Click the “Sequencer” button.
2. This is the Sequence Control.



(a) Sequence Library

(b) Selected Sequence

(c) List of steps in selected sequence

(d) Select Bioreactor

(e) Load the selected sequence onto the selected Bioreactor, in preparation for running

(f) Running Sequence information for selected Bioreactor, or if no sequence is running, displays the loaded sequence

(g) Prompt to user

(h) Delete selected sequence

(i) Copy selected sequence from one specified slot to another in the library

(j) Edit selected sequence

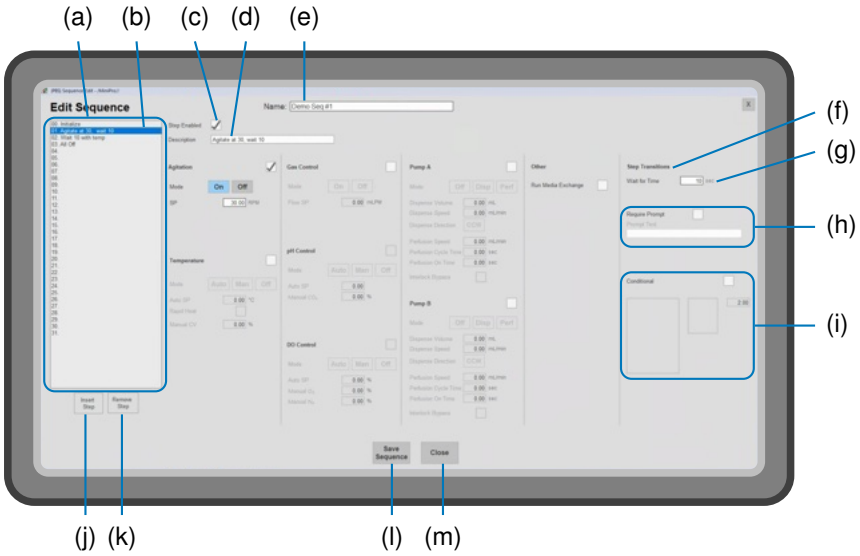
(k) Start loaded sequence on selected Bioreactor

(l) Skip current step of running sequence

(m) Hold - pauses the running sequence. When clicked, turns into a “Resume” button which resumes the running sequence.

(n) Stop current sequence

3. This is the Editor.



(a) List of steps

(b) Selected step

(c) Whether the selected step is enabled

(d) A manually-entered description of what the step does

(e) Name of sequence being edited

(f) After setting all parameters and running all phases specified for this step, all the specified Step Transitions are checked, and the sequence proceeds to the next step when all specified Step Transitions are met

(g) Wait this many seconds

(h) A prompt will appear at the end of the step that an operator has to dismiss before the next step will run

(i) The configured condition is met

(j) Insert a new blank step before the selected step

(k) Remove selected step

(l) Save sequence

(m) Go back to Sequence Control menu

4. Configure the sequence as desired, and save it.

Running sequences

1. Click the “Sequencer” button.
2. Select the desired Bioreactor.
3. If a sequence is already running, stop it by clicking the “Abort” button.
4. Select the desired sequence, load it by clicking the “Load” button, and run it by clicking the “Start” button.

Generating Reports

Along with the instructions below for generating reports through the Display Client, the SQL Server Management Studio application installed on the PBS-MiniPRO can be used to read the database contents with SQL calls and generate reports. Refer to the documentation for that software for more information.

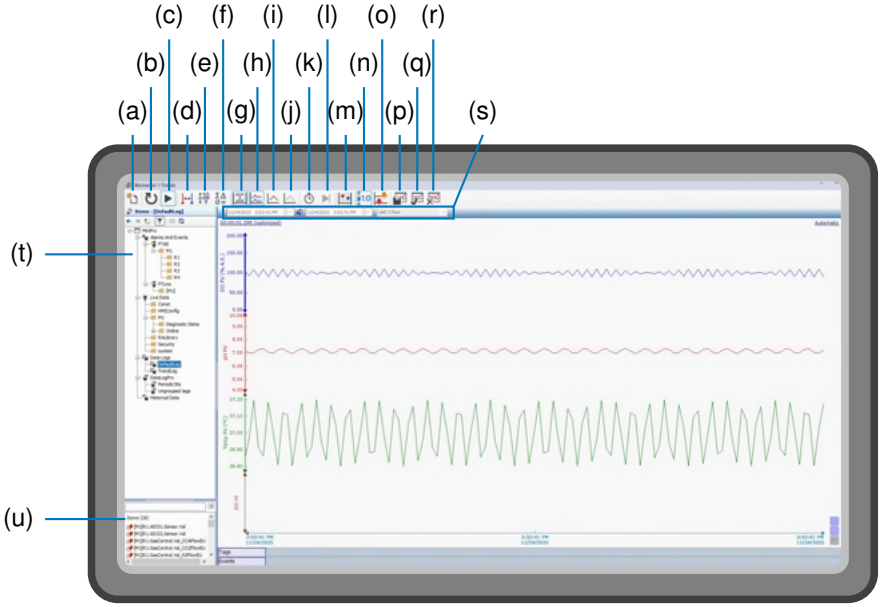
Reports via TrendPro

TrendPro allows generating reports of one or more specified parameters by timespan. This can include recorded Process Data. It can also include specific alarm types.

For further instructions on using TrendPro, see documentation such as the “FactoryTalk View Site Edition User’s Guide.”

1. To save new templates or save edits to existing templates, log in as a user in the “Maintenance” permission group or higher. Generating reports does not require any particular permission.
2. To open an empty TrendPro template, click the “Trends” button in the Software Header (see “PBS-MiniPRO Software - Header and Footer” on page 24).
   To open a TrendPro template showing the DO, pH, Temperature, and Alarms graphs for a particular Bioreactor, click the “Trends” button in the Bioreactor Overview (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).

3. This is the TrendPro interface.

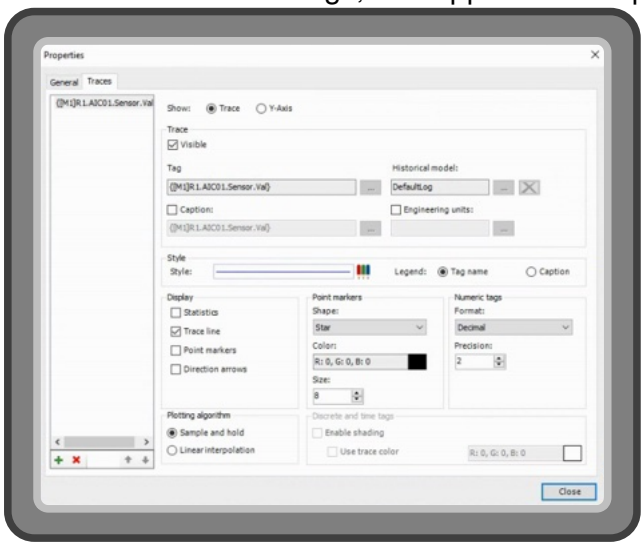


(a) New template
(b) Refresh displayed data
(c) Live Mode - show currently generated data instead of pulling recorded data from database
(d) X-axis Cursors
(e) Y-axis Cursors
(f) Statistics
(g) Automatic Scales
(h) Stack Tag Y-axes
(i) Single Trace
(j) Hide Trace
(k) New Time Period
(l) Next Time Period
(m) Rubberband Zoom
(n) Alarm Cursor
(o) Stack Event Y-axes
(p) Save Template
(q) Apply Template - open a saved template
(r) Delete Template
(s) Timespan selector
(t) Potential data sources - Data Logs → DefaultLog is the default (see “Configuring Logger Settings” on page 57)
(u) Individual tags that can be used as Traces - click and drag them onto the graph

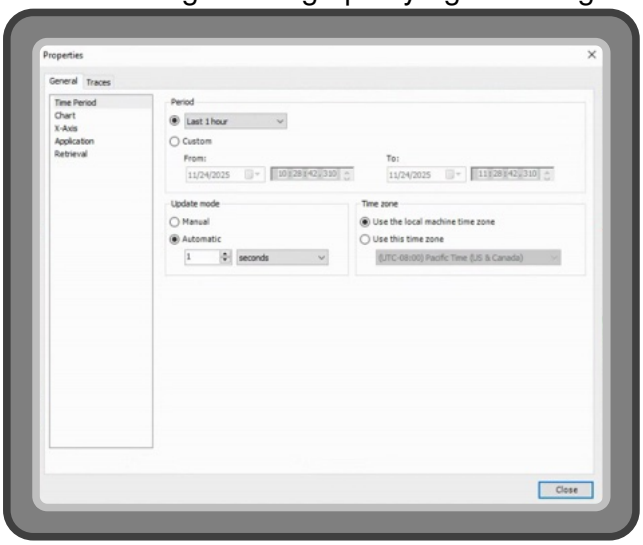
4. Setting up templates for the parameters you are interested in allows you to more easily generate reports and compare data for different cell culture runs. Create, save, delete, and open templates using the button described above.

5. Individual data series on the graph are called “Traces.” You can add additional traces to the graph by either clicking and dragging from the list of tags in the lower left-hand corner, or right-clicking the graph and selecting “Properties” → “Traces.” That menu is also how you delete and configure individual traces.

   For information about tags, see Appendix 3 on page 181.



6. You can configure the graph by right-clicking it and selecting “Properties.”



   (a) “Time Period” allows you to change the time period the graph displays, along with how it updates, and what time zone to use.

   (b) “Chart” allows you to configure the appearance of the graph like the title, margins, etc.

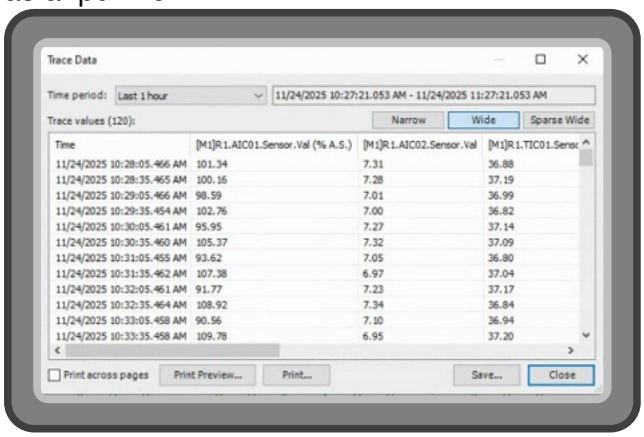
   (c) “X-Axis” allows you to configure the name, time mode, and tick marks

   (d) “Application” allows you to configure the styles of the lines on the graph, and what dimming a trace looks like

   (e) “Retrieval” allows you to configure how the data is retrieved from the database - there are many ways to custom configure the sampling rather than using the automatic optimized settings

7. To save an image of the graph, right-click the graph and select “Capture Image...” then select the destination, name, and file format (it can save as .png, .gif, .jpg, or .bmp).

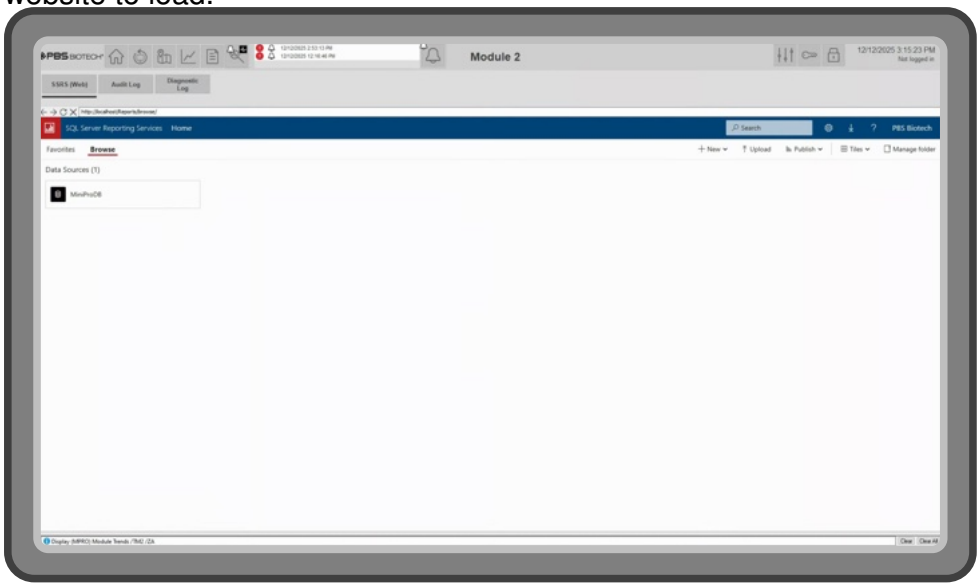
8. To save a table of the data points being graphed, right-click the graph and select “Trace Data...” - it is recommended to then select “Wide” before clicking “Save” so each tag gets its own column. When saving, file format options include comma delimited .csv or tab delimited .txt. You can also print the table of data points, or using the “Print...” option save as a .pdf file.



Process Data Reports via SQL Server Reporting Services

This is a data analysis tool created by Microsoft for power users, IT, and/or enterprise applications. Along with being accessible directly through the Display Client, it can also be accessed from another computer on the network because it runs on a server on the Control Module.

1. Click the “Reports” button in the Software Header (see “PBS-MiniPRO Software - Header and Footer” on page 24).
2. If it does not select “SSRS (Web)” click that button, and wait for the website to load.

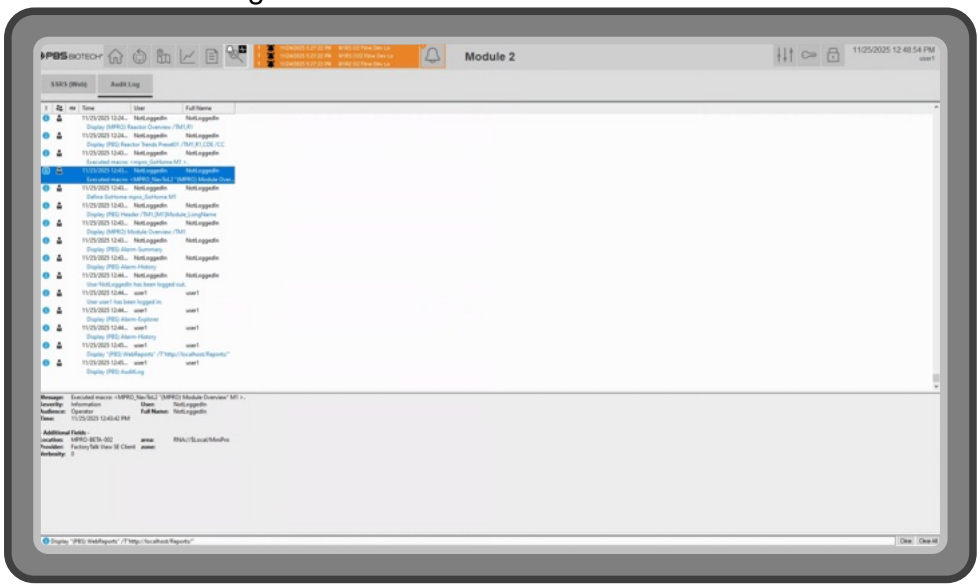


3. Because this is a standard Microsoft tool, information on how to use it can be accessed by clicking the “?” icon, or from Microsoft.

Audit Log

The “Audit Log” menu shows Events from users.

1. Click the “Reports” button in the Software Header (see “PBS-MiniPRO Software - Header and Footer” on page 24).
2. Click the “Audit Log” button.



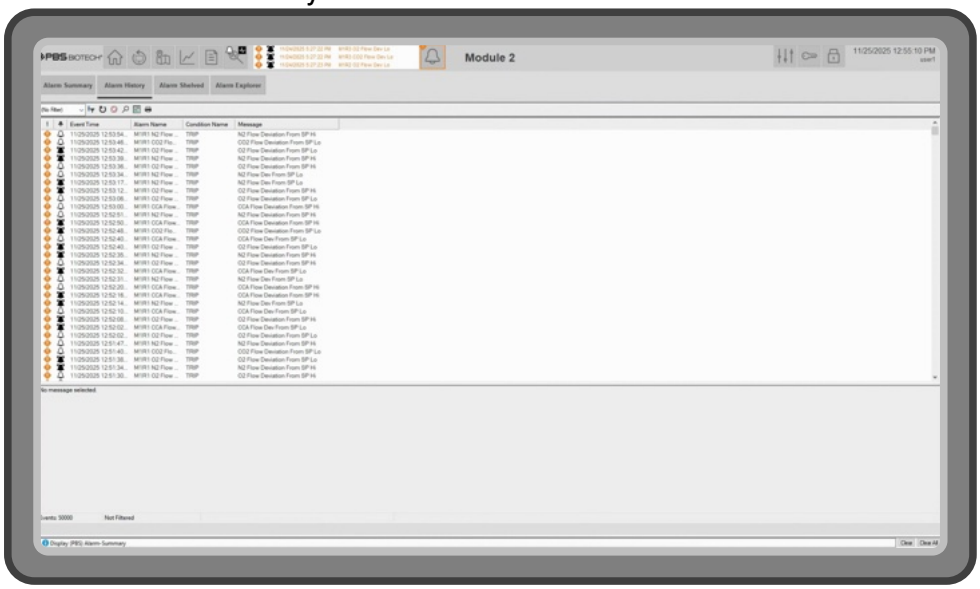
3. Click an event to see more information about it.

To export the audit events for a batch, see “Generating Batch Audit Reports” on page 127. Alternatively, operators can use the FactoryTalk “Diagnostics Viewer” application installed on the PBS-MiniPRO. Refer to the help documentation for that software for more information.

Alarms Reports via Alarm Menu

1. Click the “Alarms” button in the Software Header (see “PBS-MiniPRO Software - Header and Footer” on page 24).

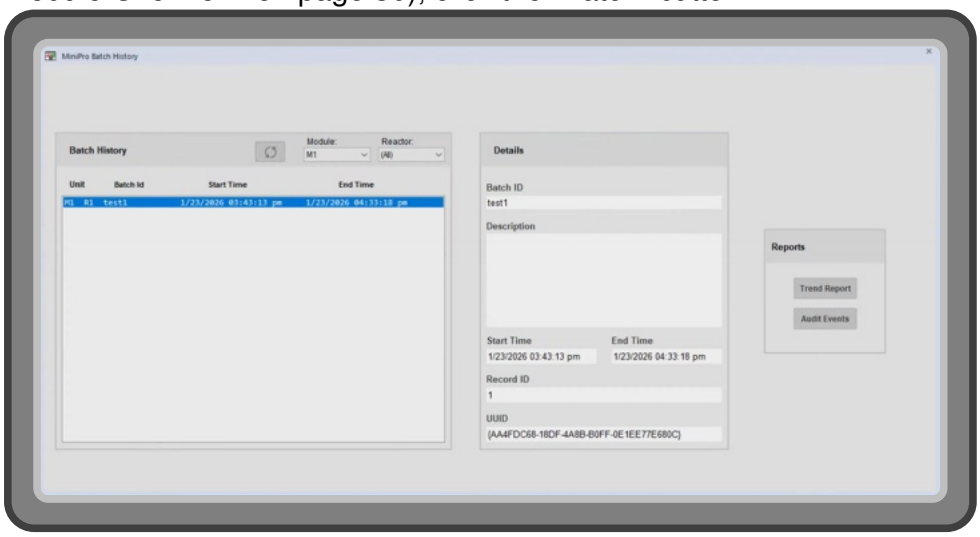
2. Click the “Alarm History” button.



