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HyPerforma 2:1 Single-Use Bioreactor (S.U.B.) User's Guide

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Contents

Appendices

Warnings, safety, and warranty information

Thank you for purchasing this high-quality Thermo Scientific equipment. We have included safety information in this guide, based on our knowledge and experience. It is important, however, for you to work with your Safety Management personnel to ensure that this equipment is integrated into your safety practices. Please take some time to perform your own job safety analysis in order to identify and control each potential hazard.

WARNING: Read and understand this user's guide before operating the equipment.

The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) is designed to be operated under traditional eukaryotic cell culture conditions. A general understanding of bioreactor systems and their operation is important prior to using the system for the first time. Read and understand the user's guide before operating; failure to do so could result in injury and potential loss of product.

WARNING: Hazardous voltage inside.

The mixer motor, motor controller and control panel all have electrical components. There is a risk of electrical shock and injury. Disconnect power before opening electrical components. Service should be performed by Thermo Fisher Scientific service personnel only. Thermo Fisher Scientific recommends using standard lockout procedures when working on electrical components. The main breaker on the electrical box may be locked out.

WARNING: Static electricity may build up in BPCs.

- BioProcess Containers (BPCs) may act as insulators for electrostatic charge. If electrostatic charge is transferred to a BPC, the charge may be stored in the BPC and/or the product inside. This phenomena varies by product and use; therefore, it is the sole responsibility of the end user to ensure a hazard assessment is conducted and the risk of electrostatic shock is eliminated.
- Where applicable, a product contact stainless steel coupler may be grounded to the frame to dissipate electrostatic build up from the material within a BPC. It is good practice to dissipate electrostatic buildup by grounding all BPCs prior to coming in contact with them. When working with BPCs, the use of non-conductive materials, such as non-conductive gloves, is recommended.

WARNING: Rotating parts—entanglement hazard.
Rotating and moving parts can cause injury. Keep hands away from moving parts during operation.

- Do not operate this equipment unless the supplied guarding is in place and properly functioning.
- It is the responsibility of the end user to assess this equipment and ensure that equipment and safeguards are in good working condition, and that all operators are trained and aware of entanglement hazards and associated protective devices, such as hazard signs and guarding.

WARNING: Use ladders and elevated platforms with caution.
A few operations, such as loading a BPC into a large S.U.B., may require the use of a ladder or platform. Before use, ensure the ladder has been inspected and weight-rated for its user. When using a ladder or platform, be sure it is stable, maintain three points of contact, and make sure the steps are clean.

WARNING: Follow lockout/tagout procedures.
To prevent injury, when servicing equipment, use your company's lockout/tagout procedures to isolate electrical, mechanical, pneumatic, hydraulic, chemical, thermal, gravitational, or any other potential energy and protect workers from the release of hazardous energy.

WARNING: Use caution with hazardous chemicals or materials.
Personnel servicing equipment need to know the hazards of any chemicals or materials that may be present on or in the equipment. Use general hazard communication techniques such as Safety Data Sheets, labels, and pictograms to communicate any hazards.

WARNING: Potential confined space.
Operators may enter larger S.U.B. systems. Evaluate this equipment against your confined space standards and procedures.

WARNING: Burst hazard—air under pressure.
The S.U.B. BPC chamber is under slight pressure under normal operating conditions. Normal passive venting prevents any excess of pressure building up within the chamber. Chamber pressure and inlet line pressure should be monitored for proper settings.

- Contents under pressure
- Do not exceed 0.03 bar (0.5 psi) BPC pressure
- Do not exceed 0.34 bar (5 psi) inlet pressure
- Ensure vent filter is properly positioned and working properly

WARNING: Hot surface—do not touch.
The heating jacket is designed to heat the inner vessel wall. Normal operating conditions generate heat and could create hot surfaces.

- Hot surface inside
- Contact with surfaces may cause burns
- Do not touch while in operation

WARNING: Pinch hazard.
The motor lift on 1,000 and 2,000 L S.U.B.s can be raised and lowered using the handheld controller. Caution should be used when changing the position of the motor to avoid pinching an operator or causing damage to the equipment or the BPC.

WARNING: Tipping hazard. The vessel should only be moved by pushing using the provided handles or at the mid-point of the vessel.
If pulled or moved too quickly, the vessel can tip, potentially leading to damage to equipment or injury to personnel. To reduce the risk of tipping, the vessel should only be moved slowly over smooth, flat surfaces by at least two qualified personnel. During movement, any locking feet should be retracted, and casters should be in the unlocked position. The vessel should not be moved by pulling of any kind.

WARNING: The Thermo Scientific HyPerforma Single-Use Bioreactor may not be installed in a potentially explosive atmosphere as set forth in the applicable EU ATEX Directive.
It is the responsibility of the end user to review and understand the potential dangers listed in the ATEX 2014/34/EU guidelines.

Protective earth grounding

Protective earth grounding must be verified prior to plugging the S.U.B. into any electrical outlet. Ensure the receptacle is properly earth grounded.

Environmental conditions

- Operating: 17°C to 27°C; 20 to 80% relative humidity, non-condensing
- Storage: –25°C to 65°C
- Installation category II (over voltage) in accordance with IEC 664
- Altitude Limit: 2,000 meters

Electrical connections

Power should be supplied by a non-GFCI 15 amp circuit. Ground faults occur when current is leaking somewhere, in effect, electricity is escaping to the ground. **Electrocution can occur when the human body serves as the path for the leakage to the ground.** A ground fault circuit interrupter (GFCI) senses the current flowing to the ground and switches off the power (trips the GFCI) in a fraction of a second at currents well below those that are considered dangerous. Due to the sensitivity of GFCIs to electrical leakage (a few mA), it is recommended that the S.U.B. is NOT plugged into a GFCI outlet.

