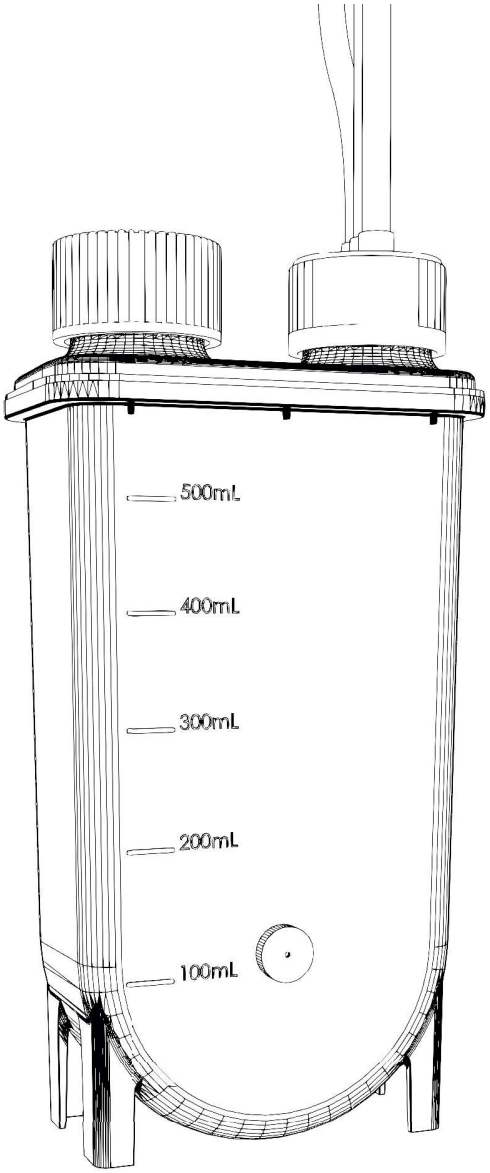
USER MANUAL

PBS-0.5 Mini Vessel with 60µm Perfusion Filter
Set-Up and Operation



VERTICAL-WHEEL BIOREACTORS

PBS Biotech 2025

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GETTING STARTED

Table of Contents and Materials

Key Materials

Materials and Equipment Quality Compliance Reminder: Ensure the condition of all materials and their packaging are intact and sterile. Contact customer.service@pbsbiotech.com with any shipping or quality concerns regarding the PBS-0.5 Mini Vessels with 60µm Perfusion Filter.

A. PBS-0.5 Mini Vessel with 60µm Perfusion Filter (P/N: IA-0.5-D-201)

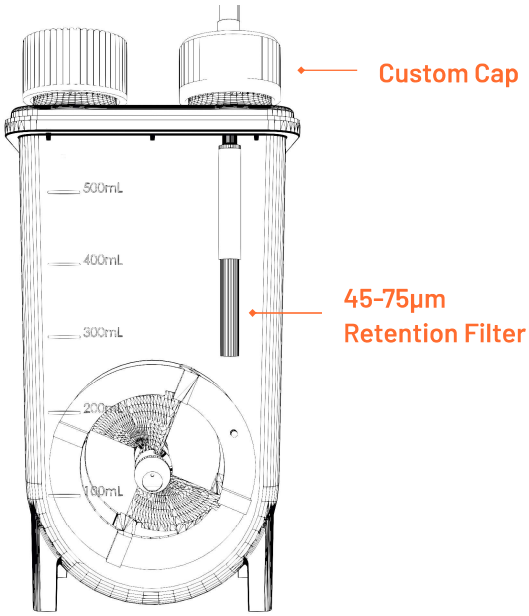


Figure 1. Close-up illustration of PBS-0.5 Mini Vessel with 60µm Perfusion Filter built into custom cap.

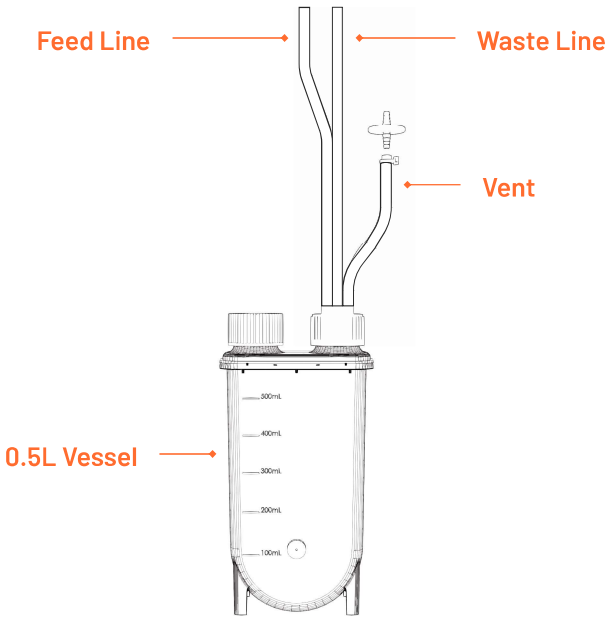


Figure 2. Illustration of the Vent, Feed, and Waste line extending from the custom cap.

