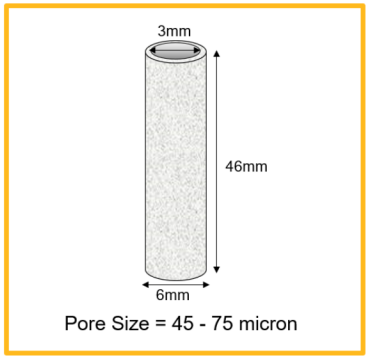
USE OF 60 MICRON PLASTIC (PE) RETENTION FILTER FOR PERFUSION MX WITH PBS-3 VERTICAL-WHEEL BIOREACTOR

DESCRIPTION: 60 MICRON PLASTIC (PE) RETENTION FILTER (5-PACK)

PART#: IC01036

NOTE: Product is not gamma irradiated. Autoclaving of perfusion assembly by the user is recommended prior to use to reduce risk of culture contamination. For Research Use Only.

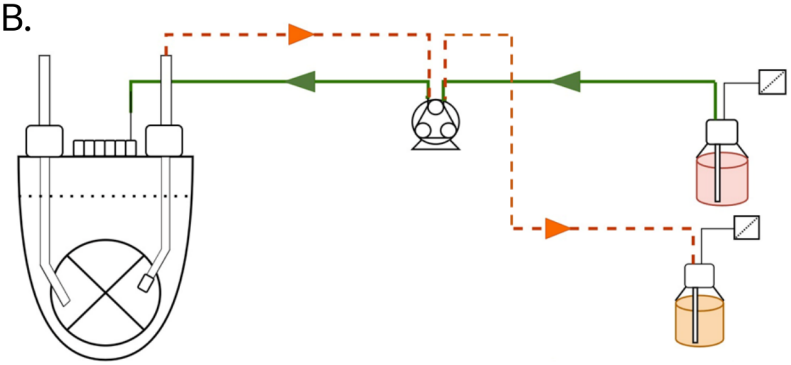
A.



DESCRIPTION

Retention filters are a critical component for performing perfusion medium exchange (MX) for Pluripotent Stem Cell (PSC) culture (as aggregates or on microcarriers) in Vertical-Wheel Bioreactors. The guidance herein provides materials and methods recommended for PBS-3 perfusion system assembly using a **60 Micron Plastic (PE) Retention Filter (Part#IC01036)**.

B.



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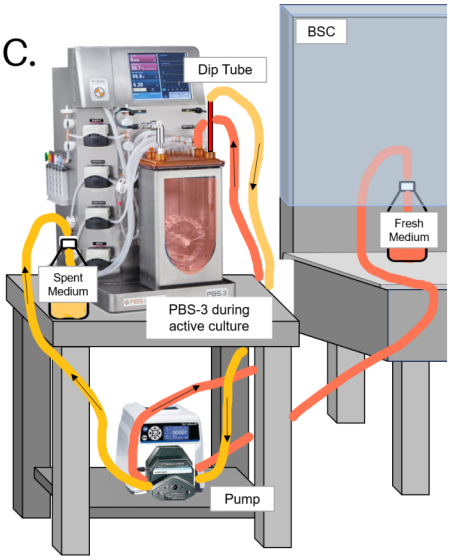

Figure 1) (A) Illustration of **60 Micron Plastic (PE) Retention Filter.** Dimensions may vary slightly. (B) Perfusion schematic of the PBS-3. Key components: media & waste reservoirs, peristaltic pump, dip tube, retention filter, and tubing. (C) Image depicts an example of what this perfusion MX set-up might look like in a laboratory setting.

MATERIALS

Recommended Materials for Perfusion MX Set-Up Using PBS-3 Vertical-Wheel Bioreactor

