KrosFlo® TFDF® Lab System

User Guide

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INSPIRING ADVANCES IN BIOPROCESSING

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Abbreviations

AC	Alternating current
CE	Conformitée Européenne
C	Concentration
D	Diafiltration
DV	Diafiltration volume
CF	Concentration factor
cm	Centimeter
FAS	Field Application Scientist
Hz	Hertz
in	Inches
kg	Kilogram
lbs	Pounds
LMH	Liters/Meters^2/Hour
LPM	Liters per minute
PCV	Percent Cell Volume
PE	Pressure Sensor
PID	Proportional, Integral and Derivative
PSI	Pounds per square inch
PV	Process variable
rpm	Reps per minute
SP	Desired setpoint
TFDF	Tangential Flow Depth Filtration
TMP	Transmembrane Pressure
UL	Underwriters Laboratories
VT	Volumetric throughput

1. Introduction

The KrosFlo® TFDF®Lab System provides a complete solution for the separation of cells from media during cell culture processes. The technology combines hardware, software and a single-use filter to achieve the filtration result. Please direct questions regarding specific applications of the technology to your regional sales representative or field application scientist.

This User Guide provides a reference document for your KrosFlo® TFDF®Lab System and is updated on a regular basis. For the latest version of the document, please visit www.repligen.com/resources. It is highly recommended that the installation process be executed by a trained Repligen engineer. For further support with troubleshooting or process optimization, please contact your local Repligen Field Application Scientist.

2. Welcome

Thank you for choosing the KrosFlo® TFDF®Lab System for your laboratory. This innovative KrosFlo® TFDF®Lab System provides a complete solution for the separation of cells from media during cell culture processes with superior flux performance, scalability and ease of use.

Figure 1. KrosFlo® TFDF®Lab System

3. About this document

Several user attention phrases are used throughout this manual. Each phrase should draw the following level of attention:

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information
IMPORTANT	Indicates information necessary for proper instrument operation
CAUTION	Cautions users regarding potentially hazardous situations in regard to user injury or damage to the instrument if the information is not heeded
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded

4. Safety precautions

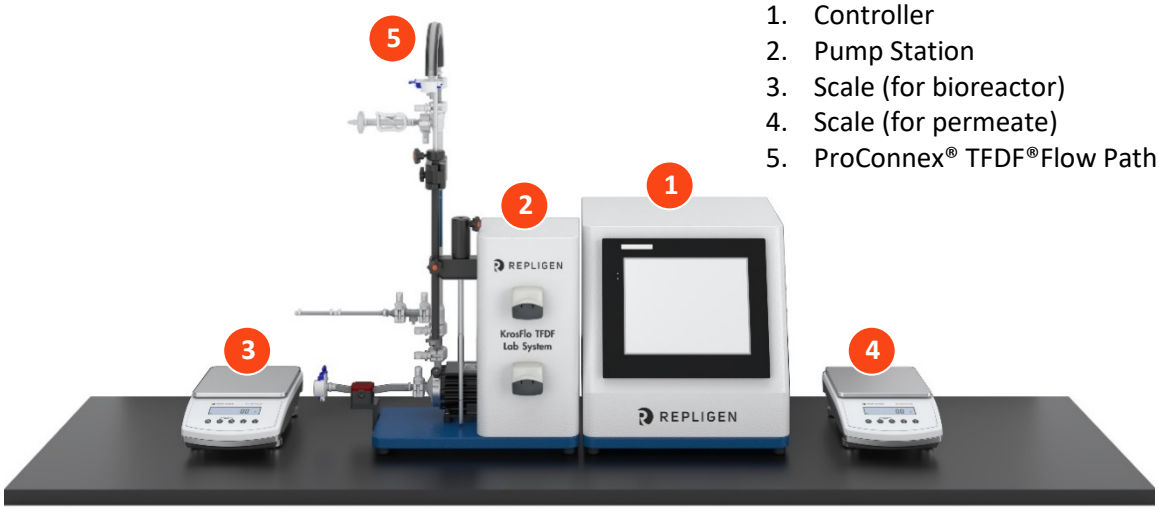
Table 2. Explanation of symbols

Symbol	Description
Caution 	Risk of danger. Consult Operating Instructions for nature of hazard and corrective actions. Potentially hazardous situation which, if not avoided, may result in property/equipment damage
Caution 	Risk of crushing. Keep fingers away from rotor while pump is in operation. Stop pump before loading or unloading tubing
Caution 	Hot surface. Do not touch
Caution 	Risk of electric shock, consult Operating Instructions for nature of hazard and corrective actions
Safety Alert Symbol 	Hazard to personnel is present, the SAS is omitted when the hazard is related to property/equipment damage only
Danger 	Imminently hazardous situation which, if not avoided, will result in death or serious injury
Warning 	Pay attention to the magnetic forces when handling the magnetic levitating centrifugal pump-head. Avoid other magnets or metal parts as contamination from physical damage or cracks may arise from the magnetic attraction. Specifically pay attention to the magnetic forces when handling two pump-heads at the same time

Table 3. Instrument safety labels

Symbol	Description
Danger 	High voltages exist and are accessible. Use extreme caution when servicing internal components. Remove power from the pump before any cleaning operation is started
Warning 	Remove power from the pump before attempting any maintenance.
Warnings 	Tubing breakage may result in fluid being sprayed from pump. Use appropriate measures to protect operator and equipment Turn drive off before removing or installing tubes Fingers or loose clothing could get caught in drive mechanism
Cautions 	Power must be turned off before connecting the external remote-control cable to prevent damage to the drive Do not contaminate the lubricant in the container, on the shaft or on the seal with foreign material. Failure to observe this precaution may result in damage to the seal and premature failure of the seal No foreign matter should be allowed under the gasket on the back of the front plate or under the heads of the screws. Failure to observe this precaution may result in leakage during washdown of the drive
Caution 	To avoid electrical shock, the power cord protective grounding conductor must be connected to the ground. Not for operation in wet locations as defined by EN61010-1
Warning 	High impeller magnetic field strength The magnetic levitating centrifugal pump head contains a rotor with a rare earth magnet with high field strength. Pacemakers may be influenced, and magnetic forces may lead to contusions. Keep distance between pump and pacemakers, and handle pump heads with care
Caution 	Pay attention to the magnetic forces when handling the magnetic levitating centrifugal pump head. Avoid other magnets or metal parts as contamination from physical damage or cracks may arise from the magnetic attraction. Specifically pay attention to the magnetic forces when handling two pump-heads at the same time
Caution 	Keep fingers away from rotor while pump is in operation. Stop pump before loading or unloading tubing

Figure 2. KrosFlo® TFDF®Lab System Components



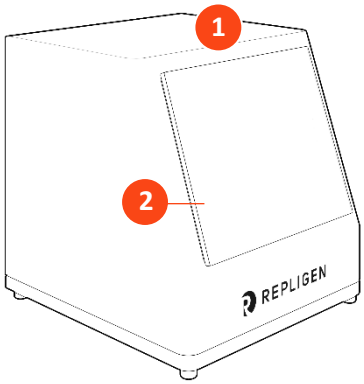
1. Controller
2. Pump Station
3. Scale (for bioreactor)
4. Scale (for permeate)
5. ProConnex® TFDF®Flow Path

4.1 Controller

Users change parameters and monitor processes through the interface of the Controller. A 12-inch touch screen enables user input, such as a change in pump speed and displays a diagram of the system for visual analysis. The Wizard feature within the KrosFlo® TFDF®Software, assists running an optimum process. Based on just a few user specifications, the KrosFlo® Wizard feature generates run parameters for simple and accurate operation.

Figure 3. Controller

1. 12” Touchscreen interface
2. Control panel



4.2 Pump Station

The Pump Station transfers all liquids during the TFDF®process. A low shear and digitally controlled magnetic levitating centrifugal style pump delivers feed stock from the bioreactor vertically through the lumen of the TFDF®filter and then back into the bioreactor. The Permeate (top) peristaltic pump transfers permeate from the filter enclosure to the permeate reservoir. The Diafiltration/Aux peristaltic pump (bottom) transfers buffer from a diafiltration reservoir to the bioreactor during the diafiltration stage. The overall process is monitored using multiple sensors:

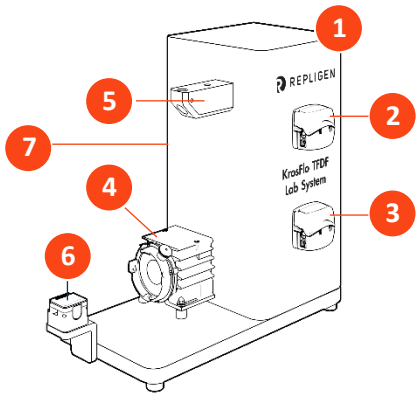
- Three in-line single-use pressure sensors for feed, permeate and retentate
- A non-invasive, clamp-on ultrasonic retentate flow meter

Optional sensors (purchased separately from the system) include:

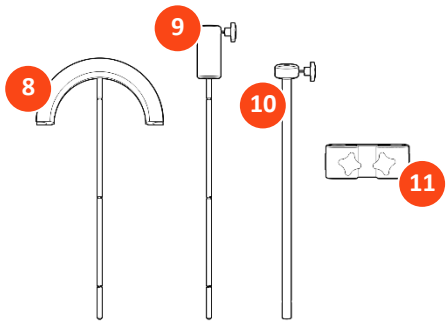
- An in-line single-use pressure sensor for a secondary filter
- An in-line single-use pressure sensor for a guard/sterile filter
- An in-line turbidity monitor

Figure 4. Pump Station

1. Pump Station
2. Permeate peristaltic pump
3. Diafiltration/Aux peristaltic pump
4. Magnetic levitation pump
5. Stand mount
6. Flow meter
7. Control panel



8. Tubing guide rod
9. Extension rod
10. Sleeve
11. Filter clamp

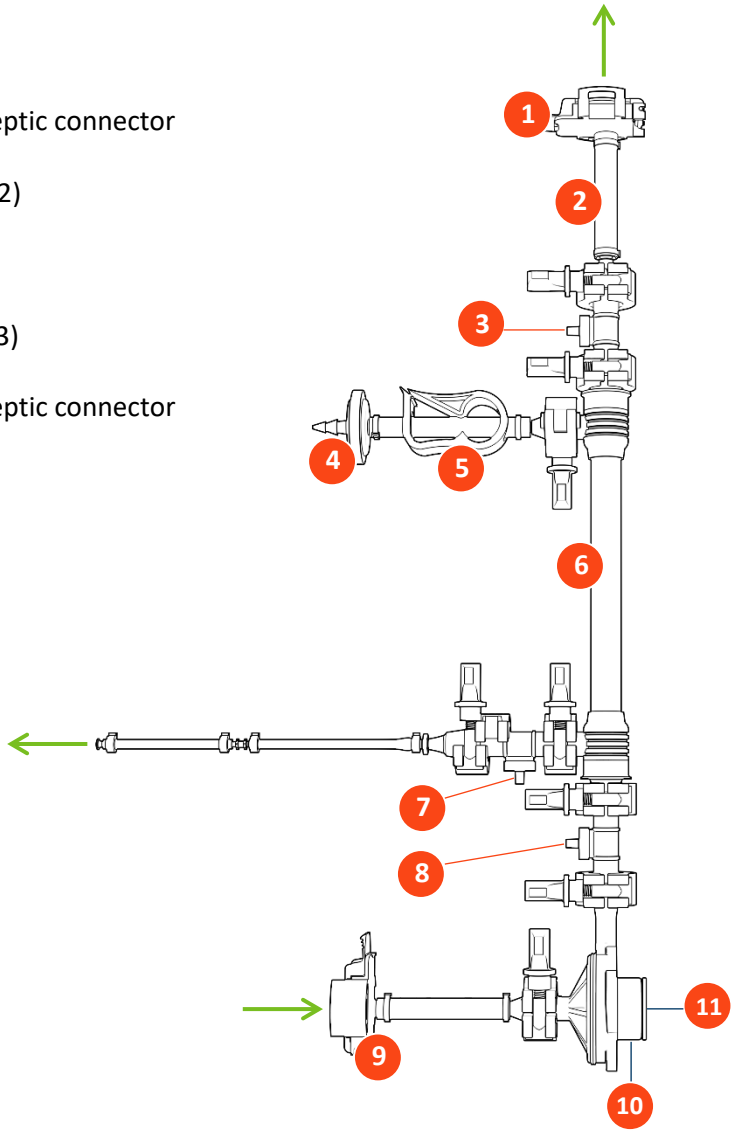


4.3 TFDF®Filter

The TFDF®Filter is a tubular depth filter available in process development to pilot and production scales. The filter functions within an enclosure with ports that deliver feed stock and remove retentate and permeate. All filter sizes larger than the trial size are available as ProConnex® TFDF®Flow Paths only, which may be configured from a library of components. Each ProConnex® TFDF®Flow Path arrives as a fully closed, irradiated device that is ready-to-use. No flushing of the filter is required.

Figure 5. ProConnex® TFDF®Flow Path

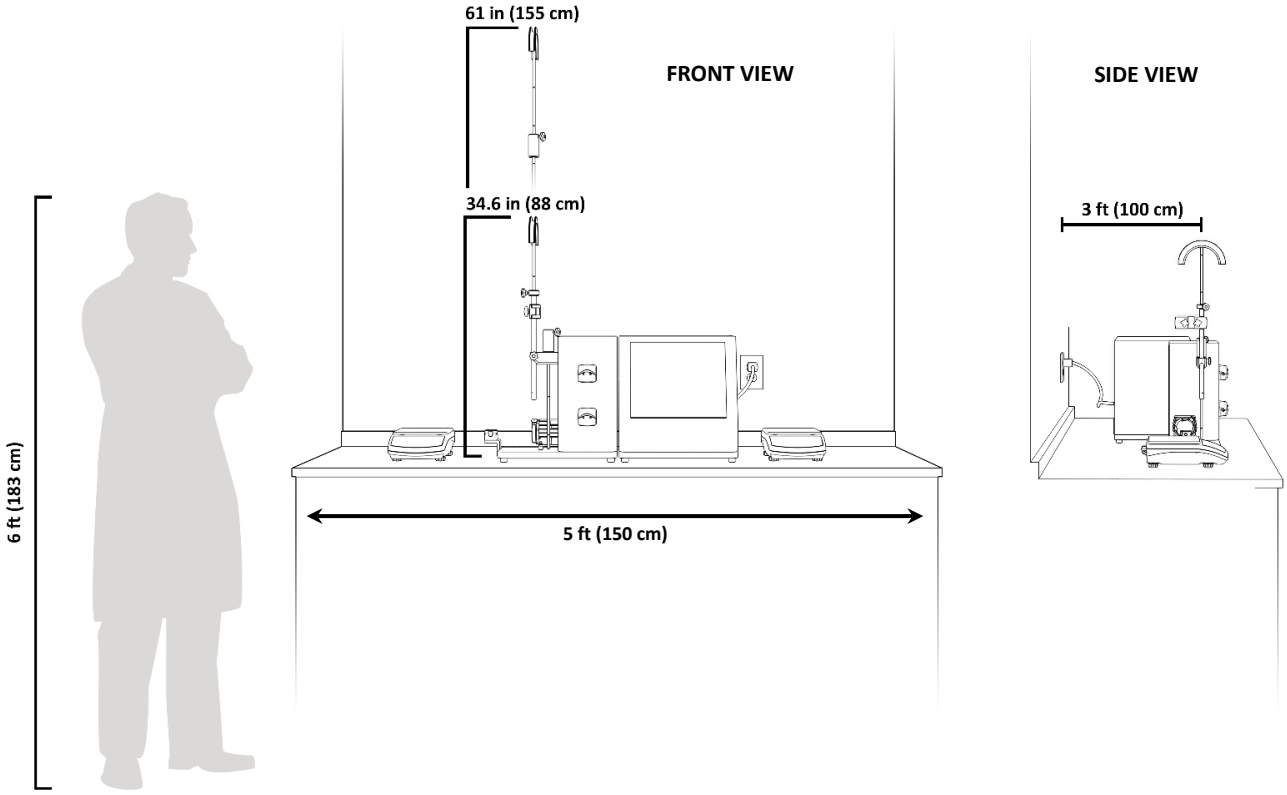
1. CPC AseptiQuik® genderless aseptic connector
2. Retentate tubing
3. Retentate pressure sensor (PE02)
4. Vent port
5. Pinch clamp
6. TFDF®filter enclosure
7. Permeate pressure sensor (PE03)
8. Feed pressure sensor (PE01)
9. CPC AseptiQuik® genderless aseptic connector
10. Magnetic pump head
11. Ferromagnetic fixation disc



5. Set-up

5.1 Space requirements

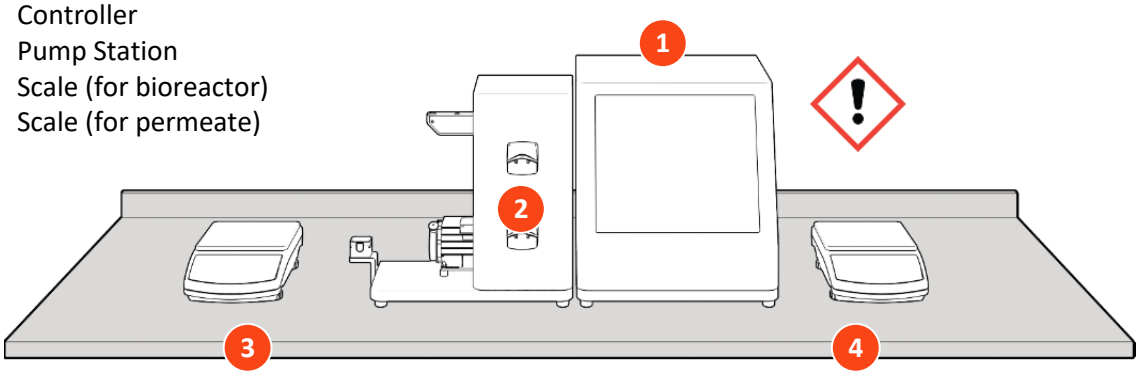
Figure 6. Bench space needed



5.2 System arrangement

Figure 7. Arranging the system on the bench

1. Controller
2. Pump Station
3. Scale (for bioreactor)
4. Scale (for permeate)

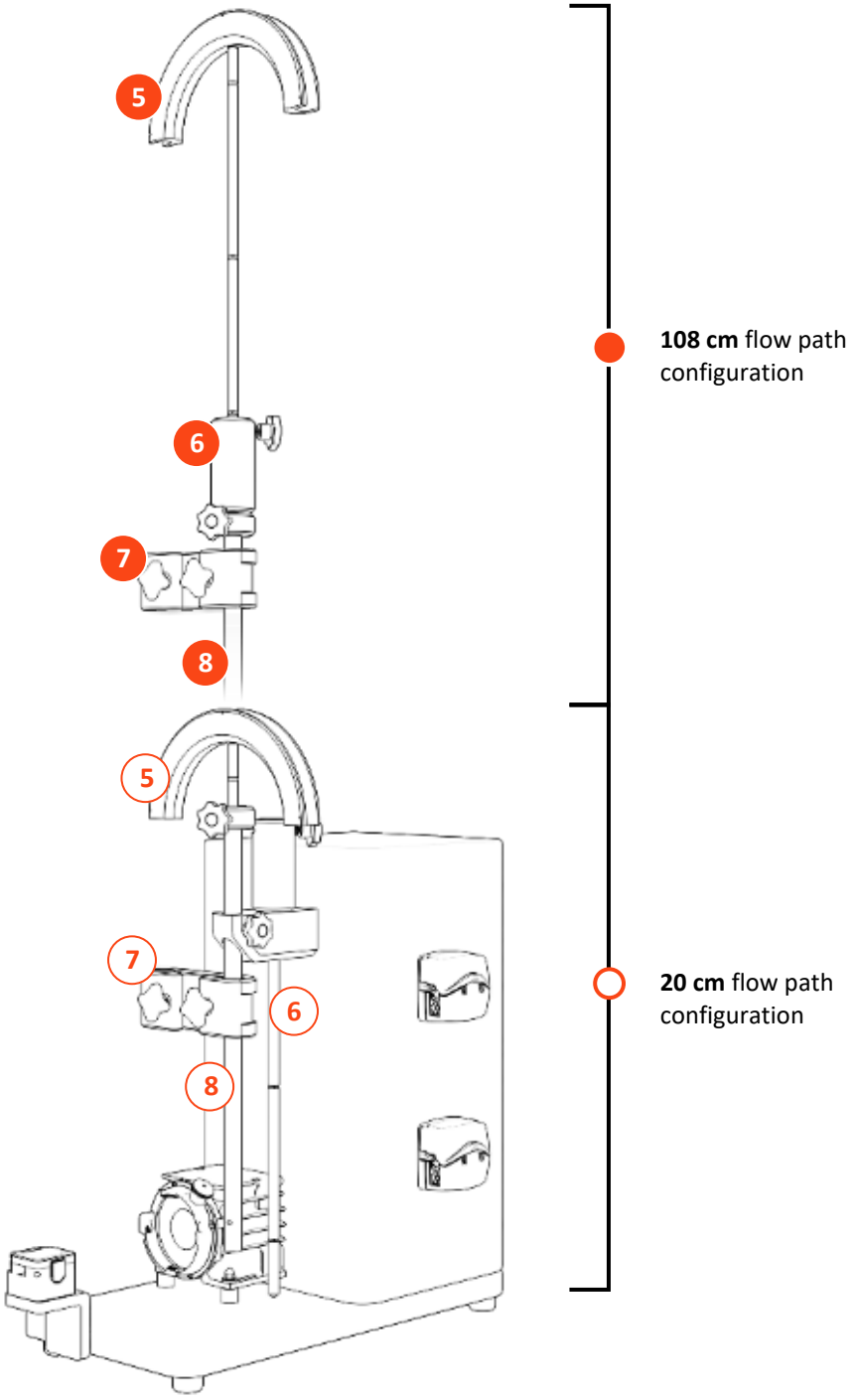


WARNING! The Controller weight is 36 lbs. Two people are recommended to lift the Controller out of the box and place it on the bench top.

5.3 Stand assembly

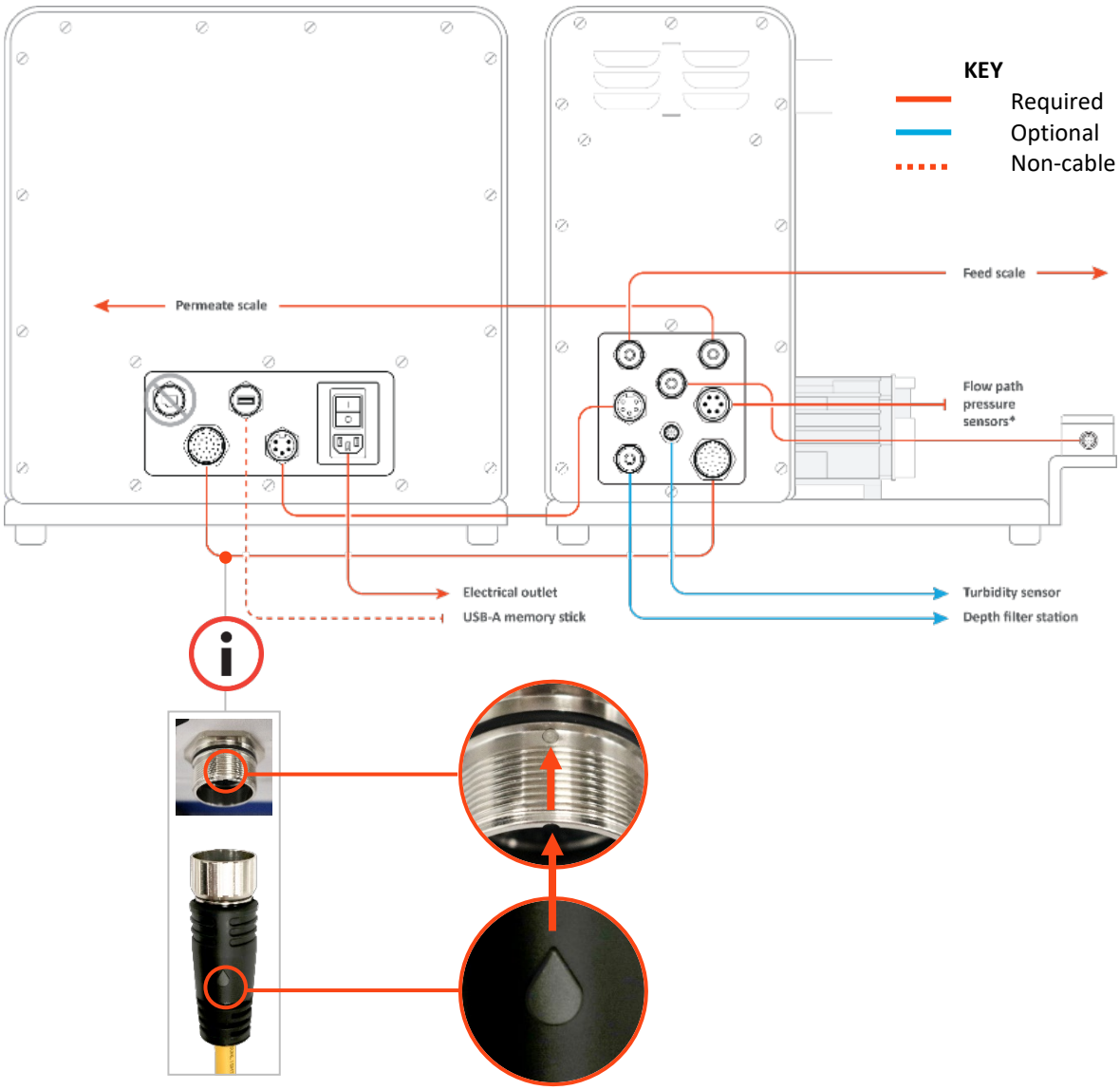
Figure 8. Assembling the stand

5. Tubing guide rod
6. Extension rod (required only for 108 cm flow path)
7. Filter clamp
8. Sleeve



5.4 System cable connections

Figure 9. System connections



Note: *Use tear drop to align pins with socket. Communication cable pins are delicate.*

5.4.1 Optional connections

1. Connect the **718 Minifast Turbidity sensor cable** from the Pump Station to your turbidity sensor.
2. Connect the longer **M12 Eurofast depth station flow meter cable** from the Pump Station to your depth station.