B. Other materials to consider:

No.	Quantity	Name
1	2	PBS Mini Base Unit P/N: FA-UNI-B-501 (One for inside of incubator and one for performing manipulations inside the BSC)
2	1	Dual-head Precision Flow Rate Pump with tubing holders compatible with either MasterFlex® L/S® 24 tubing or 3/16" ID x 5/16" OD silicone tubing
3	N/A	≥36-inch section of tubing to be used for pump calibration (should be the same type of tubing that will be placed in the pump for perfusion)
4	1	Rolling cart ~1 m high
5	500 mL	Water
6	1	Scale
7	2	Beakers (≥500 mL)
8	1	500 mL Pyrex Bottle
9	2	Weldable Transfer Caps (Figure 5)
10	N/A	Cell Culture Medium
11	1	Incubator
12	1	Biological Safety Cabinet

Source materials from your preferred supplier. Materials specific to pump operations may not be included. Please reference your pump operating instructions prior to start.

PROCEDURE 1

Setup and Operations

Procedure 1

Performing Pump Set-Up and Calibration

This procedure describes the process of calibrating and programming a pump to achieve the desired flow rate prior to connecting it to the PBS-0.5 Mini Vessel with 60µm Perfusion Filter.

Note

- *The PBS-0.5 Perfusion Vessel requires a dual-headed peristaltic pump designed for small and precise volume manipulations. Two pumps can be used if they can be programmed to use the exact same flow rate, however, minor differences in flow rate may result in vessel over- or under- filling over time.*
- *The PBS-0.5 Perfusion Vessel includes more than one type of silicone tubing for operation with a variety of pump brands. Cross-reference the information in Figure 4 with your pump manufacturer to confirm compatibility prior to start. Pump calibration is performed non-aseptically and requires a separate section of tubing not included with the PBS-0.5 Perfusion Vessel. The tubing used to calibrate the pump should be the exact same type of tubing that will be installed in the pump to execute perfusion.*

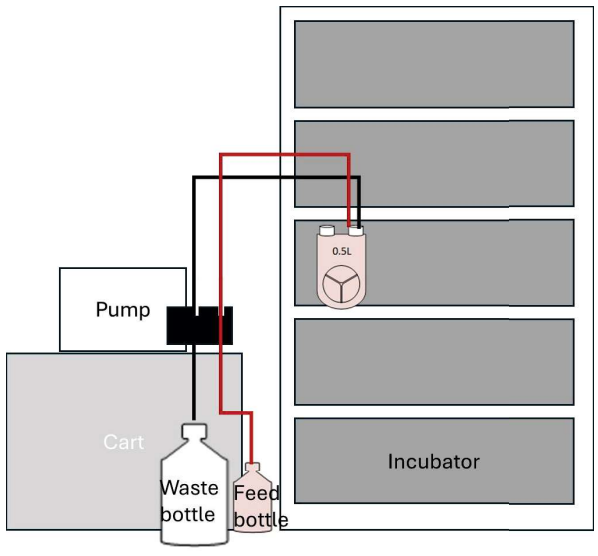


Figure 3. Positioning of incubator, pump, and cart.

Step 1 Place the pump, scale, and two beakers (≥500mL) on a bench or rolling cart near the incubator with the pump heads facing the incubator ***(Figure 3).***

Step 2 Perform pump set-up according to the pump operating instructions (install dual pump heads, plug in, turn on).

Step 3 Identify the silicone tubing type that is compatible with your pump ***(Figure 4).***

Step 4 Install a section of tubing ≥36 inches long into one of the pump heads. Ensure the pump head is securely closed on the tubing.

Step 5 Place empty 500mL beaker on scale.

Step 6 Place the pump outlet end of the tubing section in the empty beaker, avoiding kinks or blockages along the line and so that it won't be jerked out of the beaker by the pump. Secure the end of the tubing in the beaker.

Step 7 Place the inlet end of the tubing section into a second beaker filled with ~500mL water, avoiding kinks or blockages along the line so that it won't be jerked out of the beaker by the pump. Secure the end of the tubing fully submerged in the water.