Sections	Materials	Vendor	Part#
Spent Medium Line	Millex®-FG, 0.20 µm, hydrophobic PTFE, 50 mm, HB/HB	Thomas Scientific	1211K78
	Transfer Tubing, C-Flex®, Clear, 1/4" ID x 3/8" OD	VWR	MFLX06422-10
	Reducing Connector, Polypropylene, 1/4" x 3/16"	VWR	MFLX40622-32
	L/S® High-Performance Precision Pump Tubing, Platinum-Cured Silicone, L/S 15	VWR	MFLX96410-15
	Fitting, Polypropylene, Straight, Hose Barb Union, 3/16" ID	VWR	MFLX40622-57
	Transfer Tubing, BioPharm Platinum-Cured Silicone, 3/16" ID x 5/16" OD	VWR	MFLX95702-09
	Pure-Fit® TC Tube Clamp, Assembled	VWR	75831-124
	Dip Tube Assembly for PBS-3 (Included with PBS-3 Bioreactor)	PBS Biotech	IA-3-BA-007
	60 Micron Plastic (PE) Retention Filter (5-Pack)	**PBS Biotech**	**IC01036**
Fresh Medium Line	Millex®-FG, 0.20 µm, hydrophobic PTFE, 50 mm, HB/HB	Thomas Scientific	1211K78
	Transfer Tubing, C-Flex®, Clear, 1/4" ID x 3/8" OD	VWR	MFLX06422-10
	Reducing Connector, Polypropylene, 1/4" x 3/16"	VWR	MFLX40622-32
	Transfer Tubing, BioPharm Platinum-Cured Silicone, 3/16" ID x 5/16" OD	VWR	MFLX95702-09
	Pure-Fit® TC Tube Clamp, Assembled	VWR	75831-124
	Fitting, Polypropylene, Straight, Hose Barb Union, 3/16" ID	VWR	MFLX40622-57
	L/S® High-Performance Precision Pump Tubing, Platinum-Cured Silicone, L/S 15	VWR	MFLX96410-15
	Reduction Coupler 3/16" ID x 1/8" ID in Non-Animal Derived Polypropylene	Eldon James	C3-2PP
	Transfer Tubing, C-Flex® ADCF, 1/8" ID x 1/4" OD	VWR	MFLX06432-50
Medium Reservoirs	Transfer Tubing, Platinum-Cured Silicone, 1/4" ID x 3/8" OD	VWR	MFLX98906-08
	Ace Glass Cap, GI45, Swivel (2) Hose Connectors For Use With 6-9mm Tubing, Duran	Ace Glass	7634-01
	PYREX® Round Media Storage Bottles, with GL45 Screw Cap	Corning	1395-XX
Pump	L/S® Digital Drive, Computer-Compatible, 0,1 to 600 rpm; 115/230 VAC	VWR	MFLX07551-20
	L/S® Easy-Load® II Pump Head for High-Performance Precision Tubing, PPS Housing, SS Rotor	VWR	MFLX77200-62
	L/S® Easy-Load® II Pump Head Mounting Hardware, Two Pump Heads, Stainless Steel	VWR	MFLX77200-02

To order, please reach out to respective vendor

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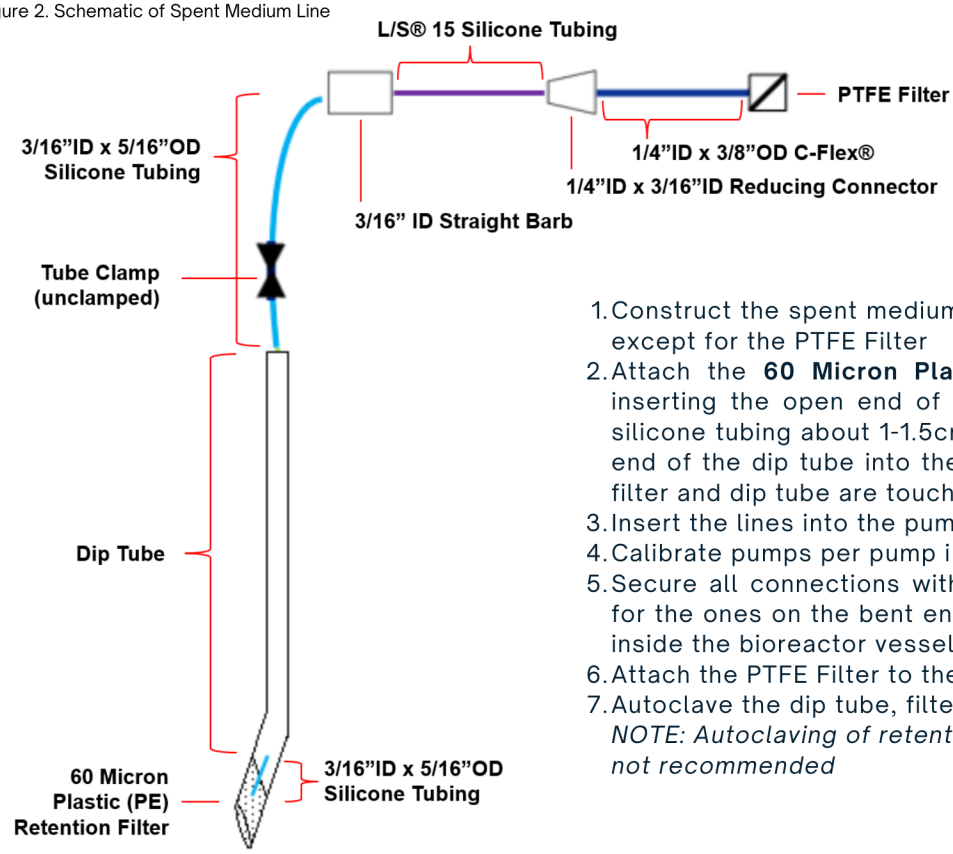
CONSTRUCTION OF SPENT MEDIUM LINE