Water jacket vessel information

S.U.B. hardware unit with water jacket has been designed to be operated with water as the heat transfer medium with temperatures not exceeding 50°C (122°F) under less than 1 MPa (150 psig) operating pressure. For the utmost safety it is recommended that the S.U.B. be operated at 75 psig or less.

Note: The S.U.B. BPC operating limits for temperature are 5 to 40°C. The internal pressure should not exceed 0.03 bar (0.5 psi). The water jacket is not required to be registered, inspected and stamped with the Code U symbol per section U-1(c)2(f) of the ASME Boiler and Pressure Vessel Code and/or European Pressure Equipment Directive (PED) 97/23/EC. Upon request, a Declaration of Conformity, PED Sound Engineering Practices can be made available.

Use of agitation speed governors and safety interlocks

Agitation speed governors set up on the bioreactor controller are used to limit the maximum mixing speed, according to pre-defined liquid volumes. Safety interlocks, which stop agitation when the volume in a S.U.B. drops below defined limits, and speed-based governors prevent damage to the drive shaft in the bioreactor. Agitation speed governors and safety interlocks typically prevent the hazardous conditions listed below.

- Operating the motor at any speed while loading the drive shaft
- Operating the agitator when volumes are less than 20% of a system's working volume
- Operating the agitator above recommended speeds based on qualified power input to volume (P/V) thresholds

The hazardous conditions above must be avoided in order to ensure qualified reliability. Using safety interlocks and agitation speed governors eliminates the chance of human error, which could reduce system reliability. Both the amount of liquid in the vessel and the amount of power applied to the impeller have an impact on the applied deflection on the shaft. Excess deflection and/or mixer speed may damage the drive shaft.

For more information about using P/V and safety interlocks in 2,000 L bioreactor systems, see section 3.6.5 of this publication.

Warranty information

Any warranties, if applicable, covering this equipment exclude: (a) normal wear and tear; (b) accident, disaster or event of force majeure; (c) your misuse, fault or negligence; (d) use of the equipment in a manner for which it was not designed; (e) causes external to the equipment such as, but not limited to, external puncturing, power failure, or electrical power surges; (f) improper storage and handling of the equipment; (g) use of the equipment in combination with equipment or software that we did not supply; (h) equipment sold to you as 'used' products; (i) contact with improperly used or unapproved chemicals or samples; (j) installation, removal, use, maintenance, storage, or handling in an improper, inadequate, or unapproved manner, such as, but not limited to, failure to follow the documentation or instructions in the deliverables or related to the equipment, operation outside of stated environmental or other operational specifications, or operation with unapproved software, materials, or other products; (k) manufacture in accordance with requirements you gave us; (l) installation of software or interfacing or use of the equipment in combination with software or products we have not approved; (m) use of the deliverables or any documentation to support regulatory approvals; (n) the performance, efficacy or compatibility of specified components; and (o) the performance of custom equipment or products or specified components or achievement of any results from the equipment, specified components or services within ranges desired by you even if those ranges are communicated to us and are described in specifications, a quote, or a statement of work. **ADDITIONALLY, ANY INSTALLATION, MAINTENANCE, REPAIR, SERVICE, RELOCATION OR ALTERATION TO OR OF, OR OTHER TAMPERING WITH, THE EQUIPMENT PERFORMED BY ANY PERSON OR ENTITY OTHER THAN US WITHOUT OUR PRIOR WRITTEN APPROVAL, OR ANY USE OF REPLACEMENT PARTS WE HAVE NOT SUPPLIED, WILL IMMEDIATELY VOID AND CANCEL ALL WARRANTIES WITH RESPECT TO THE AFFECTED EQUIPMENT. IF THE EQUIPMENT IS TO BE USED IN THE UNITED STATES, WE MAY VOID YOUR WARRANTY IF YOU SHIP THE EQUIPMENT OUTSIDE OF THE UNITED STATES.**

Use restrictions

You must use this equipment in accordance with our documentation and if applicable, with our other associated instructions, including without limitation, a “research use only” product label or “limited use” label license. This equipment is intended for research use or further manufacturing in bioprocessing applications and not for diagnostic use or direct administration into humans or animals, we do not submit the equipment for regulatory review by any governmental body or other organization, and we do not validate the equipment for clinical or diagnostic use, for safety and effectiveness, or for any other specific use or application.

Seismic guidance

The buyer of the equipment is responsible to ensure country specific codes and seismic values are assessed for suitability of equipment installation and safety at the designated site. In addition, it is the buyer’s responsibility to assess the building structure for the designated equipment to ensure correct seismic anchoring and tethering designs for both the equipment and facility. It is highly recommended that the buyer consult with a local, licensed third party architecture and engineering firm to provide the buyer with correct engineering analysis and stamped documentation prior to equipment installation at the facility. In addition the buyer will be responsible for rigging and anchoring of the equipment to a specified, fixed location. Thermo Fisher can assist with establishing compliant seismic anchoring and tethering designs for purchased equipment based on building and country codes upon request at an agreed upon fee.

It is also noted that movable equipment (i.e. non-fixed or caster mount) is exempt from seismic design requirements according to ASCE 7-16, Chapter 13, section 1.4. Although these units are exempt from the seismic design requirements of ASCE 7, it should be noted that such equipment is susceptible to overturning during a seismic event. Therefore, it is the responsibility of the buyer to address seismic safety for movable equipment at the designated facility.

How to use this guide

Scope of this publication

This user's guide contains information about the standard Thermo Scientific HyPerforma 2:1 S.U.B. systems, including hardware, components, product design verification methods, installation, operation, and specifications. It is intended for use by people who may or may not have experience with Thermo Scientific systems, but who have some knowledge of bioproduction processes and large-scale mixing systems.