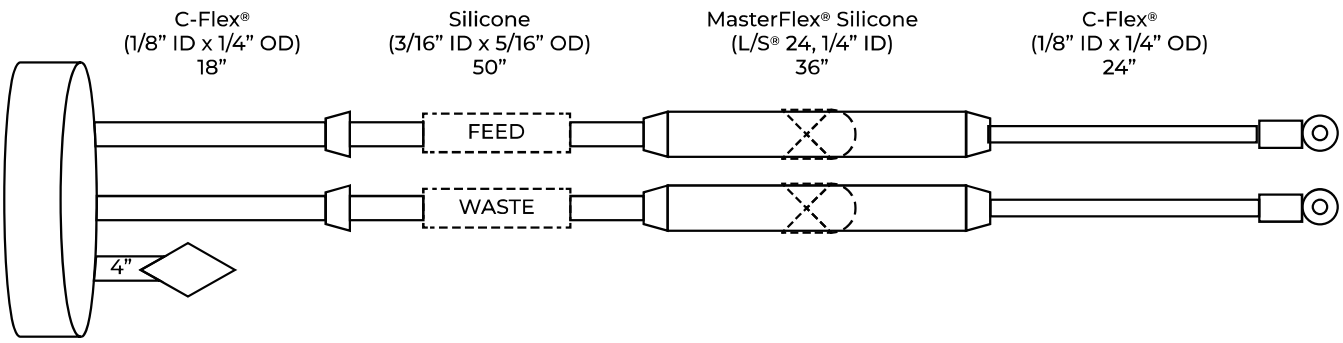
Step 8 Confirm that the pump rotation direction is correct, such that when the pump is turned on water will flow from the full beaker to the empty one.

Step 9 Start the pump to prime the line with water according to the pump operating instructions.

Step 10 Stop pump when there are no bubbles in the line and the first drops have appeared in the beaker on the scale.

Step 11 Tare the scale.

Step 12 Perform pump calibration according to pump's operating instructions.



	Silicone	Masterflex® Silicone	C-Flex®
Supplier	N/A	VWR	N/A
Part #	N/A	MFLX96410-24	N/A
Description	Platinum-Cured Silicone	High-Performance Precision Pump Tubing, Platinum-Cured Silicone	Thermoplastic Elastomer
Dimensions	3/16" ID, 5/16" OD	1/4" ID, L/S® 24	1/8" ID, 1/4" OD
Durometer	50, Shore A	45-55, Shore A	59, Shore A

Figure 4. Identifying Tubing Sections on the PBS-0.5 Mini Vessel with 60µm Perfusion Filter. The feed medium and waste medium lines are the same, but the waste medium line is connected to the 60µm filter within the PBS-0.5 vessel.

PROCEDURE 2

Batching the Vessel with Medium and Connecting Perfusion Tubing Lines

Procedure 2

Batching the Vessel with Medium and Connecting Perfusion Tubing Lines

This procedure describes the process of batching the vessel with medium and connecting the perfusion tubing lines to their respective containers, then setting up the vessel in the incubator prior to inoculation. The vessel, BSC, incubator, pump, perfusion feed bottle, and perfusion waste bottle should all be close to each other ***(Figure 3 on page 07).***

Note

- *Work on one tubing line at a time to avoid clutter in BSC.*

Step 1 Inside BSC, attach transfer cap (Figure 5) to empty sterile bottle (this step is not necessary if you are using a bioprocess bag with weldable tubing as your perfusion waste container).

Step 2 Outside of BSC, weld the transfer cap tubing (or the bioprocess bag tubing) to the end of the PBS-0.5 Perfusion Vessel waste line.

Step 3 Label the bottle (or bioprocess bag) "waste" and place it on the cart.

Step 4 Under the BSC, prepare an appropriate volume of cell culture medium (typically ~495 mL) in a sterile bottle and attach a transfer cap.

Step 5 Outside the BSC, weld the transfer cap tubing to the end of the feed line of the PBS-0.5 perfusion cap. Label the bottle "feed" and place it on the cart. Install the pumpable section of feed line tubing into one of the pump heads such that when the pump is turned on, liquid will flow out of the feed medium container and into the PBS-0.5. Close the pump head securely.

Step 6 Bring the empty PBS-0.5 Perfusion Vessel into the BSC (do not spray vent cap with ethanol) and place on Mini Base Unit inside BSC for stability.

Step 7 Turn the pump on to fill the vessel with medium to desired working volume. The feed bottle and feed line tubing should be empty after this step (this may require manipulation of the feed medium container and feed line tubing).