5.5 ProConnex® TFDF®Flow Path

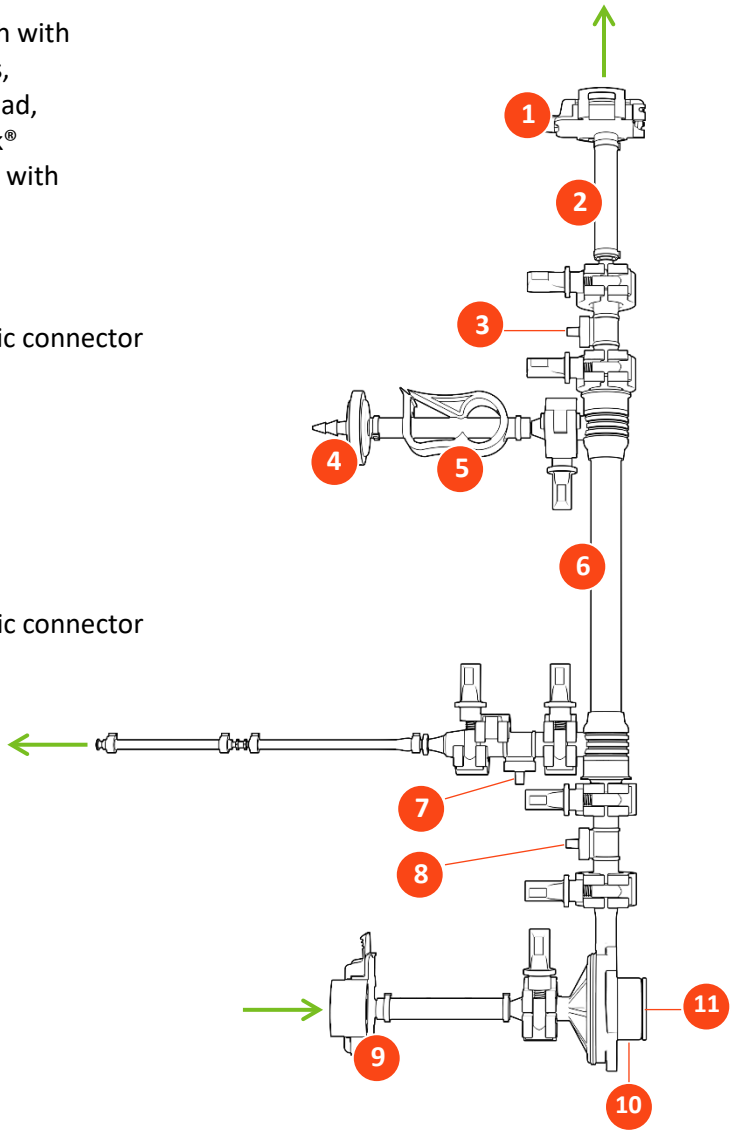
Table 4. ProConnex® TFDF®Flow Path specifications

Type	Filter	Flow path	Flow path	Flow path	Flow path
Surface Area (cm^2)	3	3	150	1500	6000
Recommended process volume	< 1 L	< 1 L	< 50 L	< 500 L	< 2000 L
Filter length (cm)	20	20	108	108	108
Effective length (cm)	2.2	2.2	108	108	108
Number of tubes	1	1	1	10	40

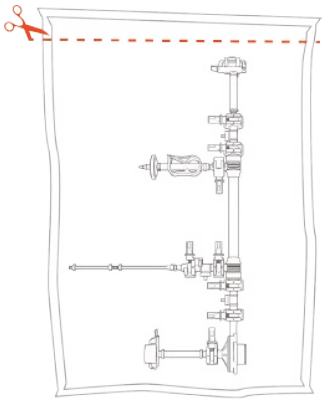
Figure 10. ProConnex® TFDF®Flow Path configuration

Example ProConnex® TFDF®flow path with filter, filter enclosure, tubing, clamps, pressure sensors, magnetic pump head, vent filter and genderless AseptiQuik® connectors. Configurations may vary with your requirements.

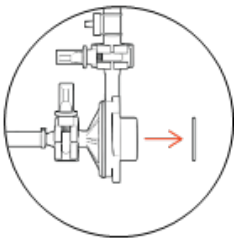
1. CPC AseptiQuik® genderless aseptic connector
2. Retentate tubing
3. Retentate pressure sensor (PE02)
4. Vent port
5. Pinch clamp
6. TFDF®Filter enclosure
7. Permeate pressure sensor (PE03)
8. Feed pressure sensor (PE01)
9. CPC AseptiQuik® genderless aseptic connector
10. Magnetic pump head
11. Ferromagnetic fixation disc



5.6 Install flow path



Unbox the flow path and remove the ferromagnetic disc.

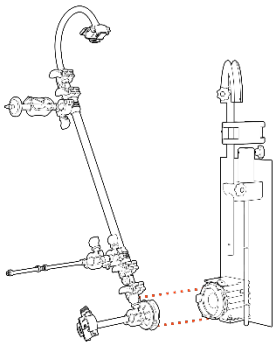


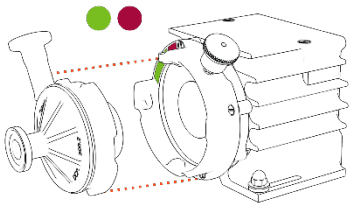
Do not cut any tubing or clamps

Note: *Remove the ferromagnetic disc before proceeding to the next step.*

5.6.1 20 cm flow path installation

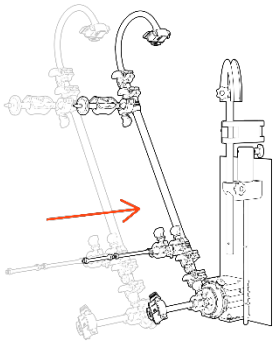
1.

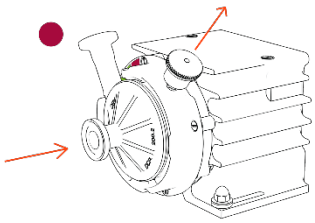




Align pump head.

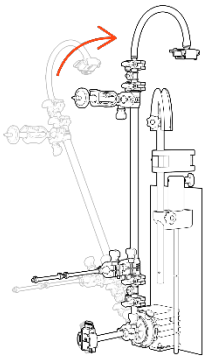
2.

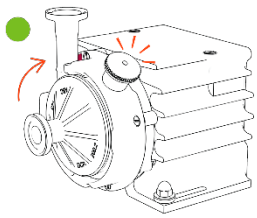




Pull locking pin and **insert** pump head.

3.

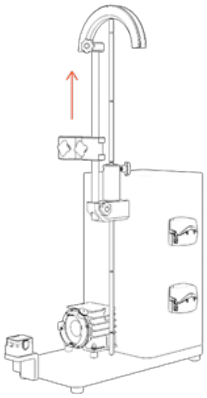




Rotate filter up (locking pin will click).

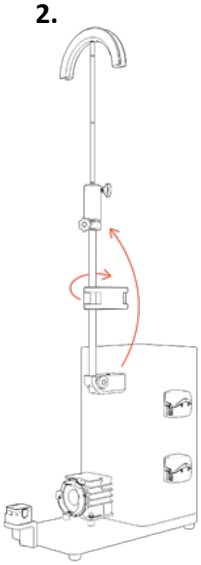
5.6.2 108 cm flow path installation

1.



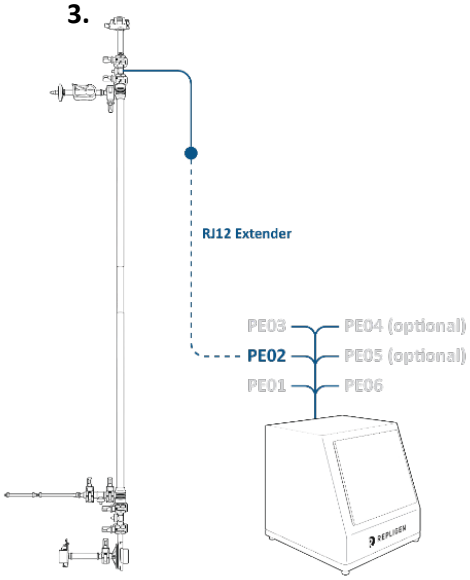
Transfer tubing guide rod to extension rod and raise sleeve to maximum height.

2.



Transfer extension rod (with tubing guide rod) to sleeve and rotate clamp so that it is out of the way during installation.

3.

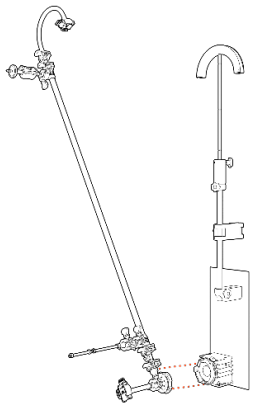


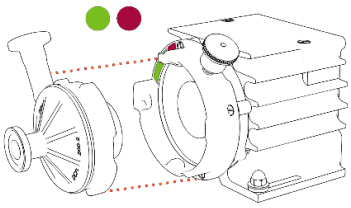
Connect retentate pressure sensor with RJ12 extender to PE02 *This is much easier to connect before the flow path is installed due to its height.*

Note: *RJ12 cables are not supplied with the ProConnex® assemblies and must be purchased separately.*

Note: *Extender available from Repligen support.*

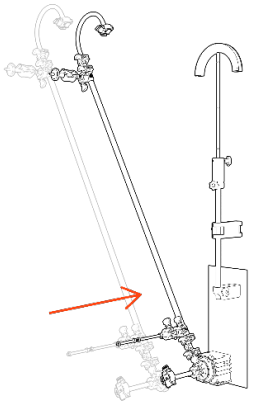
4.

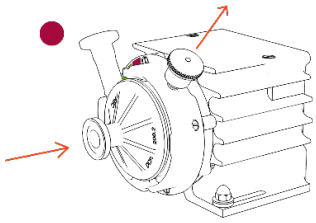




Align pump head

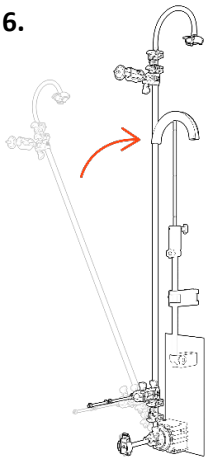
5.

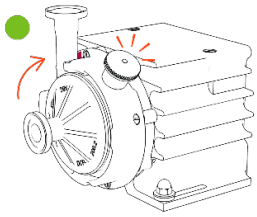




Pull locking pin and **insert** pump head

6.

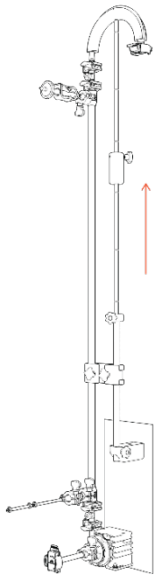




Rotate filter up (locking pin will click)

7. **Secure flow path** with clamp and raise extension rod so that guide rod is at retentate tubing height.

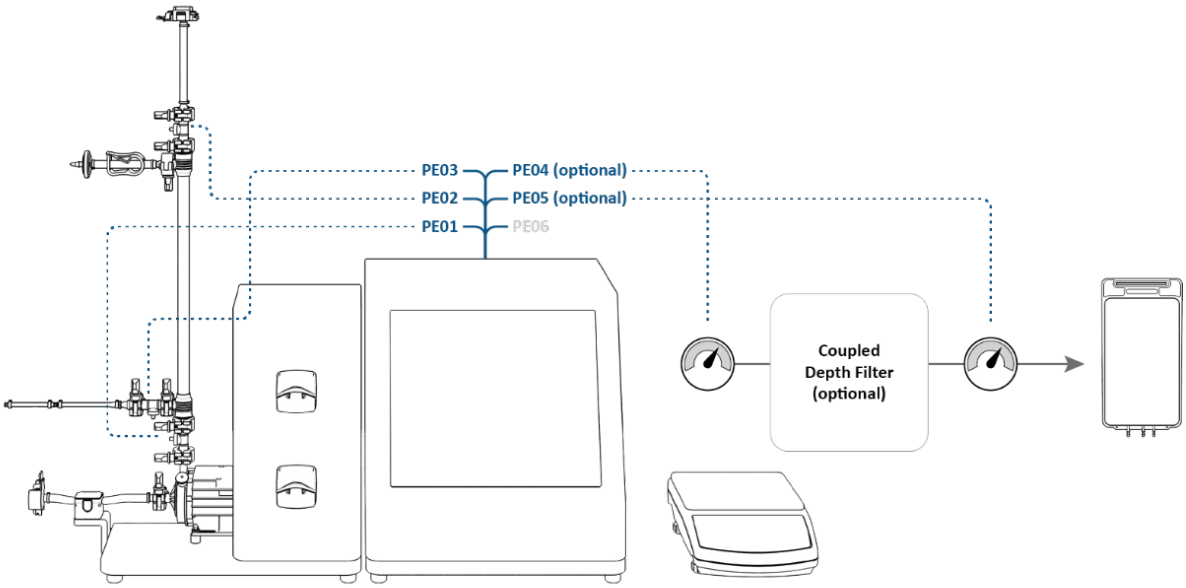
Make final adjustments as necessary.



5.7 Flow path pressure sensor and tubing connections

5.7.1 Flow path pressure sensor connections

Figure 11. Connecting pressure sensors



Connect pressure sensors:

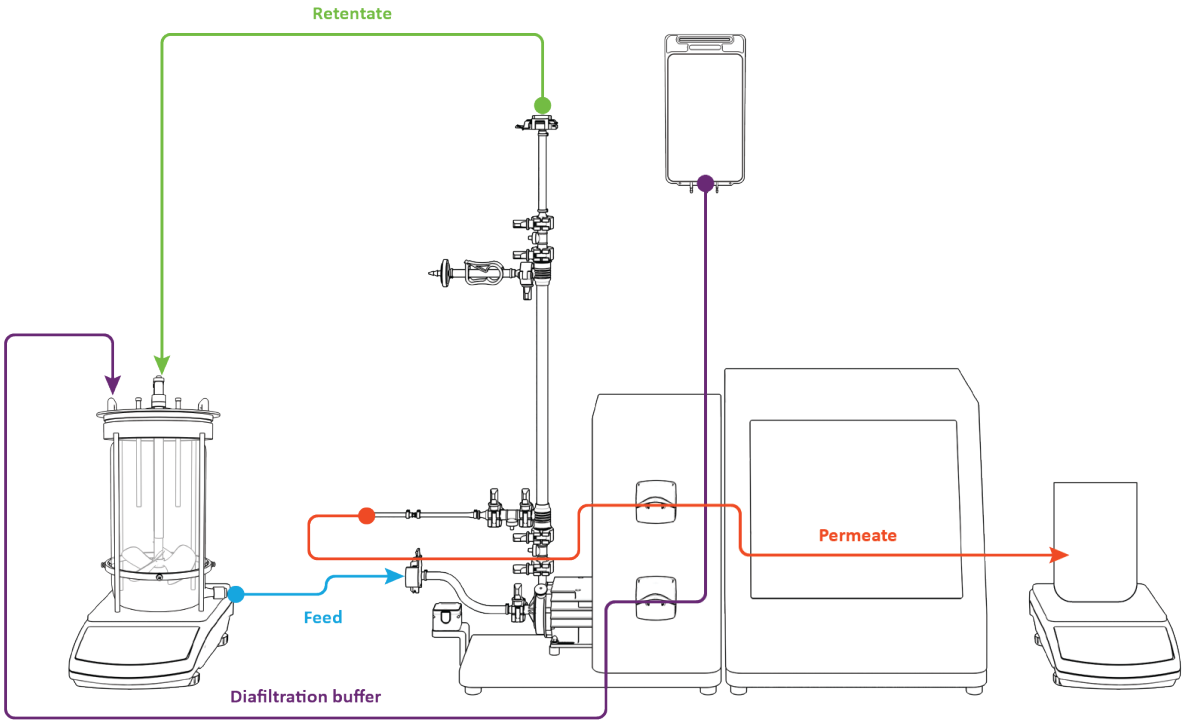
- **PE01** to **Feed**
- **PE02** to **Retentate** (for **108 cm flow path**: already connected in Installation step 3)
- **PE03** to **Permeate**
- **PE04** to **Secondary filter** (OPTIONAL)
- **PE05** to **Guard/Sterile filter** (OPTIONAL)
- **PE06 NOT USED**

Connect in-line turbidity sensor to permeate line (OPTIONAL).

Note: *In-line turbidity sensor cables are not supplied with the system and must be purchased separately.*

5.7.2 Tubing connections

Figure 12. Making tubing connections



Make tubing connections:

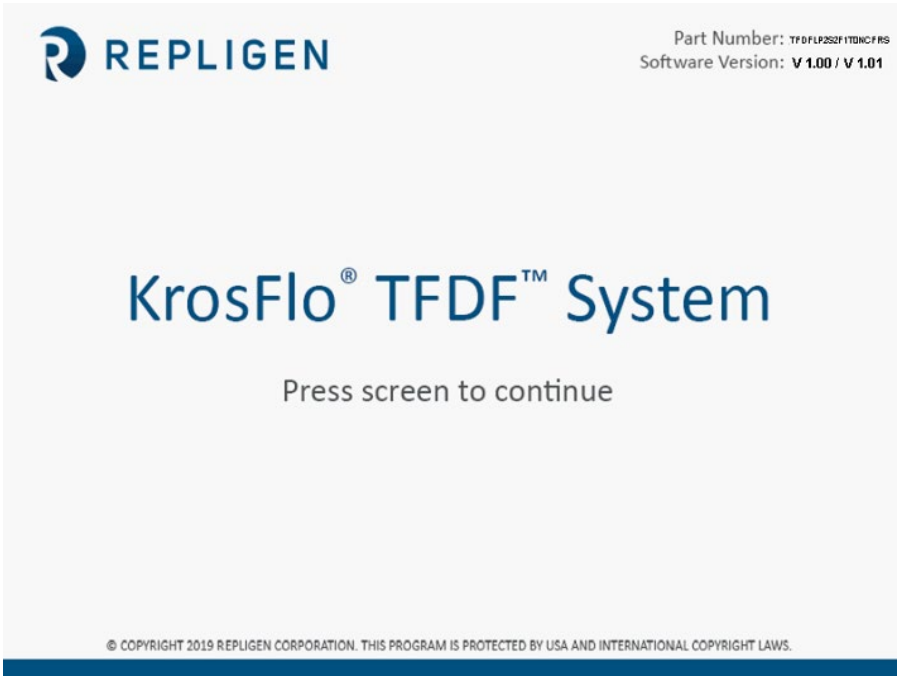
1. Route **retentate tubing** over **tubing guide** (stand adjustments may be required).
2. Route **permeate tubing** through **top peristaltic pump.**
3. Route **diafiltration buffer tubing** through **bottom peristaltic pump.**
4. **Feed line *not installed*** in flow meter (enables easier priming).

6. Getting started

6.1 Initial startup

Turn the KrosFlo® TFDF®Lab System on using the power switch located on the left rear panel of the main enclosure. Once the system has booted up the information screen shown below will display. Touch the screen to continue.

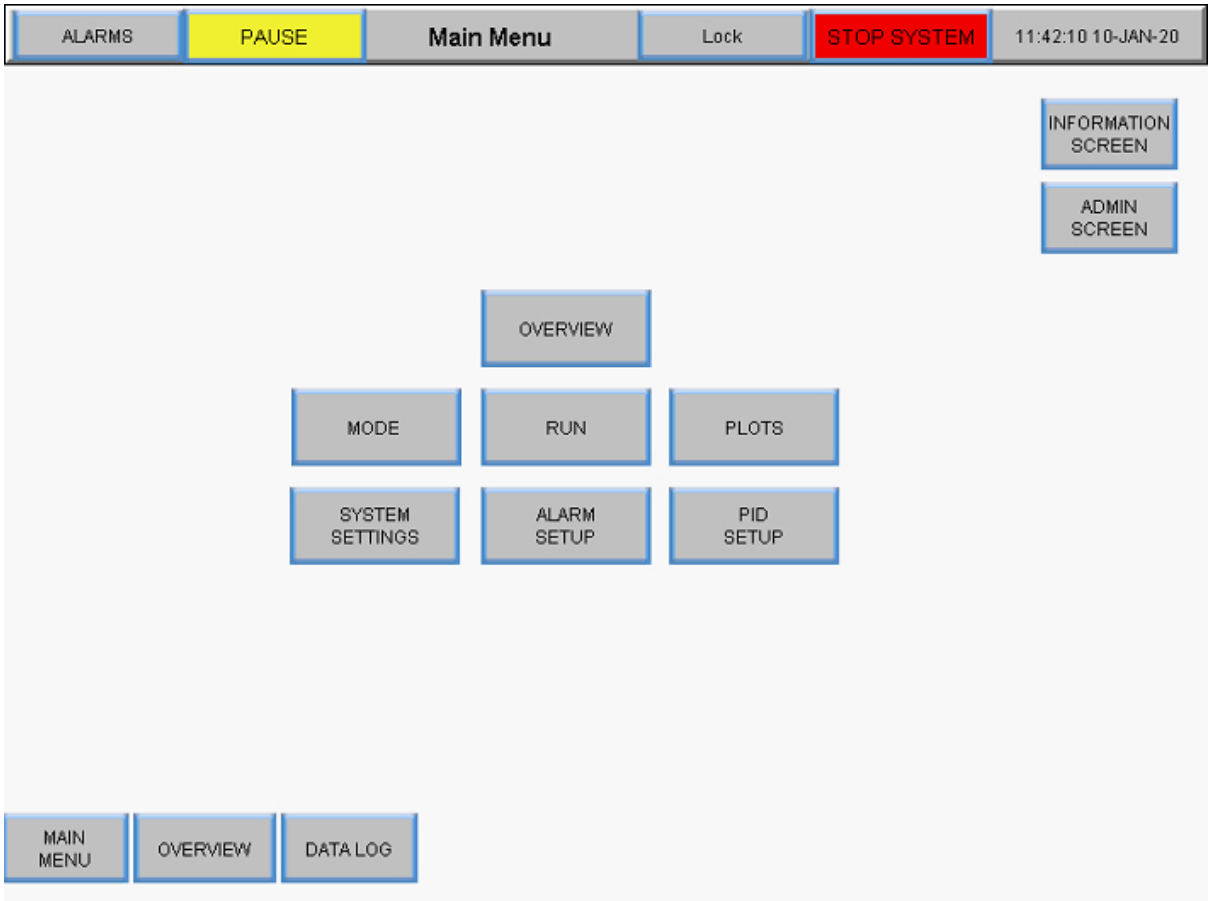
Figure 13. Touchscreen display



6.2 Touchscreen

The KrosFlo® TFDF®Lab System is controlled and operated through the touchscreen. After the initial system start up, the Main Menu screen displays:

Figure 14. Main Menu screen

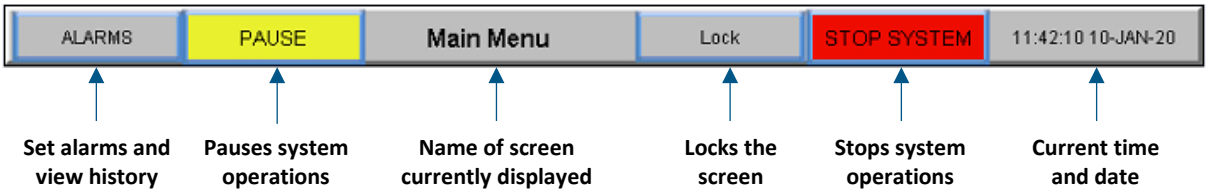


The buttons in the Main Menu Screen provide access to different operational and set-up screens. To navigate to a different screen, simply touch the button.