Document change information

Revision	Date	Section	Change made	Author
1.4	05/2016	--	Initial Release	S. Jelus
B	12/2016	How to Use This Guide	Added How to Use This Guide section	E. Hale
B	12/2016	4.2	Fixed Electrical Power Supply Requirement in Specifications section	E. Hale
C	12/2016	Warnings and Safety	Added information about safety interlocks to Warnings and Safety section	S. Jelus
C	12/2016	2.2	Added serial number information and photo of ends of multiple-section drive shafts	S. Jelus/E. Hale
C	12/2016	3.6.4	Added warning note about agitation rate and volume requirements, and the use of safety interlocks	S. Jelus/E. Hale
C	12/2016	5.1.2	Added measurement to Table 1.10 for 2,000 L drive shafts and cross-reference to Appendix D	S. Jelus
C	12/2016	3.4	Added information about 2-piece drive shaft and a note about position of impeller tubing inside the BPC	S. Jelus/E. Hale
C	12/2016	3.4	Added serial number information and photo of ends of multiple-section drive shafts	S. Jelus/E. Hale
C	12/2016	3.4	Added a note about not pushing drive shaft straight into the assembly when loading	S. Jelus/E. Hale
C	12/2016	3.4	Added information and Figure 2:105 to illustrate proper insertion of drive shaft	S. Jelus/E. Hale
C	12/2016	4.2	Added information about 2-piece drive shaft to 2,000 L specifications	S. Jelus/E. Hale
C	12/2016	4.2	Added ceiling height requirements for 2-piece drive shaft and detail about mixing speed to 2,000 L specifications	S. Jelus/E. Hale
C	12/2016	4.5	Added drive shafts as accessories	S. Jelus/E. Hale
C	12/2016	Appendix D	Added Appendix D—2,000 L S.U.B. Agitator Operation and Maintenance Guidelines	S. Jelus/E. Hale
D	02/2017	Appendix D	Removed Table D.1 in Appendix D	E. Hale
D	02/2017	3.6.5	Moved sections from Appendix D to new Agitation Rate Calculations section	E. Hale

Document change information (continued)

Revision	Date	Section	Change made	Author
D	02/2017	3.3	Changed “500–1,000L Electric Resistive Heater” to “500–1,000 L Volumes” in BPC Loading section	E. Hale
D	02/2017	3.4	Changed “BPC Loading 2,000L Water Jacket” to “BPC loading 2,000 L volume” in BPC loading section	E. Hale
D	02/2017	3.4	Moved “Securing access doors” step after port alignment step in 2,000 L BPC loading	E. Hale
D	02/2017	Appendix E	Moved drive shaft log from Appendix D to Appendix E	E. Hale
D	02/2017	3.3	Moved “Securing access doors” step from 50–250 L BPC loading to 500–1,000 L BPC loading	E. Hale
D	02/2017	6.2	Updated addresses, phone numbers, and email address	E. Hale
D	04/2017	Warnings and safety	Updated “Use of Agitation speed governors and safety interlocks” in Warnings and safety	E. Hale
D	04/2017	4.2	Changed “Maximum mixing rate” to “Agitation speed range” in hardware specifications	E. Hale
D	04/2017	4.2	Added “Minimum acceleration and deceleration rate” to 2,000 L hardware specifications	E. Hale
D	04/2017	4.5	Corrected part numbers for 2,000 L S.U.B. drive shafts	E. Hale
D	04/2017	3.6.3	Updated media fill instructions	E. Hale
D	04/2017	5.1.2	Updated drive shaft replacement intervals for 20 and 40 W/m^3 P/V in "Drive shaft longevity and replacement"	E. Hale
D	05/2017	Warnings and safety	Added potentially explosive atmosphere (ATEX) warning	E. Hale
D	05/2017	3.2, 3.3, 3.4	Added step about removing plastic insert in the thermowell before inserting RTD	E. Hale
D	05/2017	2.1, 3.1	Updated 2,000 L load cells and unlocking instructions	E. Hale
D	05/2017	Chapter 1, 4.2	Removed electric resistive heater options for 500, 1,000, and 2,000 L systems	E. Hale
D	05/2017	4.4	Corrected format for units of measurement in BPC specifications	E. Hale
E	06/2018	Warnings and safety	Replaced the images for the following warning labels: "Read and understand the user's guide..." and "Entanglement hazard"	K. Leeman
E	06/2018	Warnings, safety, and warranty information	Added warranty information and use restrictions	K. Leeman
E	06/2018	3.6.5	Revised Graph 1.2 by changing 2,000 L line to 750 L, and 1,000 L line to 375 L	K. Leeman
E	06/2018	3.6.5	Changed footnote in Table 1.7 from "40 W/m^3" to "> 20 W/m^3"	K. Leeman
E	06/2018	3.6.5	Added information about protection against drive shaft instability, including a graph depicting regions of potential agitator harmonics and cavitation for liquid working volumes of the 2,000 L S.U.B.	K. Leeman
E	06/2018	3.6.5	Added 20–40% fill agitation rates for all S.U.B. sizes to Table 1.8	K. Leeman
E	06/2018	3.6.5	Changed first footnote in Table 1.9 from "40 W/m^3" to "> 20 W/m^3"	K. Leeman
E	06/2018	5.1.2	Removed 2,000 L row from Table 1.10	K. Leeman

Document change information (continued)