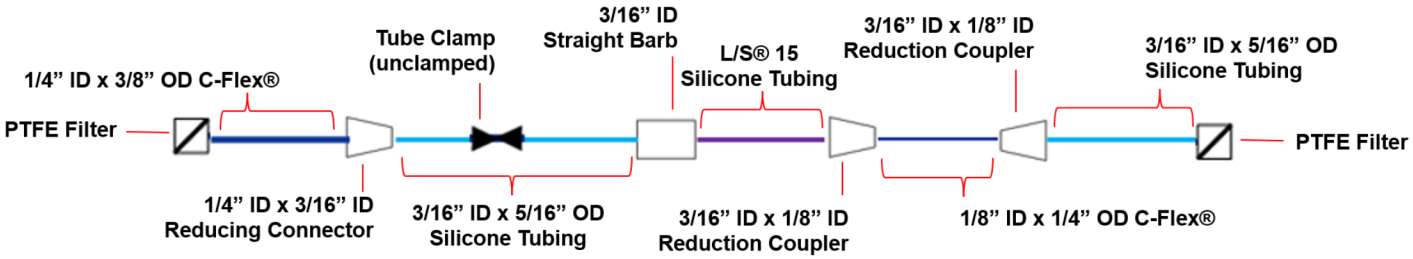
Figure 2. Schematic of Spent Medium Line



1. Construct the spent medium line as illustrated in Figure 2, except for the PTFE Filter
2. Attach the **60 Micron Plastic (PE) Retention Filter** by inserting the open end of the filter into one end of the silicone tubing about 1-1.5cm deep, then insert the angled end of the dip tube into the other side of the tubing until filter and dip tube are touching
3. Insert the lines into the pumps to test tubing lengths
4. Calibrate pumps per pump instructions and fully dry line
5. Secure all connections with autoclavable zip ties except for the ones on the bent end of the dip tube which will be inside the bioreactor vessel
6. Attach the PTFE Filter to the end of the C-Flex® Tubing
7. Autoclave the dip tube, filter, and line together
   NOTE: Autoclaving of retention filter more than once is not recommended

CONSTRUCTION OF FRESH MEDIUM LINE

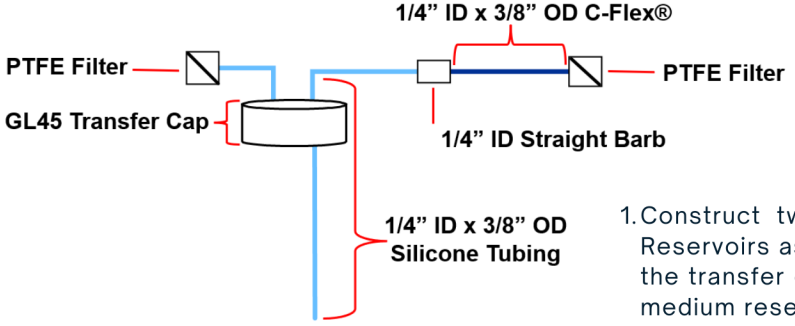
Figure 3. Schematic of Fresh Medium Line



1. Construct the Fresh Medium Line as illustrated in Figure 3
2. Insert the L/S® 15 tubing into the pump to test lengths
3. Calibrate the pump per pump instructions and fully dry line
4. Zip tie all connections
5. Attach the PTFE Filters to the end of the C-Flex® and silicone tubing
6. Autoclave the line
   NOTE: Autoclaving of retention filter more than once is not recommended

CONSTRUCTION OF MEDIUM RESERVOIR CAP CONFIGURATION

Figure 4. Medium Reservoir Cap



1. Construct two caps for the Fresh Medium and Spent Medium Reservoirs as illustrated in Figure 4, making sure the inner tube of the transfer cap is long enough to remove all medium in the fresh medium reservoir