Step 8 Transfer the PBS-0.5 into the incubator (the incubator door should not be closed tightly, such that the tubing is not clamped).

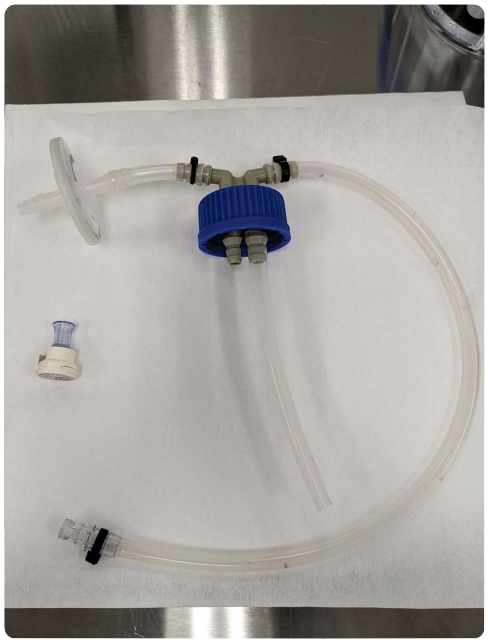


Figure 5. Example of Transfer Cap (GL45 cap with two elbow connectors). The first elbow connector (left) is connected to a 0.45um filter via ~2" of silicone tubing on the outside of the cap and empty on the inside of the cap and is for venting. The second elbow connector (right) is connected to a length of silicone tubing that can be connected to weldable tubing via MPC connector or a straight tubing connector on the outside of the cap, and a length of silicone tubing that reaches the bottom of the bottle it will be installed in on the inside (see Figure 6 for example), and is for liquid transfer.

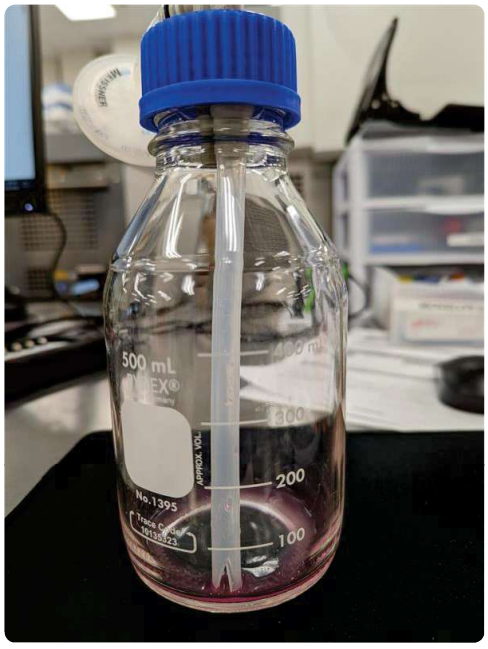


Figure 6. Example of Transfer Cap installed on bottle, with inner tubing reaching to the bottom of the bottle.

PROCEDURE 3

Programming and Initiating the Pump

Procedure 3

Programming and Initiating the Pump

This procedure describes the process of calculating the perfusion flow rate to program on the pump.

Note

- *The pump settings will change depending on the working volume of the vessel and the target medium exchange rate (using the maximum working volume of the vessel is recommended).*
- *Use the Perfusion Flow Rate Calculations at the end of this guide to calculate pump parameters for your desired medium exchange regime.*
- *50% medium exchange via discrete medium removal and addition uses the same medium volume as 0.5 VVD perfusion in 24 hours.*
- *For very slow flow rates (<1 mL/min), it may be necessary to cycle the pump on and off to achieve the desired VVD. For example, to achieve 0.5 VVD in the PBS-0.5 at a 500 mL working volume, a pump that flows at 1 mL/min should continuously cycle on for 2 minutes and off for 9.52 minutes. This is still considered continuous perfusion because it is removing and adding media at the desired rate over the span of 24 hours.*

Step 1 Determine the required pump flow rate for perfusion in units of mL/min by converting the desired vessel volumes per day (VVD) using this equation, rounding flow rate up to the nearest tenth:

$$X \ \frac{Vessel\ Volumes}{Day} \times \frac{1\ Day}{1440\ mins} \times \frac{Y\ mL}{Vessel\ Volume} = Z \ \frac{mL}{min}$$

X = Desired medium exchange rate in vessel volumes per day (VVD) Y = Bioreactor working volume (in mL)

Z = Pump flowrate (in mL/min)

Example

Bioreactor Scale	Bioreactor Working Volume (mL)	Pump Flow Rate for 0.5 VVD (mL/min)	Pump Flow Rate for 1 VVD (mL/min)
PBS-0.5	500mL	0.1736	0.3472
PBS-3	3,000mL	1.0417	2.0833
PBS-15	15,000mL	5.2083	10.4167
PBS-80	80,000mL	27.7778	55.5556

Step 2 Program the pump to flow at the calculated flow rate.