Revision	Date	Section	Change made	Author
E	06/2018	5.1.2	Under "Drive Shaft Longevity and Replacement," added "of cumulative use" after "we recommend replacing your drive shaft every 360 days." In the second sentence of the second paragraph, verbiage was changed to "...every 180 days of cumulative use." In the first sentence of the note, added "at < 50% working volume"	K. Leeman
E	06/2018	3.4	Replaced Figure 2.105 with an image to reflect the deep pocket impeller change	K. Leeman
E	06/2018	3.1	Revised Table 1.3 to reflect the recommended heating times for S.U.B.s	K. Leeman
E	06/2018	3.6.4	Added note to Tables 1.8 and 1.9 about system recommended speed/ volume control parameters	K. Leeman
E	06/2018	5.1.2	Added Table 1.11 and related note describing 2-piece drive shaft operating parameters for 2,000 L S.U.B.s	K. Leeman
E	06/2018	4.2	Updated "Operating temperature" in specifications for all sizes to "Ambient to 40 ± 0.5°C (104 ± 0.9°F)"	E. Hale
E	06/2018	--	Reformatted using new template and reorganized chapters/content	E. Hale
E	06/2018	4.2	Corrected ceiling height requirement for 2,000 L S.U.B. 4-piece drive shaft loading, and added noise level to specifications for all S.U.B. sizes	E. Hale
E	06/2018	5.2	Added FAQ about excessive residue buildup in condenser bag	E. Hale
E	06/2018	How to use this guide	Added "Abbreviations/acronyms" section	E. Hale
E	06/2018	1.2.3, 3.4.2	Added side-mounted condenser system illustration and information	E. Hale
E	06/2018	3.2.1	Updated image of media ground clip connection for 50–250 L BPC loading	E. Hale
E	06/2018	--	Removed references to 4-piece drive shafts for 2,000 L S.U.B.s	E. Hale
E	08/2018	Warnings, safety, and warranty information	Added seismic guidance	K. Leeman
F	11/2018	Warnings, safety, and warranty information	Added emphasis to "Electrical connections" section, changed "certified personnel" to "Thermo Fisher Scientific service personnel," and updated ATEX warning	E. Hale
F	11/2018	--	Removed references to metal probe clips	E. Hale
F	11/2018	2.1.3, 3.6.4, 4.3	Updated text about and images of the E-Box	E. Hale
F	11/2018	How to use this guide	Changed "Input into Thermo Scientific publications" section to "Questions about this publication"	E. Hale
F	11/2018	Appendices	Removed Appendix B (AC-Tech variable speed drive settings) and renamed Appendices C, D, and E to Appendices B, C, and D	E. Hale
F	11/2018	2.2.3, Various	Removed section 2.2.3 (Attaching the cable management system arm) and edited images showing the arm	E. Hale
F	12/2018	3.1.4, 3.6.4	Edited sentence (3.1.4) and reworded step #2 (3.6.4)	E. Hale
F	12/2018	4.2	Added tolerance to "Agitation speed range" in all specifications	E. Hale
F	12/2018	3.7.1	Updated expected accuracy in "Mixing speed verification" to ± 1.5 rpm or 1% of setpoint, whichever is greater	E. Hale

Document change information (continued)

Revision	Date	Section	Change made	Author
G	10/2019	4.2, Various	Minor revisions and updated cart length demention on Figure 4.10	T. Golightly
G	06/2020	--	Minor formatting revisions	T. Golightly
G	06/2020	Warnings, safety, and warranty information	Added Warnings for Pinch Hazard and Tipping Hazard	T.Golightly
G	06/2020	3.2.1	Removed former Step 13, and removed former Figure 3.11	T. Golightly
G	06/2020	3.2.1	Added a CAUTION note below Step 12 for the BPC loading instructions	T.Golightly
G	06/2020	3.3.1, 3.4.1	Added a CAUTION note to the BPC loading instructions	T. Golightly
H	11/2020	4.2	Corrected the overall width, length, and height in Tables 4.2 and 4.4	E. Hale
H	11/2020	4.2	Replaced Figures 4.7–4.10 with updated dimensions	T. Golightly
H	11/2020	1.3.1, 4.4	Updated "Finesse" and "PreSens" sensors to "Hamilton" sensors in Tables 1.1 and 4.23	T. Golightly
H	11/2020	4.4	Removed the "PreSens and Finesse" sensors and replaced with "Hamilton" sensors in Table 4.23	T. Golightly

Questions about this publication

If you have any questions or concerns about the content of this publication, please contact **technicaldocumentation@thermofisher.com** and your Thermo Fisher Scientific sales team.

Related publications

Please contact your local sales representative for information about the related publications listed below.

Publication	Description
Thermo Scientific HyPerforma 2:1 S.U.B. Validation Guide (DOC0016)	Information about validation procedures
Thermo Scientific HyPerforma 2:1 S.U.B. Data Sheets (for various sizes)	Product descriptions and ordering information

Abbreviations/acronyms

Refer to the list below for definitions of the abbrieviations and acronyms used in this publication.

BPC	BioProcess Container
DO	Dissolved oxygen
ETP	Equipment Turnover Package
GFCI	Ground fault circuit interrupter
HMI	Human machine interface
ID	Inner diameter
IEC	International Electrical Code
OD	Outer diameter
PED	Pressure Equipment Directive
PID	Proportional integral derivative
P/V	Power input to volume
RTD	Resistance temperature detector
STR	Stirred tank reactor
S.U.B.	Single-Use Bioreactor
TCU	Temperature control unit
VFD	Variable frequency drive

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HyPerforma 2:1 Single-Use Bioreactor overview

Chapter contents

1.1 Introduction to the Single-Use Bioreactor

The Thermo Scientific™ HyPerforma™ Single-Use Bioreactor (S.U.B.) has been designed as a single-use alternative to conventional stirred tank bioreactors currently utilized in eukaryotic cell culture. Based on years of accepted stirred tank reactor (STR) design, the S.U.B. emulates STR scalability and operating parameters, yet it has the unique advantage of being a single-use device. Ease of setup with respect to system operation, and integration into existing facilities makes the S.U.B. an attractive alternative to its conventional STR counterpart.