3. Filter alarms, if desired.
4. Click the “Print” button to print the filtered alarms. This can also be used to print to a .pdf file.

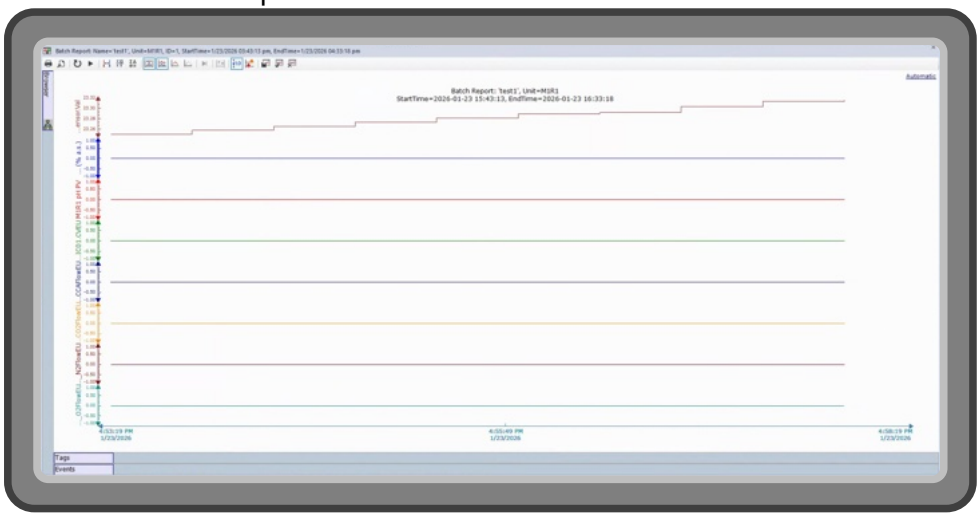
Generating Batch Trend Reports

1. From the Base Module Overview (see “PBS-MiniPRO Software - Base Module Overview” on page 30), click the “Batch” button.



2. Select the desired batch.

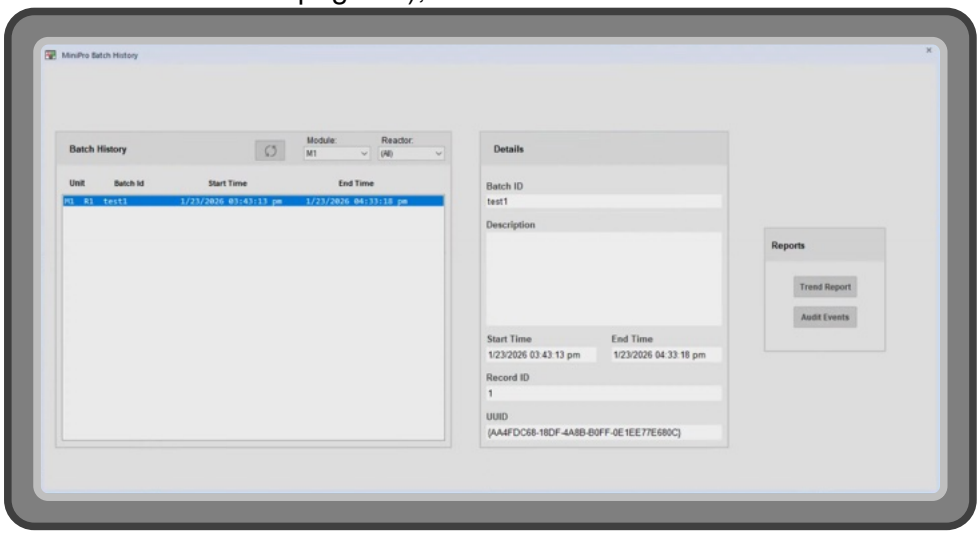
3. Click the “Trend Report” button.



4. This will open a TrendPro report for the time period the batch ran, containing the Temperature PV, DO PV, pH PV, Agitation PV, heater duty, Air flow, CO2 Flow, N2 Flow, and O2 Flow. For further instructions on how to use TrendPro reports, see “Reports via TrendPro” on page 120.

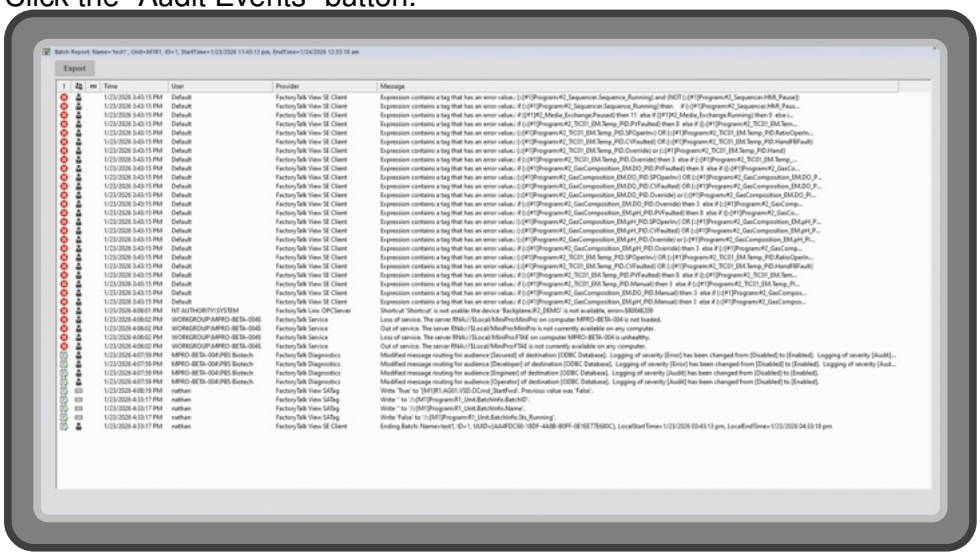
Generating Batch Audit Reports

1. From the Base Module Overview (see “PBS-MiniPRO Software - Base Module Overview” on page 30), click the “Batch” button.



2. Select the desired batch.

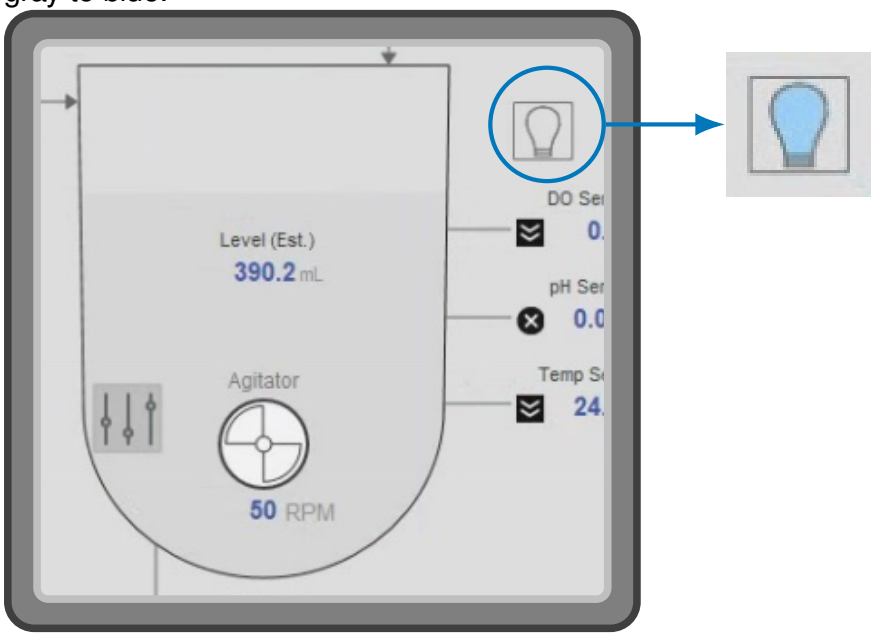
3. Click the “Audit Events” button.



4. Click the “Export” button. The path to the generated report will appear next to the “Export” button.

Light

1. Navigate to the Bioreactor Overview menu for the specific Bioreactor (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).
2. Click the lightbulb icon to turn the light on. The button will change from gray to blue.



3. Click the lightbulb icon to turn the light off. The button will change from blue to gray.

Restart and Shutdown

Users can restart or shut down the Control Module from the Windows Explorer. Note that the Base Module(s) will continue running as long as they have power. Because there is no data buffering on the Base Module(s), it is recommended that the Control Module is only restarted or shut down between runs and after turning off all controllers.

After performing a clean shutdown, the Control Module can be restarted by reconnecting power, or if it's still plugged in, by using the power switch.

To Reboot:

1. Using the keyboard, enter WIN+D to navigate to the Windows Desktop.
2. Initiate Reboot from the Start Menu.
3. Wait for Windows to completely reboot. It will launch the Display Client when it boots up again.

To Shutdown:

The following power-off procedure **MUST** be used when removing power:

1. Using the keyboard, enter WIN+D to navigate to the Windows Desktop.
2. Initiate Shutdown from the Start Menu.
3. Wait for the software to shut down and display "No Signal Detected" (or similar) on the monitor.
4. Wait an additional 10 seconds (or longer).
5. Unplug the power cord, or set the power switch to "Off."

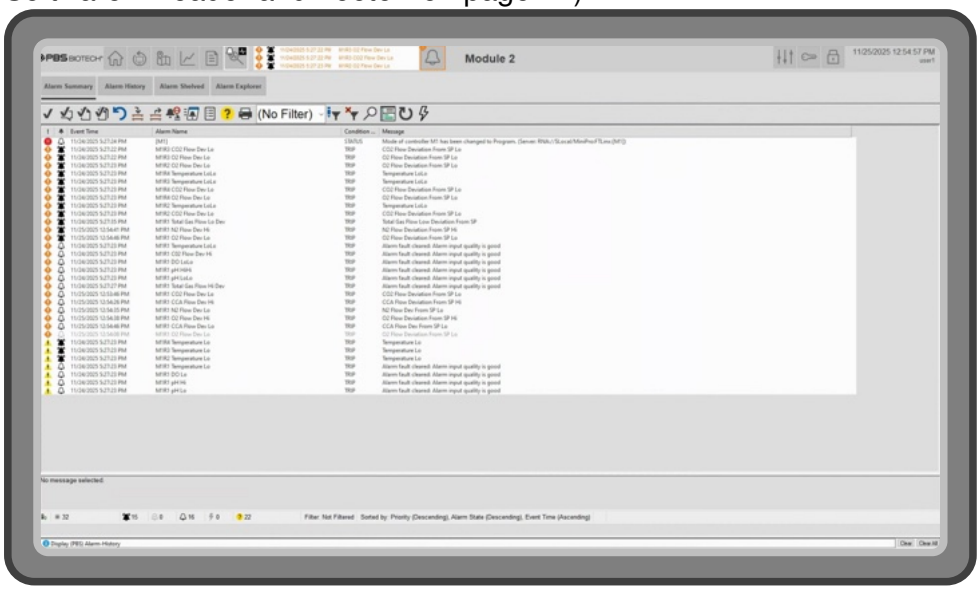
NOTICE Powering off the Control Module without following the correct power-off procedure risks corrupting files that are critical for bioreactor system operation, including loss of historical data and user account information.

Alarms

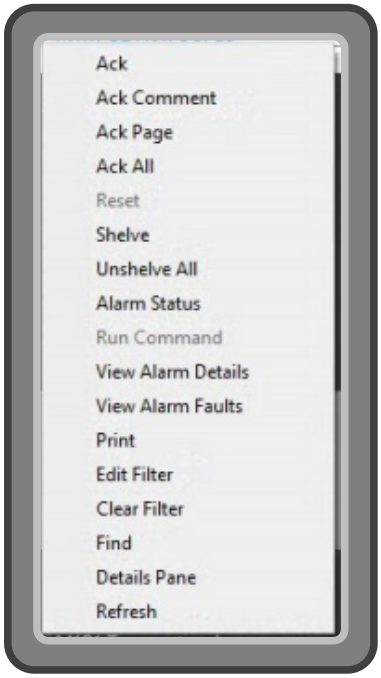
To acknowledge alarms:

1. Log in as a user with the "AlarmAck" permission.

2. Click the “Alarms” button in the Software Header (see “PBS-MiniPRO Software - Header and Footer” on page 24).



3. Right-click an alarm which has not been acknowledged:



(a) To acknowledge it without adding a comment, select “Ack.”

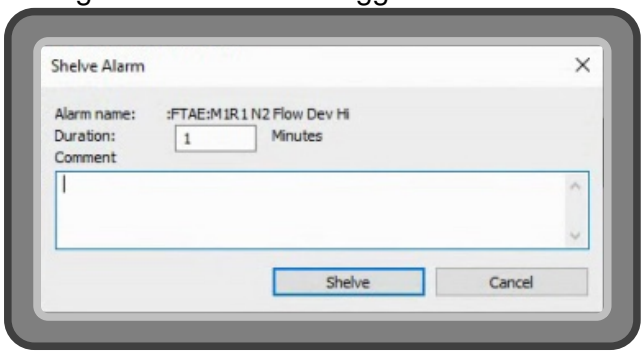
(b) To acknowledge it and add a comment, select “Ack Comment,” enter a comment, and click “Acknowledge.”

(c) To acknowledge all alarms displayed on the page without adding a comment, select “Ack Page.”

(d) To acknowledge all the alarms without adding a comment, select “Ack All.”

4. After acknowledging an alarm, its information will include which user acknowledged it and when. Note that if the alarm is still in its trigger state, it will still be displayed in the “Alarm Summary” menu. The “Alarm History” menu shows all alarm events.

5. This menu can also be used to shelve and unshelve alarms. This requires the “AlarmShelve” permission. Right-click the alarm and select “Shelve,” then if desired adjust the Duration (default is 1 minute) and/or enter a comment. After the Duration has passed, the alarm will stop being “Shelved” and go back to either being “In Alarm” or “Normal.” Shelving an alarm is the same thing as temporarily suppressing it (see “Alarms” on page 161). While an alarm is shelved, it shows up in the “Alarm Shelved” list. Alarms can also be shelved while they are not triggered, so an operator can shelve an alarm, perform an operation that would normally trigger the alarm, and then finish the operation without having nuisance alarms triggered.



For definitions of all alarms, see Appendix 2 on page 175.

Settings

WARNING! There are many settings PBS Biotech Technical Support does not recommend users change. For a complete list of all settings, their definitions, and whether PBS Biotech Technical Support recommends changing them, see Appendix 1 on page 168.

To change settings:

1. Log in to the Display Client as a user in the “Maintenance” group (for changing Alarms-related settings) or “Engineer” group (for changing all settings).
2. Navigate to the relevant faceplate for the setting you wish to modify.
3. Change the value of the setting as needed.

Other Calibrations

The calibrations which users will have to perform before and during a batch run are described, with instructions, in the sections under “Before Starting a Batch Run” on page 63, and “Starting a Run” on page 73. Additional calibrations can be performed, but such calibrations should only be performed after consulting

with PBS Biotech Technical Support. For more information on calibrations, see “Calibrating/Configuring Sensors” on page 154.

Display Client

The software interface of the PBS-MiniPRO is the Display Client. It is automatically launched when the Control Module is turned on.

Interlocks

To prevent unsafe conditions or conditions that would hinder the growth of cells, the software interlocks the controllers when certain conditions are met.

Conditions Causing Interlocks								
Interlocked Controls		**Agitation**	**Temperature**	**Level**	**Process Stop Button**	**Vessel**	**Main Gas**	**Pump Cover**
	Agitation			Estimated Level < Level Lo Lo value - 5 mL	Process Stop Button has been pushed			
	Temperature	Agitation Controller is Off	Temperature PV > Temperature Sensor Hi Hi value or Temperature PV > Temperature PID Hi Hi value	Estimated Level < Level Lo Lo value - 5 mL	Process Stop Button has been pushed	No vessel detected		
	Gases				Process Stop Button has been pushed			
	pH and DO				Process Stop Button has been pushed		Gas Totalizer is Off	
	Pump A			Estimated Level > Level Hi Hi value*	Process Stop Button has been pushed			Pump cover is open
	Pump B			Estimated Level < Level Lo Lo value*	Process Stop Button has been pushed			Pump cover is open

* The Media Exchange Phase is configured to ignore these interlocks.

To see the active interlocks for a particular controller, click the controller's faceplate, then the "Interlock Information" button.

Agitation

The Vertical-Wheel® impeller is magnetically coupled to a motor in the bioreactor which controls agitation output.

The agitation controller has two user modes:

- Off mode
- Manual mode

Off Mode

No power is supplied to the motor.

Manual Mode

User selects an RPM value which the motor is configured to deliver.

Output Ranges

For agitation control range, see “Agitation Control Range” on page 46.

Relevant Custom Settings

Each setting’s definition is included in Appendix 1, which starts on page 168.

Agitation EM Settings (page 168)

- Speed Deviation

Interlocks

The agitation motor will not turn on if the Estimated Level is less than the Level Lo Lo value minus 5 mL; this is so Pump B gets interlocked before Agitation gets interlocked.

The agitation motor will not turn on if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

When the agitation motor is running and then becomes interlocked, and the interlock condition is no longer true/goes away, the agitation motor will not automatically restart; a user will have to turn the motor back on.

Temperature

The temperature PV, reported in degrees celsius (°C), is determined by the built-in temperature sensor, which is positioned on the right side of the sleeve.