6.3 Screen navigation

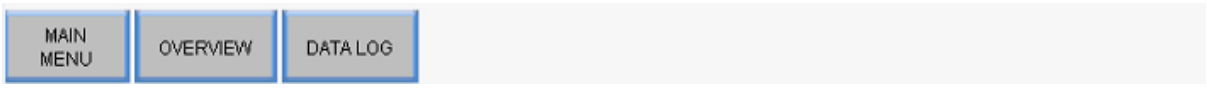
The menu bar displays at the top of all system screens.

Figure 15. Menu bar



Screen navigation options display on the bottom of all system screens. The options shown vary from screen to screen.

Figure 16. Screen navigation



6.4 Screen saver

The system is programmed with a screen saver that comes on after 30 minutes of inactivity. This does not affect operation in any way. When the screen saver is active, the display will be black. Just touch the display screen to view the active system screen.

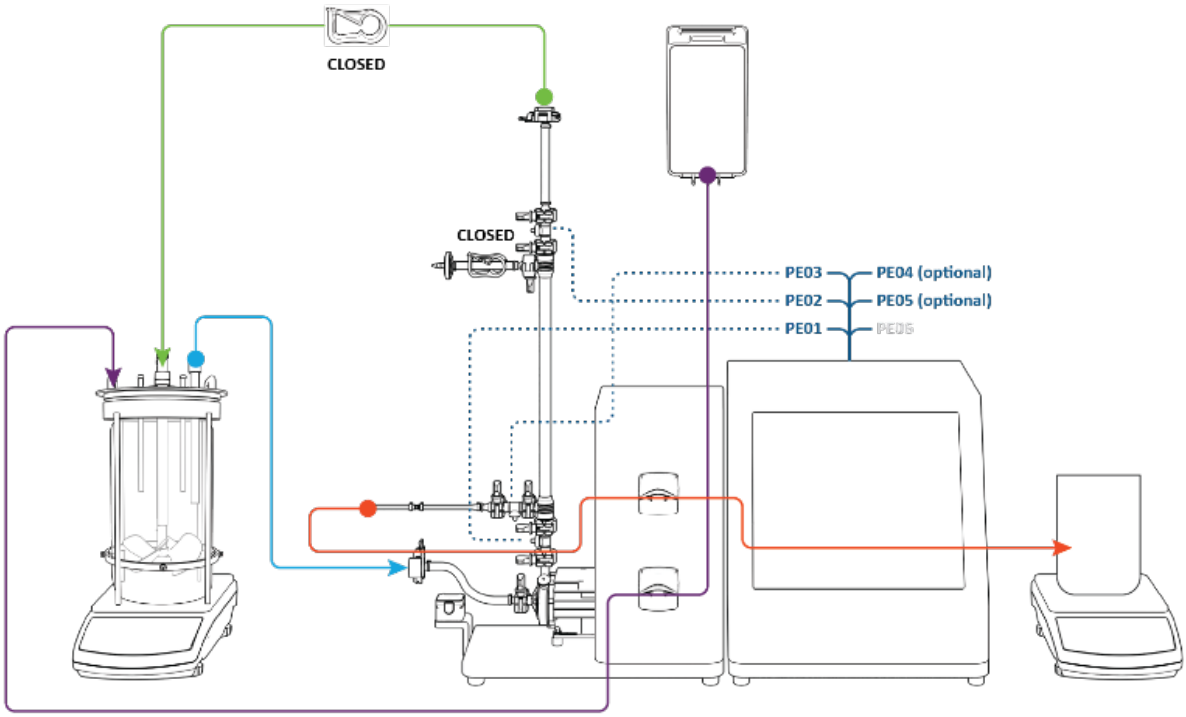
7. Running the system

7.1 Magnetic levitation pump priming

Priming the magnetic levitation pump is **required for top fed bioreactors** because an external force is required to draw liquid up and out of the bioreactor through the tubing and into the magnetic levitation pump. Priming is typically not required for bottom fed bioreactors.

7.1.1 Pump priming set-up

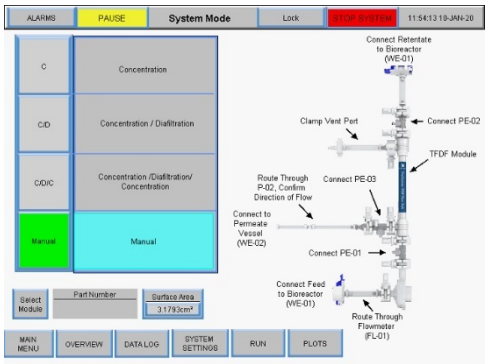
Figure 17. Set-up before priming



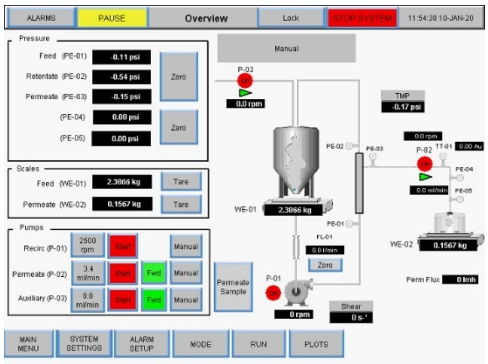
Verify the following set-up steps have been executed prior to pump priming:

- **Feed pressure sensor** connected to **PE01**
- **Retentate pressure sensor** connected to **PE02**
- **Permeate pressure sensor** connected to **PE03**
- **Vent line** clamped **closed**
- **Retentate line** clamped **closed**
- **Feed line *not installed*** in flow meter
- **Permeate line** routed through **top peristaltic pump**

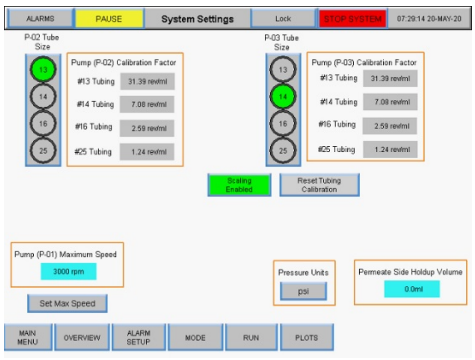
7.1.2 Pump priming process



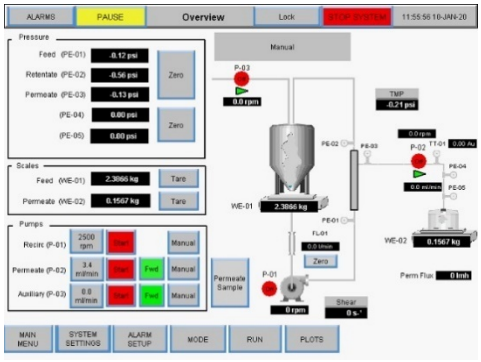
1. Press **Manual**
   Press **Overview**



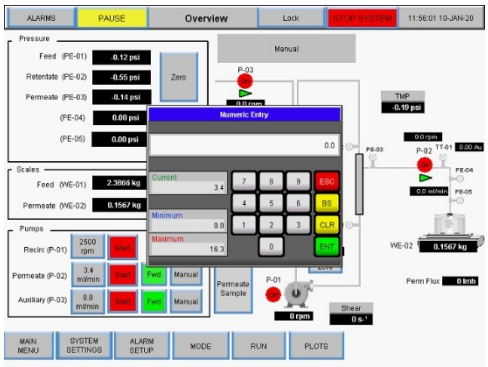
2. Toggle Permeate pump P-02 to **FWD**
   Button will show green
   Press **System Settings**



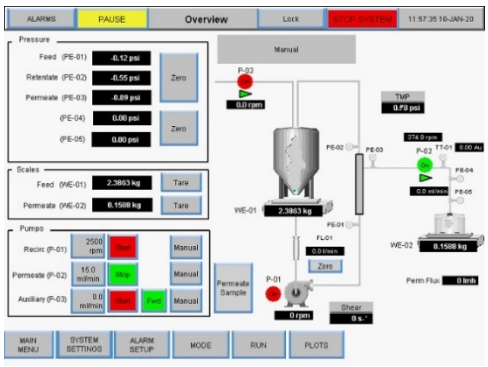
3. Select **peristaltic tubing size**
   Example with #13 shown
   Press **Overview**



4. Press Permeate pump **P-02 flow rate**



5. Input **flow rate value** in mL/min
   Press **ENT**



6. Press P-02 **Start**
   Process graphic will flash green

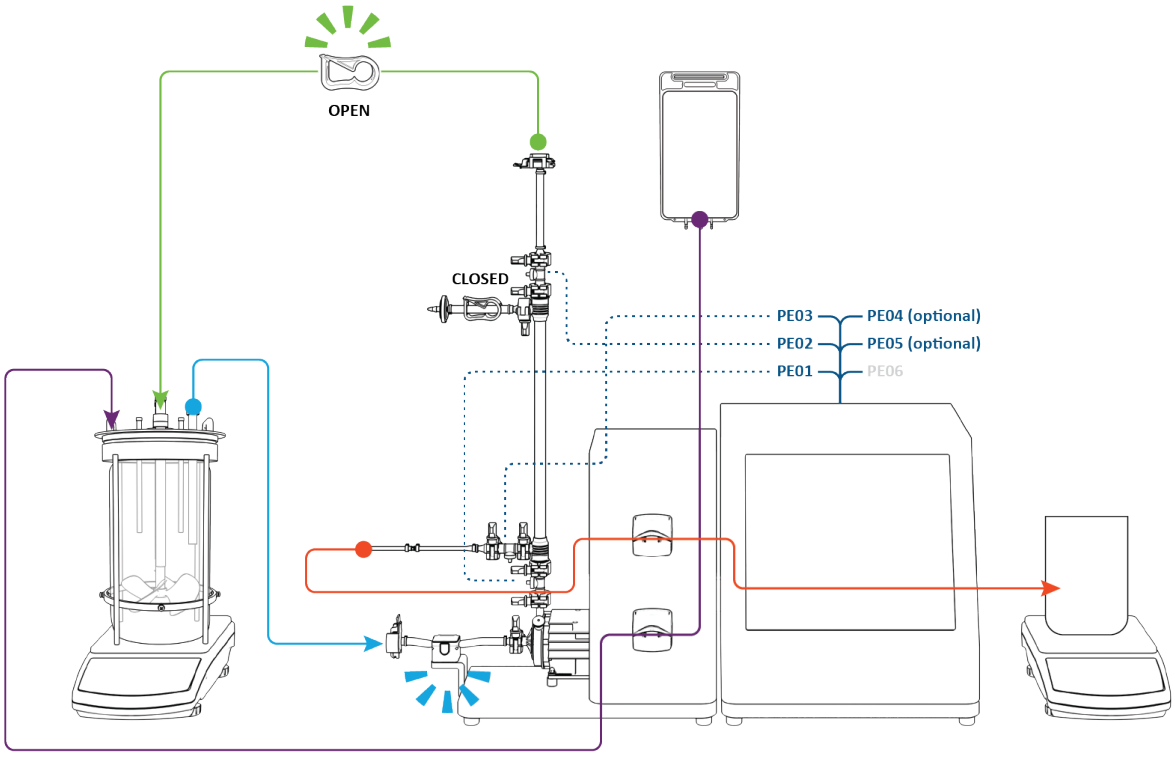
7. Permeate peristaltic pump P-02 will turn on. Fluid will flow from the bioreactor into the feed line and pump chamber. **Stop the peristaltic permeate pump P-02 when fluid can be seen in the feed pressure sensor above the pump chamber.**

IMPORTANT It is critical that no fluid enters into the TFDF®Filter element during priming.

8. Open clamp on retentate line.
9. Route **feed line** through **flow meter**.

Priming is complete.

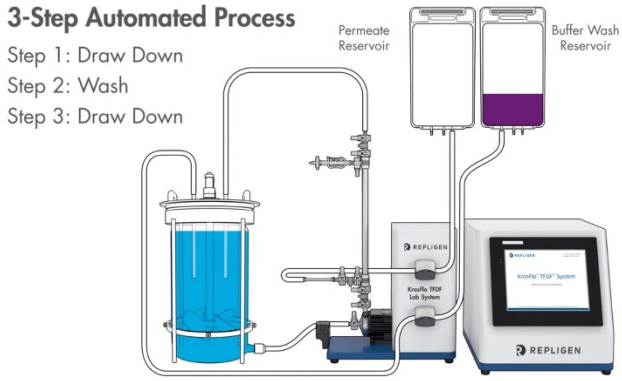
Figure 18. Primed system



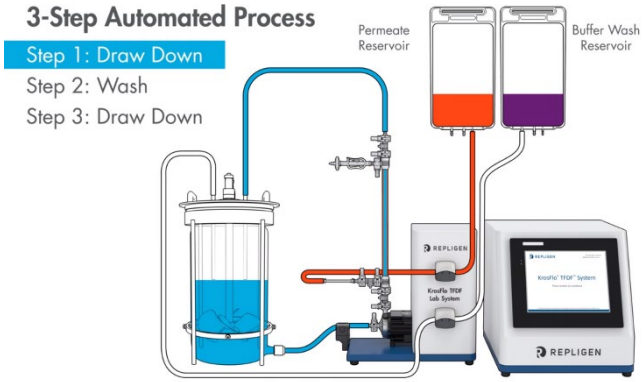
7.2 KrosFlo® TFDF®process

The typical KrosFlo® TFDF®experiment is a three-step process consisting of a draw down step, a wash step and finishing with a second draw down step.

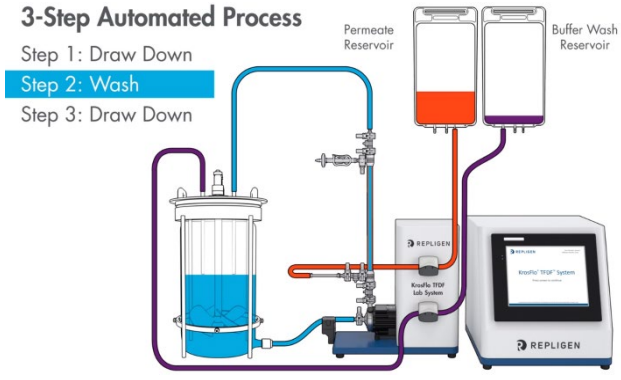
Set-up: At set-up, the cell culture feed starts in the bioreactor (blue). The permeate reservoir is empty and the buffer wash (purple) reservoir contains a volume equal to approximately **50%** of the cell culture feed volume.



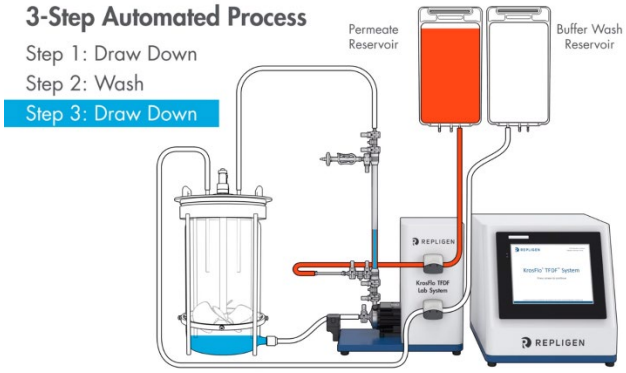
Step 1, Draw Down: The cell culture feed stock is pumped from the bioreactor through the lumen of the TFDF®filter. Retentate from the filter (blue) circulates back to the bioreactor while permeate from the filter (red) is routed to the permeate reservoir (red) with the permeate peristaltic pump operating in the clockwise direction. At the end of Step 1, approximately **50%** of the original cell culture volume has been transferred to the permeate reservoir and the cell culture feed has been effectively concentrated.



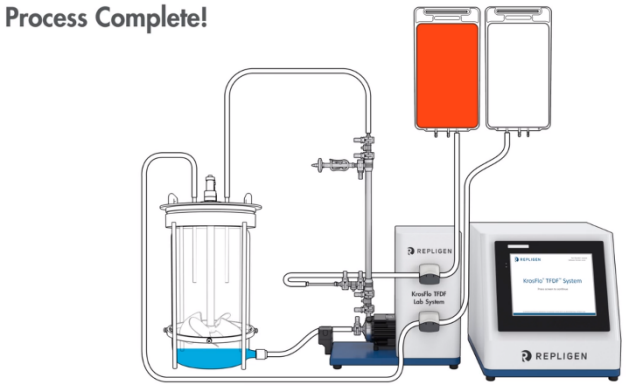
Step 2, Wash: Diafiltration/Wash buffer (purple) is pumped into the bioreactor while circulation of the cell culture feed stock continues. Permeate continues to accumulate in the permeate reservoir. At the end of Step 2, the permeate reservoir volume reaches approximately **100%** of starting cell culture volume. TMP is expected to decrease slightly.



Step 3, Draw Down: Introduction of wash buffer is discontinued, and the cell culture feed continues to circulate through the filter with retentate returning to the bioreactor and permeate routed to permeate reservoir. At the end of Step 3, clarification of the initial bioreactor feed stock had completed and the permeate reservoir volume had reached approximately 110 - 120% of the initial cell culture feed volume.



Completion: Measure turbidity and product concentration in the permeate reservoir to calculate turbidity reduction and yield. Disconnect the permeate reservoir and store for the next operation. Dispose of the filter and flow path according to your internal requirements.

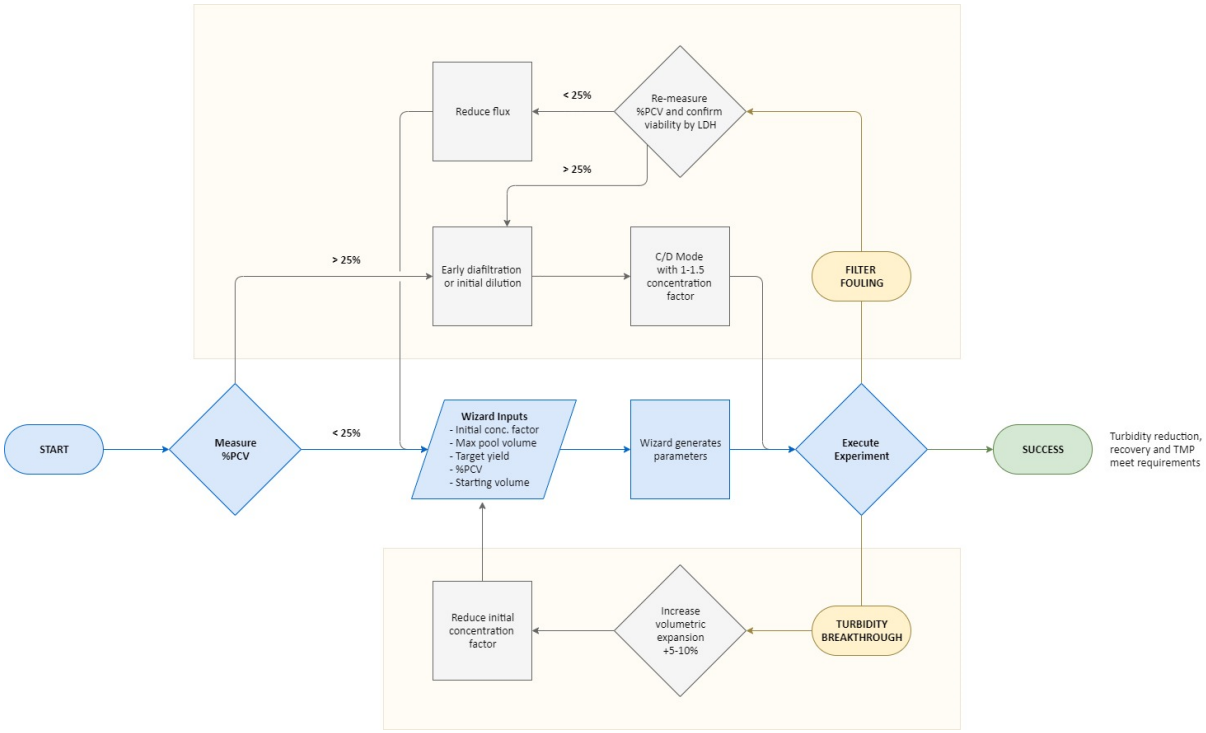


7.3 Sample and process information

Table 5. Sample and process

Parameter	Wizard feature requirement	Description
Percent Cell Volume (%PCV)	✓	• Values ~>20% may require extended diafiltration or initial dilution of feed with buffer/media. • Maximum value ~35 - 40%
Starting Volume	✓	• Choose volume representative of scaled/scalable process
Filter surface area		• Choose size representative of scaled/scalable process
Cell Density		• Minor impact on permeate quality when viability > 75% • Increasing significance with lower viabilities
Percent Viability		• < 75% may increase permeate turbidity • < 75% may require increased diafiltration
Feed stock turbidity		• Critical measurement for initial feed characteristic • Used to determine TFDF®turbidity reduction
Crossflow rate	✓	• Fixed value at 2L/min/fiber
Target yield	✓	• Typically 90 - 95% • Targeting higher yields may increase permeate turbidity • Targeting higher yields may increase diafiltration buffer requirement
Maximum final pool volume	✓	• Typically 110 - 120% of starting cell culture feed volume • Increasing may increase yield with challenging samples
Initial concentration factor	✓	• Fold concentration of starting cell culture feed • Typically 2X when %PCV < 15% • Decreasing may improve yield or breakthrough with challenging samples
Maximum time		• Maximum allowed process time • Typically calculated by Wizard feature or automated mode
Permeate Flux rate		• 650 LMH recommended standard value • Can be optimized based on specific feed characteristics • > 650 LMH possible with high viability & low %PCV
Diafiltration buffer volume		• Volume of diafiltration buffer wash in liters • Increasing volume may reduce fouling and increase yield with challenging samples

Figure 19. Experiment workflow



Running a KrosFlo® TFDF®experiment is made simple by the Wizard feature. Enter just 5 inputs to automatically generate run parameters:

- %PCV
- Max pool volume
- Target yield
- Starting volume
- Initial concentration factor

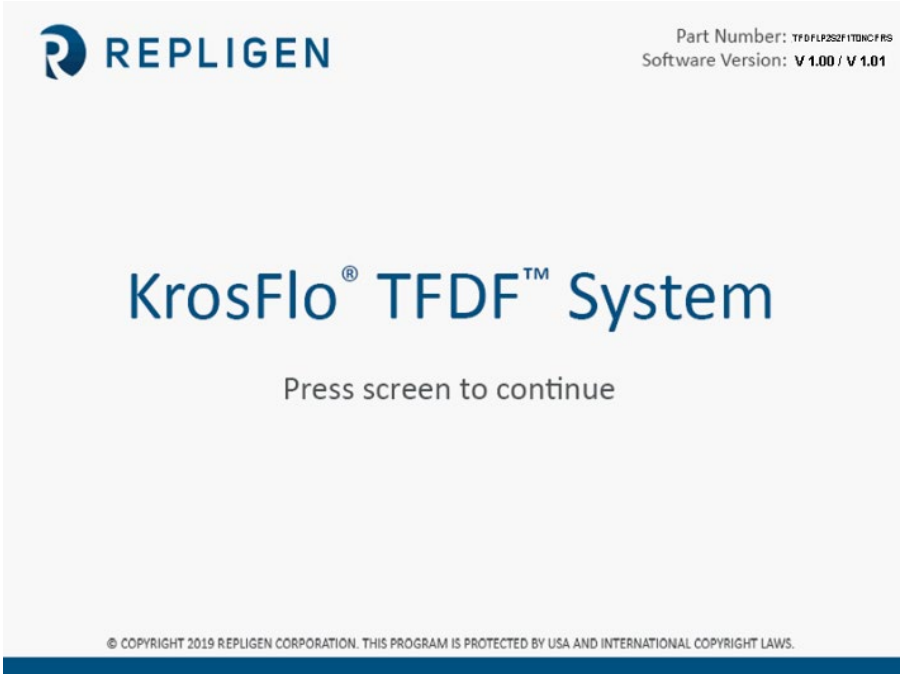
Typically, the most important experimental parameter is the %PCV. While all samples have their unique properties, it is generally found that samples with a %PCV below 25% may be run directly. Samples with %PCV > 25 may require either early diafiltration or an initial dilution of the feed material in buffer or media. Most samples meet recovery and turbidity reduction expectations with Wizard feature derived parameters and without optimization. If turbidity breakthrough is observed, it is recommended to increase volumetric expansion by 5 - 10% and reduce the initial concentration factor. If filter fouling is observed, it is recommended to verify the %PCV measurement. If %PCV is confirmed to be < 25%, reduction of the flux rate should be considered. If the %PCV is greater than 25%, trials with either early diafiltration or an initial dilution are potential steps towards improvement. If early diafiltration is implemented, recommended starting parameters are running in C/D mode with a concentration factor between 1 - 1.5.