Critical design parameters such as height-to-diameter ratios, mixer design and location, and typical control system interfaces have been maintained. A key element to the single-use design is the plastic (polyethylene) impeller with a bearing/seal assembly linking to an external mixer drive. Quick setup and changeover allows for faster turnover in cell culture runs over traditional reusable systems.

The S.U.B. system consists of the following primary components:

1. **Outer support container** with water jacket heating system, or resistive heater for 50, 100, and 250 L systems
2. **S.U.B. BioProcess Container (BPC)**, which is supplied gamma irradiated
3. **Control system for units with AC motors** for agitation
4. **Direct drive agitation mixing assembly** with an AC or DC motor, drive shaft, and impeller

Figure 1.1. 50–500 L S.U.B.s.

The **outer support container** is engineered and fabricated to fully support each BPC and allow easy access for operation. It is a stainless steel vessel that holds and supports the BPC. The outer support container contains the mixing drive and water jacketed or resistive tank on casters (2,000 L S.U.B.s are not on casters). Water jacketed heating is an option for all tank sizes, and resistive heating is available for 50, 100, and 250 L tanks. The drive shaft is detachable and reusable, and is inserted into the BPC through the mixing assembly and into the bearing port. Load cells are standard on 1,000 and 2,000 L systems, and are optional for smaller systems.

The **BPC** includes the impeller assembly, sparger, vent filter inlet/outlet ports, probe integration ports, filling, dispensing, and sampling ports. Each BPC comes fully assembled and gamma irradiated. The materials are fully qualified for biological product contact per USP Class VI plastics. Each assembly is manufactured under cGMP and is supported by qualification and validation information. No reuse cleaning is required. Innovative, proprietary technology allows for the integration of the mixing shaft and pH and dissolved oxygen (DO) probes, and the resistance temperature detector (RTD). The probe and temperature interfaces are comparable to traditional systems with the design allowing for simple aseptic connections. Integrated spargers are built into the BPC through universal ports.

The Thermo Scientific S.U.B. utilizes an open architecture design for the **control system**, allowing for integration with customer systems or with third-party controllers for feed pumps, mass flow controls, and human-machine interface (HMI) screens. Controls for agitation are integrated into the S.U.B., with pH/DO probes and controls being supplied by the user or a third-party integrator. HyPerforma S.U.B. systems require a temperature control unit selected and supplied by the end user or by Thermo Fisher Scientific.

This user's guide covers the setup, operation, maintenance, and troubleshooting of all 2:1 S.U.B. systems in the following volumes—50, 100, 250, 500, 1,000, and 2,000 L.

Note: This guide is for S.U.B. systems that operate at a minimum working volume of 50% (also known as 2:1 mixing). If you are using a S.U.B. system capable of operating at 20% working volume (5:1 mixing), refer to the HyPerforma 5:1 Single-Use Bioreactor User's Guide (DOC0022).

1.2 Hardware characteristics

1.2.1 S.U.B. hardware components

Figures 1.2 and 1.3 below illustrate all available components of a water-jacketed 500 L S.U.B. system. **Note:** 50, 100, and 250 L systems do not have a BPC loading door, and use a one-piece drive shaft.

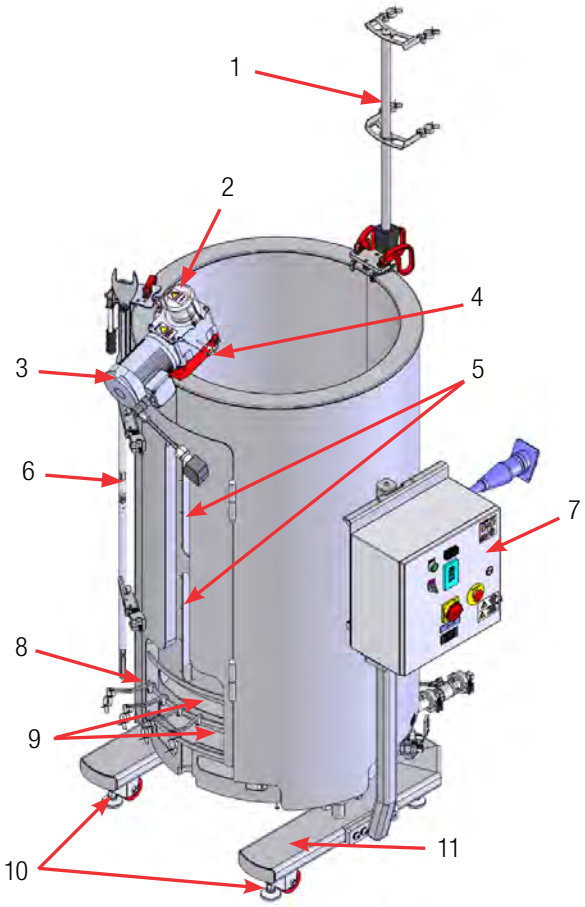


Figure 1.2. Front/side view of 500 L S.U.B.