The temperature controller has three user modes:

- Off mode
- Manual mode
- Auto mode

Off Mode

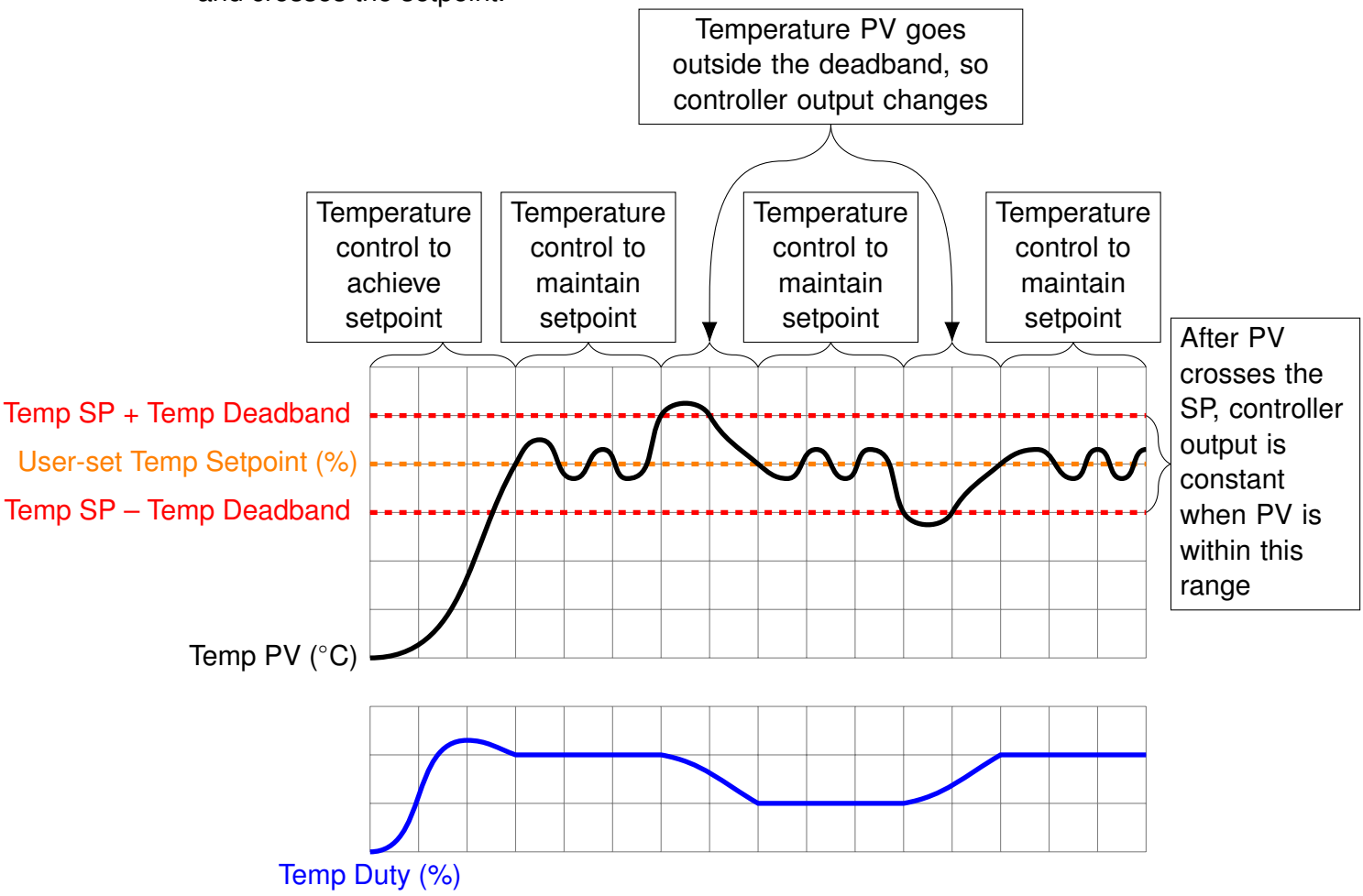
The main heater is off.

Manual Mode

User selects a main heater duty as a percentage of its maximum power.

Auto Mode

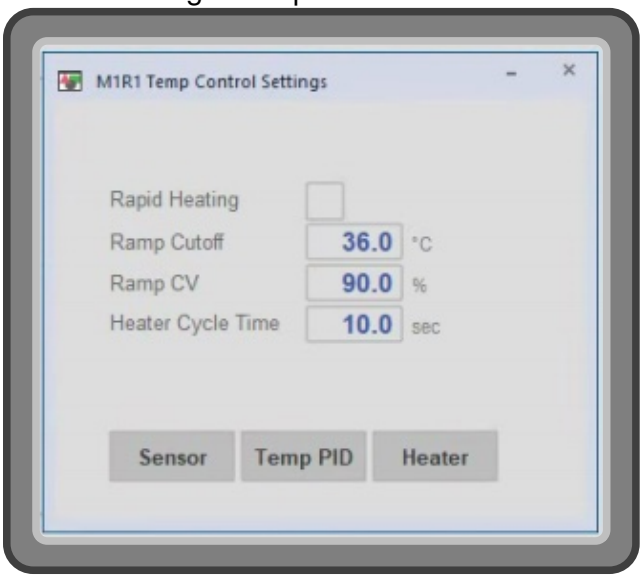
User selects a setpoint in °C. A PID controller adjusts the main heater duty to attempt to achieve the setpoint. After the Temperature PV enters the deadband and has crossed the setpoint, the controller output is kept constant while the Temperature PV is within the Temperature setpoint ± the Temperature deadband. If the Temperature PV deviates outside of the deadband, the controller output will change until the Temperature PV re-enters the deadband and crosses the setpoint.



This type of deadband, where control doesn't hold steady immediately when the PV is within the deadband but only does when the PV crosses the Setpoint is called a "zero crossing" deadband. This deadband behavior is enabled when the "Temp Deadband" is set to a nonzero value. The deadband should not be changed by anyone who is not knowledgeable about control strategy.

Rapid Heating

The Rapid Heating feature in Temperature Auto mode allows the operator to set a CV (the “Ramp CV” setting) for the Temperature controller to use while ramping up to the setpoint, instead of relying on the PID to determine the CV. Once the PV is greater than or equal to the “Ramp Cutoff” setting (which should be set to be lower than the setpoint, to prevent overshoot), the PID takes over to calculate CV. Check the “Rapid Heating” checkbox in the “Temp Control Settings” faceplate to enable this feature.



Output Ranges

For temperature control range, see “Temperature Control Range” on page 46.

The recommended main heater duty output range is 0 – 100%.

Relevant Custom Settings

Each setting’s definition is included in Appendix 1, which starts on page 168.

Temp Control Settings (page 168)

- Rapid Heating
- Ramp Cutoff
- Ramp CV
- Heater Cycle Time

Interlocks

The main heater will not turn on if the agitation controller is Off. This is to avoid creating temperature gradients in the vessel while heating.

The main heater will not turn on if the temperature PV is greater than the Temperature Sensor Hi Hi value, or greater than the Temperature PID Hi Hi value. This protects the run against a sensor error or an improperly entered setpoint. Note that the Temperature PID Hi Hi value is not associated with any enabled alarms, but it is still used for this interlock.

The main heater will not turn on if the estimated Level is less than the Level Lo Lo value minus 5 mL. This prevents damage to the disposable or its contents when the bioreactor system cannot properly control temperature at low volumes, and is set up so Pump B gets interlocked before the main heater does.

The main heater will not turn on if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

The main heater will not turn on if there is no vessel detected. This is to avoid heating the air in the vessel sleeve or exposing operators to hot surfaces.

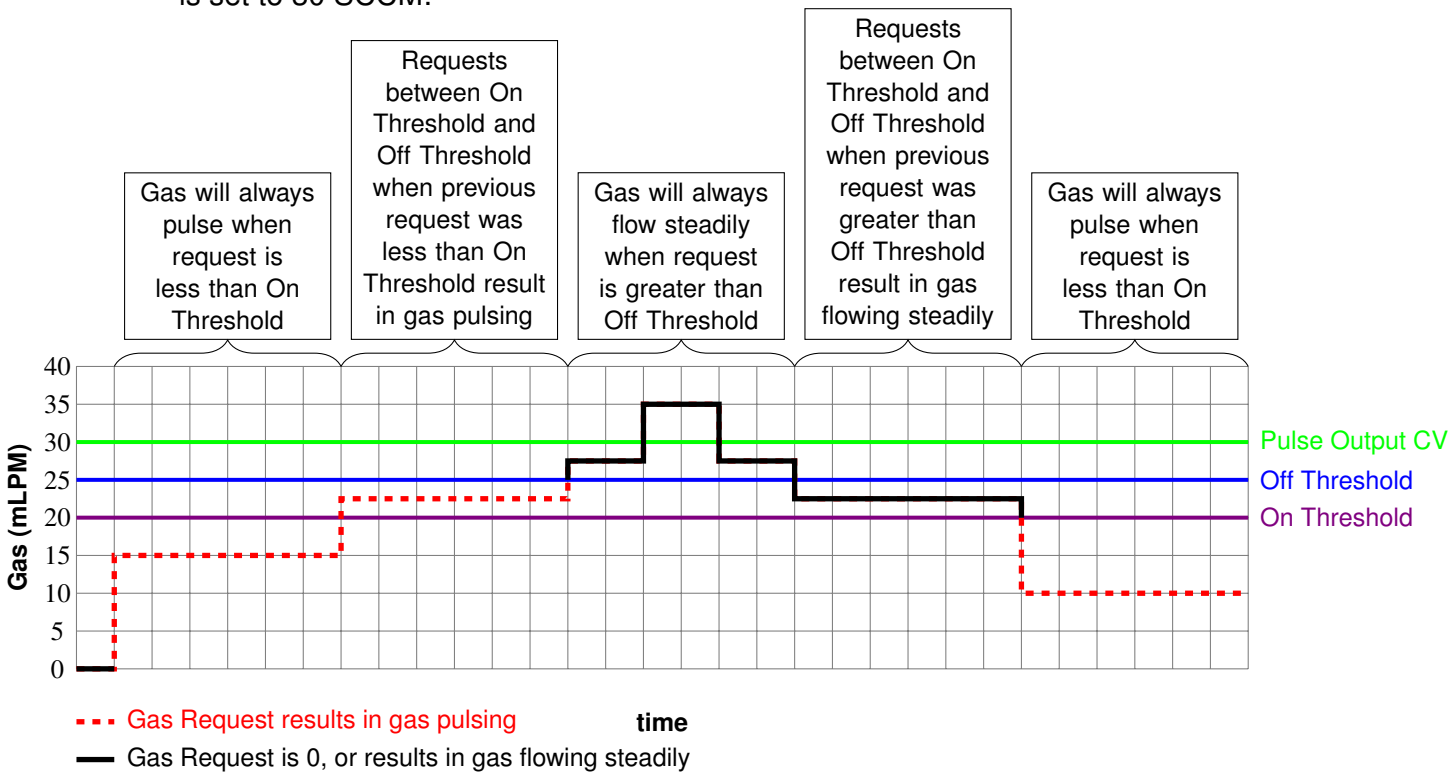
Main Gas

The main gas PV, reported in milliliters per minute (mLPM), is determined by reading the feedback voltages from the four gas mass flow controllers (MFCs): Air, N_2, O_2, and CO_2. Main gas only has two modes: Off, where no gas flows, and Manual, where the gas flows at the rate requested by the user.

The gas flow rate ranges for the MFCs are defined in “Gas Flow Rate Range” on page 46. If the requested flow rate for an individual gas is less than the MFC’s minimum flow rate, the MFC will “pulse” its output to meet the request. A requested flow rate of the MFC’s “Off Threshold” value or higher will always result in a steady flow being delivered. A requested flow rate of the MFC’s “On Threshold” value or lower will always result in the MFC delivering “pulses” of gas at the MFC’s “Pulse Output CV” value which over time average out to deliver the requested flow rate.

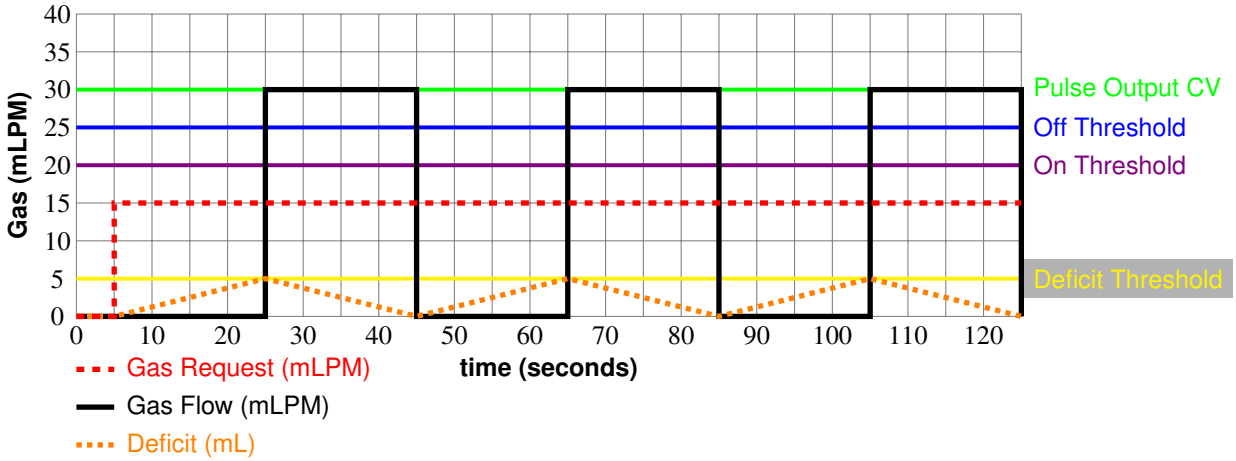
If the requested flow rate is between the “On Threshold” and “Off Threshold” values, then whether the MFC pulses will depend on which direction it entered the deadband from. If the MFC had been pulsing because the earlier request was below the “On Threshold” value, it will continue to pulse while the request is between the “On Threshold” and “Off Threshold” values. If the MFC had been flowing steadily because the earlier request was above the “Off Threshold” value, then it will continue to flow steadily between the “On Threshold” and “Off Threshold” values.

For example, the following chart shows which requested gas flow rates will result in the gas flowing steadily versus pulsing when the “On Threshold” is set to 20 SCCM, the “Off Threshold” is set to 25 SCCM, and the “Pulse Output CV” is set to 30 SCCM:

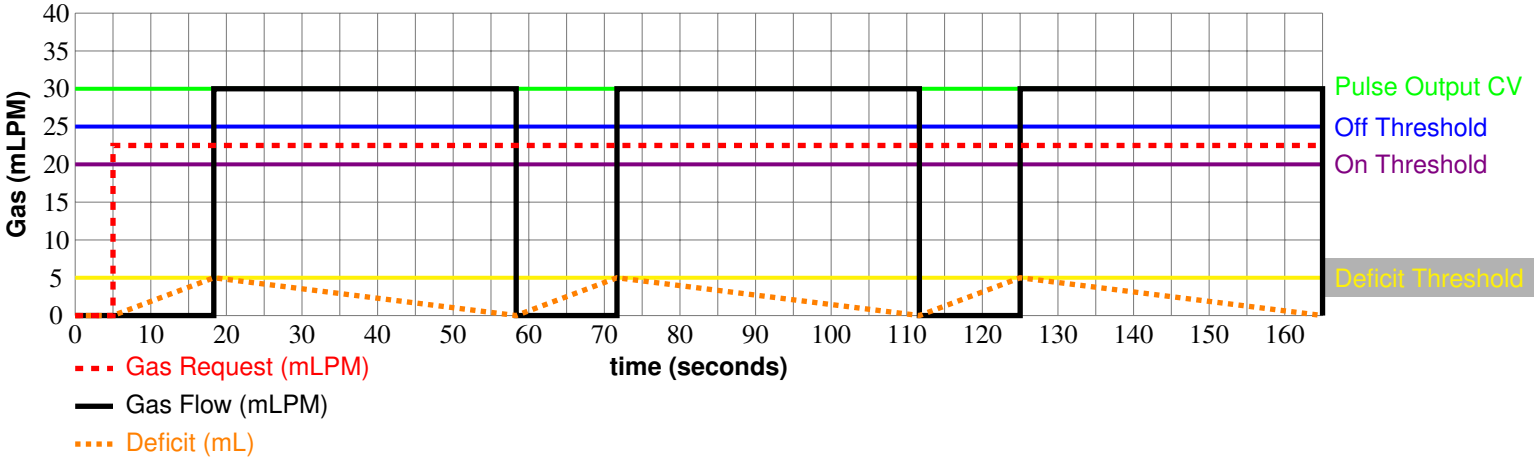


As the MFC's actual flow rate continues to be zero while having a nonzero requested flow rate, the software calculates a "Deficit" in volume of gas flowed. Once this deficit reaches the "Deficit Threshold" the MFC turns on and will flow at the "Pulse Output CV" value until the deficit is back to 0 mL, at which point the MFC will turn off.

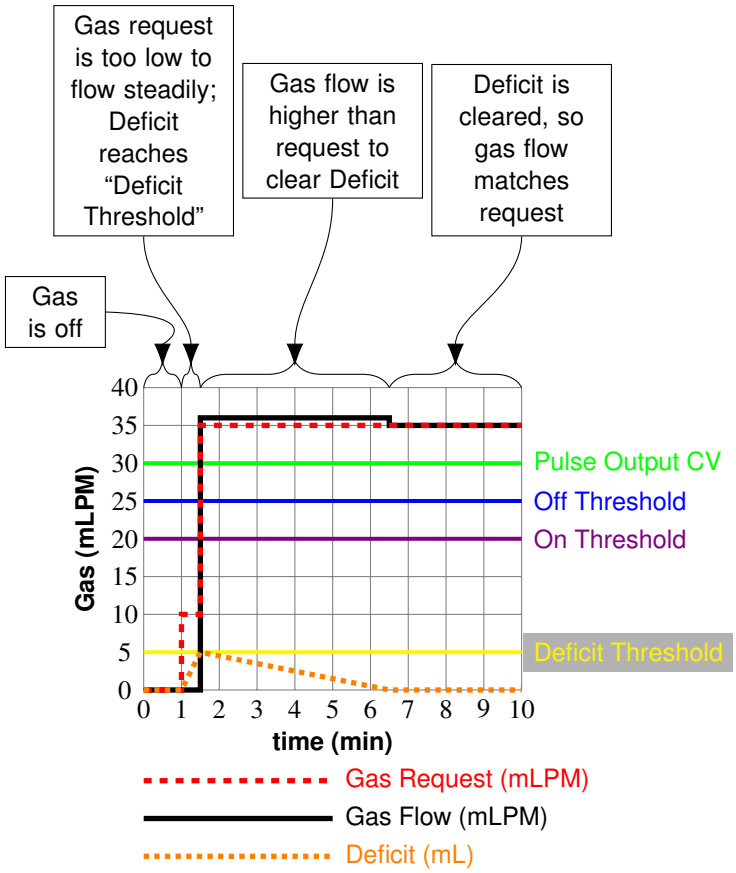
For example, if the MFC's "Pulse Output CV" is set to 30 SCCM, and the requested flow rate is 15 mLPM (which is less than the "On Threshold" value of 20 SCCM), the MFC will pulse at 30 mLPM for half the time, and be off for half the time, and this effectively delivers 15 mLPM. The following example shows the timing for when the "Deficit Threshold" is set to 5 mL:



A higher flow request (in the below example, between the "On Threshold" and "Off Threshold" values) resulting in the MFC pulsing will result in the Deficit hitting the "Deficit Threshold" value more quickly, and it taking longer to clear the Deficit while the MFC is pulsing:



If the gas starts to flow steadily while the Deficit is nonzero, the MFC will actually flow at a slightly higher rate than the flow request until the Deficit is cleared. It will flow the flow request plus the “Overflow Payoff Rate.” The following example shows what will happen if the MFC starts to flow steadily when the Deficit initially was equal to the “Deficit Threshold” of 5 mL, and the “Overflow Payoff Rate” is 1 SCCM:



The software prioritizes the gas composition to meet the pH and DO controller requests in the following order:

1. CO_2
2. O_2
3. N_2
4. Air (remainder of request)

Relevant Custom Settings

Each setting’s definition is included in Appendix 1, which starts on page 168.