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CONSTRUCTION OF COMPLETE PERFUSION SYSTEM

1. While in the BSC, once probes are installed in PBS-3 vessel per the PBS-3 manual instructions, insert the sterilized dip tube on the spent medium line into the bioreactor
2. With the compression fitting loosened, adjust the height of the dip tube to the center or just below the center of the wheel and tighten
3. Install the vessel into the PBS-3 and continue with set-up per the PBS-3 manual instructions
4. To connect the Spent Medium Reservoir, bring a bottle and transfer cap into the BSC, insert transfer cap into bottle
5. Outside the BSC, weld the 1/4"ID x 3/8"OD C-Flex® portion of the spent medium line to the 1/4"ID x 3/8"OD C-Flex® portion of the Spent Medium Reservoir cap
6. Select an addition line above the liquid level and weld the 1/8"ID x 1/4"OD portion of the addition line to the 1/8"ID x 1/4"OD portion of the fresh medium line
7. To construct the Fresh Medium Reservoir, bring an empty bottle and transfer cap into the BSC. put the cap onto empty bottle
8. Outside of the BSC, weld the 1/4"ID x 3/8"OD portion of the fresh medium line to the 1/4"ID x 3/8"OD portion of the transfer cap
9. Leave the Fresh Medium Reservoir in the BSC and connect to the PBS-3
10. Install the L/S®15 silicone tubing section on the spent medium line in the rear pump head, ensuring the bioreactor side of the line is on the suction and Reservoir side is on the discharge end of the pump
11. Install the L/S®15 silicone tubing section of the fresh medium line in the front pump head, ensuring the Fresh Medium Reservoir is on the suction side of pump and bioreactor is on discharge side of the pump
12. Continue the PBS-3 set-up per PBS-3 manual to initiate culture

INITIATE PERFUSION MX

1. Replace empty bottle on fresh media line with bottle containing fresh medium in the BSC
2. Before starting media exchange, put pump into continuous mode and set flow rate to 50mL/minute
3. Remove waste line from back pump head, resting line on top of pump head
4. Press start to prime the line until first drop of medium enters the vessel, stop pump
5. Change flow rate to desired flow rate (See Figure 5D)
6. Put spent medium line back into the rear pump head and start the pump
7. After run concludes, dispose of retention filter (additional autoclaving may deform plastic)

PUMP HEADS AND FLOW RATES

B.

C.

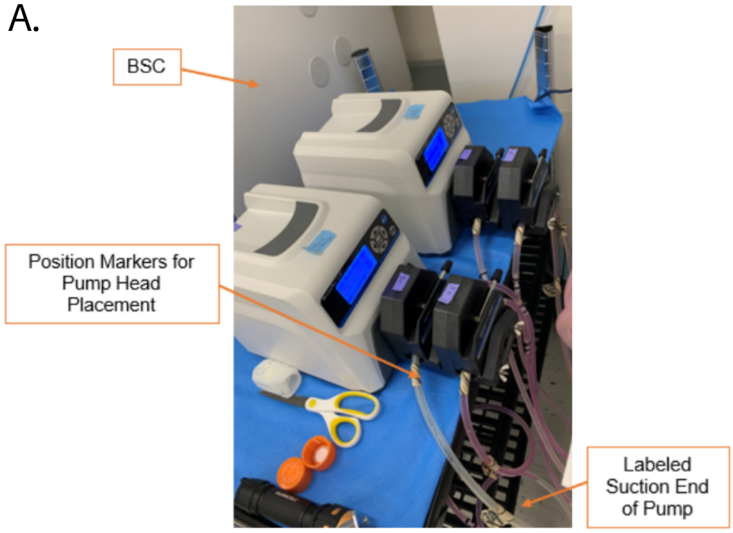
D.

PBS-3 Flowrates	
MX Rate (VVD)	**Pump Flowrate (mL/min)**
0.5	1.042
1	2.083
2	4.167

Flowrates assume 3L working volume and MX period of 24-hours

Figure 5. (A) Example of what pump set-up may look like in lab setting. (B) L/S® Digital Drive, Computer-Compatible, 0,1 to 600 rpm; 115/230 VAC (VWR Part#MFLX07551-20) (C) L/S® Easy-Load® II Pump Head for High-Performance Precision Tubing, PPS Housing, SS Rotor (VWR Part#MFLX77200-62). Masterflex® mounting hardware is required to mount two pump heads on one pump drive (VWR Part#MFLX77200-02). (D) Ideal Flow Rates based on recommended materials, working volume, and MX period.

A.



RELATED PRODUCTS

Product	Part Number
PBS-3 Vertical-Wheel Bioreactor, SUS	IA-3-B-711
PBS-3 Single-Use Vessel, Floored, SUS	FA-3-D-704-L
PBS-3 Single-Use Vessel, Floored, SUS	GA-3-D-714
PBS-3 Dip Tube Assembly	IA-3-BA-007
60 Micron Plastic (PE) Retention Filter (5-Pack)	IC01036

For more information, please contact your account manager at **sales@pbsbiotech.com**.

To place an order, please contact customer service at **customer.service@pbsbiotech.com**.

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