8. Overview of system screens and functions

8.1 Information screen

The Information screen displays after the KrosFlo® TFDF®Lab System is powered up. It provides information including system part number and software version.

Figure 20. Information screen

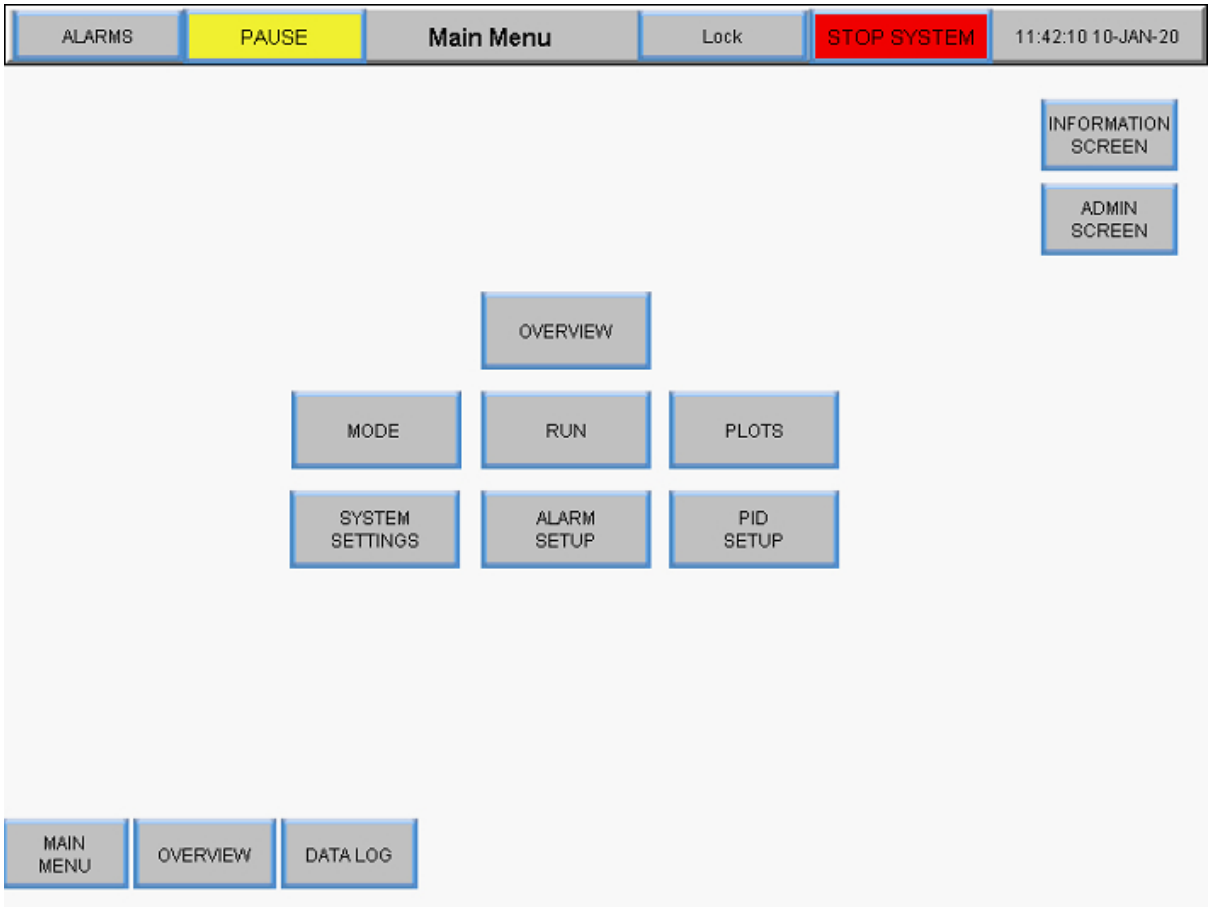


8.2 Main Menu screen

The buttons in the Main Menu screen provide access to all of the system's operational and set-up screens. To navigate to a specific screen, simply touch the button.

- **Overview:** Shows all the available auxiliary inputs (aux pumps, pressure sensors, scales, flow meters, turbidity meter) and their live values, available options on the screen will change depending on what automation mode is selected
- **Mode:** Allows the user to select an automation mode and select a filter part number
- **Run:** Allows the user to input run setpoints, parameters, and use the Wizard feature
- **Plots:** Shows live graphs and plots of the experiment
- **System Settings:** Allows the user to set pressure units, calibration factors, and max RPM of the main pump
- **Alarm Set-up:** Allows the user to set audible alarms and stop alarm setpoints
- **PID Set-up:** Allows the user to change PID values for main pump and aux pumps

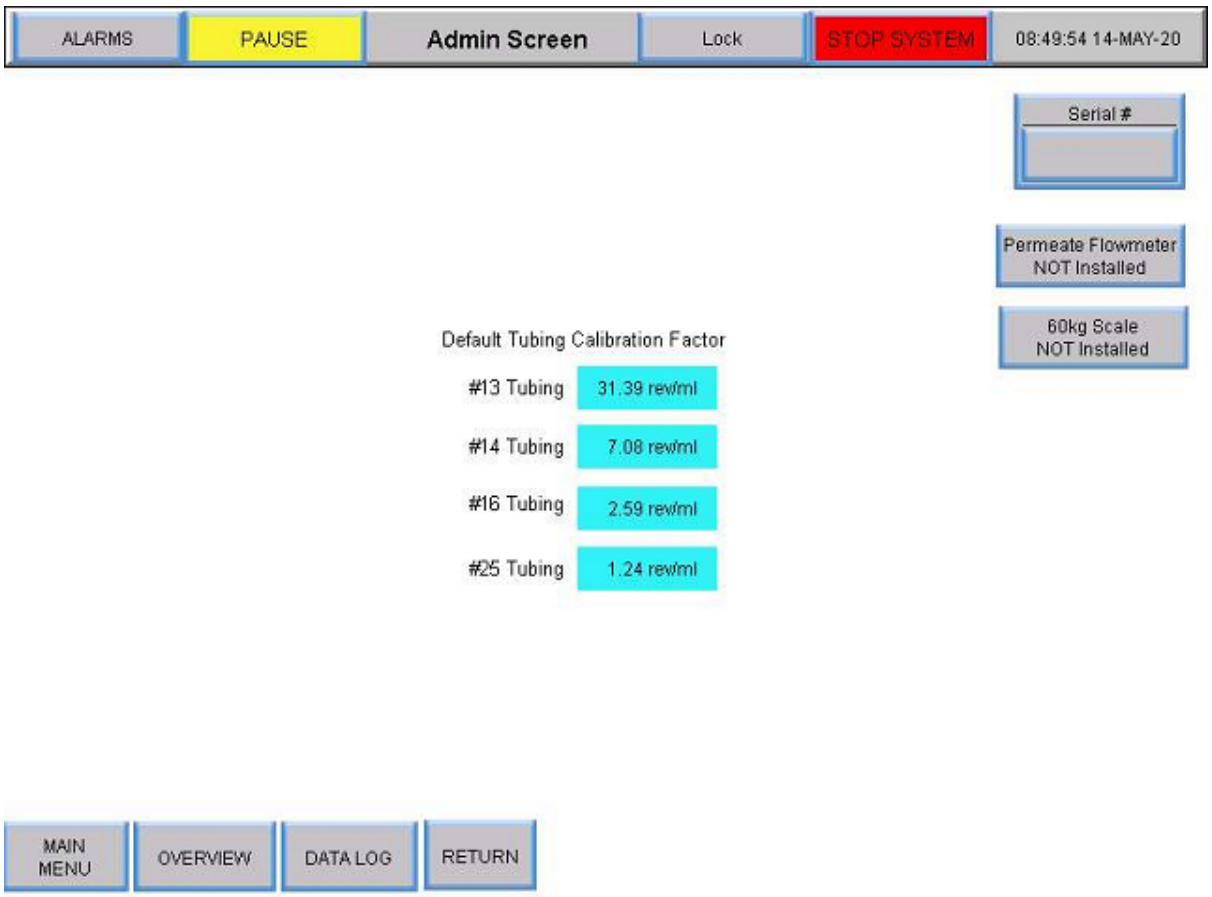
Figure 21. Main Menu screen



8.3 Admin screen

The Admin screen allows users to adjust default tubing calibration factors, change the system serial number and update the permeate flow meter installation status. To access this screen, select the **Admin Screen** button in the Main Menu screen.

Figure 22. Admin screen



8.3.1 Changing the default tubing calibration factors

The calibration factor converts pump rotations to a volumetric flow rate. Default values are included with the system. Values specific for your unit and tubing require measuring volume transfer over a period of time with a set pump rpm. Changing default values is done at the Admin level only. To change a tubing calibration factor setting, select one of the blue fields and enter a new default value. Setting the calibration factor here sets the default for operation.

Users can restore tubing calibration factors to default values using the **Reset Tubing Calibration** button in the System Settings screen. For more information, see the System Settings Screen section.

8.3.2 Changing the system serial number

Select **Serial #** and enter the new system serial number. Once the number is updated, the new value will display under Serial #.

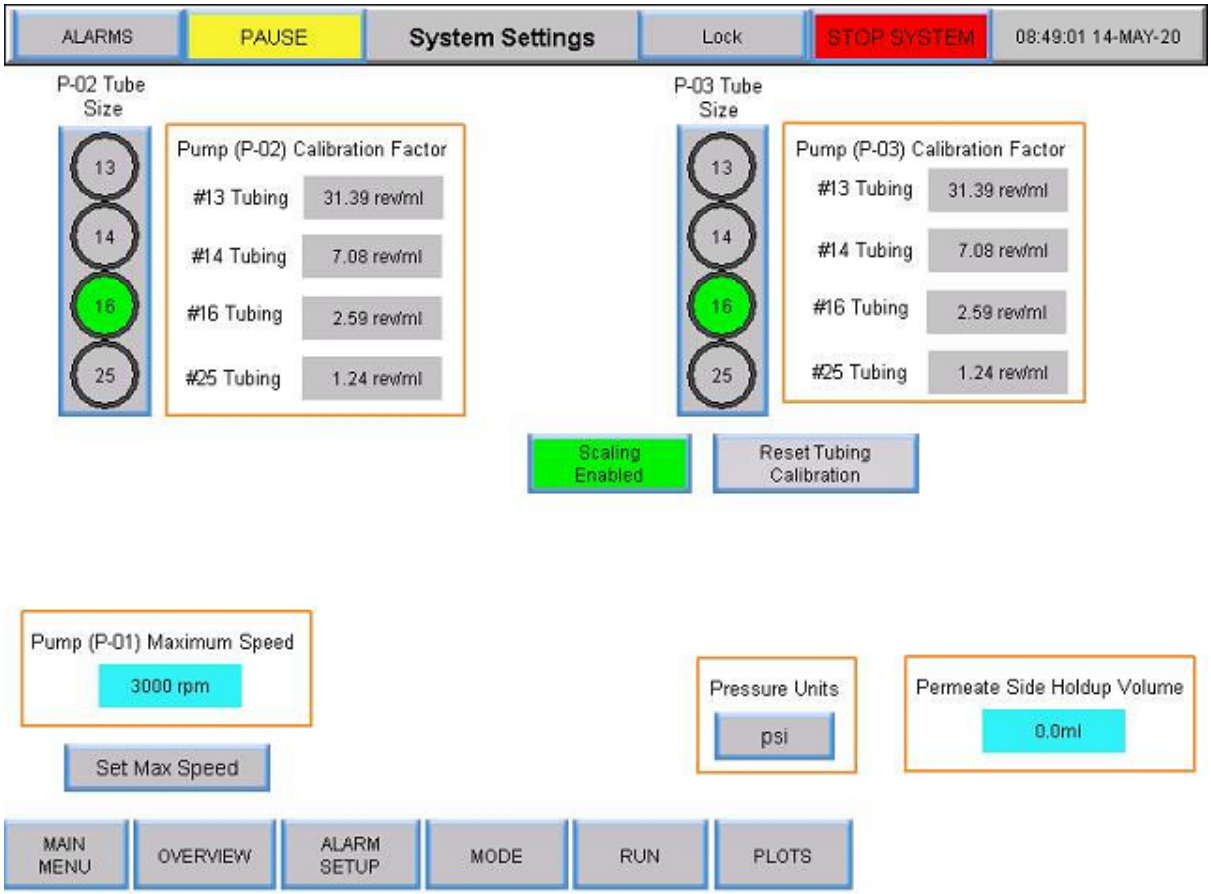
8.3.3 Updating the permeate flow meter installation status

To change flow meter installation status, toggle the **Permeate Flow meter** button between Installed and NOT Installed.

8.4 System Settings screen

The System Settings screen lets users set pressure units, tubing sizes and calibration factors, product vessel capacity and pump speeds. To access this screen, select the **System Settings** button in the Main Menu screen.

Figure 23. System Settings screen



8.4.1 Setting tubing sizes

1. Confirm the size of the tubing that will be used by noting the size printed directly on the tubing itself.
2. Select a circle button under the P-02 or P-03 Tube Size column to view size options. The button will turn green.
3. Options to select from will automatically start with #13 tubing. Select the desired tubing size.

8.4.2 Scaling

Select the **Scaling** button to toggle between Scaling Enabled and Scaling Disabled.

- When scaling is enabled (green), calibration factors are applied, and the user can enter a flowrate
- When scaling is disabled (red), the system will control the pump speed. Users can enter an RPM instead of a flowrate. Users can also select tubing calibration factors in the System Settings Screen and enter values that will be used instead of default values

8.4.3 Changing the tubing calibration factors for a run (not default)

1. Select a grey box next to a tubing size under the Pump (P-02) or Pump (P-03).
2. Choose a flow rate/rpm and measure the output on a scale.
3. Add the rpm/ml number in the respective box for calibration.

The following table shows the available flow rates for the KrosFlo® TFDF®Lab system.

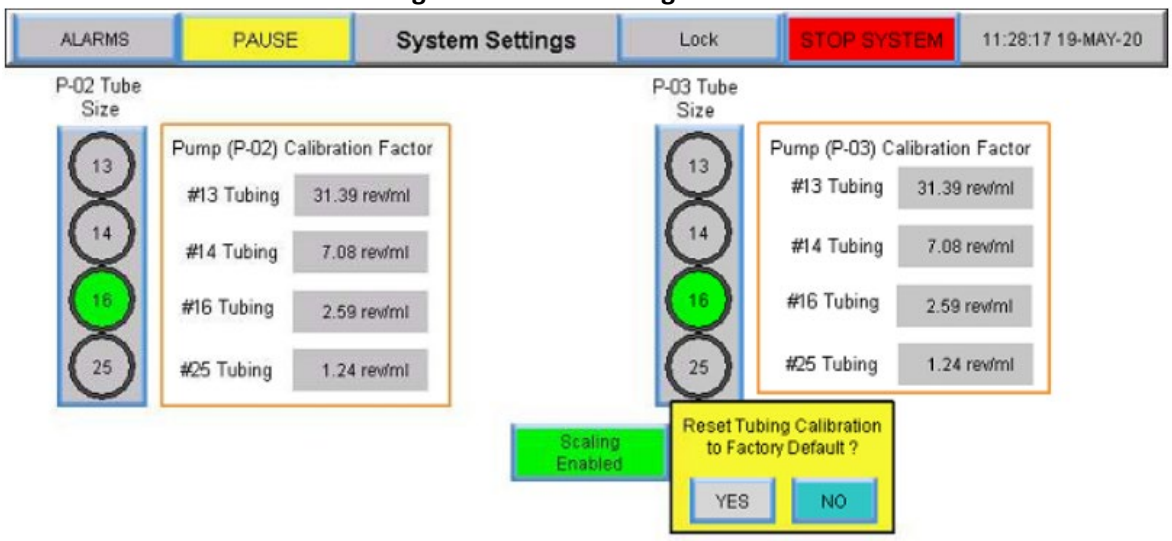
Table 6. KrosFlo® TFDF®Lab System permeate tubing flow rates

Tubing size	Low range (ml/min)	High range (ml/min)
#13, 0.76 mm	0	16.3
#14, 0.89 mm	0	57.0
#16, 1.52 mm	0	190.0
#25, 2.79 mm	0	340.0

8.4.4 Resetting Tubing Calibration factors

1. Select the **Reset Tubing Calibration** button.
2. Select **YES** to set the tubing calibration factors back to factory default settings.

Figure 24. Reset Tubing Calibration



Note: *Default tubing calibration factor settings can be set in the admin screen.*

8.4.5 Setting the maximum magnetic levitating recirculation/feed pump (P-01) speed

Select the **blue** box under Pump (P-01) Maximum Speed and enter a value. 2500 rpm is recommended for most applications.

8.4.6 Setting pressure units

Select the button box under pressure units to toggle between psi or mBar.

8.4.7 Setting the Permeate Side Hold-up Volume

Select the **blue** box under Permeate Side Hold-up Volume and enter a value.

8.5 PID loop settings

The **proportional–integral–derivative controller** (**PID controller** or **three-term controller**) is a control loop mechanism employing feedback between the systems pump and its sensors. A PID controller continuously calculates an *error value* as the difference between a desired setpoint (SP) and a measured process variable (PV) and applies a correction based on proportional, integral, and derivative terms (denoted *P*, *I*, and *D* respectively).

The distinguishing feature of the PID controller is the ability to use the three *control terms* of proportional, integral and derivative influence on the controller output to apply accurate and optimal control. The controller attempts to minimize the error over time by adjustment of a *control variable*, such as the speed of a pump, to a new value determined by a weighted sum of the control terms.

In this model:

1. **Term P** is proportional to the current value of the SP - PV error *e(t)*. For example, if the error is large and positive, the control output will be proportionately large and positive, taking into account the gain factor "K". Using proportional control alone will result in an error between the setpoint and the actual process value, because it requires an error to generate the proportional response. If there is no error, there is no corrective response.
2. **Term I** accounts for past values of the SP - PV error and integrates them over time to produce the **I** term. For example, if there is a residual SP - PV error after the application of proportional control, the integral term seeks to eliminate the residual error by adding a control effect due to the historic cumulative value of the error. When the error is eliminated, the integral term will cease to grow. This will result in the proportional effect diminishing as the error decreases, but this is compensated for by the growing integral effect.
3. **Term D** is a best estimate of the future trend of the SP - PV error, based on its current rate of change. It is sometimes called "anticipatory control", as it is effectively seeking to reduce the effect of the SP - PV error by exerting a control influence generated by the rate of error change. The more rapid the change, the greater the controlling or dampening effect.
4. **Tuning** - The balance of these effects is achieved by loop tuning to produce the optimal control function. The tuning constants are shown below as "K" and must be derived for each control application, as they depend on the response characteristics of the complete loop external to the controller. These are dependent on the behavior of the measuring sensor, the final control element (such as a control valve), any control signal delays and the process itself. Approximate values of constants can usually be initially entered knowing the type of

application, but they are normally refined, or tuned, by "bumping" the process in practice by introducing a setpoint change and observing the system response.

To access this screen, select the **PID Set-up** button in the Main Menu screen.

Values for Proportional, Integral and/or Derivative loop settings can be added for Recirc/Feed pump control, Reactor volume control and Permeate flow control. The default values are optimized for stable control and are recommended. To add or change values, select a blue box and enter a value.

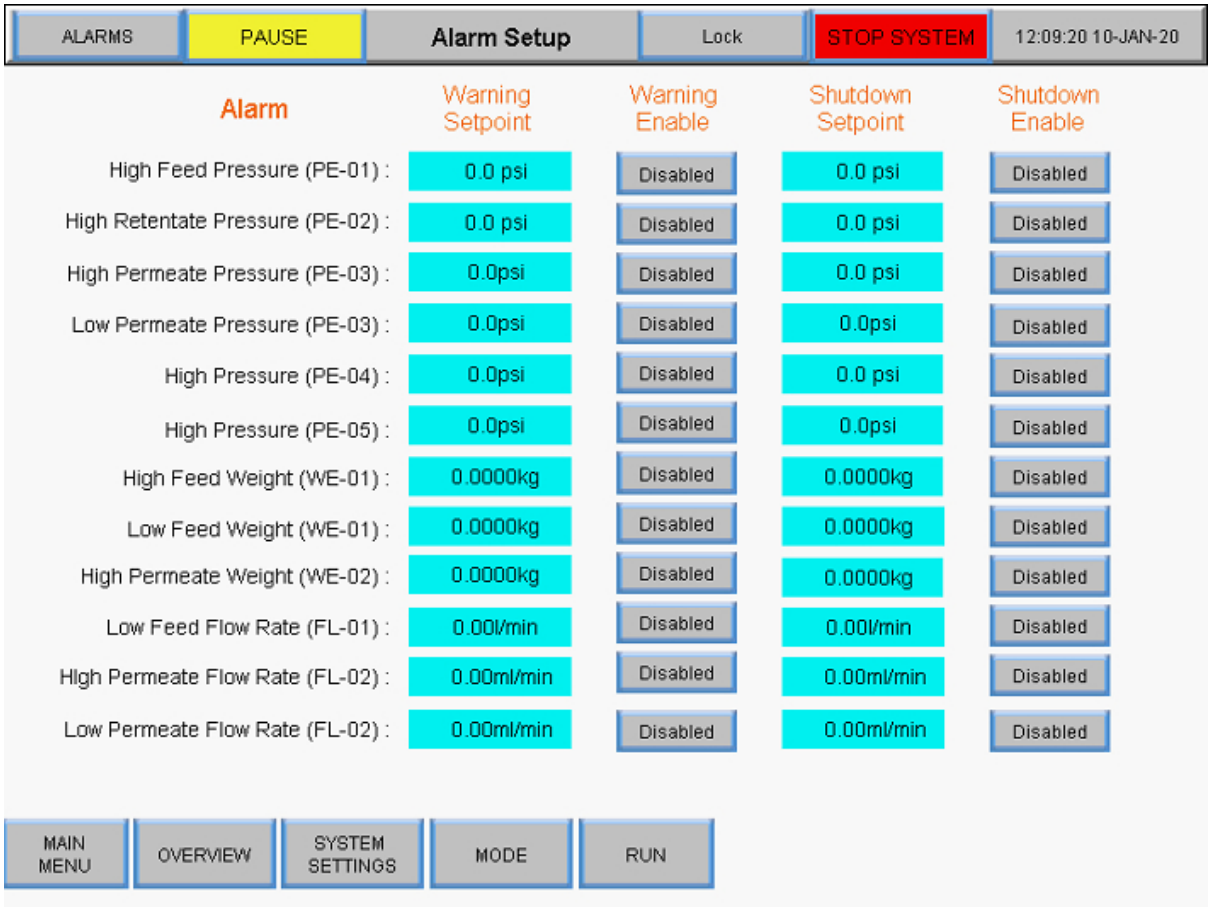
Figure 25. PID loop settings screen

8.6 Alarms

8.6.1 Alarm Set-up screen

The Alarm Set-up screen displays all configurable alarms for the KrosFlo® TFDF®Lab System. These alarms are designed to help protect the system and users during operation. To access this screen, select **Alarms** in the menu bar.

Figure 26. Alarm Set-up screen



The following system alarms are available:

1. High Feed Pressure (PE-01): Monitors if the feed pressure into the filter has increased due to obstruction in the fibers in the filter or the tubing going into the filter. Check for bent tubing.
2. High Retentate Pressure (PE-02): Monitors if the retentate pressure on the tubing exiting the filter is increased. Check for bent tubing or obstructions in the tubing lines back to the recirculation vessel.
3. High Permeate Pressure (PE-03): Monitors if the permeate pressure on the filtrate side is high due to an obstruction or bent tubing.
4. Low Permeate Pressure (PE-03): Indicates a fouled filter. Terminate the run if near completion or reduce the flux to finish the run.
5. High Pressure (PE-04) – Indicates a fouled secondary filter. Replace the filter
6. High Pressure (PE-05) – Indicates a fouled secondary filter (for example, the sterile guard filter). Replace the filter.
7. High Feed Weight (WE-01): Used to make sure the recirculation vessel does not overfill.
8. Low Feed Weight (WE-01): Used to make sure the recirculation vessel does not run dry.
9. High Permeate Weight (WE-02) – Used to make sure the permeate vessel does not overfill.