Gas Control Settings (page 169)

- High Deviation
- Low Deviation

- CO2 Minimum
- O2 Minimum
- N2 Minimum
- CO2 Maximum
- O2 Maximum
- N2 Maximum

Gas Control EM Advanced (page 172)

- Midpoint

CO2 Pulse (page 169)

- Pulse Output CV
- Off Threshold
- On Threshold
- Deficit Threshold
- Overflow Payoff Rate
- Enabled/Disabled

O2 Pulse (page 170)

- Pulse Output CV
- Off Threshold
- On Threshold
- Deficit Threshold
- Overflow Payoff Rate
- Enabled/Disabled

N2 Pulse (page 171)

- Pulse Output CV
- Off Threshold
- On Threshold
- Deficit Threshold
- Overflow Payoff Rate
- Enabled/Disabled

Air Pulse (page 171)

- Pulse Output CV
- Off Threshold
- On Threshold
- Deficit Threshold
- Overflow Payoff Rate
- Enabled/Disabled

Interlocks

Gases will not flow if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

Dissolved Oxygen

The dissolved oxygen PV is reported as a percent of Air Saturation [(%) or (DO%)] and is determined by a DO sensor. A measurement of 100% DO does not mean the media is fully saturated with Oxygen, but instead that the media is fully saturated with Air. Because the DO sensor is fluorometric, shining a bright light directly onto the DO sensor spot can affect the DO PV.

The DO is controlled by varying the N_2 and O_2 gas flow as a percentage of main gas flow. The DO PV is lowered by increasing the % N_2 composition, and is raised by increasing the % O_2 composition. To understand how the software

determines which gases to flow, see “Main Gas” on page 138.

The DO controller has three user modes and one sensor error mode:

- Off mode
- Manual mode
- Auto mode
- Sensor error mode

Off Mode

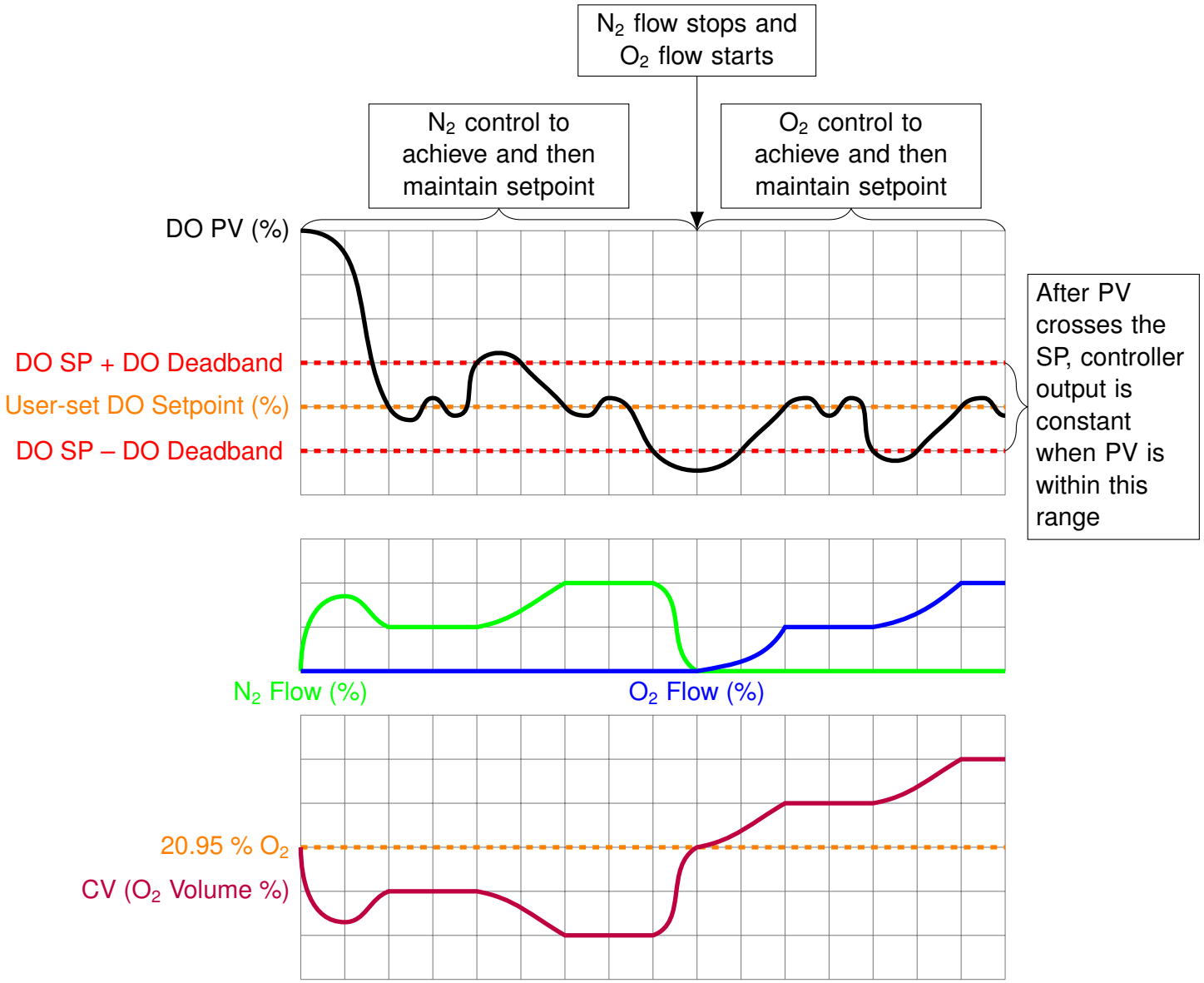
N_2 and O_2 are 0% of main gas flow.

Manual Mode

User selects N_2 and/or O_2 flow as a percentage of main gas flow.

Auto Mode

User selects a setpoint in units of % dissolved oxygen, which the software achieves by adjusting N_2 flow and O_2 flow. The gases are both output by a split-range PID loop. Which gas flows is determined by the CV (Control Variable). If the CV is less than 20.95 % O_2 (the percent of Oxygen in Air), the controller outputs N_2. If the CV is greater than 20.95 % O_2, the controller outputs O_2. After the DO PV enters the deadband and has crossed the setpoint, the controller output is kept constant while the DO PV is within the DO setpoint ± the DO deadband. If the DO PV deviates outside the deadband, the controller output will change until the DO PV re-enters the deadband and crosses the setpoint.



This type of deadband, where control doesn't hold steady immediately when the PV is within the deadband but only does when the PV crosses the Setpoint is called a "zero crossing" deadband. This deadband behavior is enabled when the "DO Deadband" is set to a nonzero value. The deadband should not be changed by anyone who is not knowledgeable about control strategy.

This is an "exclusive split range" deadband, because N_2 and O_2 cannot flow at the same time.

Sensor Error Mode

When DO is in Auto Mode and the DO sensor's raw value goes outside the valid range, or there is an IO fault from the DO sensor, the DO controller outputs the last known good CV. Note that this is only intended to preserve short term stability – users should still take appropriate action in the event of sensor failure.

Output Ranges

The recommended N_2 output is 0 - 100% of main gas flow. The N_2 MFC output is stated in "Gas Flow Rate Range" on page 46. N_2 "pulsing" at the minimum value takes effect if the N_2 % called for represents less than the MFC's minimum flow rate.

The O_2 MFC output is stated in "Gas Flow Rate Range" on page 46. O_2 "pulsing" at the minimum value takes effect if the O_2 % called for represents less than the MFC's minimum flow rate.

For a full explanation of MFC pulse logic, see "Main Gas" on page 138.

Interlocks

N_2 and O_2 will not flow if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

N_2 and O_2 will not flow if the Gas Totalizer is Off.

pH

The pH PV is determined by a pH sensor. The sensor head uses internal temperature compensation to provide more accurate pH readings.

The pH is controlled by varying the CO_2 flow in % composition of main gas flow. Increasing CO_2 flow decreases pH PV. To understand how the software

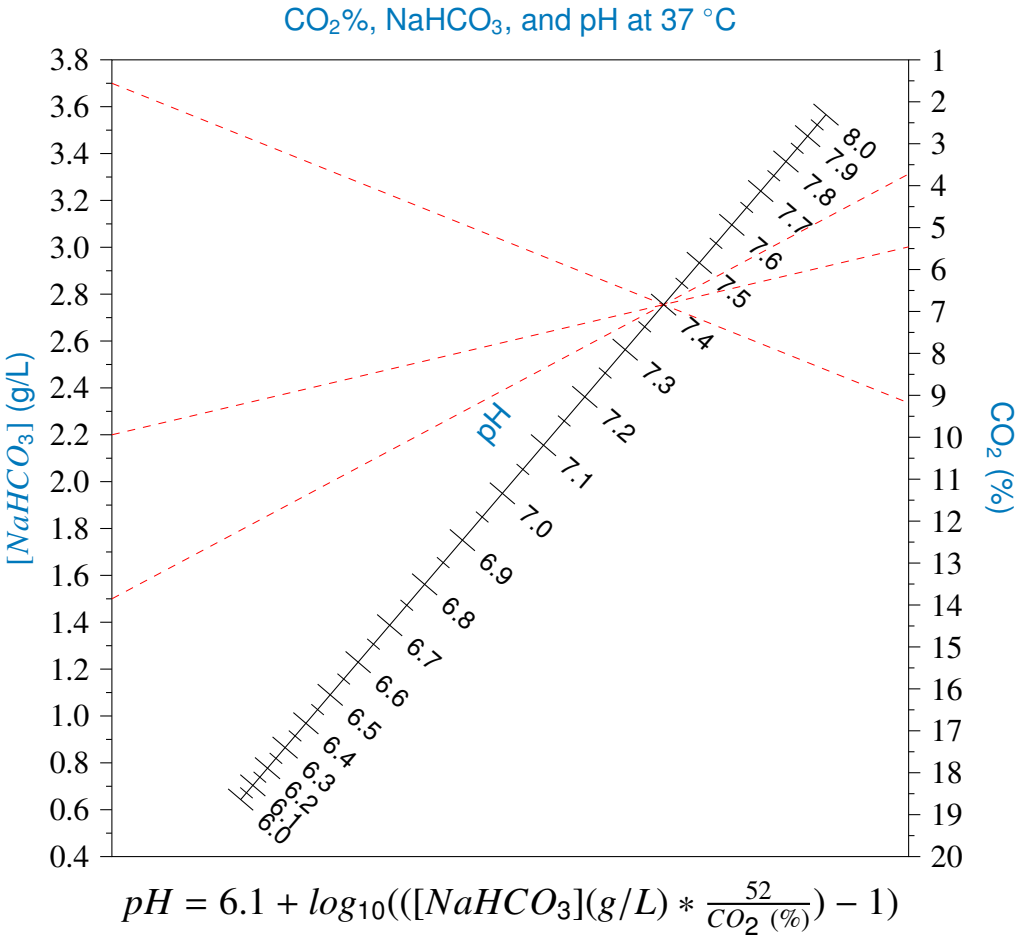
determines which gases to flow, see “Main Gas” on page 138.

Before inoculating (i.e. when there is no metabolic activity), the pH has a predictable relationship with the concentration of sodium bicarbonate ($NaHCO_3$) in the medium and the % CO_2 composition. Below the following chart is the equation to calculate the resulting pH from a known concentration of sodium bicarbonate and a known % CO_2 composition. However, the following chart can be simpler to use.

To find the pH that would result from a known concentration of sodium bicarbonate and a known % CO_2 composition, draw a straight line between the points on the sodium bicarbonate and CO_2 axes. The line will cross the pH axis at the pH value. In fact, the chart can be used to find the third variable if any of the other two are known.

For example, if the medium being used has a sodium bicarbonate concentration of 3.7 g/L and the desired pH is 7.4, draw a straight line between those points on the corresponding axes, and extend the line to the CO_2 axis. You can see that a % CO_2 composition of just over 9% will result in the desired pH.

You can also see that to get the same pH using sodium bicarbonate concentrations of 2.2 g/L and 1.5 g/L will require % CO_2 compositions of about 5.5% and 3.5%, respectively.



The pH controller has three user modes and one sensor error mode:

- Off mode
- Manual mode
- Auto mode
- Sensor error mode

Off Mode

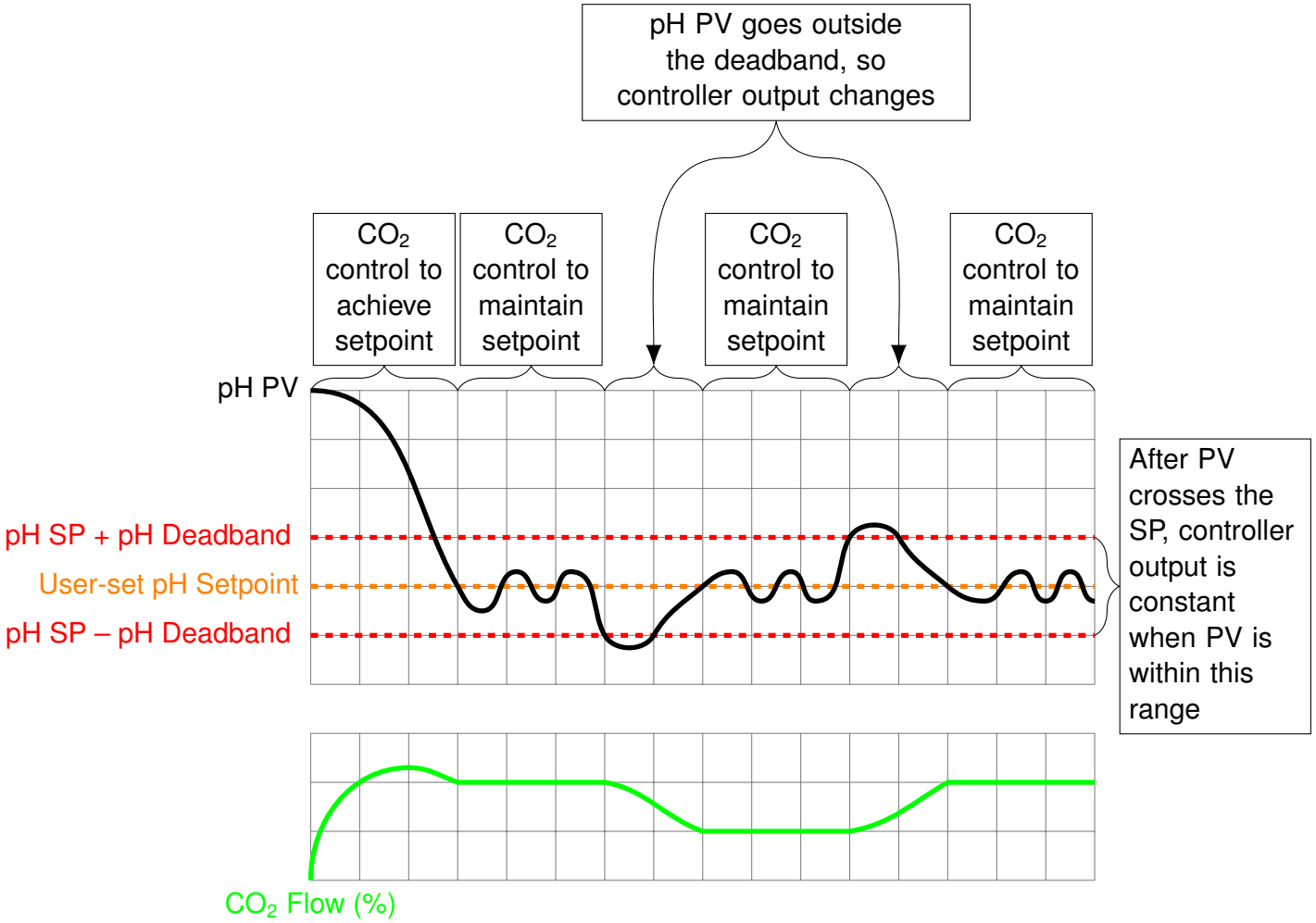
CO_2 is 0% of main gas flow.

Manual Mode

User selects a CO_2 flow in % composition of main gas flow.

Auto Mode

User selects a setpoint in pH units. The software achieves the setpoint by adjusting CO_2 flow. Once the pH PV becomes less than the setpoint, the controller output is kept constant while the pH PV is within the pH setpoint ± the pH deadband.



This type of deadband, where control doesn't hold steady immediately when the PV is within the deadband but only does when the PV crosses the Setpoint is called a "zero crossing" deadband. This deadband behavior is enabled when the "pH Deadband" is set to a nonzero value. The deadband should not be changed by anyone who is not knowledgeable about control strategy.

Sensor Error Mode

When pH is in Auto Mode and the pH sensor's raw value goes outside the valid range, or there is an IO fault from the pH sensor, the pH controller outputs the last known good CV. Note that this is only intended to preserve short term

stability – users should still take appropriate action in the event of sensor failure.

Output Ranges

The recommended CO_2 output is 0 - 100% CO_2 composition of main gas flow. The CO_2 MFC output is stated in “Gas Flow Rate Range” on page 46. CO_2 “pulsing” at the minimum value takes effect if the CO_2 % called for represents less than the MFC minimum flow rate. For a full explanation of MFC pulse logic, see “Main Gas” on page 138.

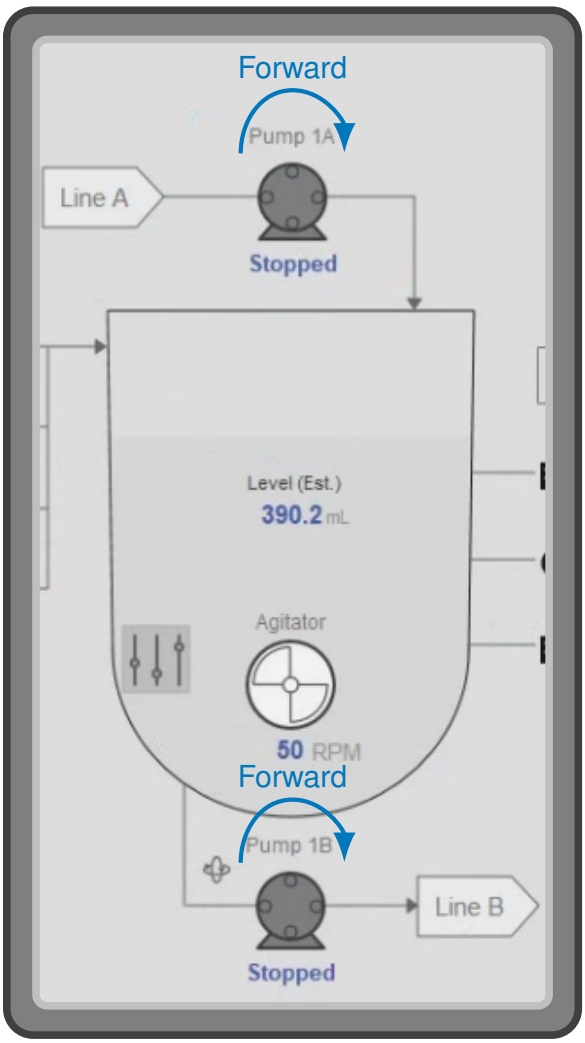
Interlocks

CO_2 will not flow if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

CO_2 will not flow if the Gas Totalizer is Off.