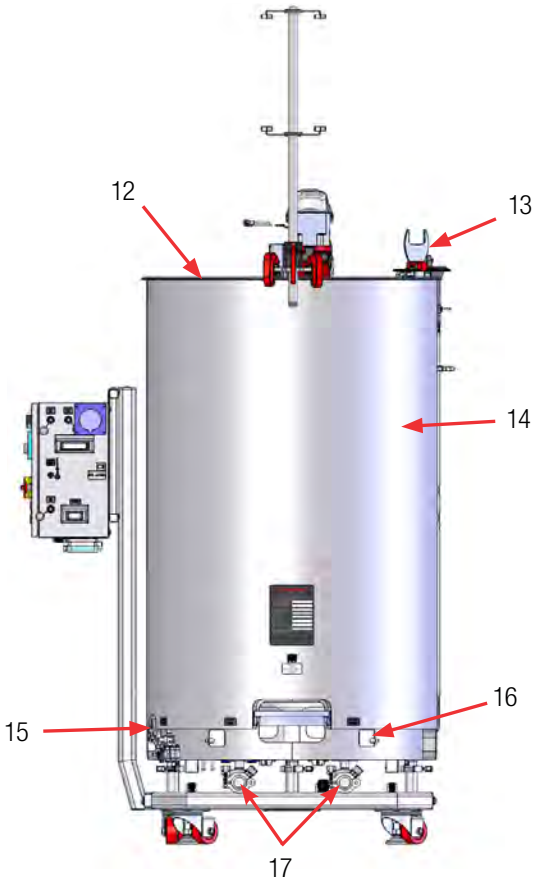


Figure 1.3. Back view of 500 L S.U.B.

1. Exhaust vent filter holder
2. Mixing assembly with shield
3. Mixer motor
4. Bearing port receiver with clamp
5. Liquid sight windows
6. Drive shaft, stored
7. Electrical control panel (E-Box), optional
8. Probe hanger bracket
9. Probe access windows
10. Leveling casters
11. Cart assembly
12. 0.95 cm (3/8 in.) Dimpled water jacket (not present in resistive 50, 100, and 250 L S.U.B.s)
13. Standard tool set: 10 mm (3/8 in.) x 16.9 Nm (150 in-lb.) square torque wrench, load cell and motor cap lockout wrench
14. Stainless steel outer support container
15. Bleed valve
16. Bottom cutouts/pins for BPC attachment/alignment
17. Quick connect water inlet/outlet ports (for water-jacketed S.U.B.s only)

Figures 1.4 and 1.5 below illustrate all available components of a 2,000 L S.U.B. system. **Note:** 1,000 L systems have a cutout instead of a back access door. See section 4.1.3 for a complete illustration of a 1,000 L S.U.B.

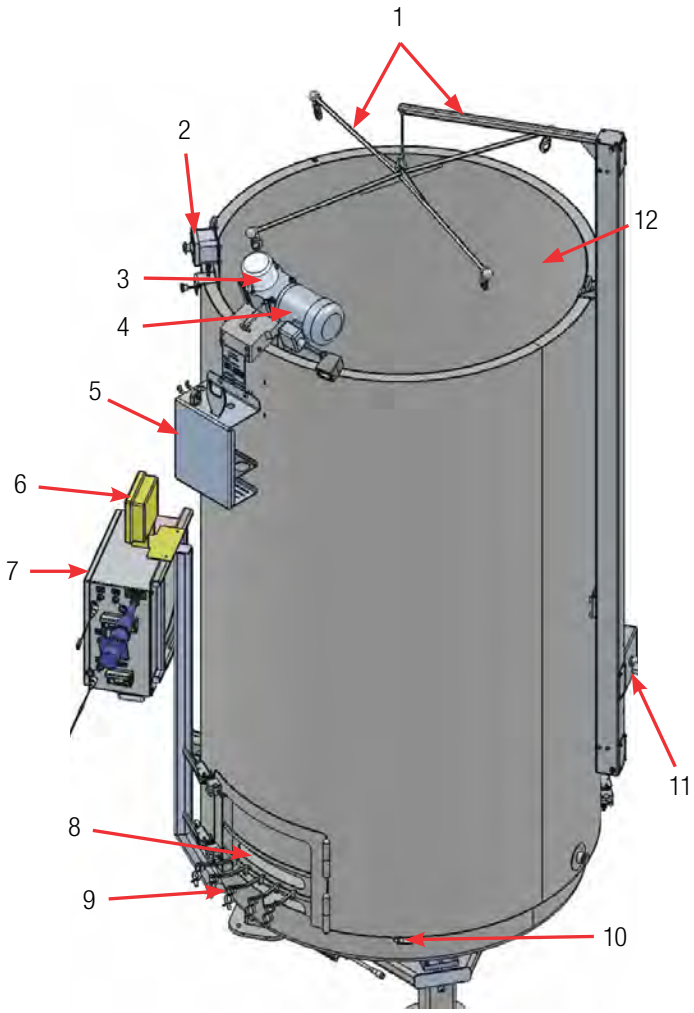


Figure 1.4. Front/side view of 2,000 L S.U.B.

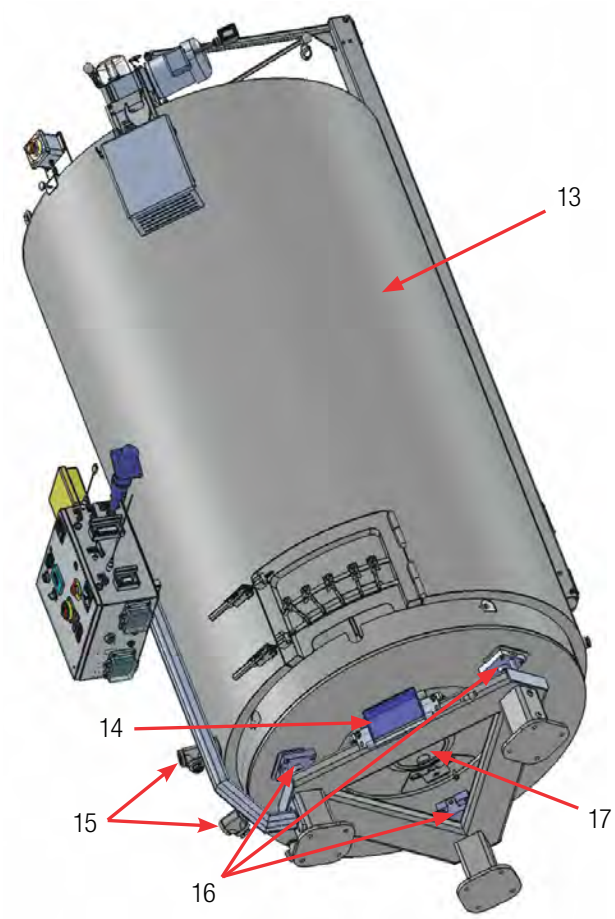


Figure 1.5. Side/bottom view of 2,000 L S.U.B.