10. High Feed Flow Rate (FL-01) – Indicates a high flow rate which can shear cells.
11. Low Feed Flow Rate (FL-01) – Indicates a low-flow issue which could rapidly foul the filter due to insufficient crossflow.
12. High Permeate Flow Rate (FL-02) – Indicates flux is too fast which could foul the filter.
13. Low Permeate Flow Rate (FL-02) – Indicates flux is too low which could be indicative of a fouled filter or obstruction in the permeate line.

Alarms have two categories:

1. **Warning Alarms** – These display as a flashing orange starburst approximately once per second. A beep will also sound when an alarm setpoint is reached. The system will continue to run if a warning alarm is triggered but will indicate active alarm.
2. **Shutdown Alarms** – These shut down the sequence, but not the full system. For example, the recirculation/feed pump will stay on, the permeate pump will stop, and the diafiltration pump will stop to maximize the opportunity to recover the run.

High alarms will trigger when the process value rises to the stored setpoint or above. Low alarms will trigger when the process value falls to the stored setpoint or below. The system is equipped with a short delay to avoid low alarms during startup conditions.

To modify an alarm threshold value, select the appropriate blue box in the Warning or Shutdown Setpoint column and enter the desired value. Users can enable or disable an alarm by toggling the grey **Warning Enable** or **Shutdown Enable** buttons.

If an alarm triggers, a red blinking Alarm Reset button will display in the bottom right corner of the system screen and will remain until the issue is resolved. It can be cleared once the system is operating below alarm conditions. Selecting the **Alarm Reset** button will silence the alarm and reset the flashing button.

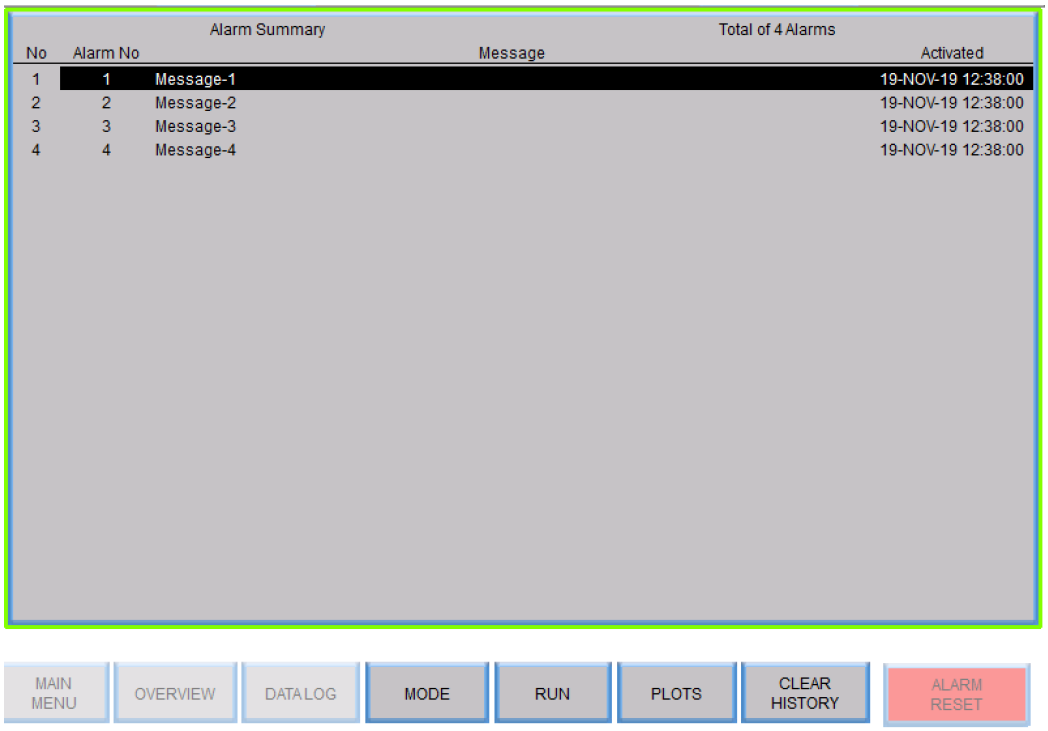
Figure 27. Alarm Reset button

8.6.2 Alarm History

The Alarm History screen logs a complete history of any configured alarm that was triggered by the system. To access this screen, select **Alarms** in the menu bar.

To erase the list of prior alarms, select **Clear History** button.

Figure 28. Alarm History screen

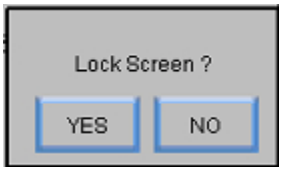


8.7 Lock screen

The Lock option on the KrosFlo® TFDF®System allows users to lock the screen for cleaning without inadvertently affecting system operation.

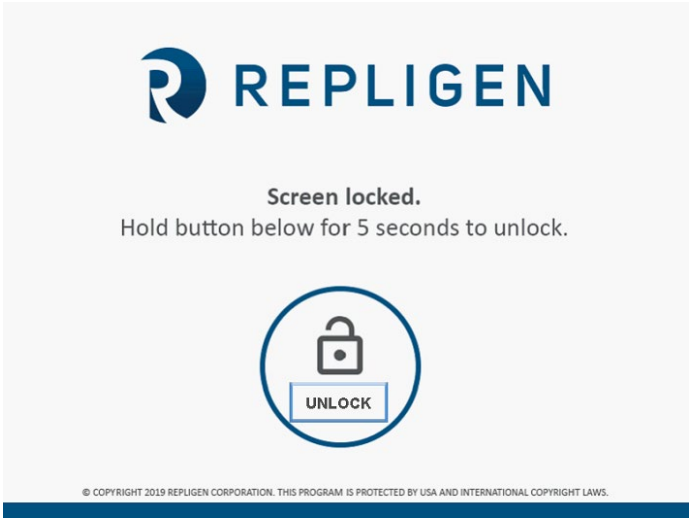
1. Select Lock in the menu bar. The following message will display:

Figure 29. Lock screen message



2. Select **Yes**. The Lock Screen will display:

Figure 30. Lock screen



To unlock the screen, press and hold the **Unlock** button for five seconds.

8.8 Data logging

This screen allows users to transfer data from the KrosFlo® TFDF®System to a USB drive. To access this screen, select **Data Log** from the Main Menu screen.

Figure 31. Data logging screen

To record process data, insert a USB drive into the USB port of the Controller. Data logging activates automatically with detection of a USB drive.

When the data is logging, the USB Ready button will be green and display “On”. When the data is writing, the USB Writing button will be green display “On” for a short time. When data logging is

active and a USB drive is not installed, the following error message will appear on the top of the screen: **RTE-004: Log buffer memory is full**.

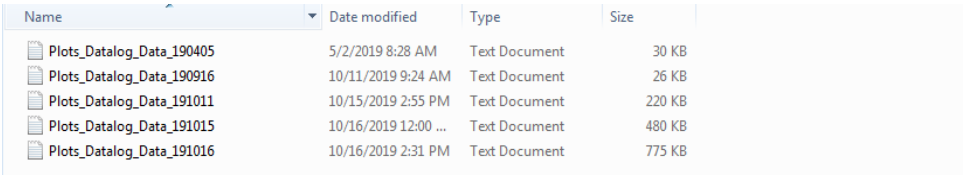
To remove the USB Drive, select the **USB Eject** button.

IMPORTANT: Insert the USB drive into the system prior to a run. Run data will not be recorded if the USB drive is not present when the run starts.

8.8.1 Experimental data

Experimental settings and measured values are stored on a USB for all modes. The resolution with which a measured parameter is saved is 30 seconds. The data log is saved daily with the date (YYMMDD) at the end of the file name.

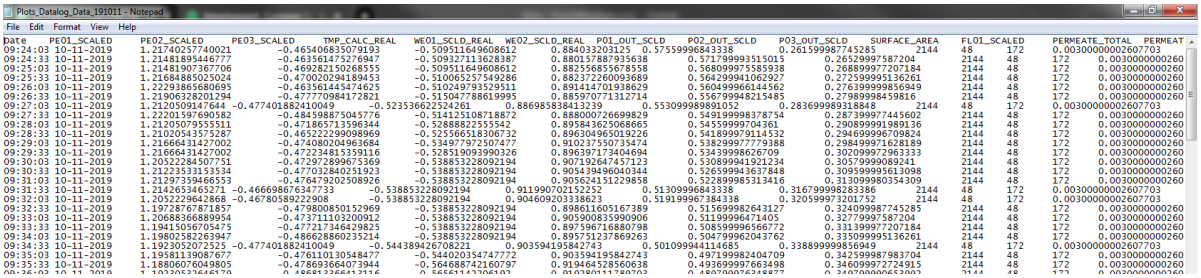
Figure 32. Data Log Files



Recorded data includes the following columns:

- Timestamp (hh:mm:ss)
- Pressure sensors (PSI)
  - PE01 (Feed)
  - PE02 (Retentate)
  - PE03 (Permeate)
  - PE04 (Secondary Filter)
  - PE05 (Sterile/Guard Filter)
- TMP Calculated: (Feed pressure + Retentate pressure)/2 - Permeate pressure
- Scale readings (kg)
  - Feed scale
  - Retentate scale
- Module surface area (calculated from selected filter PN): (Fiber Count * Pi * Effective Length * Fiber size)
- Flow meters
  - Feed flow measurement
  - Permeate flow measurement (optional)
  - Permeate totalizer (optional, calculated using the detected flow rate / run duration)
- Turbidity meter (optional)
- Concentration factor (CF) calculation: Starting vol. / (Starting vol. - (Feed wt at start - (Current feed wt – Perm. Hold-up))
- Diafiltration volume (DV) calculation: (Permeate total – Perm wt at start of D mode) / (Starting vol. - (Feed wt at start – Feed wt at start of D mode))

Figure 33. Recorded data example



Plots_Datalog_Data_191011 - Notepad

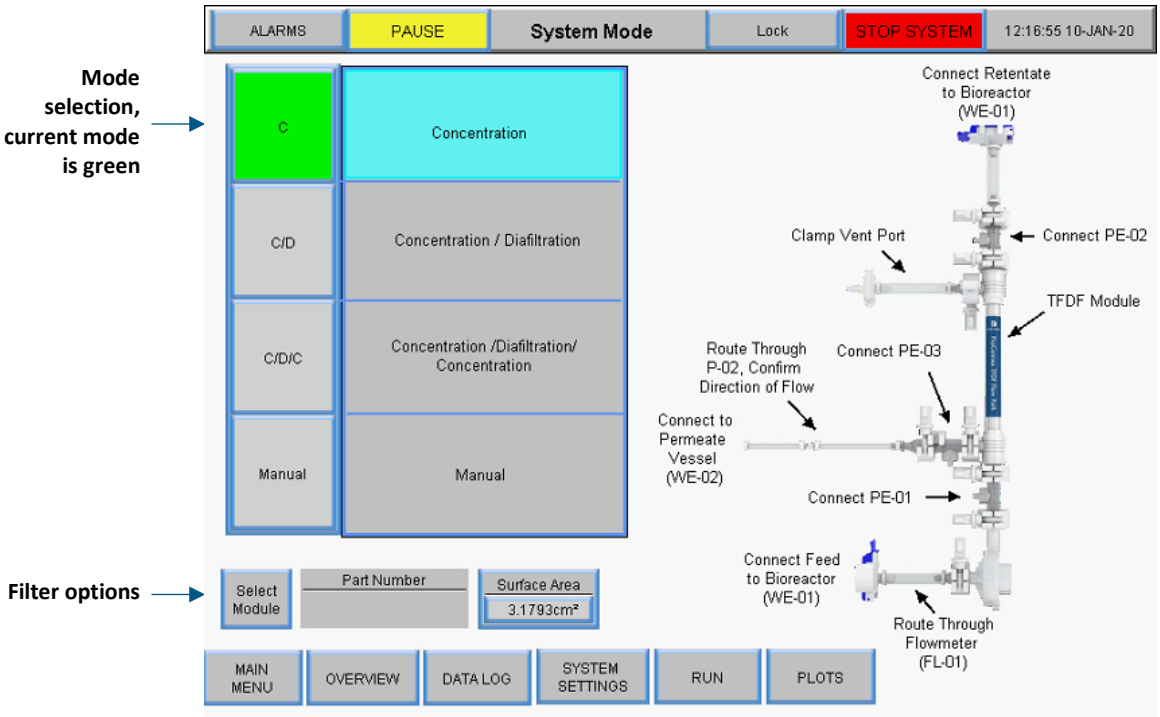
File Edit Format View Help

Date	PE01_SCALED	PE02_SCALED	PE03_SCALED	TMP_CALC_REAL	WE01_SCLD_REAL	WE02_SCLD_REAL	P01_OUT_SCLD	P02_OUT_SCLD	P03_OUT_SCLD	SURFACE_AREA	FL01_SCALED	PERMEATE_TOTAL	PERMEAT
09:24:03 10-11-2019	1.21740257740021	-0.46540683507919	-0.46540683507919	-0.46540683507919	-0.5095116496086	0.884033203125	0.575599968433	0.261599987745	2144	48	172	0.00300000002607	
09:24:33 10-11-2019	1.21481895446777	-0.46356147527694	-0.46356147527694	-0.46356147527694	-0.5093271136283	0.880157887935	0.571799993515	0.26529997587	2144	48	172	0.00300000002607	
09:25:03 10-11-2019	1.21481907367706	-0.46928215026855	-0.46928215026855	-0.46928215026855	-0.5095116496086	0.882556855678	0.568099975585	0.26889997720	2144	48	172	0.00300000002607	
09:25:33 10-11-2019	1.21684885025024	-0.47002029418945	-0.47002029418945	-0.47002029418945	-0.5100652575492	0.882372260093689	0.564299941062927	0.27259999513626	2144	48	172	0.00300000002607	
09:26:03 10-11-2019	1.22293865680695	-0.46356144547462	-0.46356144547462	-0.46356144547462	-0.5102497935295	0.891414701938629	0.560499966144562	0.27619999985694	2144	48	172	0.00300000002607	
09:26:33 10-11-2019	1.21906328201294	-0.47777094817282	-0.47777094817282	-0.47777094817282	-0.5150477886619	0.885970771312714	0.55679994821548	0.27989998459816	2144	48	172	0.00300000002607	
09:27:03 10-11-2019	1.21205091476440	-0.47740188241004	-0.47740188241004	-0.47740188241004	-0.5235366225242	0.886985838413239	0.553099989891052	0.28369998931884	2144	48	172	0.00300000002607	
09:27:33 10-11-2019	1.22201597690582	-0.48459887054776	-0.48459887054776	-0.48459887054776	-0.5141251087188	0.888000726998	0.549199998378754	0.28739997744560	2144	48	172	0.00300000002607	
09:28:03 10-11-2019	1.21205079555511	-0.47186571359634	-0.47186571359634	-0.47186571359634	-0.5288882255554	0.895843625068665	0.545599997043610	0.29089999198913	2144	48	172	0.00300000002607	
09:28:33 10-11-2019	1.21020543575287	-0.46522229909896	-0.46522229909896	-0.46522229909896	-0.5255665183067	0.896304965019226	0.541899979114532	0.29469999670982	2144	48	172	0.00300000002607	
09:29:03 10-11-2019	1.21666431427002	-0.47408020496368	-0.47408020496368	-0.47408020496368	-0.5349779725074	0.910237550735474	0.538299977779388	0.29849997162818	2144	48	172	0.00300000002607	
09:29:33 10-11-2019	1.21666431427002	-0.47223481535911	-0.47223481535911	-0.47223481535911	-0.5285190933900	0.896397173404694	0.534399986267090	0.30209997296333	2144	48	172	0.00300000002607	
09:30:03 10-11-2019	1.20522284507751	-0.47292889675354	-0.47292889675354	-0.47292889675354	-0.5388532280921	0.907192647457123	0.530899941921234	0.30579999089241	2144	48	172	0.00300000002607	
09:30:33 10-11-2019	1.21223533153534	-0.47703284025192	-0.47703284025192	-0.47703284025192	-0.5388532280921	0.905439496040344	0.526599943637848	0.30959999563098	2144	48	172	0.00300000002607	
09:31:03 10-11-2019	1.21297359466553	-0.47647920250892	-0.47647920250892	-0.47647920250892	-0.5388532280921	0.905624151229858	0.522899985331416	0.31309998034309	2144	48	172	0.00300000002607	
09:31:33 10-11-2019	1.21426534652710	-0.46669867634773	-0.46669867634773	-0.46669867634773	-0.5388532280921	0.911990702152252	0.519099968433388	0.31679998283386	2144	48	172	0.00300000002607	
09:32:03 10-11-2019	1.20522296428680	-0.46780589222908	-0.46780589222908	-0.46780589222908	-0.5388532280921	0.904609203338623	0.519199967384338	0.32059997320175	2144	48	172	0.00300000002607	
09:32:33 10-11-2019	1.19728767871857	-0.47980850152969	-0.47980850152969	-0.47980850152969	-0.5388532280921	0.898611605167389	0.515699982643127	0.32409998774528	2144	48	172	0.00300000002607	
09:33:03 10-11-2019	1.20688366889954	-0.47371103200912	-0.47371103200912	-0.47371103200912	-0.5388532280921	0.905900835990906	0.51199996471405	0.32779997587204	2144	48	172	0.00300000002607	
09:33:33 10-11-2019	1.19415056705475	-0.47721734642982	-0.47721734642982	-0.47721734642982	-0.5388532280921	0.897596716880798	0.508599996566772	0.33139997720718	2144	48	172	0.00300000002607	
09:34:03 10-11-2019	1.19802582263947	-0.48662886023521	-0.48662886023521	-0.48662886023521	-0.5388532280921	0.895751237869263	0.504799962043762	0.33509999513626	2144	48	172	0.00300000002607	
09:34:33 10-11-2019	1.19230320072525	-0.47740188241004	-0.47740188241004	-0.47740188241004	-0.5443894267082	0.903594195842743	0.501099944114685	0.33889999858949	2144	48	172	0.00300000002607	
09:35:03 10-11-2019	1.19581139087677	-0.47611013054847	-0.47611013054847	-0.47611013054847	-0.5440203547477	0.903594195842743	0.49719998240470	0.34259997983704	2144	48	172	0.00300000002607	
09:35:33 10-11-2019	1.18806076049805	-0.47869366407394	-0.47869366407394	-0.47869366407394	-0.5646887421607	0.919464528560638	0.49369999766394	0.34609997272491	2144	48	172	0.00300000002607	

8.9 System Mode

The System Mode screen lets users operate and monitor the system and select and manage its different operational modes. To access this screen, select **Mode** from the Main Menu Screen.

Figure 34. System Mode screen



The KrosFlo® TFDF®System has four operational modes:

1. **Concentration Mode:** Automated filtration mode where retentate is concentrated to a certain concentration factor.
2. **Concentration/Diafiltration Mode:** Automated filtration mode where retentate is concentrated to a certain concentration factor, then run in diafiltration mode where retentate volume is kept constant through addition of buffer/media.
3. **Concentration/Diafiltration/Concentration Mode:** Automated filtration mode where retentate is concentrated to a certain concentration factor, then run in diafiltration mode where retentate volume is kept constant through addition of buffer/media, and finally retentate is concentrated again to a final concentration factor.
4. **Manual Mode:** Open mode where the user can start/stop any pump(s), tare scales, sensors.

To select an operational mode, select a mode button. The mode currently selected will be green. Each mode has a Run Setpoints and Overview screen.

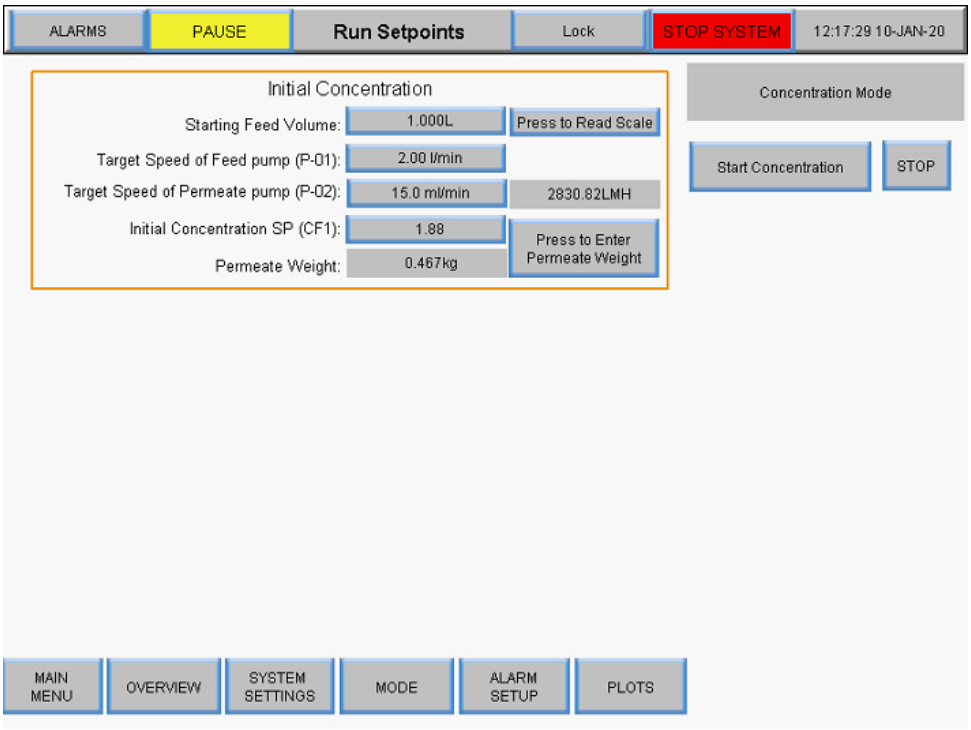
8.10 Concentration, Concentration/Diafiltration and Concentration/Diafiltration/ Concentration Modes

8.10.1 Run Setpoints screen

For the automated modes, the Run Setpoints screen allows manipulation of select setpoints or options for the operational mode. To access this screen, select the **Run** button at the bottom of the System Mode Screen.