Estimated Level

The Estimated Level is calculated by net flow of pump totalizers. For both pumps, clockwise is considered ‘Forward.’ For standard operation, Pump A is designated for addition and Pump B for removal. Flowing either pump clockwise increases its totalizer, and flowing counter clockwise decreases its totalizer. The Estimated Level is Manual + A - B.



For the estimated level to work properly, the user must perform a level adjustment to 0 mL with an empty vessel. After filling the vessel with medium, before turning any controls on, the user should perform a level adjustment to the filled volume if the Estimated Level reported by the software is significantly different from the actual volume in the vessel.

The working level range of the PBS-MiniPRO is 300 – 500 mL. Below the minimum, the Vertical-Wheel® impeller is not fully covered and may not function optimally, but certain processing steps may be performed with volumes as low as 100 mL. Above the maximum there is the danger of overfilling the vessel, causing overflow.

Pumps

Functions

Switches Each pump has a corresponding switch on the Base Module that can be used to operate the pump without the software. The pump will flow at the “Hand Speed” setting.

Manual Use the pump menu to turn a pump on clockwise to a user-set flow rate. Users can either use the “Start CW” button to start the pump, and the “Stop Motor” button to stop it, or they can hold down the “Jog CW” button to run the pump and let go of it when they want the pump to stop.

Dispense The user uses the pump menu to set a direction, flow rate, and volume. The pump then flows in that direction at that flow rate until the requested volume is delivered, or the user stops the pump.

Perfusion The user uses the pump menu to set a flow rate, cycle time, and on time. The pump turns on for the ‘On Time’ at the ‘Speed’ or flow rate, then turns off for the remainder of the ‘Cycle Time.’

Relevant Custom Settings

Each setting’s definition is included in Appendix 1, which starts on page 168.

Pump Settings (page 172)

- Pump A Hand Speed
- Pump B Hand Speed

Interlocks

Reasons for interlocks

Pump A will not turn on if the Estimated Level is greater than the Level Hi Hi value. This is to prevent overfilling the vessel.
Pump B will not turn on if the Estimated Level is less than the Level Lo Lo value. This is to prevent inadvertently emptying the vessel.
The Media Exchange Phase is configured to ignore both these interlocks.

Both pumps will not turn on if the Process Stop Button has been pushed. This acts similarly to an emergency stop.

A pump will not turn on if its pump cover is open.

Using a pump interlocked due to Estimated Level

If Pump A is interlocked due to the Estimated Level being above the Level Hi Hi value, you have the following options to get around the interlock:

- Use Pump B instead
- Adjust the Estimated Level, especially if its current value is not accurate (see “Level Adjustment to Filled Volume” on page 86)
- Temporarily shelve or suppress or disable the Level Hi Hi alarm
- Temporarily increase the Level Hi Hi value

If Pump B is interlocked due to the Estimated Level being below the Level Lo Lo value, you have the following options to get around the interlock:

- Use Pump A instead
- Adjust the Estimated Level, especially if its current value is not accurate (see “Level Adjustment to Filled Volume” on page 86)
- Temporarily shelve or suppress or disable the Level Lo Lo alarm
- Temporarily increase the Level Lo Lo value

Additional information about interlocks

When a pump is running and then becomes interlocked, and the interlock condition is no longer true/goes away, the pump’s motor will not automatically restart; a user will have to turn the motor back on. This is not true for Perfusion; if the pump was running in Perfusion mode and becomes interlocked, and the interlock condition goes away, the pump will automatically start running again.

Additional Perfusion Information

An overview of how the Perfusion function works is in the “Perfusion” entry of the “Functions” subsection on page 151.

It is important to note that the Cycle Time cannot be less than the On Time. Also note that both pumps need to be configured and started. Over time, even when both pumps are set to the same perfusion settings, the Estimated Level may drift slightly due to small decimal rounding, and this may require periodically adjusting the Estimated Level. If, over time, it is determined that one pump is flowing slower than the other, the operator can either adjust the cycle time and/or on time of one of the pumps (which may require periodically adjusting the Estimated Level to keep the PV within alarm thresholds), or recalibrate one of the pumps.

It is recommended to use a ‘Speed’ of 1.0 mLPM because the pump flowrate can be less accurate at speeds below 0.5 mLPM, and 1.0 mLPM is a more convenient number to use for calculations. It is recommended to use an ‘On Time’ of 60 seconds primarily for convenience in calculations, but additionally

because shorter ‘On Times’ can make it difficult for an operator to confirm that liquid is actually pumping successfully - poor welds, kinks in tubing, or accidentally leaving a tubing line clamped can all potentially go unnoticed if the ‘On Time’ is too short.

The necessary ‘Cycle Time’ to achive a desired Vessel Volume per Day (VVD) based on the working vessel volume, ‘Speed,’ and ‘On Time’ can be calculated from the equation below the following chart. However, the following chart can be simpler to use, especially if the ‘Speed’ is set to 1.0 mLPM and ‘On Time’ is set to 60 seconds.

Cycle Time (sec)
(for Speed=1 mL/min and On Time=60 sec)

		Working Volume (mL)				
VVD (Vessel Volume per Day)		**300**	**350**	**400**	**450**	**500**
	0.1	2880	2469	2160	1920	1728
	0.2	1440	1234	1080	960	864
	0.3	960	823	720	640	576
	0.4	720	617	540	480	432
	0.5	576	494	432	384	346
	0.6	480	411	360	320	288
	0.7	411	353	309	274	247
	0.8	360	309	270	240	216
	0.9	320	274	240	213	192
	1.0	288	247	216	192	173

$$Cycle\ Time\ (sec) = \frac{Speed\ (mL/min) * On\ Time\ (sec)}{Working\ Volume\ (mL) * VVD} * \frac{1440\ minutes}{day}$$

Main Light

The PBS-MiniPRO has a white LED light to illuminate the contents of the vessel. It can be turned on and off through the software. This light source does not impact the DO sensor spot or pH sensor spot and therefore the DO or pH PVs, but other sources of light might and therefore operators should use caution when using other light sources.

Calibrating/Configuring Sensors

Pre-Calibration Medium Conditioning Strategy

After adding cell culture medium but before inoculating with cells, the DO and pH sensors must be calibrated. It is true that the calibration parameters for the specific vessel being used should have been entered before adding cell culture medium (see “Enter Vessel Calibration Information” on page 63). However, a further calibration is necessary because the vessel-specific calibration parameters are only an estimate; they can be affected by the sterilization process, or by shipping or handling. Before the DO and pH sensors are calibrated, their reported measurements should not be considered accurate or reliable. In order to calibrate these sensors, the cell culture medium first needs to be conditioned, and the reported sensor PVs need to equilibrate. The controls should be set so the medium is in the ideal condition for the cells being cultured.

One reason for this is that if the medium is already in the ideal condition for the cells being cultured, the operators will not have to wait any time between calibrating the DO and pH sensors and inoculating with the cells. For example, if the operator were to set pH in Auto mode before calibrating, with a setpoint of pH=7.4, the software will adjust the percent of CO_2 in the headspace until the pH sensor reports a measurement of 7.4. However, because the sensor has not been calibrated yet, and the vessel-specific calibration parameters are only an estimate (which is why calibrating them is necessary), the actual pH of the media in the vessel is very unlikely to be 7.4. If it is actually 7.5 or 7.3, for example, then after performing a calibration, the operator will have to wait to inoculate until the software adjusts the percent of CO_2 in the headspace and the pH sensor again reports a measurement of 7.4. Otherwise the bioreactor wouldn’t be an optimal environment for the cells. But if the operator knew that the cell culture medium will have a pH of 7.4 from running 5% CO_2 in the headspace, the operator could set pH to Manual mode at 5% CO_2 to equilibrate (see the “$NaHCO_3$, CO_2%, and pH at 37 °C” chart on page 146 for more information). After equilibration and before calibration, it would not matter if the pH sensor reports 7.3 or 7.5, because the operator would calibrate it to be 7.4 (after verifying by taking a sample and measuring its pH). The operator could then immediately inoculate because the bioreactor would already be an optimal environment for the cells.

A second reason is that the DO and pH sensors should be calibrated to a measurement that is as close as possible to the condition the software will be controlling to during the cell culture run. This will minimize inaccuracy in the reported sensor measurement. Calibrating to a measurement which will not actually be controlled to introduces unnecessary inaccuracy in the reported

sensor measurement. While the software uses a straight line to scale from the raw input minimum and maximum to the scaled minimum and maximum, no sensor is 100% perfect, and there are inaccuracies. In the above example, when the operator put pH in Auto mode with a setpoint of 7.4 before calibrating, let's say that the actual PV was 7.3. When the operator then calibrates the pH to read 7.3, the sensor's reading at that single point has less inaccuracy than it would for any other pH PV. But because the operator intends to control the pH at 7.4 during the cell culture run via Auto mode, it would be in their best interest to manually control pH to conditions that will result in the pH being 7.4 before that first calibration. This can be accomplished by using the "$NaHCO_3$, CO_2%, and pH at 37 °C" chart on page 146 and setting the CO_2% by putting pH in Manual mode. Then, the operator could calibrate the pH reading to equal 7.4, and the running condition will match the PV where sensor inaccuracy has been minimized.

The agitation and main gas controls need to be on, so the contents of the vessel are mixed homogenously, the Temperature is not interlocked, and the gases flowing through the headspace are able to efficiently diffuse into the medium. The agitation can be set to control to a higher RPM during this stage than when the bioreactor is inoculated, to speed up the process of conditioning the medium. Similarly, setting main gas flow to a higher flow rate during this stage than when the bioreactor is inoculated will also speed up the process of conditioning the medium. Or, operators could instead choose to set the main gas flow to a lower flow rate, to minimize gas use before inoculation. This may require additional time to condition the medium. The temperature control should be set to the temperature optimal for the cells. For most applications, this is 37 °C. This is important because the temperature of the medium has an effect on both the DO and pH of the medium.

It is recommended to condition the medium before these first calibrations by controlling DO and pH in Manual mode, rather than Auto mode. As explained above, this saves time and also minimizes calibration inaccuracy. Additionally, if DO is controlled in Auto mode, then the operator would be required to measure the DO of a sample to use as a reference when performing the calibration. For both pH and DO, regardless of whether the controller is in Auto or Manual mode, care must be taken when taking the sample and measuring it to ensure accuracy, as off-gassing can result in the sample's gas composition changing to be different from that of the medium. This is especially difficult to avoid for the DO. For more information, see "Sampling" on page 157. This off-gassing can lead to the measurement of the sample not being accurate, and calibrations being performed to inaccurate or non-representative reference measurements can result in the calibrated sensor measurements being less accurate.

While it is technically possible to use DO and/or pH in Auto mode when conditioning the medium before performing the ‘high-point’/‘one-point’ calibrations, it is not recommended for the reasons explained above. Before inoculation, the gas composition of the headspace has a reliable and predictable effect on the gas composition of the medium. This means that putting DO and pH in Manual mode allows the operator to directly control the actual DO and pH of the medium. This allows the operator to use a very reliable and accurate reference when performing the ‘high-point’/‘one-point’ calibrations after the medium is conditioned and the sensor readings equilibrate.

Which Sensors Can Be Calibrated

It is possible to perform calibrations on the following sensors. Their calibration scaling minimum and maximum values can also be manually entered; however, this should not be done without consulting PBS Biotech Technical Support.

Dissolved Oxygen

For a single-use DO sensor, users should perform a ‘high-point’ calibration before inoculation. It is generally not recommended that users perform additional ‘high-point’ calibrations during a run. Users should not perform any other type of calibrations, or manually enter calibration scaling minimum and maximum values, without consulting PBS Biotech Technical Support.

This is because before inoculating with cells, the gas composition of the headspace has a reliable and predictable effect on the actual DO of the medium, so the operator can directly control the DO by putting pH and DO in Manual mode. This is the best reference to use when calibrating the DO sensor.

After inoculating with cells, the cells’ oxygen consumption additionally affects the actual DO of the medium, and so it can no longer be determined only based on the gas composition of the headspace. This means that a reference sample will have to be taken and measured, and the DO would have to be calibrated to that reference measurement. Taking the sample and handling it afterwards can introduce additional air to the sample and result in off-gassing, so the sample’s gas composition no longer matches that of the media in the vessel, and the measured DO of the sample is not representative of the DO of the media in the vessel. For more information, see “Sampling for DO Measurement” on page 159. Calibrating to an inaccurate or non-representative reference measurement can result in the calibrated sensor measurements being less accurate.

Additionally, for most applications, the DO sensor drift is minimal throughout a cell culture run. If sensor drift is suspected to be an issue for a process, it needs to be confirmed by isolating as many variables as possible when taking reference samples. Contact Applications Engineering at app.eng@pbsbiotech.com for additional information. If sensor drift is confirmed to be an issue for a process, the methods of collecting a sample and measuring it need to be confirmed to change the DO of the sample as little as possible for the reference measurement to be reliable. For more information, see “Sampling for DO Measurement” on page 159.

pH

For a single-use pH sensor, users should perform a ‘one-point’ calibration before inoculation, and throughout a run if the measured pH of a sample shows that the sensor has drifted. Users should not perform any other type of calibrations, or manually enter calibration scaling minimum and maximum values, without consulting PBS Biotech Technical Support.

Temperature

The PBS-MiniPRO is shipped with its temperature sensor already calibrated. Users should not calibrate the temperature sensor without consulting PBS Biotech Technical Support.

Sampling

For instructions to take a sample manually, see “Take Sample” on page 95.

Do not attempt to combine samples for different types of analyses, for example measuring the pH of a sample that is going to be processed for cell counts. This will introduce more variability and error into the cell count. Each sample should be unique.

When validating any sampling and measurement method, multiple samples should be taken and compared to understand the inherent variation.

Sampling for Cell Counting

Sample should be representative of the culture – A representative sample should have the same proportion of healthy cells as in the vessel. If the cells are growing in aggregates or microcarrier clumps, those amounts and morphologies should be reflected in the sample as well.

Volume – A sample of 5 mL or larger is recommended for cell counts.

Sample Method – Factors which impact how representative a sample is are the shear force the sample is subjected to as it is taken, the speed at which the sample is taken, and the location in the vessel from which the sample is taken. Passing a sample through a pump or small connectors can subject the sample to shear force and affect cell health/viability, and affect the size/shape of aggregates or microcarrier clumps. Sampling speed and location can impact the number of total cells contained in a sample.

Handling Sample – For viability counts, the sample should be handled and processed as quickly and gently as possible to avoid artificially increasing the percent of dead or unhealthy cells.

Counting Cells in Sample – Users should validate their cell count method by taking multiple samples and comparing them, to understand the inherent variation.

Sampling for pH Measurement

Concerns – As a sample is removed and manipulated, CO_2 in the cell culture medium can be stripped out and replaced with air, which will increase the pH. A sample which is left to sit in ambient conditions will also experience off-gassing, which will have the same effect as actively stripping out the CO_2. The reverse can also happen; cellular metabolic activity may continue in the sample, causing the CO_2 and/or lactic acid to be higher than the concentration in the vessel.

Volume – A sample of sufficient size for the offline pH meter to read it should be taken.

Sample Method – Minimize turbulence and air exposure while taking the sample. The bioreactor tubing is gas permeable, so as soon as liquid leaves the vessel environment it is changing from its in situ conditions.

Handling Sample – Minimize turbulence and air exposure while handling the sample - perform the measurement as quickly as possible.

Measuring Sample – Getting a measurement as quickly as possible should be the priority. A dedicated benchtop pH meter will give a measurement more quickly than a metabolite analyzer, for example. The sampling and pH measurement methods can also be validated. Before inoculating with cells, when the % CO_2 composition is manually set and the pH PV has stabilized, the measured pH should match the expected pH from the "$NaHCO_3$, CO_2%, and pH at 37 °C" chart on page 146, given the % CO_2 composition and sodium bicarbonate concentration of the medium. Taking a sample can introduce air and strip out CO_2, causing the sample's measured pH to be higher than that in the actual bioreactor. Comparing the sample's measured pH to the theoretical pH based on the

% CO_2 composition and sodium bicarbonate concentration of the medium is a good way to verify that the samples are being taken and handled appropriately.

Sampling for DO Measurement

Sampling for measuring the DO can be done regularly, but DO calibration should only be performed if DO sensor drift has been confirmed to be a significant issue. It's possible to introduce significant error by measuring the DO of a sample, and this concern should be weighed against concerns about sensor drift.

Concerns – As a sample is removed and manipulated, it will be rapidly equilibrating to the gas composition of atmospheric air, which will result in the DO of the sample rapidly approaching 100%. A sample which is left to sit in ambient conditions will also experience off-gassing, which will have the same effects as actively stripping out the other gases. Cellular metabolic activity might also continue in the sample, causing the O_2 to be lower than that of the media in the vessel.

Volume – A sample of sufficient size for the offline gas analyzer to read it should be taken.

Sample Method – Minimize turbulence and air exposure while taking the sample. The bioreactor tubing is gas permeable, so as soon as liquid leaves the vessel environment it is changing from its in situ conditions.