1. Bag lift assembly
2. Auxiliary emergency stop (E-Stop)
3. Mixing assembly with shield
4. Mixer motor
5. Standard tool set
6. Load cell display
7. Electrical control panel (E-Box)
8. Probe access window
9. Probe clips
10. Bottom cutouts/pins for BPC alignment
11. Pneumatic bag lift control
12. Water jacket
13. Stainless steel outer support container
14. Load cell summing block
15. Quick connect water inlet/outlet ports
16. Load cells (3)
17. Sparge plate access

1.2.2 S.U.B. system features

The S.U.B. is designed for system mobility and easy integration, and utilizes a straightforward operator interface. The following sections give general descriptions of S.U.B. hardware features.

Agitation

If your system uses an AC motor and a Thermo Scientific electrical control panel (E-Box), the stirring speed is adjusted by using the keypad interface on the control panel. The agitation control interface utilizes a digital display to indicate stirring speed in units of revolutions per minute (rpm). Power is supplied to the motor by a two-position power switch. The up and down arrows on the agitation keypad adjust the stirring speed. If your 50, 100, 250, or 500 L system has a DC motor and is integrated and managed by a third-party controller, agitation is managed by the controller. Thermo Fisher Scientific does not provide electrical control for units with DC motors.

Bioreactor control system

The S.U.B. is designed to integrate with existing bioreactor control systems in their numerous configurations. The S.U.B. control system supplied with the Thermo Scientific E-Box manages the agitation process parameters. Parameters of pH and DO, gas management, feed addition, and base addition control must be managed by an external controller supplied by the end user or a third-party integrator.

Temperature

The S.U.B. can be operated within the temperature range from ambient to 40°C. For 50, 100, and 250 L systems with resistive heaters and Thermo Scientific E-Boxes, temperature setpoints can be adjusted via the temperature controller located on the front panel of the S.U.B. E-Box. This controller is pre-programmed to avoid overshoot during heat-up, and to maintain a target temperature of ± 0.5°C based on the set value display. The process temperature is measured by means of a supplied resistive temperature detector (RTD) (pt-100) that is inserted into the thermowell of the S.U.B. BPC. Water jacket system temperature control is maintained through the temperature control unit (TCU).

Heating performance

Heating times for the S.U.B. systems vary based on operating liquid volume and temperature, ambient or heating fluid temperature, sparger rate, and mixing rate. Users should adjust process liquid staging and seeding strategies to the unique aspects of the S.U.B. Process controllers and heaters in 50–250 L resistive systems are designed to provide optimum heat transfer, and to minimize heat-up times, while maintaining the material integrity of the polymer film construction of the BPC. Refer to section 3.1.4 for expected heating times.

1.2.3 Additional system components

Drive shafts

The S.U.B. drive shaft is detachable and reusable. It is inserted into the BPC through the hollow pass-through of the motor assembly, into the bearing port, through the tubing sleeve inside the BPC, and into the polyethylene impeller. Drive shaft rods may be made of aluminum, stainless steel, or carbon fiber, depending on the size of the vessel and the strength requirements.

As a general rule, drive shafts should be replaced after 360 days of service, or as specified in Chapter 5 of this publication. Always keep a log of actual drive shaft usage. Appendix D includes a form that can be used for this purpose.

AC and DC motors

AC and DC motor options are available to help tailor the system to specific needs. The DC motor operates at a lower voltage and, when integrated with a controller system that receives sensor feedback, provides more accurate speed control through a digital program transmitter. The DC motor comes with an encoder, but does not come with a motor control option from Thermo Scientific, and must be specified by the end user.

The AC motor may be used with the Thermo Scientific E-Box, includes the variable frequency drive, and is controlled using either the provided keypad or a controller specified by the end-user.

Options and accessories

The following additional system components may or may not be installed on your S.U.B. system. To order accessories for retro-fitting to your unit, contact your sales representative.

Exhaust vent filter heaters

The exhaust vent filter heater system, which includes the heater, a controller, and power cord (Figure 1.6), is available for increased longevity of the exhaust filter on the BPC.

The heating element is fully insulated with molded silicone and secured around the filter by use of snap retainers, fully encapsulating the exhaust filters for consistent temperature regulation. Heating the filter sufficiently to eliminate the formation of condensation reduces the risk of fouling the filter membrane. The heater is factory-preset to operate between 40°C–50°C, but can easily be adjusted to the demand of the application. Temperature settings above 60°C are not recommended.

Figure 1.6. Vent filter heater.

Condenser systems (for 2,000 L units only)

The condenser system supports the effective use of 2,000 L S.U.B.s, and condenser systems with a cart assembly are also available as an auxiliary component for other S.U.B. sizes. The condenser system efficiently condenses exhaust gases and transfers condensate back into the bioreactor, preventing potential vent filter blockage and reducing fluid loss due to evaporation. It is offered in both single and double chill-plate formats. The condenser plate on condenser systems with a cart assembly is chilled by a closed bath recirculating chiller, which has sufficient capacity to cool two condenser plates simultaneously. The condenser plate on side-mounted condenser systems is chilled by a house recirculating chilling loop.