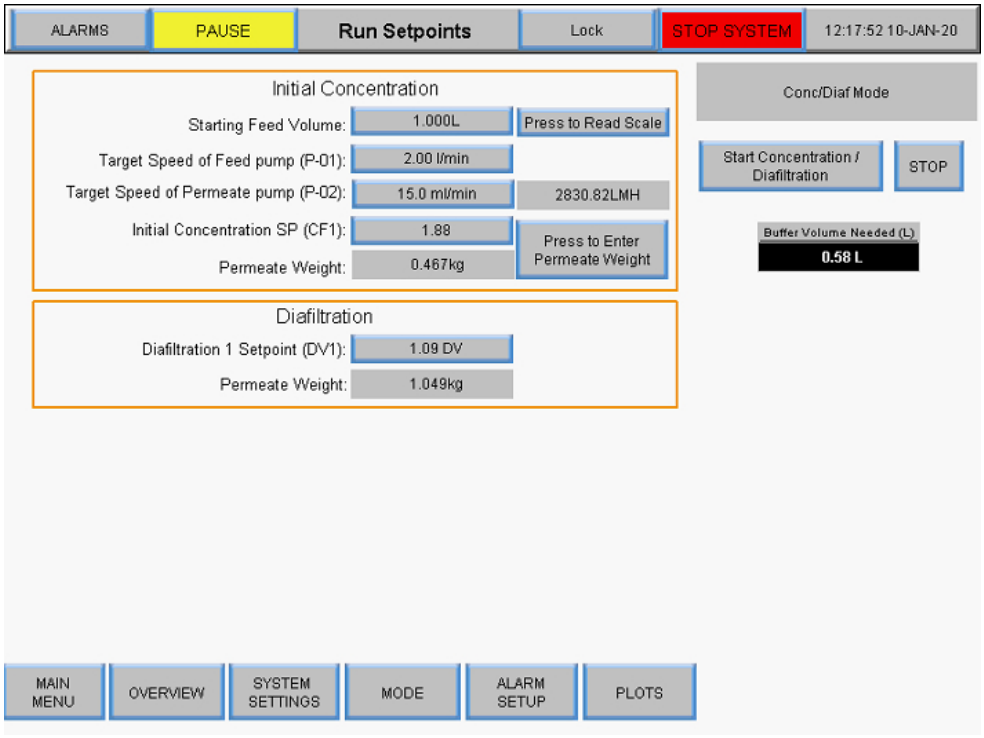
- **Concentration Mode**: Run Setpoints screen used to configure a simple draw down process. Users can select the Concentration Factor (CF) or Permeate Weight as the setpoint

Figure 35. Concentration Mode Run Setpoints screen



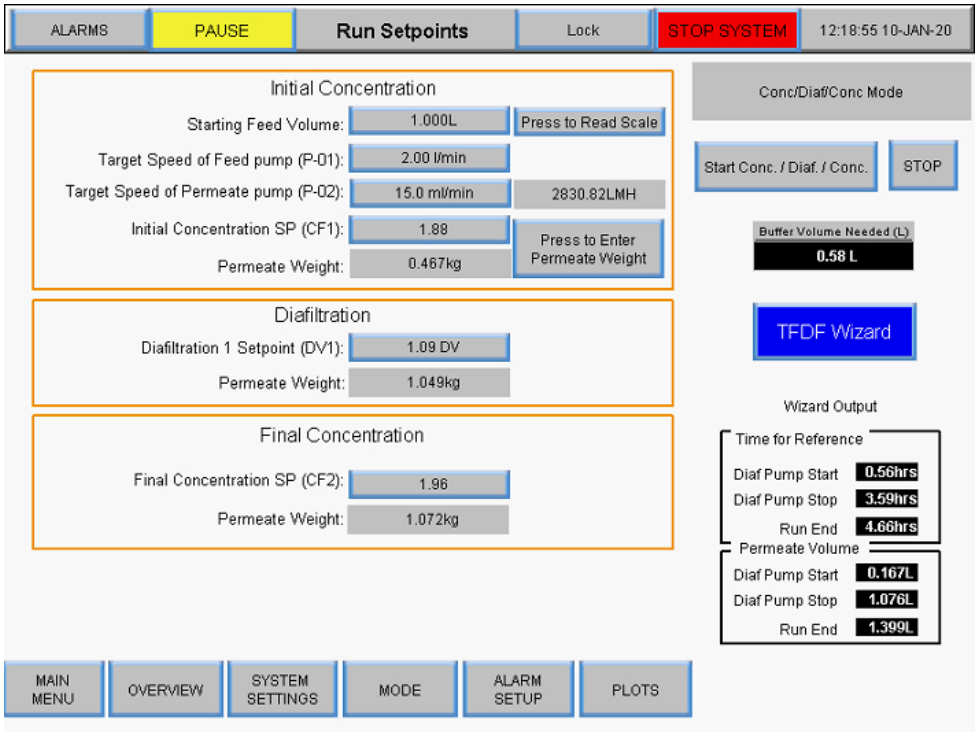
- **Concentration/Diafiltration Mode:** Run Setpoints screen is used to configure a drawdown followed by a buffer addition step, users can also use Concentration Factor (CF) or Permeate Weight as the end point for the Concentration step

Figure 36. Concentration/Diafiltration Mode Run Setpoints screen



- **Concentration/Diafiltration/Concentration Mode:** Run Setpoints Screen is used to configure an initial drawdown, buffer addition and a final drawdown, users have the same options for setpoints as the previous modes, selecting the **Start Concentration/Diafiltration/Concentration** button in this mode initiates the Wizard feature that automatically runs calculations to determine various setpoints, for more information, refer to the Wizard feature section

Figure 37. Concentration/Diafiltration/Concentration Mode Run Setpoints screen



Commands:

- **Start:** Starts the automated run at the setpoints entered
- **Stop:** Stops the run
- **Enter:** This button appears on both the Overview Screen and the Run Setpoints screen once the mode is doneselect the **Enter** button to acknowledge the automation mode is done

Initial Concentration setpoint values (all modes):

- **Target Speed of Feed pump (P-01):** The target speed of the magnetic levitating recirculation/feed pump controls the rpm of the pump with feedback from clamp-on flow meter (FL-01)
- **Target Speed of Permeate pump (P-02):** Input the target speed of the permeate pump in either ml/min or VVD, the system measures the change in weight on the permeate scale and controls the rpm of the permeate pump to match target permeate rate
- **Initial Concentration SP (CF1):** A dimensionless concentration factor used to quantify concentration of the feedstock it is the amount that the feedstock has been reduced in volume relative to the initial volume for example, if 1 L of feedstock is concentrated to 0.25L with 0.75 L padding through the filter as permeate, a 4-fold concentration has been performed, the Concentration Factor would therefore be 4
- **Permeate Weight:** This value refers to the total cumulative permeate weight at end of the final concentration step, input the target weight for the cells mass on the WE-01 scale, the system controls the rpm of P-03 to keep the mass at the target weight, tare with the empty product vessel so only the weight of the sample is read, the target should then be the weight of the sample once the recirculation line is filled

Diafiltration setpoint values (Concentration/Diafiltration and Concentration/Diafiltration/ Concentration modes):

- **Diafiltration 1 Setpoint (DV1):** A Diavolume (DV) is a measure of volume passed through the filter as permeate during the diafiltration step, it is based on the volume of diafiltration buffer introduced into the unit operation compared to the retentate volume at the start of the operation, for instance, if 5 L of feedstock are present at the beginning of diafiltration and the operation calls for 2 DV, then 10 L will pass through the filter as permeate while continuously adding buffer to maintain 5 L of retentate

Final Concentration Setpoint values (Concentration/Diafiltration/Concentration mode only):

- **Final Concentration SP (CF2):** A dimensionless concentration factor used to quantify feedstock concentration after a diafiltration has been performed it is the feedstock volume reduction factor relative to the initial starting volume, not from the start of the second concentration, for instance, if 1 L of feedstock is processed until 0.75 L has passed through to the filtrate and 0.25 L is left in the retentate, a 4-fold concentration has been performed so the Concentration Factor is 4X and the input would be 4
- **Permeate Weight:** This value refers to the total cumulative permeate weight at end of the final concentration step

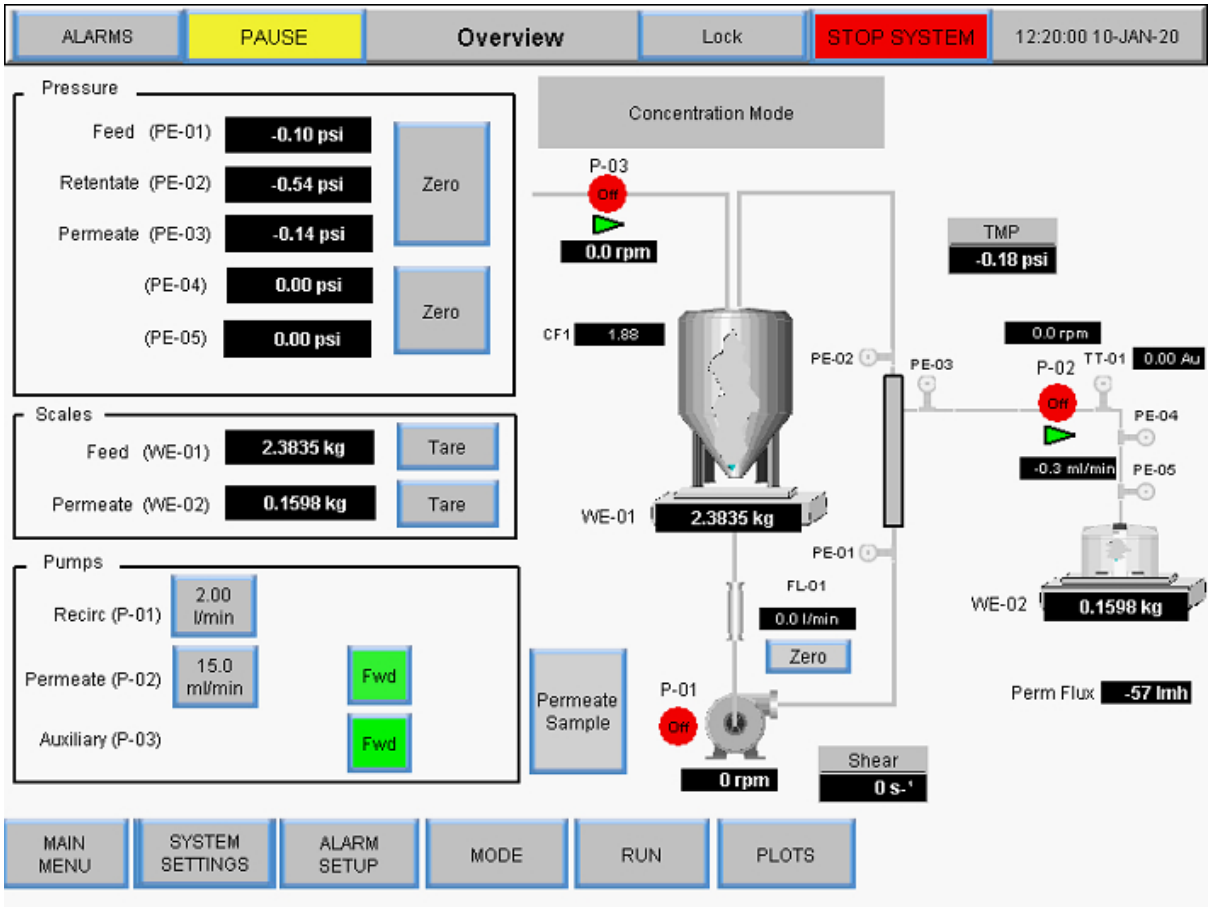
8.10.2 Overview screen

The Overview screen displays the KrosFlo® TFDF®Lab System's operational flow path and instrumentation. Process data (flow, pressure, volume) is displayed on-screen in real time. Process data outputs are displayed in the black boxes. Setpoint input data is displayed in the grey boxes. To access this screen, select the **Overview** button at the bottom of the System Mode Screen.

Concentration Mode operations:

- User input of Concentration Factor or Permeate weight for Concentration step
- Change direction of Auxiliary pumps (P-02 and P-03)
- Tare scales (WE-01 and WE-02)
- Tare pressure sensors (PE-01, PE-02, PE-03, PE-04, PE-05, and PE-06)
- Tare Flow meter (FL-01 and FL-02)

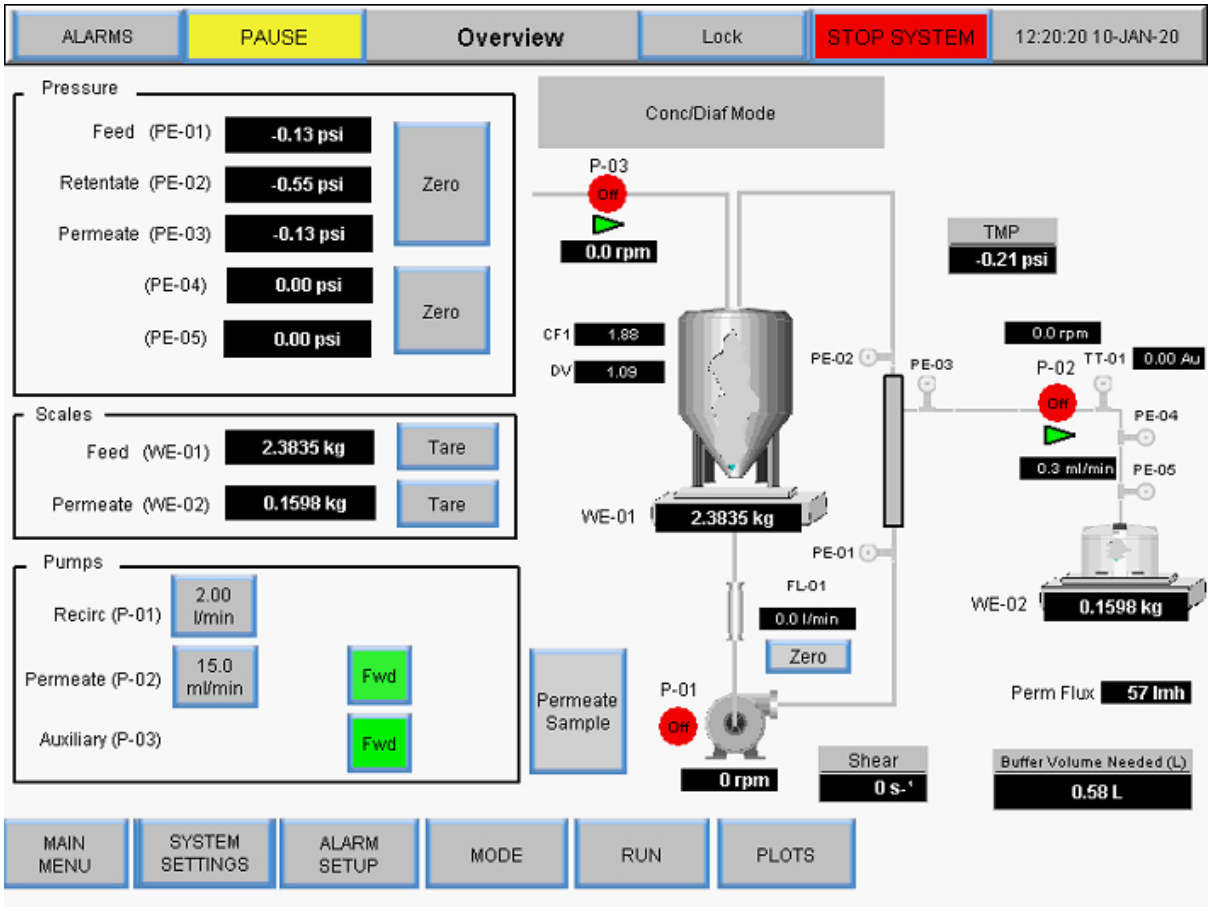
Figure 38. Concentration Mode Overview screen



Concentration/Diafiltration Mode operations:

- User input of Concentration Factor or Permeate weight for Concentration step
- User input of Diafiltration Volume or Permeate weight for Diafiltration step
- Change direction of Auxiliary pumps (P-02 and P-03)
- Tare scales (WE-01 and WE-02)
- Tare pressure sensors (PE-01, PE-02, PE-03, PE-04, PE-05, and PE-06)
- Zero Flow meter (FL-01 and FL-02)

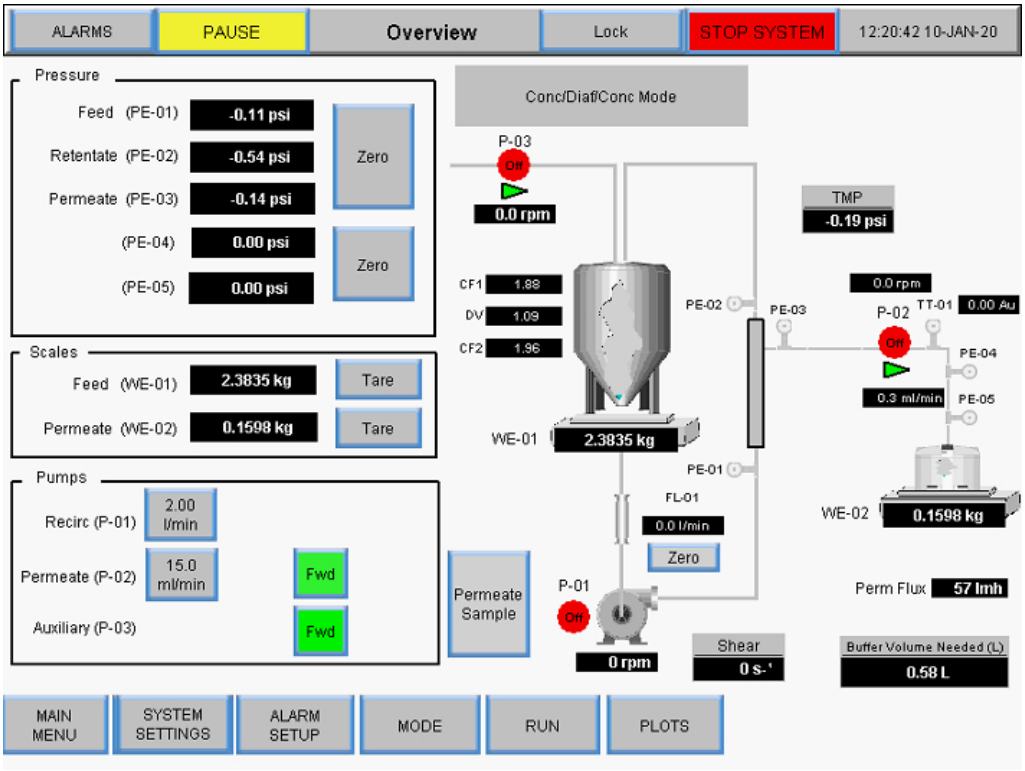
Figure 39. Concentration/Diafiltration Mode Overview screen



Concentration/Diafiltration/Concentration Mode operations:

- User input of Concentration Factor or Permeate weight for Concentration 1 step
- User input of Diafiltration Volume or Permeate weight for Diafiltration step
- User input of Concentration Factor or Permeate weight for Concentration 2 step
- Change direction of Auxiliary pumps (P-02 and P-03)
- Tare scales (WE-01 and WE-02)
- Tare pressure sensors (PE-01, PE-02, PE-03, PE-04, PE-05, and PE-06)
- Tare Flow meter (FL-01 and FL-02)

Figure 40. Concentration/Diafiltration/Concentration Mode Overview screen



8.11 Wizard feature

The Wizard feature enables the system to run the process automatically with preset setpoints. Users input five parameters, and the setpoints for Concentration 1, Diafiltration step, and Concentration 2 will be automatically calculated:

- Process volume
- PCV (Packed Cell Volume)
- Initial Concentration Factor (CF 1)
- Expected Final Yield
- Final Permeate Volume

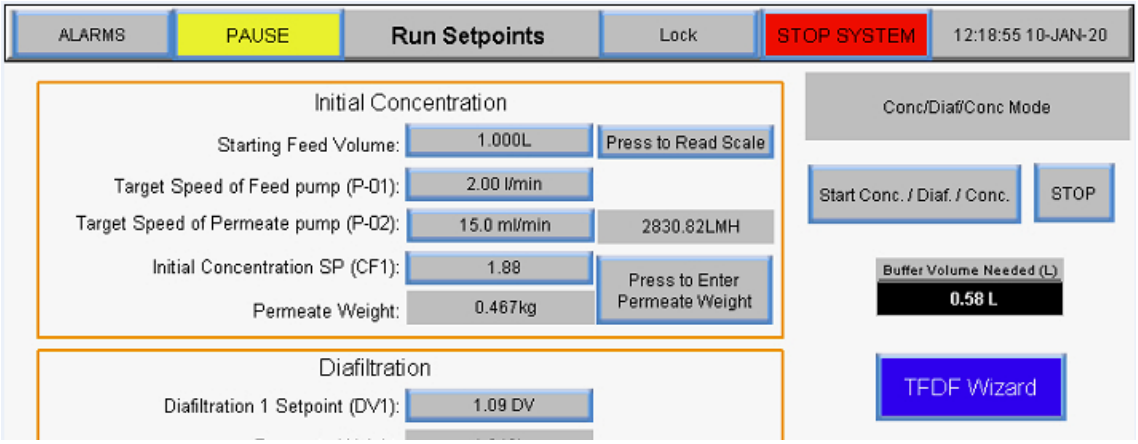
The Wizard feature is accessed in the Run Setpoints screen when the Concentration/ Diafiltration/ Concentration mode is selected. Its calculations determine:

- Diafiltration buffer volume needed
- Permeate volume at diafiltration pump start
- Permeate volume at diafiltration pump stop
- Permeate volume at run end
- Time for reference at diafiltration pump start
- Time for reference at diafiltration pump stop
- Time for reference at run end

To run the Wizard feature:

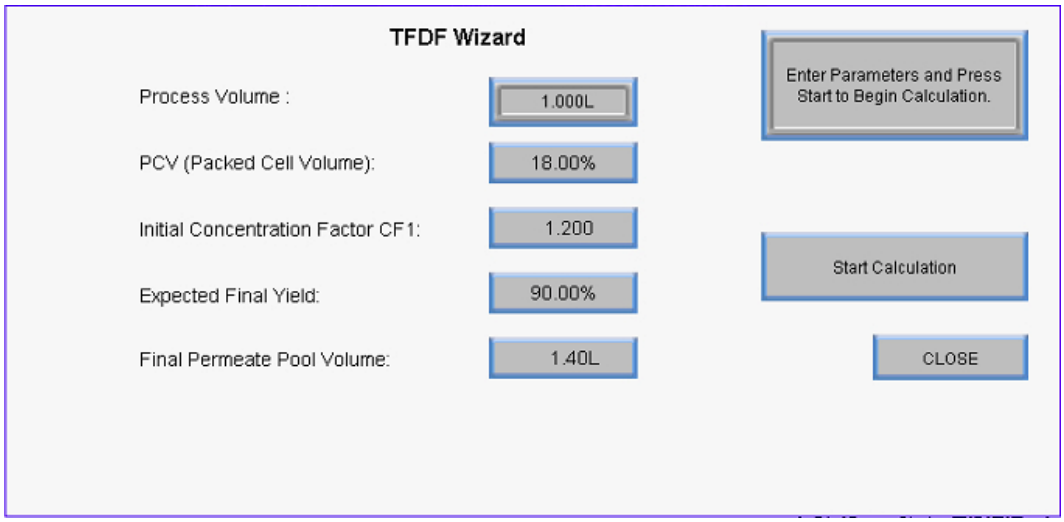
1. Select the **Start Conc./Diaf./Conc.** button in the Run Setpoints screen.

Figure 41. Starting the Wizard feature



The initial Wizard feature screen will display:

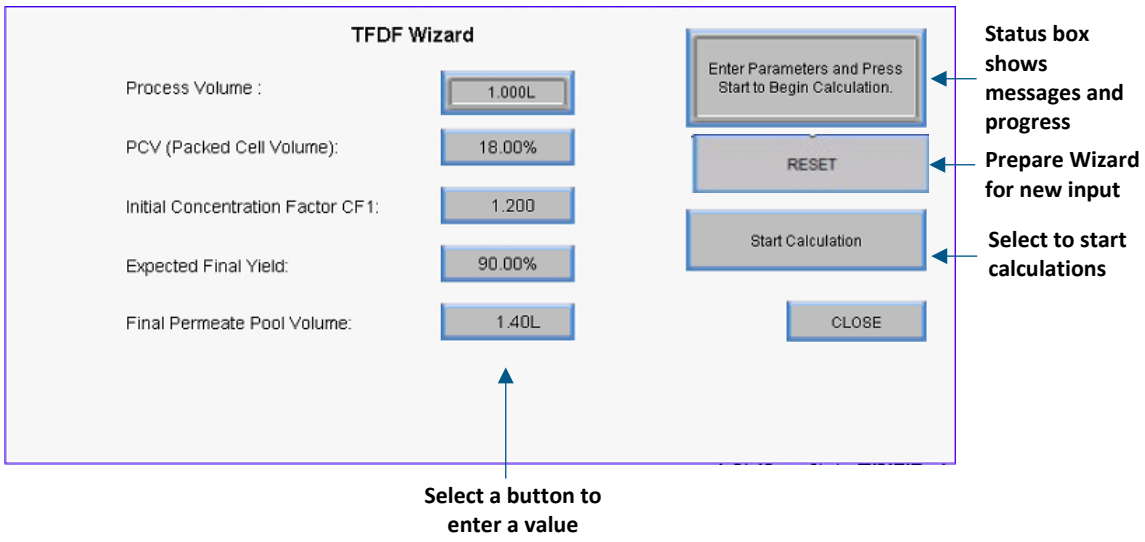
Figure 42. Initial screen



2. The Wizard feature initially displays default values. To enter a value and perform a calculation, select a settings button and enter the appropriate value. Repeat this step for any other settings that need to be calculated.