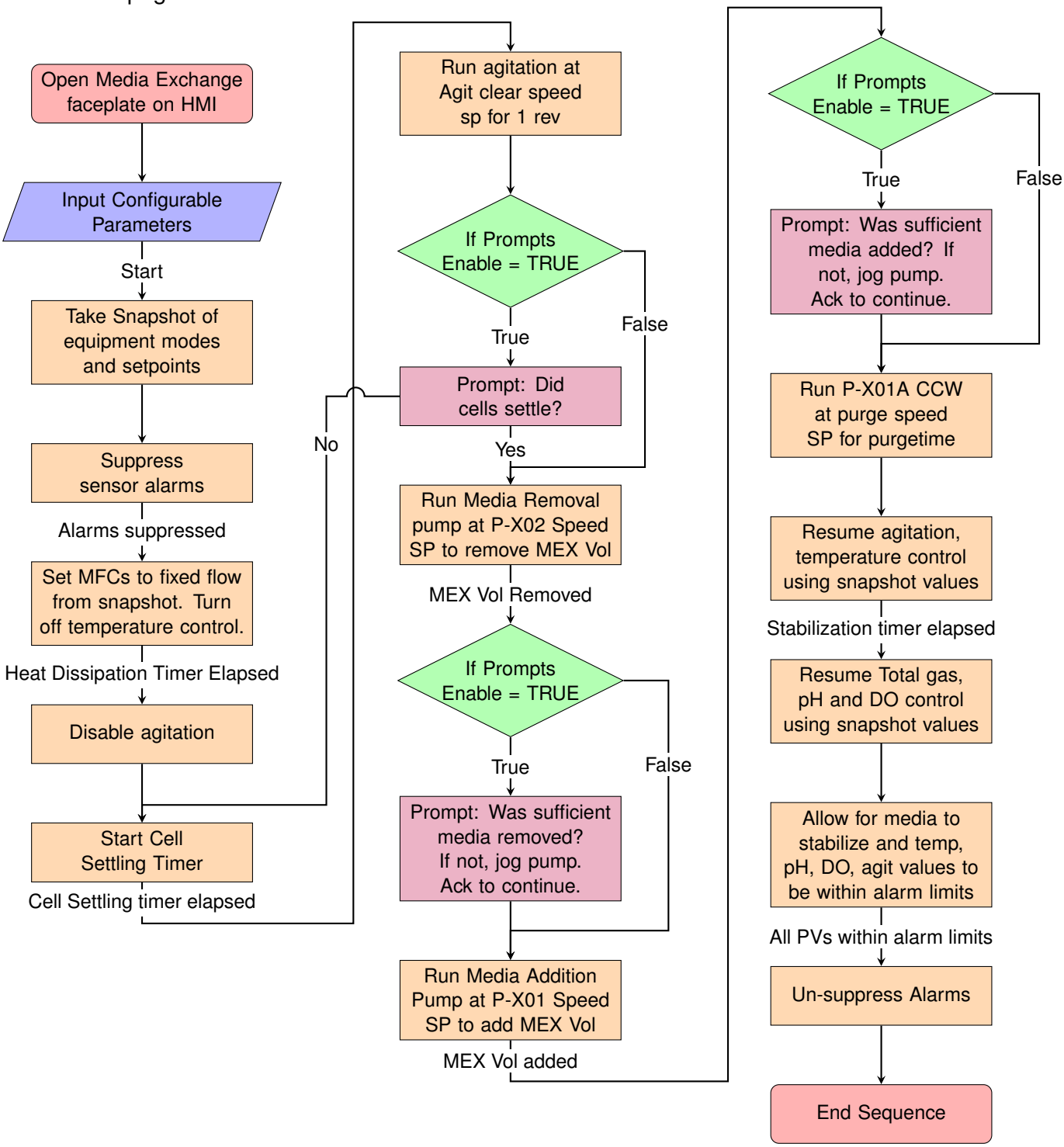
Handling Sample – Minimize turbulence and air exposure while handling the sample - perform the measurement as quickly as possible.

Measuring Sample – Getting a measurement as quickly as possible should be the priority. A blood gas analyzer will give a measurement more quickly than a metabolite analyzer, for example. The sampling and DO measurement methods can also be validated. Before inoculating with cells, when the % composition of the CO_2 and N_2 in the headspace is manually set and the DO PV has stabilized, the measured DO should match the percent of air entering the headspace. Comparing the sample's measured DO to the theoretical DO is a good way to verify that the samples are being taken and handled appropriately.

Media Exchange Phase

The Media Exchange Phase is configured to ignore pump interlocks from the Estimated Level being too high or too low. The following flowchart describes the entire process that the Media Exchange Phase goes through. For instructions on how to use the feature, see "Exchanging Medium" on page 108. For definitions of the parameters used, see "Media Exchange Phase" on

page 173.


Open Media Exchange faceplate on HMI
Input Configurable Parameters
Start
Take Snapshot of equipment modes and setpoints
Suppress sensor alarms
Alarms suppressed
Set MFCs to fixed flow from snapshot. Turn off temperature control.
Heat Dissipation Timer Elapsed
Disable agitation
Start Cell Settling Timer
Cell Settling timer elapsed
Run agitation at Agit clear speed sp for 1 rev
If Prompts Enable = TRUE
True
False
Prompt: Did cells settle?
No
Yes
Run Media Removal pump at P-X02 Speed SP to remove MEX Vol
MEX Vol Removed
If Prompts Enable = TRUE
True
False
Prompt: Was sufficient media removed? If not, jog pump. Ack to continue.
Run Media Addition Pump at P-X01 Speed SP to add MEX Vol
MEX Vol added
If Prompts Enable = TRUE
True
False
Prompt: Was sufficient media added? If not, jog pump. Ack to continue.
Run P-X01A CCW at purge speed SP for purgetime
Resume agitation, temperature control using snapshot values
Stabilization timer elapsed
Resume Total gas, pH and DO control using snapshot values
Allow for media to stabilize and temp, pH, DO, agit values to be within alarm limits
All PVs within alarm limits
Un-suppress Alarms
End Sequence

Relevant Custom Settings

Each setting's definition is included in Appendix 1, which starts on page 168.

Media Exchange Phase (page 173)

- Prompts Enabled
- Heat Dissipation Time
- Cell Settle Time
- Agitator Clear Speed
- Media Exchange Volume Type
- Media Exchange Volume
- Removal Pump Flowrate
- Addition Pump Flowrate
- Addition Pump Purge Flowrate
- Addition Pump Purge Time
- Stabilization Time
- Stabilization Timeout

Batch

Rather than manually recording the start and end dates of various runs, users can start a new batch when they start a new run and end it after harvest. This makes it easier to access the relevant data for generating a report.

Alarms

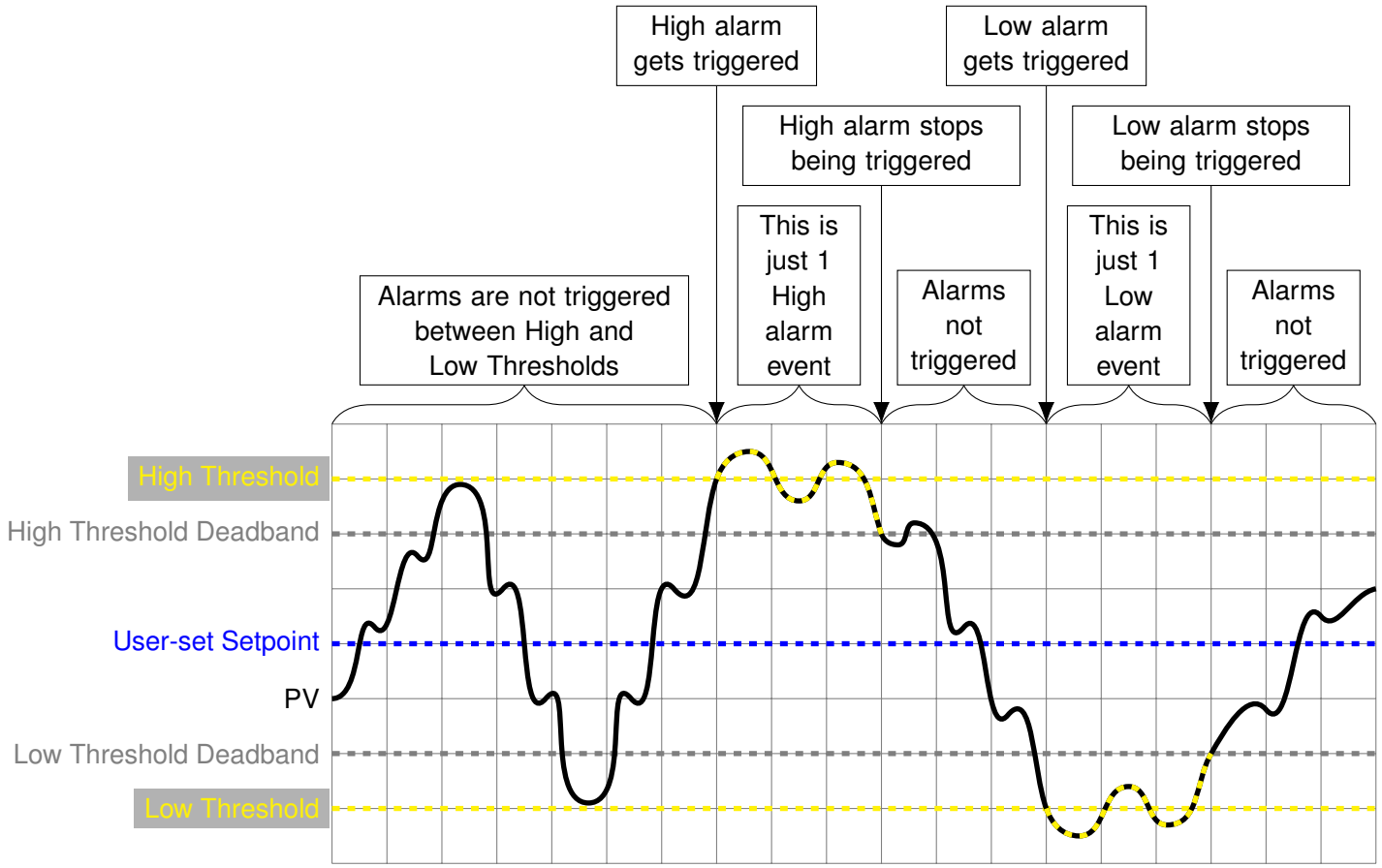
For definitions of all alarms, see Appendix 2 on page 175.

Limit alarms, Deviation alarms, and Input Failure alarms work with Thresholds for the Low Low, Low, High, High High, Low Deviation, and High Deviation conditions.

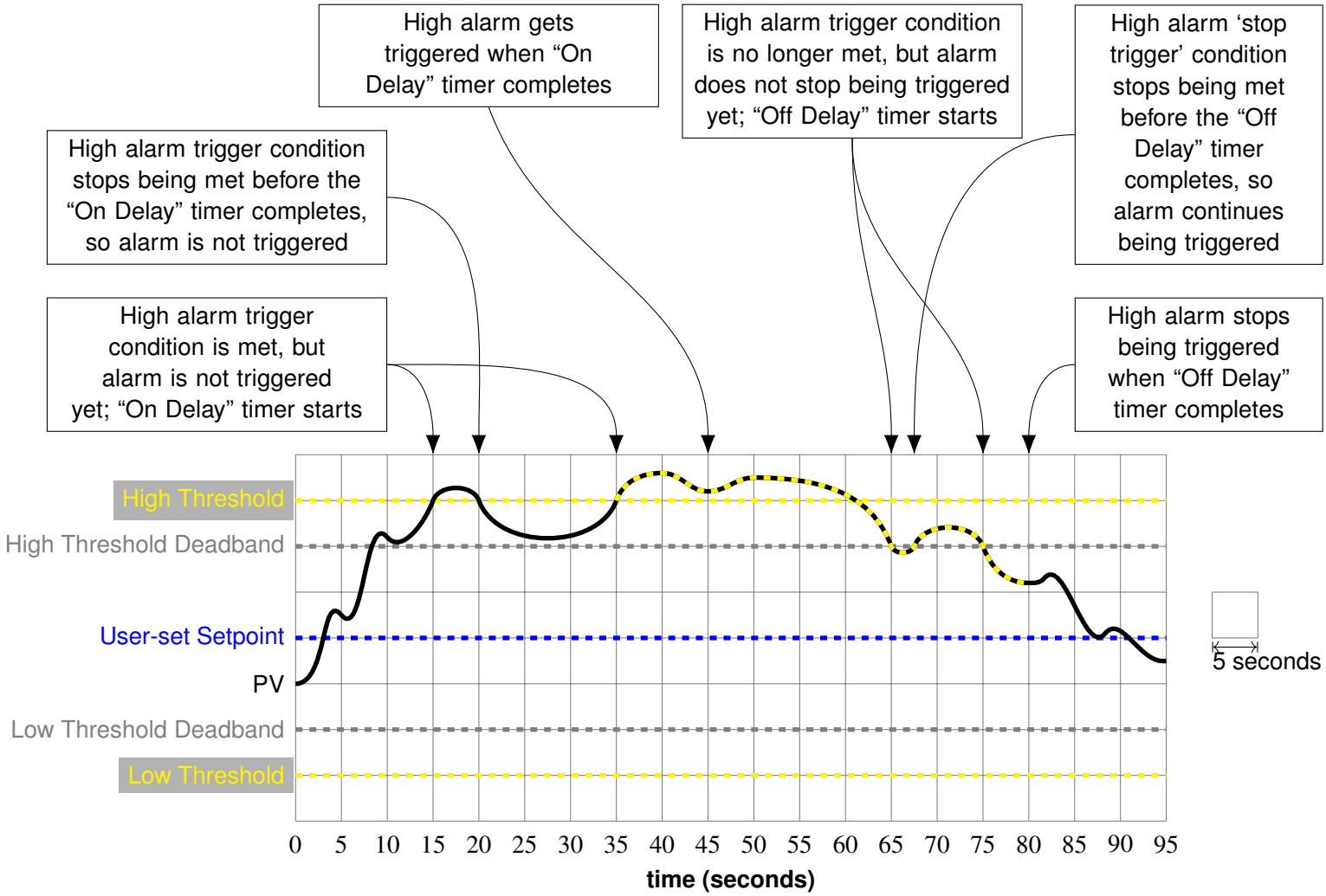
For the Low Deviation and High Deviation alarms, the Threshold is a value relative to the Setpoint. The alarm is not triggered until the PV crosses the Threshold. Then, the alarm remains triggered until the PV again crosses the Threshold.

For the Input Failure alarms, the Threshold is a constant value, and they share a Deadband. If the input signal for the pH or DO sensor falls outside a valid range, or there is an IO fault from the sensor, the sensor PV will read -9999. For 15 seconds, the software puts the sensor into "fast blink" mode, in an effort to read a valid signal with as short a delay as possible. During this time, the device will show an icon of a triangle with a question mark, indicating that the PV is uncertain. If the full 15 seconds elapse and there is still no valid signal, the Input Failure alarm is triggered. If the controller was set to Auto mode, it will change to Sensor Error mode.

For the Limit alarms (the Low Low, Low, High, and High High alarms), the Threshold is a constant value, and each Threshold also has a Deadband associated with it. The alarm is not triggered until the PV crosses the Threshold. Then, the alarm remains triggered until the PV crosses the Deadband going the other direction. For Low Low and Low alarms, the PV must be higher than the “Threshold plus Deadband” value for the alarm to no longer be triggered. For High and High High alarms, the PV must be lower than the “Threshold minus Deadband” value for the alarm to no longer be triggered. The following illustrates how the Low and High alarms work with their Thresholds and Deadbands. The Input Failure, Low Low, and High High alarms function similarly.



Also applicable to the alarms is how the “On Delay (Sec)” and “Off Delay (Sec)” settings affect the behavior. If the “On Delay (Sec)” for an alarm is set to a nonzero value, then the alarm’s trigger condition has to be met for the specified duration before the alarm will actually be triggered. If the “Off Delay (Sec)” for an alarm is set to a nonzero value, then the alarm’s trigger condition has to be over for the specified duration before the alarm will stop being triggered. The following illustrates how the High alarms work with their “On Delay (Sec)” and “Off Delay (Sec)” settings. Other alarm types with “On Delay (Sec)” and “Off Delay (Sec)” settings function similarly.



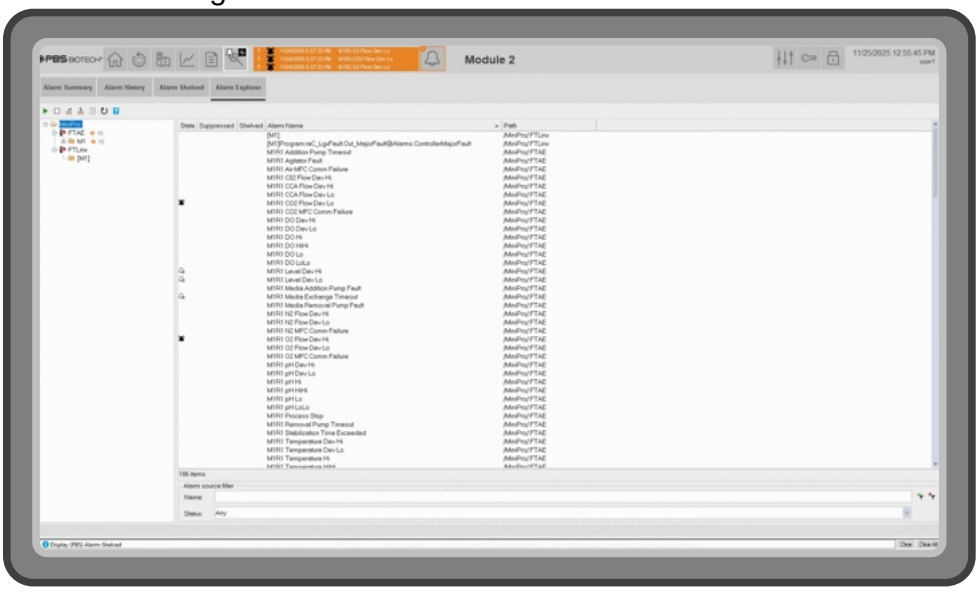
“On Delay (Sec)” is 10 seconds

“Off Delay (Sec)” is 5 seconds

The Agitation Speed Deviation alarm has a feature which allows the motor to start without alarms immediately getting triggered. This is called the “Delay (sec),” where the controller has to be on for a specified duration (the “Delay (sec)”) before the software evaluates whether an alarm trigger condition is

being met. This is separate from the “On Delay (Sec)” setting.

The “Alarm Explorer” menu can be used to see the current status of each alarm and see how it is configured. Note, however, that the configuration cannot be changed from that menu.



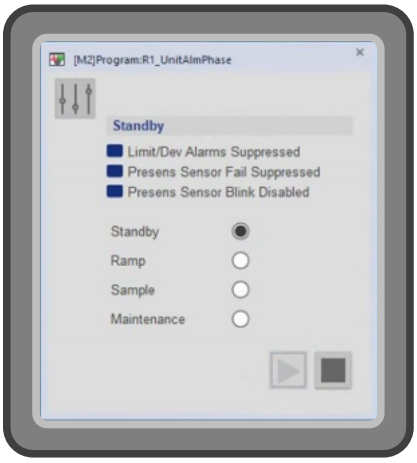
FTAE contains the vast majority of the alarms, which are Tag-Based Alarms. This means that the HMI detects the alarm condition based on the values of tags. FTLinx contains Device Alarms, or alarm firmware instructions. This means that the controller actually monitors for and generates alarms, and sends the information to the HMI.

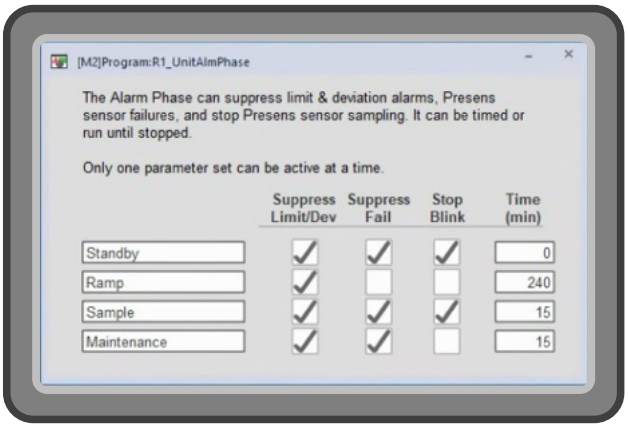
Alarm States	
In Alarm and Unacknowledged	The alarm is in its triggered state, and no user has acknowledged it.
In Alarm and Acknowledged	The alarm is in its triggered state, and a user has acknowledged it.
Normal and Unacknowledged	The alarm was in its triggered state but currently is not, and no user has acknowledged it.
Normal and Acknowledged	The alarm was in its triggered state but currently is not, and a user has acknowledged it.