PROCEDURE 4

Priming the Perfusion Feed Tubing Line and Initiating Perfusion

Procedure 4

Priming the Perfusion Feed Tubing Line and Initiating Perfusion

This procedure describes the process of priming the perfusion feed line prior to initiating perfusion in the PBS-0.5 Perfusion Vessel.

Note

- *Use the PBS-0.5 Perfusion Vessel at maximum working volume to reduce risk of medium dropping below retention filter and pulling air into the waste line*
- *Perform inoculation, sampling, and harvest as you would in the standard PBS-0.5 Vessel*

Step 1 Prepare a sufficient volume (typically the volume of medium that will be perfused for the next 24 hours + 10%) of fresh complete medium in a sterile bottle and aseptically transfer into the BSC.

Step 2 Aseptically transfer the empty bottle labelled "Feed" into the BSC.

Step 3 Aseptically swap the caps on the bottles, so that the full bottle has the transfer cap. Label the full bottle "Feed" and place it on the cart. Cover the feed bottle with aluminum foil if necessary to protect light-sensitive components.

Step 4 Turn the pump on and prime the feed line, stopping the pump when the first drops of medium are observed in the PBS-0.5 vessel. Ensure complete priming by manipulating the tubing so that the air bubbles escape the line.

Step 5 Install the pumpable section of waste line tubing into the other pump head such that when the pump is turned on, liquid will flow out of the PBS-0.5 and into the waste container. Close pump head securely.

Step 6 Turn the pump on. Visually observe medium being pulled through the perfusion filter and flowing towards the waste bottle, at the same rate that medium is flowing from the feed bottle and dripping into the PBS-0.5 through the cap. If you do not observe this, check your set-up again.

PROCEDURE 5

Changing Fresh Medium and Waste Medium Bottles

Procedure 5

Changing Fresh Medium and Waste Medium Bottles

This procedure describes how to change fresh and waste medium bottles. Bottles can be swapped by swapping the transfer caps to new bottles, or by welding on new bottles that already have transfer caps attached.

Note

- *Add enough extra medium to fresh medium bottles such that the end of the transfer cap inner tubing is always submerged in medium, considering the volume in the tubing lines*
- *Work as quickly as possible to prevent pump downtime*

Step 1 Pause the perfusion pump and clamp both the feed and waste lines securely. Do not remove the tubing from the pump heads.

Step 2 Prepare a sufficient volume of fresh medium in a sterile bottle and aseptically transfer into BSC.

Step 3 Aseptically transfer the Feed bottle into the BSC.

Step 4 Aseptically swap the caps on the bottles, so that the full bottle has the transfer cap. Label the full bottle “Feed” and place it on the cart.

Step 5 Aseptically transfer an empty, sterile bottle into the BSC.

Step 6 Aseptically transfer the full Waste bottle into the BSC.

Step 7 Aseptically swap the caps, so that the empty bottle has the transfer cap. Label the empty bottle “Waste” and place it on the cart.

Step 8 Unclamp all lines.

Step 9 Restart pump, and visually observe the medium is flowing correctly.

06 TROUBLESHOOTING

Troubleshooting

Unexpected decreases in vessel volume observed

- Check for air in the feed medium line, and confirm that the feed medium bottle is not empty. If there is air in the feed line, re-prime the line to clear all air. Some bubbles are ok, but there should not be any significant gaps with no medium in the tubing lines.
- Check that the tubing is not pinched or clamped anywhere, and that all welding spots are open.
- Recalibrate the pumps- if the culture volume is going down unexpectedly and there are no problems with the tubing, the waste line pump may have a faster flow rate than the feed line pump.

Unexpected increases in vessel volume observed

- Check for air in the waste medium line, and confirm that the cell retention filter Is not clogged.
- Check that the tubing is not pinched or clamped anywhere, and that all welding spots are open.
- Recalibrate the pumps – if the culture volume is going up unexpectedly and there are no problems with the tubing, the feed line pump may have a faster flow rate than the waste line pump.

Filter clogging due to cell debris

- Backflush the filter by briefly reversing the direction of the pump. This can be done periodically to reduce the risk of the filter clogging.

STILL HAVE QUESTIONS?

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