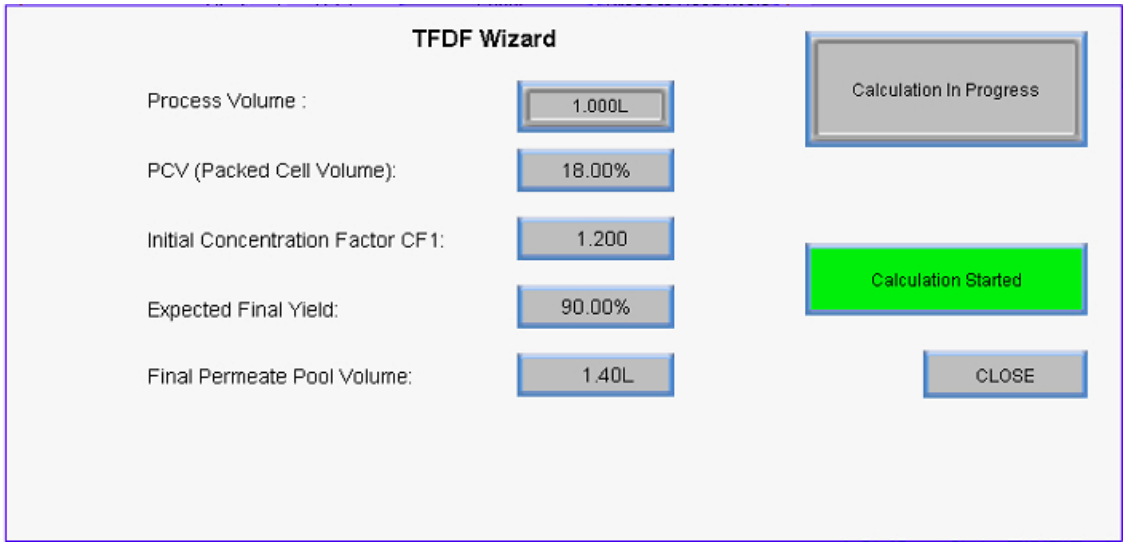
Note: *Users can enter values for one, multiple or all settings in the Wizard feature.*

Figure 43. Initial Wizard feature screen



3. Select the **Start Calculation** button. The button will turn green while the calculation is in progress and the status box will display any notifications.

Figure 44. Calculation in progress



A Calculation successful message will display once the calculation is complete.

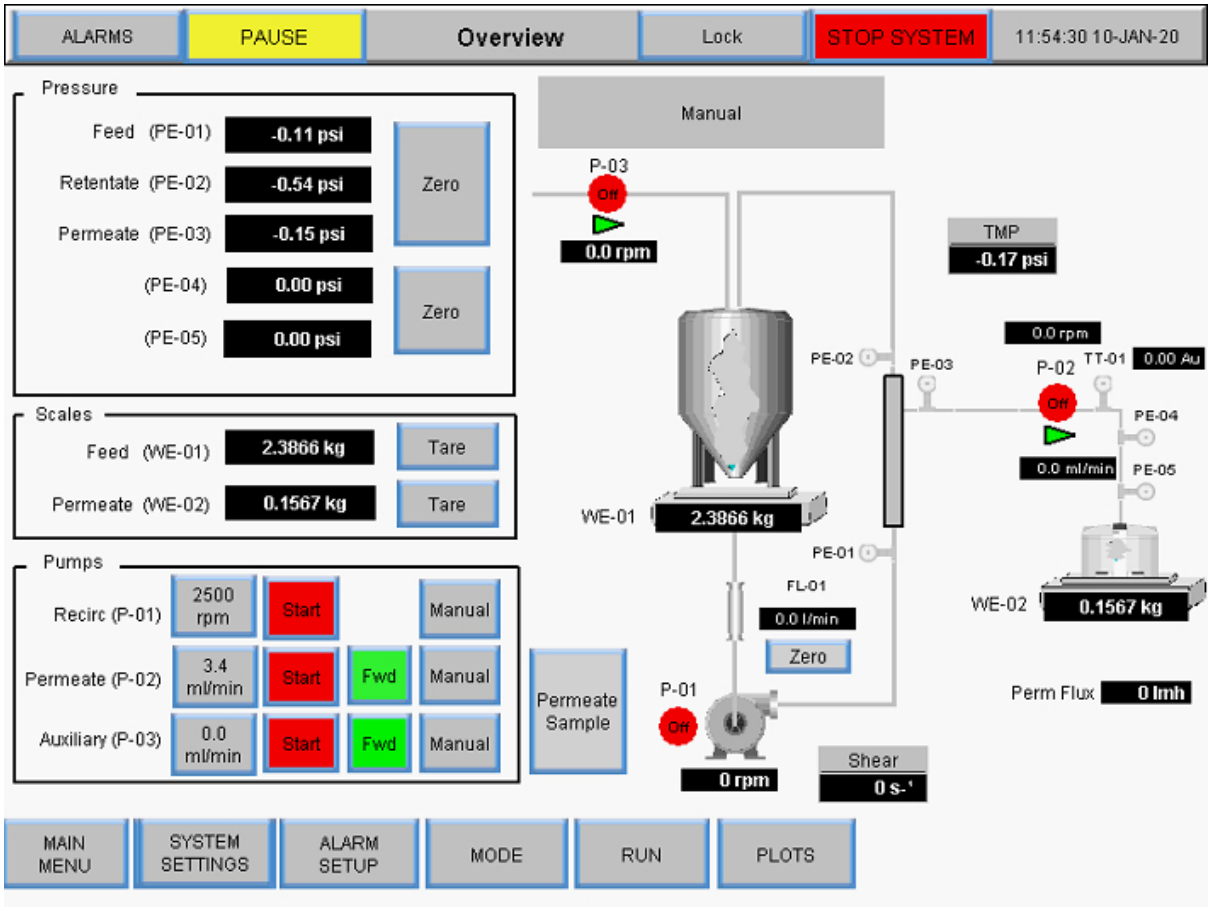
- If a Calculation Failure message displays, valid setpoint criteria could not be determined based on the input. Adjust the entered values and restart the calculation
- If a Calculation Timeout message displays, select **RESET** and restart the calculation

To exit the Wizard feature, select **Close**.

8.12 Manual Mode

In the Overview screen in Manual Mode, the pumps can be started manually by pressing the red **Start** button. Alternatively, the feed (P-01) and permeate (P-03) pumps can be put into an individual automation loop where data from either the flow meter modulates pump speed. Access this feature by toggling the **Manual** button to **Auto**. Only the individual control loop for that pump is activated and the pump will run at the setpoint entered based on the feedback from the flow meter (FL-01) for the feed pump (P-01) control or scale (WE-01) for the Aux/Diafiltration pump (P-03) control.

Figure 45. Manual Mode Overview screen



Any combination of running auxiliary components is possible in Manual Mode, there are no set points to automate:

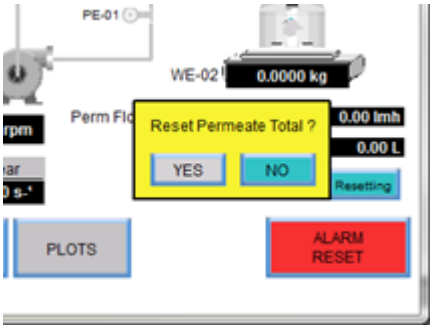
- Start/stop main pump (P-01)
- Start/stop auxiliary pumps (P-02 and P-03)
- Change direction of auxiliary pumps (P-02 and P-03)
- Tare scales (WE-01 and WE-02)
- Tare pressure sensors (PE-01, PE-02, PE-03, PE-04, PE-05, and PE-06)
- Tare Flow meter (FL-01 and FL-02)

Description of Terms:

- **Perm Flow:** Flow of permeate calculated by rpm of permeate pump
- **VT:** Volumetric Throughput - Total permeate mass/volume divided by the surface area of the filter
- **Perm Total:** Totalized Permeate volume calculated from pump rpm

- **Reset:** Resets the Permeate Total to 0.00 L. The following prompt will appear:

Figure 46. Reset prompt



- **Pause:** Press the **Pause** button during permeate vessel change-out to stop the permeate total calculation. When the permeate vessel is replaced click on the **Pause** button to resume the permeate total calculation and permeate pump
- **Shear Rate:** Calculation of the shear rate at the wall of the fiber based on the number of fibers, fiber ID and recirculation flow rate

8.12.1 Instrumentation

- P-01: Magnetic Levitating Recirculation/Feed Pump
- P-02: Permeate Pump (top peristaltic pump on Pump Station).

Note: *Forward flow direction corresponds to clockwise and to the right of the Pump Station.*

- P-03: Diafiltration/Aux Pump (bottom peristaltic pump on Pump Station)

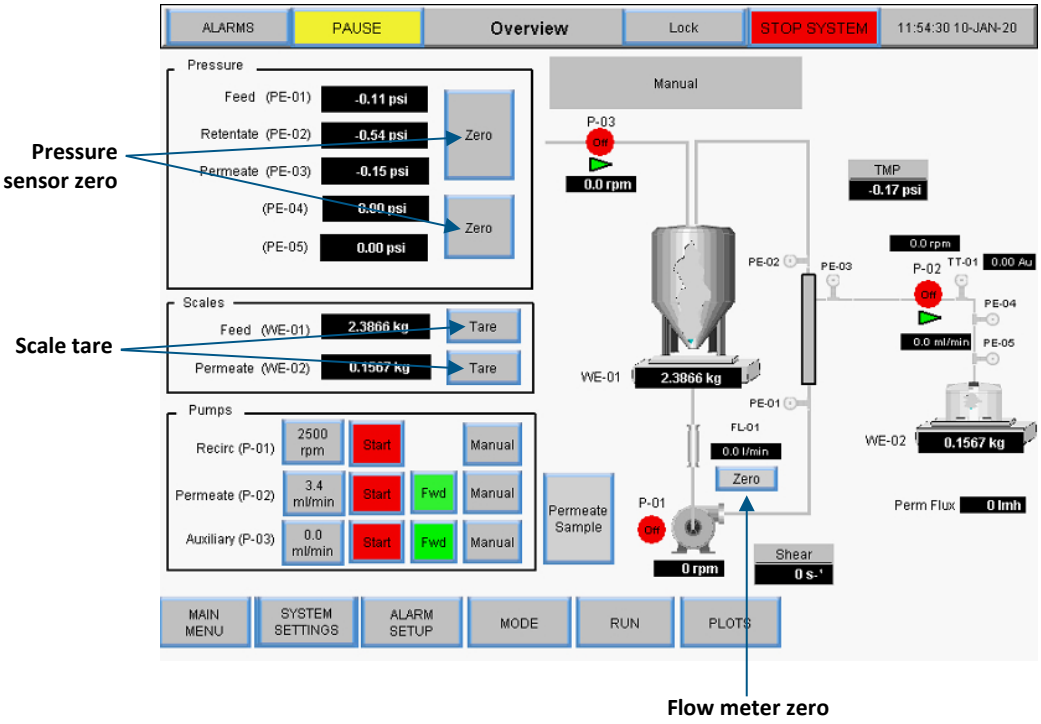
Note: *Confirm direction of flow.*

For more detailed instructions about the functionality of the overview screens please refer to the System Modes section on page 45.

Note: *All data entered and saved in the system must be inputted in this manner*

8.12.2 Taring

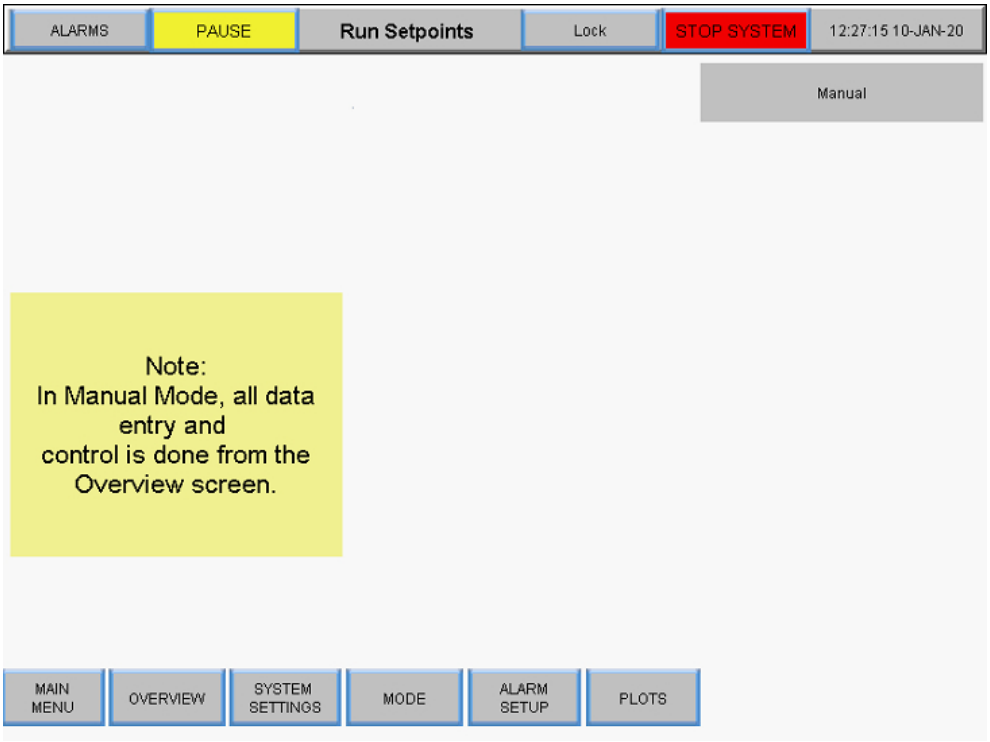
Figure 47. Manual Mode Overview screen



- **Pressure Sensor Zero:** Zeros the pressure sensor reading to 0.0 psi or bar. The zero button disappears during automation sequences to prevent accidental zeroing.
- **Scale Taring:** Tares the reading of the scales to 0.0000 kg, the tare button disappears during automation sequences to prevent accidental zeroing, other functional items are specific to the mode overview screen and described in their respective mode sections.
- **Flow meter Zero:** Zeros the flow meter located between the bioreactor and the filter, *zero the flow meter only after flow path is primed only*, the zero button disappears during automation sequences to prevent accidental zeroing
- **Reset Totalizer:** While the permeate is flowing the totalizer sums the total volume. For accuracy, reset the totalizer before beginning a process

Select **Manual** in the Run Setpoints screen to change to manual mode. In manual mode, the TFDF®setpoints will no longer be available. To operate in manual mode, return to the Overview Screen.

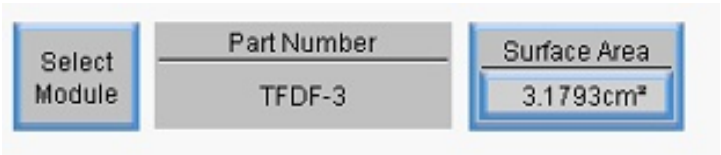
Figure 48. Manual Mode Run Setpoints screen



8.13 Selecting a filter module

The filter options on the System Mode screen let users select different filter modules for operation and displays the current selection.

Figure 49. Filter settings



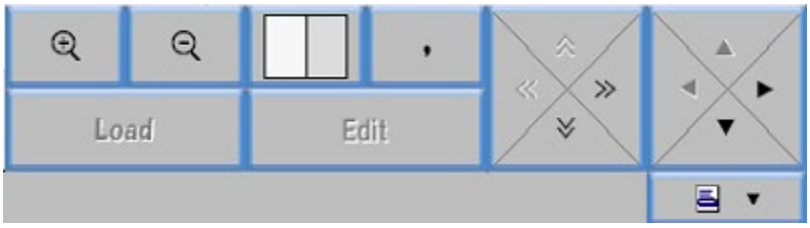
- **Select Module** – displays the filter modules currently available and lets users select the filter module best suited for their application
- **Part Number** – displays the part number for the currently selected filter module
- **Surface Area** – displays the membrane surface area as calculated by inner circumference multiplied by the length of the fiber

To view available filter modules and select one for the operational mode, touch the **Select Module** button. The Filter List Screen will display.

Figure 50. Filter List screen (left side)

To scroll through the filter module table, select the **Navigation** icon at the bottom right corner of the screen. The Navigation menu will display:

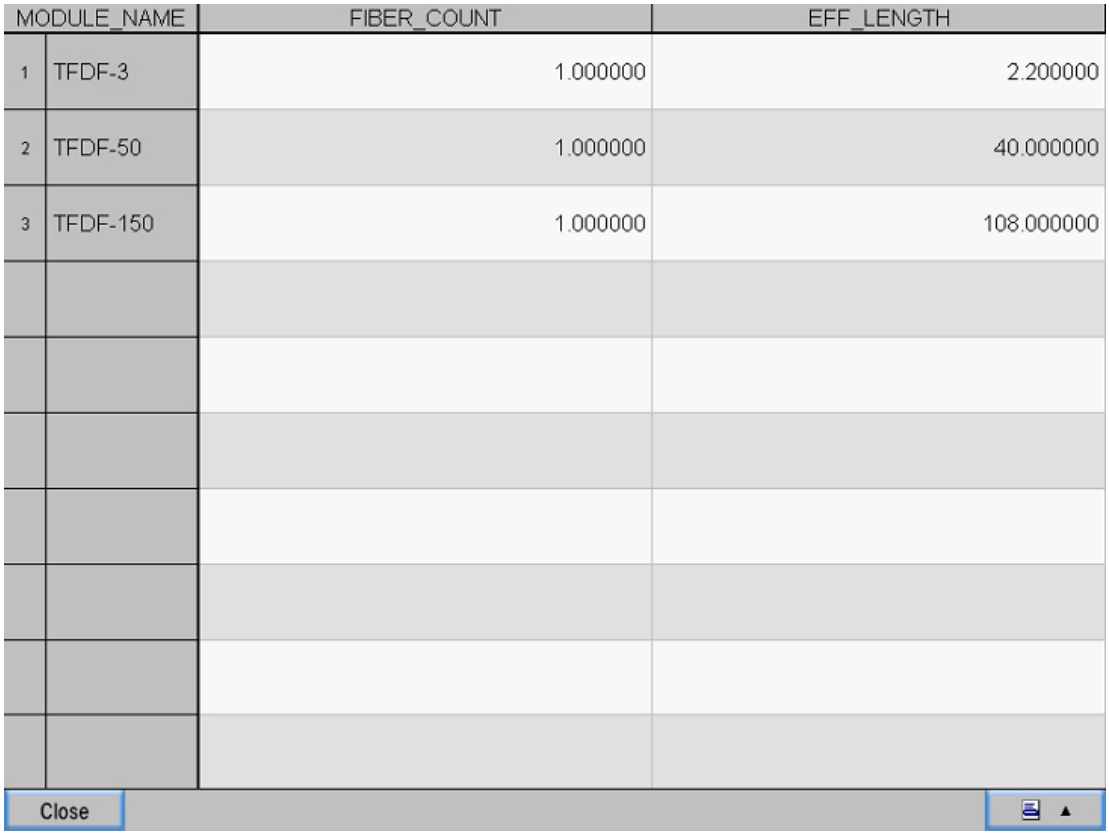
Figure 51. Navigation menu



- Select the + and – magnifying glass icons to zoom in and out
- Select the arrows to scroll left/right or up/down in the list
- Select the white/grey box to change the table view between alternating grey and white rows, alternating grey and white columns, or all white

The initial table displays the Fiber Size and Fiber Count columns. To view EFF Length, select the **Navigation** icon and then the **Right** arrow to scroll in the table.

Figure 52. Filter List screen (right side)



Select **Close** to return to the System Mode screen.

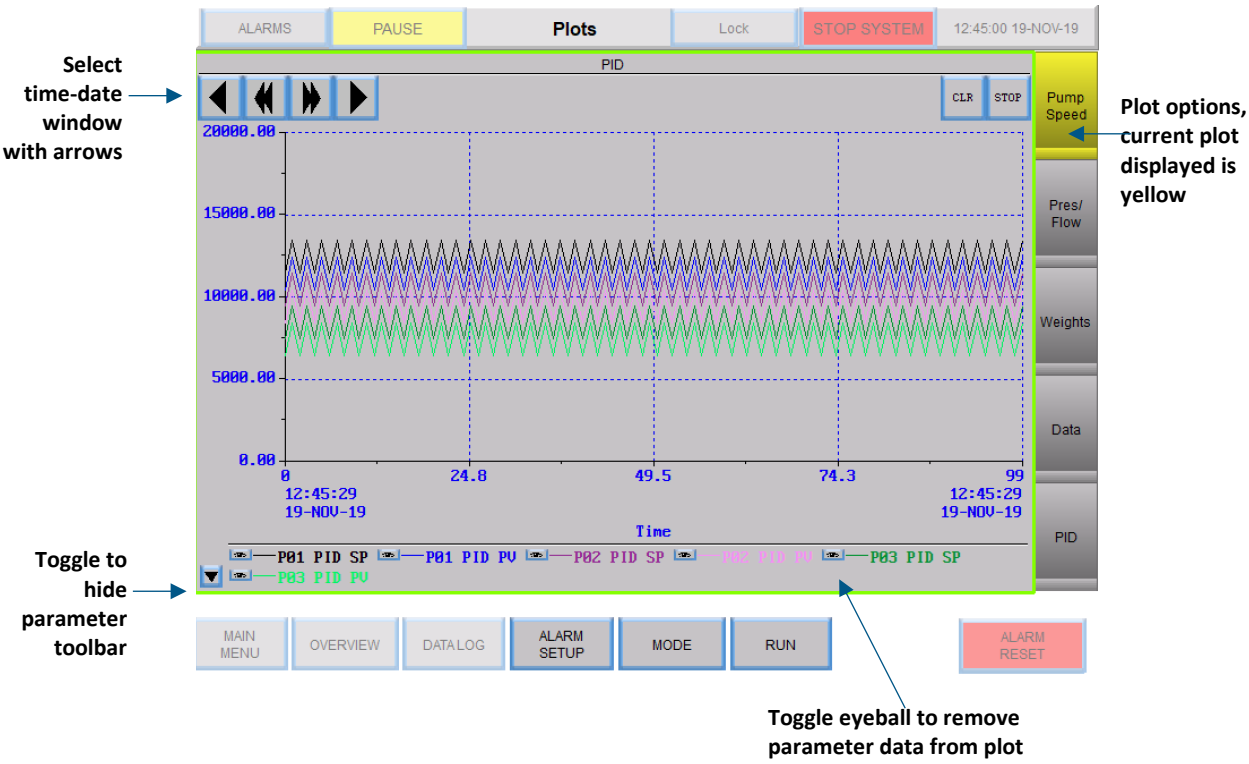
To select a specific filter module:

1. Select a row, which is associated with its respective part number, for the desired filter family.
2. Select the **Navigation** icon.
3. Select the **Load** button.
4. Select **Close**. The part number for the filter module selected will now display in the filter options in the System Mode Screen and will be used during system operation.

8.14 Plot screens

Users can view trend plots of pump speed, pressure/flow, weights, data and PID in the Plot screen. To access this screen, select **Plots** from the Main Menu screen.