Alarm States	
Suppressed	When an alarm is suppressed, it will not be displayed in the Alarm and Event Banner. An alarm which is suppressed and unacknowledged will be displayed in the Alarm and Event Summary. Suppressed alarms will be logged in the alarm history log.
Disabled	This completely stops the function of the disabled alarm. The alarm condition is no longer monitored, and notifications are not generated by the controller.
Shelved	This temporarily suppresses an active alarm. The operator specifies a duration for the alarm to remain shelved. When the duration runs out, the alarm is automatically unshelved.
Active	The alarm is in its triggered state.
Inactive	The alarm is not in its triggered state.

Alarms Phase

For extended conditions (such as while a Bioreactor is not being used for a cell culture run), the Alarms Phase can be used to suppress groups of alarms so the software ignores the alarms which would otherwise be triggered. The feature is accessed by navigating to the Bioreactor Overview menu for the specific Bioreactor, and clicking its Alarms Phase Indicator Button (see “PBS-MiniPRO Software - Bioreactor Overview” on page 32).





Alarms Phase Modes/Parameter Sets	
Standby	This is intended to be used between cell culture runs, when the Bioreactor will be idle for an extended and undetermined amount of time and no vessel will be installed.
Ramp	This is intended to be used while setting up a cell culture run, while the PVs are outside their alarm ranges because the controllers have not been on for enough time to achieve setpoint.
Sample	This is intended to be used when taking a sample from the vessel in the BSC. For more information, see “Sampling by pipette” on page 96.
Maintenance	This is intended to be used during maintenance operations, when no vessel will be installed.

Alarms Phase Options	
Suppress Limit/Dev	The DO, pH, and Temperature ‘Limit’ alarms will be ignored, even if they would otherwise be triggered by the PVs.
Suppress Fail	The pH and DO sensor failure alarms will be ignored, even if they would otherwise be triggered by the vessel being removed.
Stop Blink	The pH and DO optical sensor blinking is stopped, so no new PVs are registered.
Time (min)	When set to 0, the Alarms Phase mode/parameter set runs indefinitely from when the user clicks “Start Phase” until they click “Stop Phase.” When set to a nonzero number, the Alarms Phase mode/parameter set stops after that many minutes have elapsed.

For instructions on setting an Alarms Phase, see “Set the Bioreactor’s Alarms Phase to ‘Ramp’ Mode” on page 87.

User Accounts

Users are required to log in with an individual user name and password to access the Display Client. Users can choose to log out of the Display Client, and are logged out automatically after thirty (30) minutes of inactivity. Changes a user makes while they are logged in are recorded in the database and can be

exported in an Audit Log report.

Use of shared or generic accounts is not recommended in regulated environments or when traceability of user actions is desired. Users in regulated environments are responsible for ensuring that any such use of accounts is managed appropriately.

Users have user names, passwords, and user groups. For information on configuring users and user groups, see “Configuring Users and Groups” on page 50.

User Group Permissions

Each user is assigned to at least 1 group. Each group is granted the permissions of one or more “HMI Security Codes” which are represented by letters. And the individual permissions/security tags are assigned to one or more HMI Security Codes.

The PBS-MiniPRO default configuration has the following user groups/user roles:

Operator – Access to basic faceplate controls, setpoints, and alarm acknowledgment. Has HMI Security Code A.

Operating Supervisor – Higher-level operator access, likely including area-wide control. Has HMI Security Codes A and B.

Maintenance – Authorized to bypass devices and perform maintenance functions. Has HMI Security Codes A, B, and C.

Maintenance Supervisor – Oversees maintenance activities. Has HMI Security Codes A, B, C, and D.

Engineering – Full access to configure advanced features, PID tuning, and system parameters. Has HMI Security Codes A, B, C, D, and E.

Manager – Access to high-level system data and reporting. Has HMI Security Code F.

Administrator – Full control over security configuration, user management, and system settings. Has HMI Security Code G.

For which permissions/security tags are assigned to which HMI Security Codes, see Appendix 4 on page 185.

Custom Settings

The following settings are not settings for the PlantPAx off-the-shelf faceplates, but rather are on faceplates specific to the PBS-MiniPRO.

Refer to Rockwell's documentation for how their off-the-shelf faceplates and settings work, specifically the "PlantPAx_4_DisplayElements - proces-rm014_-en-p.pdf" and "PlantPAx_4_Instructions - proces-rm013_-en-p.pdf," which are both located in:

`C:\Users\Public\Documents\RSView Enterprise\SE\Documentation`

Agitation EM Settings

To access this faceplate from the Bioreactor Overview Menu, click "Display Advanced Properties" next to the Agitator Indicator Button.

Group Name	Setting Name Name	Definition
N/A	Speed Deviation	Triggers an Agitation Speed Deviation alarm when PV is outside of SP ± Speed Deviation. Note that this alarm does not have an entry in the Alarm Server, and therefore will not show up in the Alarms Explorer or History. The only indication it has been triggered will be on the "Speed Deviation" indicator button in the "Agitation EM Settings" faceplate.

Temp Control Settings

To access this faceplate from the Base Module Overview, click "Temp PID," then click "Settings."

Group Name	Setting Name Name	Definition
N/A	Rapid Heating	When checked, the Temperature controller uses the Rapid Heating feature when Temperature is set to Auto mode. The Rapid Heating feature is while the Temperature PV is less than the Ramp Cutoff, it uses the Ramp CV value as the CV. When the Temperature PV is greater than or equal to the Ramp Cutoff, the temperature controller uses the PID to determine CV.
N/A	Ramp Cutoff	When the Rapid Heating feature is enabled and the Temperature controller is in Auto mode, the Temperature controller outputs the Ramp CV while the Temperature PV is less than this value, and switches to using the PID to determine CV once the Temperature PV is greater than or equal to this value.
N/A	Ramp CV	When the Rapid Heating feature is enabled and the Temperature controller is in Auto mode, the Temperature controller outputs this value as the CV while the Temperature PV is less than this value.

Temp Control Settings (continued)

Group Name	Setting Name Name	Definition
N/A	Heater Cycle Time	The Temperature controller's heater cycles in this time period. If the CV were 50%, for example, the heater would alternate between being on for half of this time, and off for half of this time. It is not related to the Rapid Heating feature.

Gas Control Settings

To access this faceplate from the Base Module Overview, click "Gas Composition," then click "Settings."

Group Name	Setting Name Name	Definition
Flow Deviation Alarms	High Deviation	When the total gas flow is higher than the SP by this value, a Total Gas Flow Hi Dev alarm is triggered.
Flow Deviation Alarms	Low Deviation	When the total gas flow is lower than the SP by this value, a Total Gas Flow Lo Dev alarm is triggered.
Gas Composition Limits	CO2 Minimum	The minimum percent composition of CO_2 allowed. This applies to Auto Mode and Manual Mode.
Gas Composition Limits	O2 Minimum	The minimum percent composition of O_2 allowed. This only applies to Manual Mode, as Auto Mode uses a split-range controller and if one gas had a nonzero minimum it would simply mean that the other gas's maximum was 0.
Gas Composition Limits	N2 Minimum	The minimum percent composition of N_2 allowed. This only applies to Manual Mode, as Auto Mode uses a split-range controller and if one gas had a nonzero minimum it would simply mean that the other gas's maximum was 0.
Gas Composition Limits	CO2 Maximum	The maximum percent composition of CO_2 allowed. This applies to Auto Mode and Manual Mode.
Gas Composition Limits	O2 Maximum	The maximum percent composition of O_2 allowed. This only applies to Manual Mode, as Auto Mode uses a split-range controller and if one gas had a nonzero minimum it would simply mean that the other gas's maximum was 0.
Gas Composition Limits	N2 Maximum	The maximum percent composition of N_2 allowed. This only applies to Manual Mode, as Auto Mode uses a split-range controller and if one gas had a nonzero minimum it would simply mean that the other gas's maximum was 0.

CO2 Pulse

To access this faceplate from the Base Module Overview, click “Gas Composition,” then click “Devices,” then click “CO2 Pulse.”

Group Name	Setting Name Name	Definition
N/A	Pulse Output CV	When the requested gas flow is below the On Threshold, the MFC will pulse at this flow rate.
N/A	Off Threshold	When the requested gas flow is above this value, the MFC will flow steadily. It will also flow steadily if the request is between the On Threshold and Off Threshold if the MFC was previously flowing steadily.
N/A	On Threshold	When the requested gas flow is below this value, the MFC will pulse to deliver the requested gas. It will also pulse if the request is between the On Threshold and Off Threshold if the MFC was previously pulsing.
N/A	Deficit Threshold	When the MFC is in pulse mode, and the deficit (the amount of gas requested that has not yet been delivered) reaches this volume, the MFC will turn on at the Pulse Output CV until the deficit reaches 0.
N/A	Overflow Payoff Rate	When the deficit (the amount of gas requested that has not yet been delivered) is nonzero and the MFC is able to flow steadily, it will flow at this value above the requested flow rate until the deficit reaches 0.
N/A	Enabled/ Disabled	The MFC’s pulse feature can be disabled, so the MFC will flow steadily at any requested flow rate. The MFC is much less accurate at lower flow rates, so this should be considered before disabling the pulse feature.

O2 Pulse

To access this faceplate from the Base Module Overview, click “Gas Composition,” then click “Devices,” then click “O2 Pulse.”

Group Name	Setting Name Name	Definition
N/A	Pulse Output CV	When the requested gas flow is below the On Threshold, the MFC will pulse at this flow rate.
N/A	Off Threshold	When the requested gas flow is above this value, the MFC will flow steadily. It will also flow steadily if the request is between the On Threshold and Off Threshold if the MFC was previously flowing steadily.
N/A	On Threshold	When the requested gas flow is below this value, the MFC will pulse to deliver the requested gas. It will also pulse if the request is between the On Threshold and Off Threshold if the MFC was previously pulsing.

O2 Pulse (continued)

Group Name	Setting Name Name	Definition
N/A	Deficit Threshold	When the MFC is in pulse mode, and the deficit (the amount of gas requested that has not yet been delivered) reaches this volume, the MFC will turn on at the Pulse Output CV until the deficit reaches 0.
N/A	Overflow Payoff Rate	When the deficit (the amount of gas requested that has not yet been delivered) is nonzero and the MFC is able to flow steadily, it will flow at this value above the requested flow rate until the deficit reaches 0.
N/A	Enabled/ Disabled	The MFC's pulse feature can be disabled, so the MFC will flow steadily at any requested flow rate. The MFC is much less accurate at lower flow rates, so this should be considered before disabling the pulse feature.

N2 Pulse

To access this faceplate from the Base Module Overview, click "Gas Composition," then click "Devices," then click "N2 Pulse."

Group Name	Setting Name Name	Definition
N/A	Pulse Output CV	When the requested gas flow is below the On Threshold, the MFC will pulse at this flow rate.
N/A	Off Threshold	When the requested gas flow is above this value, the MFC will flow steadily. It will also flow steadily if the request is between the On Threshold and Off Threshold if the MFC was previously flowing steadily.
N/A	On Threshold	When the requested gas flow is below this value, the MFC will pulse to deliver the requested gas. It will also pulse if the request is between the On Threshold and Off Threshold if the MFC was previously pulsing.
N/A	Deficit Threshold	When the MFC is in pulse mode, and the deficit (the amount of gas requested that has not yet been delivered) reaches this volume, the MFC will turn on at the Pulse Output CV until the deficit reaches 0.
N/A	Overflow Payoff Rate	When the deficit (the amount of gas requested that has not yet been delivered) is nonzero and the MFC is able to flow steadily, it will flow at this value above the requested flow rate until the deficit reaches 0.
N/A	Enabled/ Disabled	The MFC's pulse feature can be disabled, so the MFC will flow steadily at any requested flow rate. The MFC is much less accurate at lower flow rates, so this should be considered before disabling the pulse feature.

Air Pulse

To access this faceplate from the Base Module Overview, click “Gas Composition,” then click “Devices,” then click “Air Pulse.”

Group Name	Setting Name Name	Definition
N/A	Pulse Output CV	When the requested gas flow is below the On Threshold, the MFC will pulse at this flow rate.
N/A	Off Threshold	When the requested gas flow is above this value, the MFC will flow steadily. It will also flow steadily if the request is between the On Threshold and Off Threshold if the MFC was previously flowing steadily.
N/A	On Threshold	When the requested gas flow is below this value, the MFC will pulse to deliver the requested gas. It will also pulse if the request is between the On Threshold and Off Threshold if the MFC was previously pulsing.
N/A	Deficit Threshold	When the MFC is in pulse mode, and the deficit (the amount of gas requested that has not yet been delivered) reaches this volume, the MFC will turn on at the Pulse Output CV until the deficit reaches 0.
N/A	Overflow Payoff Rate	When the deficit (the amount of gas requested that has not yet been delivered) is nonzero and the MFC is able to flow steadily, it will flow at this value above the requested flow rate until the deficit reaches 0.
N/A	Enabled/ Disabled	The MFC’s pulse feature can be disabled, so the MFC will flow steadily at any requested flow rate. The MFC is much less accurate at lower flow rates, so this should be considered before disabling the pulse feature.

Gas Control EM Advanced

To access this faceplate from the Base Module Overview, click “Gas Composition,” then click “Advanced.”

Group Name	Setting Name Name	Definition
DO	Midpoint	Point which if DO CV is lower, it will call N2, and if higher, it will flow O2

Pump Settings

To access this faceplate from the Base Module Overview, click either pump, then click “Settings.”

Group Name	Setting Name Name	Definition
Pump A	Hand Speed	Speed the switch on the housing will flow the pump when used

Pump Settings (continued)

Group Name	Setting Name Name	Definition
Pump A	At-Speed Threshold	N/A for PBS-MiniPRO
Pump A	At-Speed Delay	N/A for PBS-MiniPRO
Pump B	Hand Speed	Speed the switch on the housing will flow the pump when used
Pump B	At-Speed Threshold	N/A for PBS-MiniPRO
Pump B	At-Speed Delay	N/A for PBS-MiniPRO

Media Exchange Phase

To access this faceplate from the Bioreactor Overview Menu, click “MediaExchange” under “Equipment Phases.” For more information on how the feature works, see “Media Exchange Phase” on page 159.

Group Name	Setting Name Name	Definition
N/A	Prompts Enabled	Prompts for user confirmation at various points of the automated Media Exchange sequence when set to “On”
N/A	Heat Dissipation Time	Time between turning off temperature control and turning off agitation control in the automated Media Exchange sequence
N/A	Cell Settle Time	Time between turning off agitation control and running it at the “Agitator Clear Speed” value
N/A	Agitator Clear Speed	RPM the agitation runs after cells have settled, to clear any settled cells or microcarriers or aggregates off the Vertical-Wheel® impeller
N/A	Media Exchange Volume Type	Whether the automated Media Exchange sequence will exchange a specified volume (mL) of spent medium, or a percent of the total Estimated Level
N/A	Media Exchange Volume	The amount of spent medium the automated Media Exchange sequence will exchange, either in mL or percent
N/A	Removal Pump Flowrate	The speed Pump B will run while removing spent medium during the automated Media Exchange sequence
N/A	Addition Pump Flowrate	The speed Pump A will run while adding fresh medium during the automated Media Exchange sequence

Media Exchange Phase (continued)

Group Name	Setting Name Name	Definition
N/A	Addition Pump Purge Flowrate	After adding fresh medium during the automated Media Exchange sequence, Pump A will flow in reverse at this flowrate for the 'Addition Pump Purge Time,' to clear the addition line of media after filling the vessel. Note that the total volume flowed will be removed from the Level Totalizer, and the Estimated Level will have to be adjusted.
N/A	Addition Pump Purge Time	After adding fresh medium during the automated Media Exchange sequence, Pump A will flow in reverse at the 'Addition Pump Purge Flowrate' for this amount of time, to clear the addition line of media after filling the vessel. Note that the total volume flowed will be removed from the Level Totalizer, and the Estimated Level will have to be adjusted.
N/A	Stabilization Time	After resuming agitation and temperature control in the automated Media Exchange sequence, the software waits for this amount of time before proceding to resume total gas, pH, and DO control.
N/A	Stabilization Timeout	After resuming total gas, pH, and DO control in the automated Media Exchange sequence, the software waits for pH, DO, Temperature, and Agitation PVs to be within the alarm limits. If this much time elapses and any of those PVs are not within the alarm limits, a Stabilization Timeout alarm is triggered.

Alarms Definitions

Alarm names follow the MxRy convention (see “PBS-MiniPRO Base Module - Front Overview” on page 16).

For an explanation of how Alarms work, see “Alarms” on page 161.

Contact PBS Biotech Technical Support if any ‘I/O Fault’ alarm gets triggered.

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
N/A	[Mx]	Triggered from built-in diagnostics for the Base Module, usually on restarting the Control Module or Base Module	This alarm clears on its own on restart. Acknowledge it once it clears.
N/A	[Mx]Program: raC_LgxFault. Out_MajorFault @Alarms. ControllerMajor Fault	A major fault occurs on the controller	The condition causing the major fault is resolved. Contact PBS Biotech Technical Support if you encounter this alarm.
System Configuration	Mx NTP Sync Fail	Controller time synchronization fails	The software attempts to reconnect every minute if this fails. The alarm goes away when connection is restored.
N/A	Mx Process Stop	User pushes the Process Stop Button on the Base Module	User de-presses the Process Stop Button on the Base Module

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
Agitation EM Settings	MxRy Agitation Speed Deviation	Agitation has been on for at least the “Delay (sec)” time, and either Agitation PV has been below SP minus “Speed Deviation” or above SP plus “Speed Deviation” for the “On Delay (Sec)” time. Note that this alarm does not have an entry in the Alarm Server, and therefore will not show up in the Alarms Explorer or History. The only indication it has been triggered will be on the “Speed Deviation” indicator button in the “Agitation EM Settings” faceplate.	Agitation PV has been above SP minus “Speed Deviation” and below SP plus “Speed Deviation” for the “Off Delay (Sec)” time
Agitator	MxRy Agitator IO Fault	A communication failure with the Agitator	The fault is no longer true
N/A	MxRy Air Flow Dev Hi	PV has been above Threshold for 5 seconds	PV goes back within range
N/A	MxRy Air Flow Dev Lo	PV has been below Threshold for 5 seconds	PV goes back within range
Air MFC	MxRy Air MFC IO Fault	A communication failure with the MFC	The fault is no longer true
N/A	MxRy CO2 Flow Dev Hi	PV has been above Threshold for 5 seconds	PV goes back within range
N/A	MxRy CO2 Flow Dev Lo	PV has been below Threshold for 5 seconds	PV goes back within range
CO2 MFC	MxRy CO2 MFC IO Fault	A communication failure with the MFC	The fault is no longer true
DO Sensor	MxRy DO Hi	DO PV has been above the “PV High” Threshold for the “On Delay (Sec)” time	DO PV has been below the “PV High” Threshold minus “PV High” Deadband for the “Off Delay (Sec)” time
DO Sensor	MxRy DO HiHi	DO PV has been above the “PV High-High” Threshold for the “On Delay (Sec)” time	DO PV has been below the “PV High-High” Threshold minus “PV High-High” Deadband for the “Off Delay (Sec)” time

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
DO Sensor	MxRy DO Lo	DO PV has been below the “PV Low” Threshold for the “On Delay (Sec)” time	DO PV has been above the “PV Low” Threshold plus “PV Low” Deadband for the “Off Delay (Sec)” time
DO Sensor	MxRy DO LoLo	DO PV has been below the “PV Low-Low” Threshold for the “On Delay (Sec)” time	DO PV has been above the “PV Low-Low” Threshold plus “PV Low-Low” Deadband for the “Off Delay (Sec)” time
DO Sensor	MxRy DO Sensor Failure	DO raw input signal has been below the “Input Failure Low Threshold” or above the “Input Failure High Threshold,” or there is an I/O fault from the sensor, for 10 seconds	DO raw input has been above the “Input Failure Low Threshold” plus “Input Failure Deadband” and below the “Input Failure High Threshold” minus “Input Failure Deadband” for the “Off Delay (Sec)” time
N/A	MxRy N2 Flow Dev Hi	PV has been above Threshold for 5 seconds	PV goes back within range
N/A	MxRy N2 Flow Dev Lo	PV has been below Threshold for 5 seconds	PV goes back within range
N2 MFC	MxRy N2 MFC IO Fault	A communication failure with the MFC	The fault is no longer true
N/A	MxRy O2 Flow Dev Hi	PV has been above Threshold for 5 seconds	PV goes back within range
N/A	MxRy O2 Flow Dev Lo	PV has been below Threshold for 5 seconds	PV goes back within range
O2 MFC	MxRy O2 MFC IO Fault	A communication failure with the MFC	The fault is no longer true
pH Sensor	MxRy pH Hi	pH PV has been above the “PV High” Threshold for the “On Delay (Sec)” time	pH PV has been below the “PV High” Threshold minus “PV High” Deadband for the “Off Delay (Sec)” time
pH Sensor	MxRy pH HiHi	pH PV has been above the “PV High-High” Threshold for the “On Delay (Sec)” time	pH PV has been below the “PV High-High” Threshold minus “PV High-High” Deadband for the “Off Delay (Sec)” time
pH Sensor	MxRy pH Lo	pH PV has been below the “PV Low” Threshold for the “On Delay (Sec)” time	pH PV has been above the “PV Low” Threshold plus “PV Low” Deadband for the “Off Delay (Sec)” time

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
pH Sensor	MxRy pH LoLo	pH PV has been below the “PV Low-Low” Threshold for the “On Delay (Sec)” time	pH PV has been above the “PV Low-Low” Threshold plus “PV Low-Low” Deadband for the “Off Delay (Sec)” time
pH Sensor	MxRy pH Sensor Failure	pH raw input signal has been below the “Input Failure Low Threshold” or above the “Input Failure High Threshold,” or there is an I/O fault from the sensor, for 10 seconds	pH raw input has been above the “Input Failure Low Threshold” plus “Input Failure Deadband” and below the “Input Failure High Threshold” minus “Input Failure Deadband” for the “Off Delay (Sec)” time
Pump A	MxRy Pump A Drive Fault	The drive experiences and reports an abnormal condition. Information in the Diagnostics tab should be reported to PBS Biotech Technical Support.	The fault is no longer true
Pump A	MxRy Pump A IO Fault	A communication failure with the pump	The fault is no longer true
Pump B	MxRy Pump B Drive Fault	The drive experiences and reports an abnormal condition. Information in the Diagnostics tab should be reported to PBS Biotech Technical Support.	The fault is no longer true
Pump B	MxRy Pump B IO Fault	A communication failure with the pump	The fault is no longer true
N/A	MxRy Stabilization Time Exceeded	During the automated media exchange, the time it takes for Agitation, Temperature, pH, or DO to stabilize exceeds the “Stabilization Timeout” setting	Resets when the Media Exchange Phase resets
Temperature Sensor	MxRy Temperature Hi	Temperature PV has been above the “PV High” Threshold for the “On Delay (Sec)” time	Temperature PV has been below the “PV High” Threshold minus “PV High” Deadband for the “Off Delay (Sec)” time

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
Temperature Sensor	MxRy Temperature HiHi	Temperature PV has been above the “PV High-High” Threshold for the “On Delay (Sec)” time	Temperature PV has been below the “PV High-High” Threshold minus “PV High-High” Deadband for the “Off Delay (Sec)” time
Temperature Sensor	MxRy Temperature Lo	Temperature PV has been below the “PV Low” Threshold for the “On Delay (Sec)” time	Temperature PV has been above the “PV Low” Threshold plus “PV Low” Deadband for the “Off Delay (Sec)” time
Temperature Sensor	MxRy Temperature LoLo	Temperature PV has been below the “PV Low-Low” Threshold for the “On Delay (Sec)” time	Temperature PV has been above the “PV Low-Low” Threshold plus “PV Low-Low” Deadband for the “Off Delay (Sec)” time
Temperature Sensor	MxRy Temperature Sensor Failure	Temperature raw input signal has been below the “Input Failure Low Threshold” or above the “Input Failure High Threshold” for the “On Delay (Sec)” time	Temperature raw input has been above the “Input Failure Low Threshold” plus “Input Failure Deadband” and below the “Input Failure High Threshold” minus “Input Failure Deadband” for the “Off Delay (Sec)” time
Gas Control Settings	MxRy Total Gas Flow Hi Dev	Gas Flow PV has been above SP plus “High Deviation” Threshold for 10 seconds	Gas Flow PV goes below SP plus “High Deviation” Threshold
Gas Control Settings	MxRy Total Gas Flow Lo Dev	Gas Flow PV has been below SP minus “Low Deviation” Threshold for 10 seconds	Gas Flow PV goes above SP minus “Low Deviation” Threshold
Level Estimate	MxRy Vessel Level Hi	Estimated Level PV has been above the “PV High” Threshold for the “On Delay (Sec)” time	Estimated Level PV has been below the “PV High” Threshold minus “PV High” Deadband for the “Off Delay (Sec)” time
Level Estimate	MxRy Vessel Level HiHi	Estimated Level PV has been above the “PV High-High” Threshold for the “On Delay (Sec)” time	Estimated Level PV has been below the “PV High-High” Threshold minus “PV High-High” Deadband for the “Off Delay (Sec)” time

Faceplate	Alarm Name	Alarm is Triggered When:	Alarm Stops Being Triggered When:
Level Estimate	MxRy Vessel Level Lo	Estimated Level PV has been below the “PV Low” Threshold for the “On Delay (Sec)” time	Estimated Level PV has been above the “PV Low” Threshold plus “PV Low” Deadband for the “Off Delay (Sec)” time
Level Estimate	MxRy Vessel Level LoLo	Estimated Level PV has been below the “PV Low-Low” Threshold for the “On Delay (Sec)” time	Estimated Level PV has been above the “PV Low-Low” Threshold plus “PV Low-Low” Deadband for the “Off Delay (Sec)” time

Important Tags Definitions

Tag names follow the MxRy convention (see “PBS-MiniPRO Base Module - Front Overview” on page 16). If a tag is designated as “Read” in the tags table, it is one which may be desired for monitoring or datalogging. If a tag is designated as “Read/Write” in the tags table, it additionally may be desired to write to using a Recipe (see “RecipePro+” on page 114).

The periods (.) in a tag indicate the path to access the tag itself. For example, the `[M1]R1.TIC01.EM.PSet_State` tag is the Temperature Control Mode on Control Module 1 Bioreactor 1.

- To access it in the RecipePro+ Tag Browser, the path would be:
  FTLinx → M1 → Online → R1 → TIC01 → EM
  and then select `PSet_State` from the list.
- To access it in the in TrendPro Live Data, the path would be:
  MiniPro → Live Data → M1 → Online → R1 → TIC01 → EM
  and then select `PSet_State` from the list.

Some tags, such as the `[M1]R1.Ag01.VSD.OSet_SpeedRef` tag, are not displayed in the RecipePro+ Tag Browser, but can be added to a recipe by typing the tag name directly into the “Tag Set” field for an ingredient.

Data Types	
BOOL	Boolean; can have a value of 0 or 1, where 0 means “Off” or “False” and 1 means “On” or “True”
REAL	A single-precision floating-point number, occupying 32 bits of memory
DINT	Double Integer; a 32-bit signed data type to store whole numbers ranging from -2,147,483,648 to 2,147,483,647. It cannot hold decimal values.

Tag Name	Data Type	Description	Read/Write	Visible in Tag Browser
[M1]R1.AG01.VSD.OCmd_StartFwd	BOOL	Operator Command to Start Agitator Drive	Read/Write	No
[M1]R1.AG01.VSD.OCmd_Stop	BOOL	Operator Command to Stop Agitator Drive	Read/Write	No
[M1]R1.AG01.VSD.OSet_SpeedRef	REAL	Operator Setting of Agitator Setpoint (RPM)	Read/Write	No

Tag Name	Data Type	Description	Read/Write	Visible in Tag Browser
[M1]R1.AG01.VSD.Val_SpeedFdbk	REAL	Speed Feedback (actual) from Agitator Drive (RPM)	Read	Yes
[M1]R1.AIC01.AutoSP	REAL	pH Auto Mode Setpoint	Read/Write	Yes
[M1]R1.AIC01.Sensor.Val	REAL	pH PV (incl. Manual Override, if used)	Read	Yes
[M1]R1.AIC02.AutoSP	REAL	DO Auto Mode Setpoint (%)	Read/Write	Yes
[M1]R1.AIC02.Sensor.Val	REAL	DO PV (incl. Manual Override, if used) (%)	Read	Yes
[M1]R1.FIC01.Valve.Val_CVOut	REAL	Air MFC flow request (mLPM); pulsing accounted for	Read	Yes
[M1]R1.FIC01.Valve.Val_Pos	REAL	Air MFC actual flow rate from feedback (mLPM)	Read	Yes
[M1]R1.FIC02.Valve.Val_CVOut	REAL	O2 MFC flow request (mLPM); pulsing accounted for	Read	Yes
[M1]R1.FIC02.Valve.Val_Pos	REAL	O2 MFC actual flow rate from feedback (mLPM) (CV EU)	Read	Yes
[M1]R1.FIC03.Valve.Val_CVOut	REAL	CO2 MFC flow request (mLPM); pulsing accounted for	Read	Yes
[M1]R1.FIC03.Valve.Val_Pos	REAL	CO2 MFC actual flow rate from feedback (mLPM) (CV EU)	Read	Yes
[M1]R1.FIC04.Valve.Val_CVOut	REAL	N2 MFC flow request (mLPM); pulsing accounted for	Read	Yes
[M1]R1.FIC04.Valve.Val_Pos	REAL	N2 MFC actual flow rate from feedback (mLPM) (CV EU)	Read	Yes
[M1]R1.GasControl.EM.PSet_State	DINT	Gas Control Mode; 1=Off,2=On	Read/Write	Yes
[M1]R1.GasControl.OSet_CO2ManSP	REAL	pH Manual Mode CO2 CV (%)	Read/Write	Yes

Tag Name	Data Type	Description	Read/Write	Visible in Tag Browser
[M1]R1.GasControl.OSet_DOControlMode	DINT	DO Control Mode; 1=Off, 2=Auto, 3=Man	Read/Write	Yes
[M1]R1.GasControl.OSet_N2ManSP	REAL	DO Manual Mode N2 CV (%)	Read/Write	Yes
[M1]R1.GasControl.OSet_O2ManSP	REAL	DO Manual Mode O2 CV (%)	Read/Write	Yes
[M1]R1.GasControl.OSet_pHControlMode	DINT	pH Control Mode; 1=Off, 2=Auto, 3=Man	Read/Write	Yes
[M1]R1.GasControl.Out_CCAFrac	REAL	Air Gas CV Output (fraction of total gas from 0 to 1)	Read	Yes
[M1]R1.GasControl.Out_CO2Frac	REAL	CO2 Gas CV Output (fraction of total gas from 0 to 1)	Read	Yes
[M1]R1.GasControl.Out_N2Frac	REAL	N2 Gas CV Output (fraction of total gas from 0 to 1)	Read	Yes
[M1]R1.GasControl.Out_O2Frac	REAL	O2 Gas CV Output (fraction of total gas from 0 to 1)	Read	Yes
[M1]R1.GasControl.TotalFlowSP	REAL	Total Gas Flow Setpoint (mLPM)	Read/Write	Yes
[M1]R1.GasControl.Val_CCAFlowEU	REAL	Air MFC flow request (mLPM); pulsing not accounted for	Read	Yes
[M1]R1.GasControl.Val_CO2FlowEU	REAL	CO2 MFC flow request (mLPM); pulsing not accounted for	Read	Yes
[M1]R1.GasControl.Val_N2FlowEU	REAL	N2 MFC flow request (mLPM); pulsing not accounted for	Read	Yes
[M1]R1.GasControl.Val_O2FlowEU	REAL	O2 MFC flow request (mLPM); pulsing not accounted for	Read	Yes
[M1]R1.LVL01.Manual_SP	REAL	Estimated Level manual adjustment by operator (mL)	Read/Write	Yes

Tag Name	Data Type	Description	Read/Write	Visible in Tag Browser
[M1]R1.LVL01. Sensor.Val	REAL	Estimated Level PV (mL)	Read	Yes
[M1]R1.TIC01. AutoSP	REAL	Temperature Auto Setpoint (C)	Read/Write	Yes
[M1]R1.TIC01.CVEU	REAL	Temperature Heat Duty Output (%)	Read	Yes
[M1]R1.TIC01.EM. PSet_State	DINT	Temperature Control Mode; 1=Off, 2=Auto, 3=Man	Read/Write	Yes
[M1]R1.TIC01. ManualCV	REAL	Temperature Manual Mode Heat Duty CV (%)	Read/Write	Yes
[M1]R1.TIC01. Sensor.Val	REAL	Temperature PV (incl. Manual Override, if used) (C)	Read	Yes

Permissions

This table shows which Permissions are associated with which HMI Security Code(s) by default.

For an explanation of how Permissions work, see “User Group Permissions” on page 167.

Permission	Description	A (Operators)	B (Operating	C (Maintenance)	D (Maintenance Supervisor)	E (Engineering)	F (Manager)	G (Administrator)
AlarmAck	Acknowledge/Reset Alarms	✓	✓	✓	✓	✓	✓	
AlarmShelve	Shelve Alarms	✓	✓	✓	✓	✓	✓	
EnterOperSettings	Enter Setpoints and Control Variables	✓	✓	✓	✓	✓	✓	
OperateEquipment	Command Equipment in Operator Command Source	✓	✓	✓	✓	✓	✓	
ProcedureControl	Start and stop Alarms Phases	✓	✓	✓	✓	✓	✓	
ProcedureExceptions	Exception Processing (Resume; manual; Auto; Semi-Auto; Pause; Disconnect; Release) (N/A on PBS-MiniPRO)	✓	✓	✓	✓	✓	✓	
ProcedureManual Control	Manual Procedure; Sequence; and Batch Processing (Stop; Abort; Reset) (N/A on PBS-MiniPRO)	✓	✓	✓	✓	✓	✓	
RespondToPrompts	Respond to Prompts	✓	✓	✓	✓	✓	✓	
CmdClientNav	Allow Navigation Between or Off Clients (N/A on PBS-MiniPRO)		✓	✓	✓	✓	✓	✓
BypassInterlocks	Bypass Permissives and Interlocks		✓	✓	✓	✓	✓	
CmdSrcOperProg	Acquire/Lock and Release Equipment Operator Command Source		✓	✓	✓	✓	✓	
DeviceConfig Thresholds	Modify Limits and Deadbands		✓	✓	✓	✓	✓	

Permission	Description	A (Operators)	B (Operating	C (Maintenance)	D (Maintenance Supervisor)	E (Engineering)	F (Manager)	G (Administrator)
ProcedureAdvanced Exceptions	Exception Processing (Step Change; Parameter Change; Acquire; Reorder; Activate) (N/A on PBS-MiniPRO)		✓	✓	✓	✓	✓	
ProcedureChange Parameters	Configure Alarms Phases, and run “Default Alarm Setpoints” and “Default Presens Cal” recipes from their respective buttons		✓	✓	✓	✓	✓	
ProcedureChange Setpoints	Override Downloaded Setpoints (N/A on PBS-MiniPRO)		✓	✓	✓	✓	✓	
ProcedureEquipment Control	Manual Supervisory EP/EM Control (N/A on PBS-MiniPRO)		✓	✓	✓	✓	✓	
ProcedureForce Sequence	Force Steps/States (N/A on PBS-MiniPRO)		✓	✓	✓	✓	✓	
AlarmDisable	Disable Alarms		✓	✓	✓	✓		
BypassFeedback	Can Bypass Feedback			✓	✓	✓		
CmdSrcMaint	Acquire/Release Equipment Maintenance Command Source			✓	✓	✓		
CmdSrcOutOfService	Can put device in/out of service			✓	✓	✓		
DeviceConfig Diagnostics	Configure device diagnostics (N/A on PBS-MiniPRO)			✓	✓	✓		
OverrideInputs	Override Inputs			✓	✓	✓		
OverrideOutputs	Override Outputs (N/A on PBS-MiniPRO)			✓	✓	✓		
ResetAccumulators	Reset Run Time Accumulators			✓	✓	✓		
ShowFaceplate	Navigate to full faceplate (N/A on PBS-MiniPRO)			✓	✓	✓		

Permission	Description	A (Operators)	B (Operating	C (Maintenance)	D (Maintenance Supervisor)	E (Engineering)	F (Manager)	G (Administrator)
ConfigTrend	Configure Trend Properties			✓	✓	✓	✓	✓
ControllerManagement	Configure NTP Server settings in the “System Configuration” faceplate)				✓	✓		
DeviceConfigFailTimers	Modify Alarm Delay Times				✓	✓		
DeviceConfigLimits	Configure device limits				✓	✓		
DeviceConfigTimers	Configure device timers				✓	✓		
DeviceConfigTuning	Change Tuning; Inflights; and Preacts				✓	✓		
AlarmConfig	Alarm Configuration					✓		
ConfigSecurity	Change the security for a device					✓		
DeviceConfigBehavior	Change the setup of the device (Advanced)					✓		
DeviceConfigHMI	Change the configuration of the device’s HMI interface					✓		
EnableSimulation	Put Device in Simulation					✓		
SysAdmin	PBS System Admin							✓