Figure 53. Plot screen



- To view the plot for a specific set of trended data, select a button on the right of the screen, the button for the plot currently being displayed will be yellow
- Select a time-date range of the historical data using the forward and reverse arrows at the top left side of the screen
- Each plot has a set of ‘pens’ shown in the pen toolbar at the bottom of the screen, each pen represents data for a specific system parameter and has a unique trace color in the plot, to remove a parameter from the plot display, toggle the eyeball button next to the parameter, see Table 13 for a list of all parameters plotted
- To hide the pen toolbar, toggle the arrow button on the lower left of the screen

Table 7. Parameter data

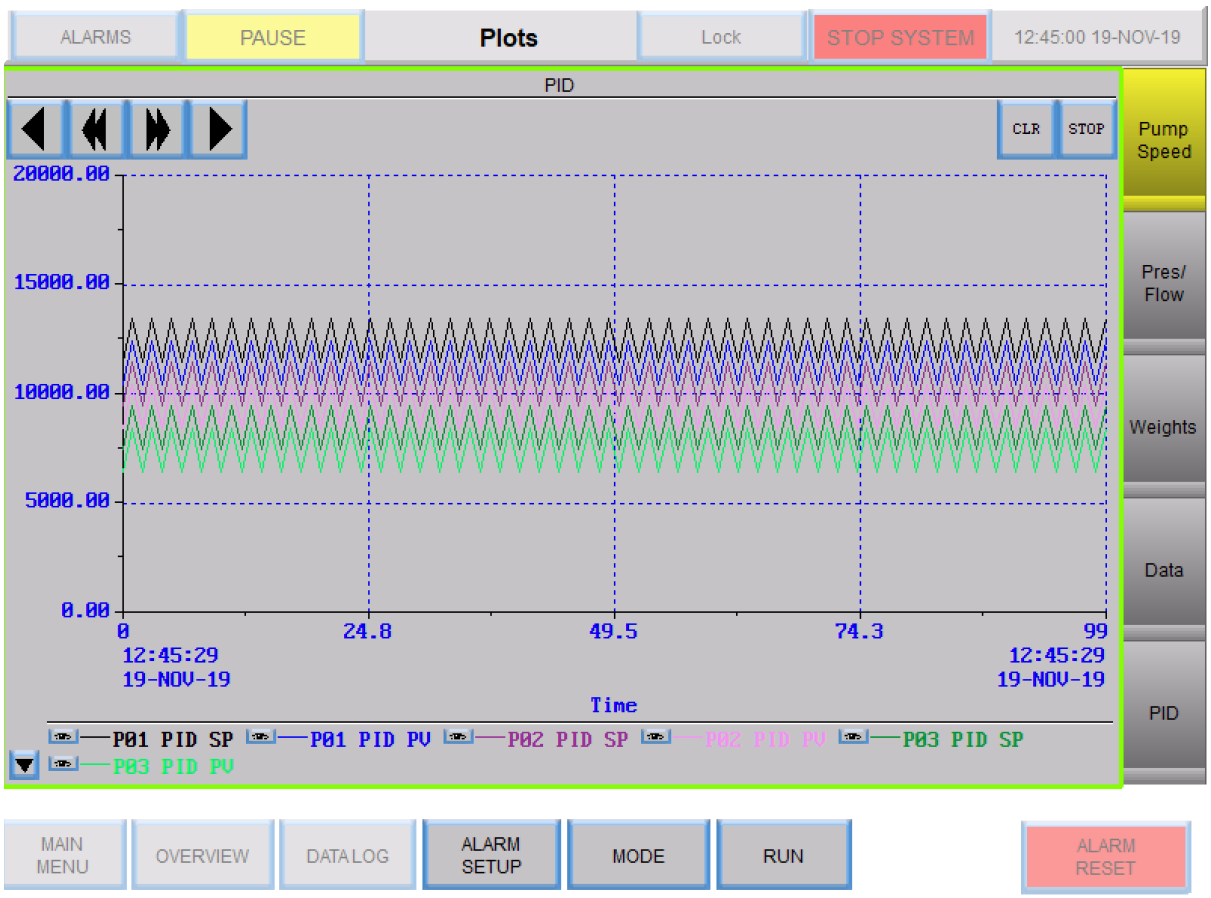
Tag Number	Parameter	Units
FL-01	Feed flow rate	LPM
FL-02	Permeate flow	ml/min
WE-01	Product vessel weight	kg
WE-02	Permeate vessel weight	kg
PE-01	Feed pressure	Psig or mbar
PE-02	Retentate pressure	Psig or mbar
PE-03	Permeate pressure	Psig or mbar
P-01 P-02 P-03	Feed pump set-point Pump speed P-02 Pump speed P-03	RPM or LPM RPM or ml/min RPM or ml/min

8.14.1 Pump Speed

Pen traces in the Pump Speed plot display trend data for the following:

- P-01 PID set point
- P-01 PID PV
- P-02 PID set point
- P-02 PID PV
- P-03 PID set point
- P-03 PD PV

Figure 54. Pump Speed plot

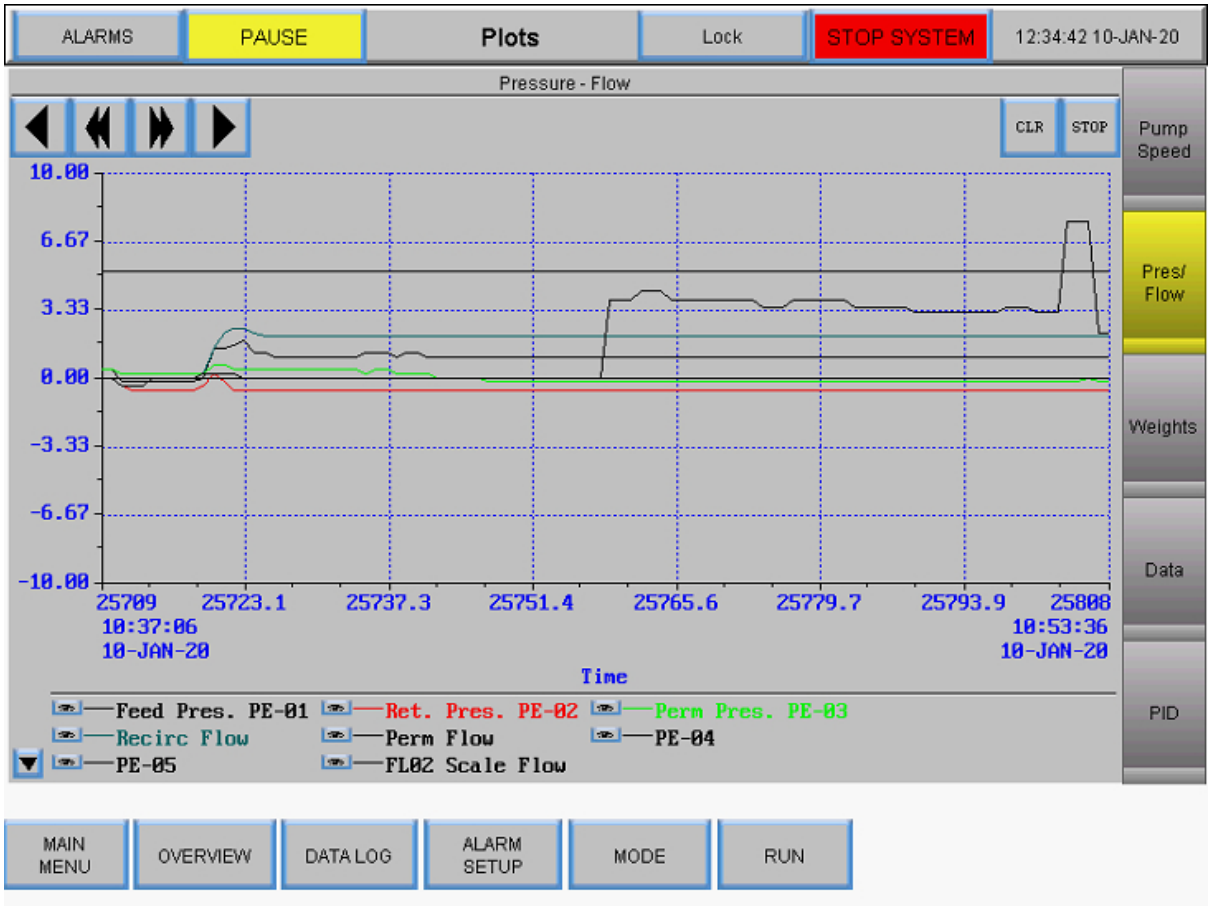


8.14.2 Pressure/Flow

Pen traces in the Pressure/Flow plot display trend data for the following:

- PE-01 Feed pressure
- PE-02 Retentate pressure
- PE-03 Permeate pressure
- Recirc/Feed flow
- Permeate flow
- PE-04 Pre-sterile filter
- PE-05 Post sterile filter
- FL-02 Scale flow

Figure 55. Pressure/Flow plot

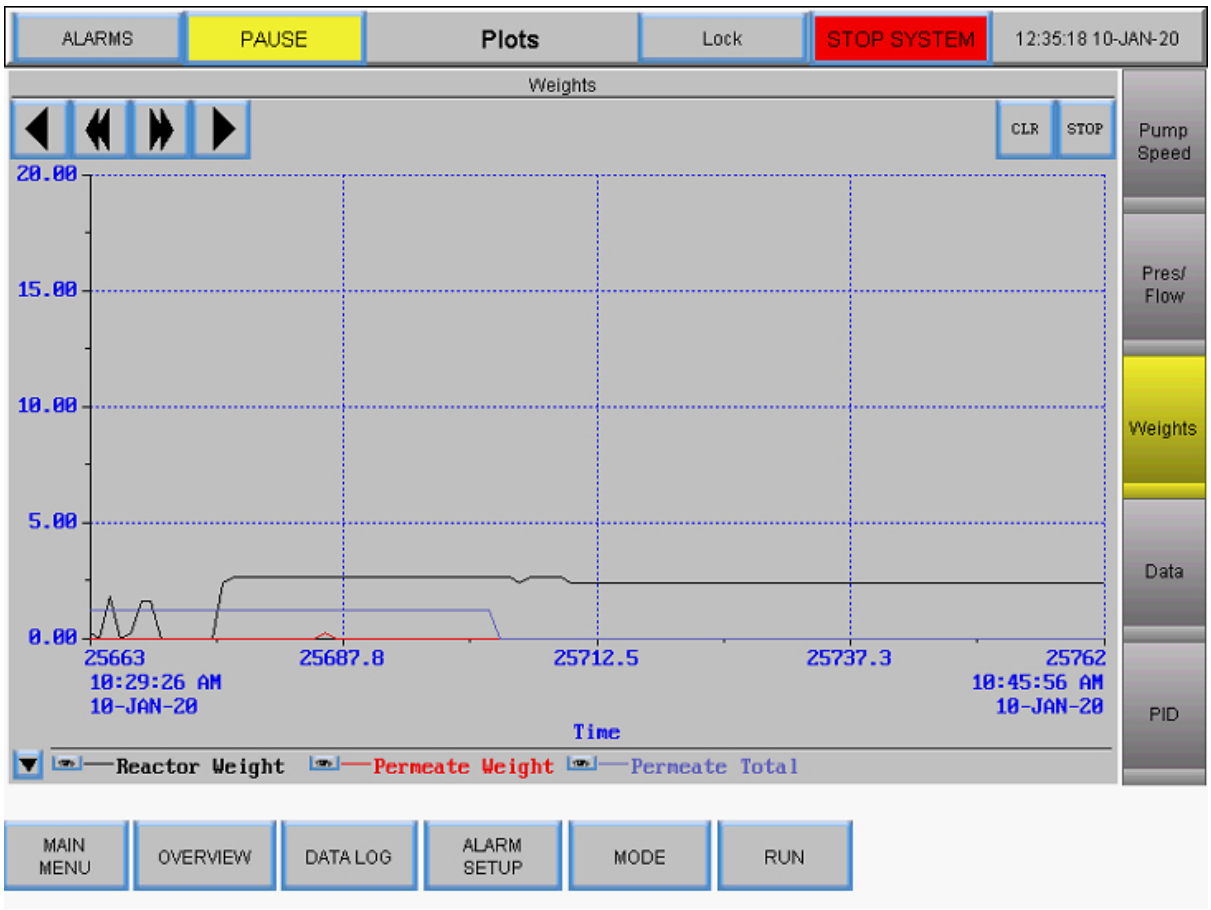


8.14.3 Weights

Pen traces in the Weights plot display trend data for the following:

- Reactor weight
- Permeate weight
- Permeate total

Figure 56. Weights plot

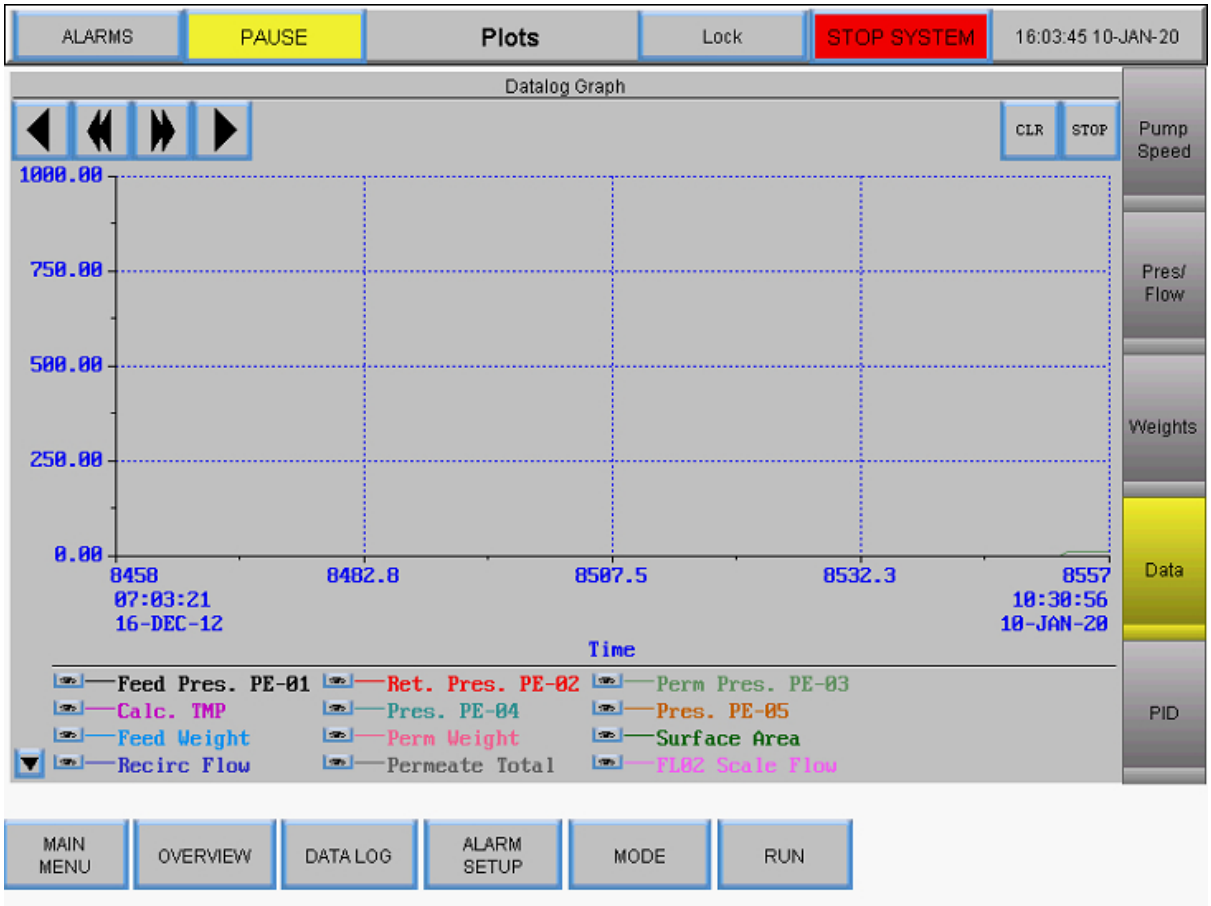


8.14.4 Data

Pen traces in the Data plot display trend data for all tracked parameters:

- PE-01 Feed pressure
- PE-02 Retentate pressure
- PE-03 Permeate pressure
- Calculated TMP
- PE-04 Pre-sterile filter pressure
- PE-05 Post-sterile filter pressure
- Feed Weight
- Permeate Weight
- Surface area
- Recirc/Feed flow
- Permeate total
- FL-02 Scale flow

Figure 57. Data plot

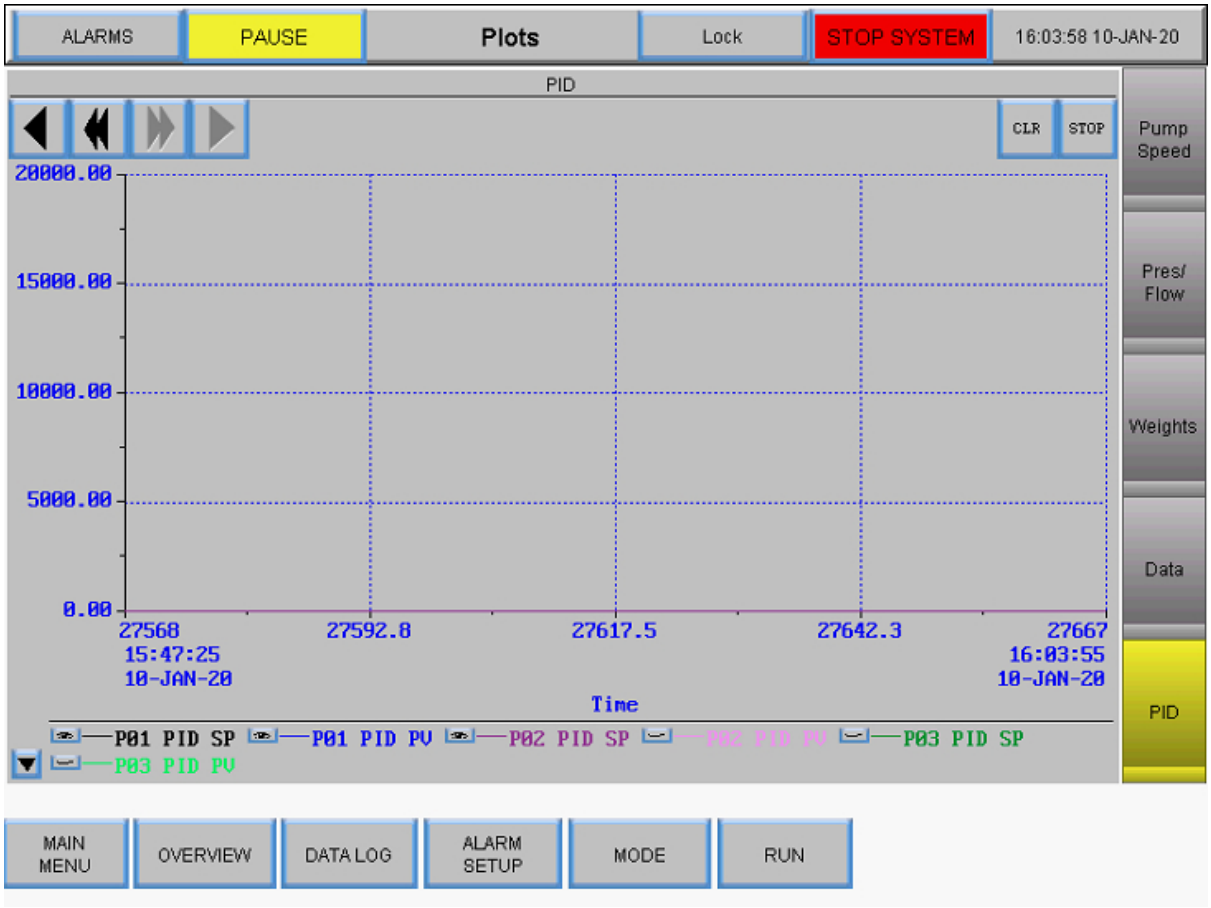


8.14.5 PID

Pen traces in the PID plot display trend data for the following:

- P-01 PID set point
- P-01 PID PV
- P-02 PID set point
- P-02 PID PV
- P-03 PID set point
- P-03 PD PV

Figure 58. PID plot



9. Troubleshooting

System will not turn on
Make sure the power cord to the Main Enclosure is connected, fully inserted into a power outlet and in the receptacle on the enclosure.

Permeate and/or media/buffer feed flow is much higher/lower than expected

1. Verify that the color code on the peristaltic tubing matches the color code for the tubing selection in the Settings Screen.
2. Confirm tubing is fed and seated properly in the peristaltic pump head.
3. Confirm that the selected tubing size is appropriate for the permeate and diafiltration flow rate.

Retentate flow is too low, not moving

1. Confirm that the magnetic levitating pump head is primed, and no air is trapped inside.
2. Make sure the pump head is seated properly in the magnetic levitating pump.
3. Inspect the pump head for clogs.
4. Inspect the filter for clogs.

Pump Station not responding
There are several different communications protocols for the Pump Station. To address most issues, shut the system down then disconnect and reconnect the 26 pin communications cable from the Pump Station to the Main Enclosure (see connect system cables sections).

Note: *the peristaltic pumps (P-02 and P-03) will work regardless of which port the station's umbilical cord is connected to on the Main Enclosure. However, the recirculation/feed pump must be connected to the correct port to function.*

Data recording message "buffer full"
This message indicates that either the USB drive is not inserted or not working.

1. Make sure the USB drive is inserted into the USB port on the right side of the Main Enclosure.
2. If there is a USB drive in place already, try another drive.
3. Go to the Data Logging Screen and make sure it is set to record.

10. Maintenance

The KrosFlo® TFDF®Lab System design is robust and intended for use with other process and lab equipment. The frame, cabinet, and pumps can be cleaned by wiping down surfaces with mild cleaning agents and/or warm water, a damp cloth or lab wipes. The display should be cleaned with computer screen cleaner and computer screen wipes.

All repairs of the system must be performed by a qualified Repligen service engineer. Opening of the system and attempted repair by the user or third party shall void the product warranty.

The KrosFlo® TFDF®Lab System is manufactured in Marlborough, MA, USA.

11. General information

11.1 Safety guidelines

Figure 59. Magnetic forces caution and warning

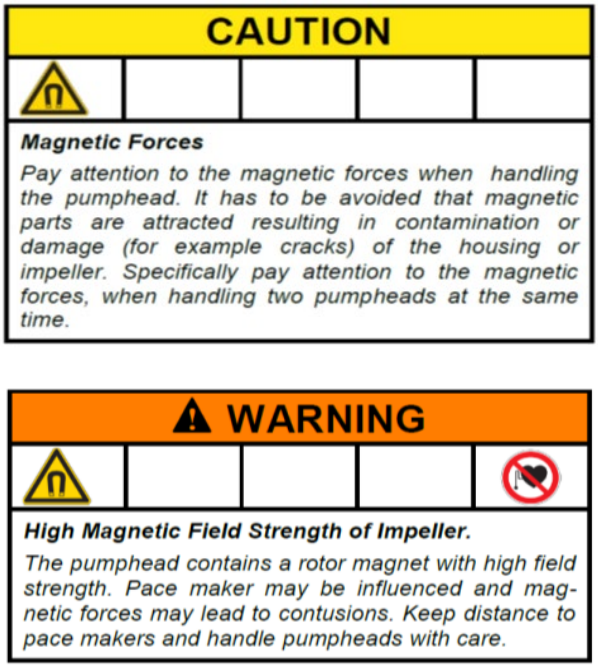


Table 8. Warning: Product use limitation

Symbol	Description
Caution 	Risk of danger. Consult Operating Instructions for nature of hazard and corrective actions This product is not designed for, nor intended for use in patient-connected applications; including, but not limited to, medical and dental use, and accordingly has not been submitted for FDA approval This product is not designed for, nor intended for use in hazardous duty areas as defined by ATEX or the NEC (National Electrical Code); including, but not limited to use with flammable liquids, consult the factory for products suitable for these types of application

11.2 System specifications

Table 9. System output

Description	Specifications
Feed / recirculating pump type	Magnetic levitating pump
Feed / recirculating pump capacity	0 - 11000 RPM, 0 - 10 LPM @ 0.0 bar, 0.0 - 21.8 psi (1.5 bar)
Diafiltration and permeate pumps type	Peristaltic
Diafiltration and permeate pumps capacity	0.1 - 100 RPM (0.01 RPM Resolution) Maximum 340 ml/min (4.8 mm ID and 1.6 mm thickness) 0.0002 - 35 ml/min/channel 3 channel, 8 rollers 14.5 psi (1.0 bar) max differential pressure
Display	Automation Direct 12” LCD touchscreen
Retentate flow meter	Ultrasonic clamp-on flow meter 0 - 8000 ml/min, 2% accuracy (±16 ml/min) Calibrated for #15 PharmaPure® tubing
Recommended process volume	1 - 50 L
Number of pressure sensors supported	5
Pressure sensor range	-14 - 30 psi (-1 - 2 bar)
TFDF®Filter surface area supported	2 - 150 cm^2
Number of scales supported	2

Table 10. System input

Description	Specifications
Power requirements	120 VAC, 10 A 230 VAC, 5 A, 50/60 Hz

Table 11. System construction

Description	Weight
Controller weight	36 lbs (16.2 kg)
Controller dimensions	16 x 13 x 21 in (40 x 33 x 53 cm)
Pump Station weight	16 lbs (7.3 kg)
Pump Station dimensions	11 x 11 x 19 in (min)/39 in (max) (28 x 28 x 48/99 cm)
Controller type	PLC
Controller and Pump Station rating	IP20
Enclosure material of construction	Delrin and powder coated/anodized aluminum

Table 12. System environment

Description	Specifications
Temperature, operating	4˚ to 40˚ C (39˚ to 104˚ F)
Humidity (non-condensing)	15% - 95% 10% - 50%
Altitude	Less than 2000 m
Noise level	< 75 dBa @ 1 meter
Pollution degree	Pollution Degree 2
Chemical resistance	Housing: Powder-coated aluminum Filter stand: Delrin and powder-coated/anodized aluminum Flow path components: polypropylene, polycarbonate, polysulfone, and C-Flex/PharmaPure® materials

11.3 System components

Table 13. System components list

Item	Included Components
Controller	Controller with housing-connected cables

Pump Station	• Pump Station attachments
	  ○ Peristaltic pumps x2
	  ○ Magnetic levitation pump
	  ○ Stand mount with locking knob
	  ○ Flow meter
	• A/C Power cables (US, UK, EU and China versions included)
	• Pump Station power cable (5 pin)
	• Controller-Pump Station communication cable (26 pin)
	• Tubing guide rod
	• Extension rod with locking knob
	• Rod sleeve with locking knob
	• Filter clamp with 2 locking knobs

Scales	Digital scales x2 Powered RS232 communication cables x2

12. Index