The condenser system protects against filter blockage by condensing out moisture prior to exhaust gases reaching the vent filters. BPCs are not intended to operate under pressure, and fouled (blocked) exhaust filters lead to bag pressurization. While vent filter heaters may prevent condensate buildup in many instances, in larger bioreactors (such as the 2,000 L S.U.B.) this becomes less effective. Condensing out the moisture first is a more reliable method for preventing liquid from reaching the filters.

The S.U.B. condenser system with cart assembly Figure 1.7) consists of the following components:

- **Cart and brackets** provide convenient means of organizing and transporting key working elements of the condenser system.
- **Chill plates** secure disposable double chamber condenser bags to cool exhaust gases. Up to two plates can be used per system.
- **Peristaltic pump**, for returning condensate to the bioreactor.
- **Temperature control unit (TCU, also referred to as a chiller)**, which circulates water to cool the condenser plate.
- **Condenser disposables** include the BPC (double-chambered bag), tubing, and exhaust filters though which the exhaust gases flow and are chilled, and in which the condensate collects and is returned to the bioreactor.

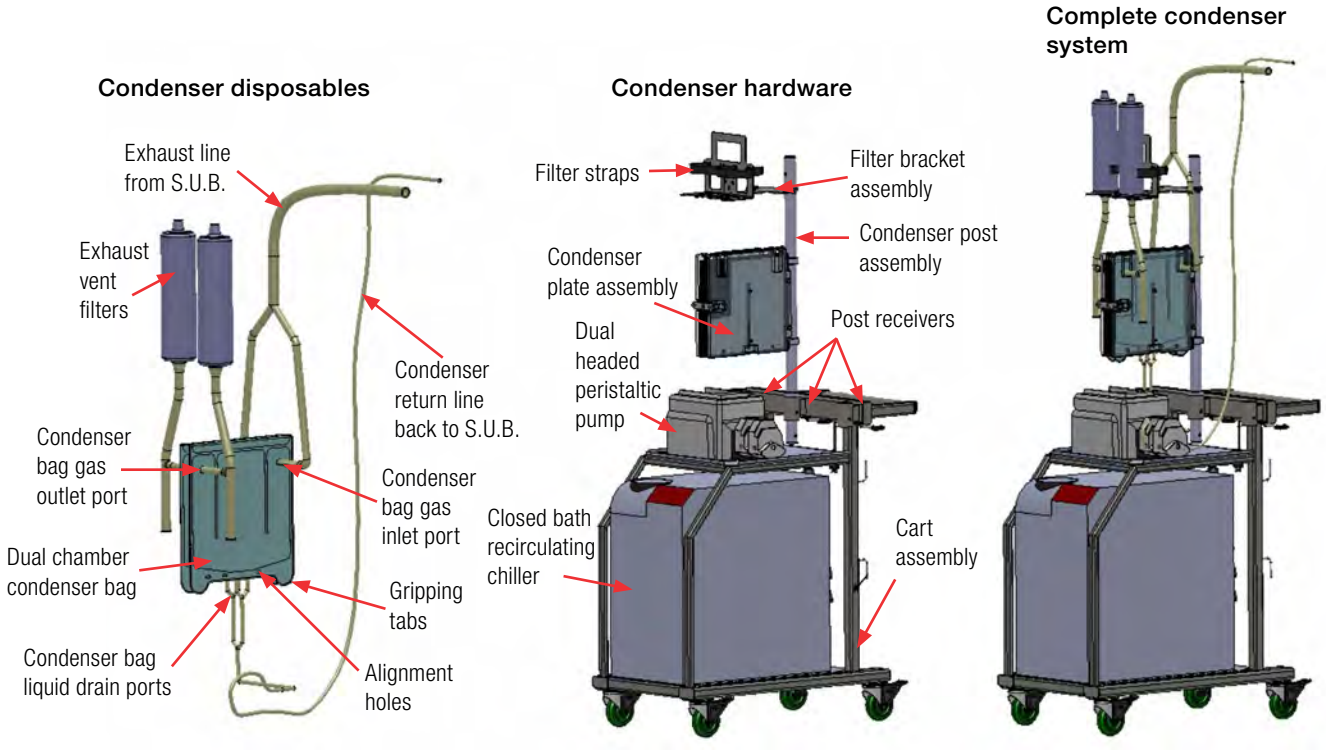


Figure 1.7. Overview of condenser system cart assembly option for 2,000 L S.U.B.s.

Side-mounted condenser systems (Figure 1.8) are only available for 2,000 L S.U.B.s, and attach directly to the outer support container.

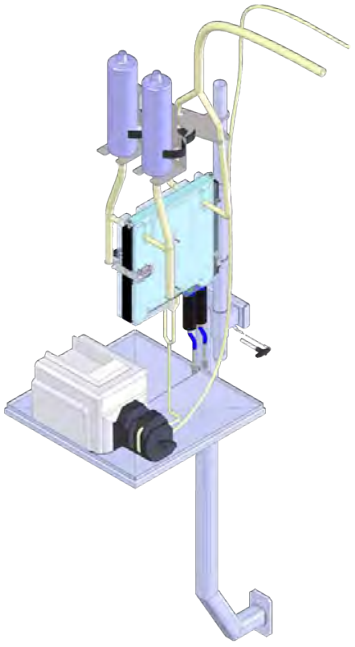


Figure 1.8. Side-mounted condenser system option for 2,000 L S.U.B.s.

Load cells

Load cells, which are used to determine the weight of the contents of a S.U.B., are installed on standard 1,000 and 2,000 L S.U.B. systems, and are available as an option for 50–500 L units. Load cell retro-fit kits can also be added to existing S.U.B. units by a certified service technician. **Note:** Load cells arrive uncalibrated. The load cell manufacturer or a qualified technician should calibrate these systems onsite. The load cell kit comes with three load cells, summing block, wiring, and a display screen with a choice of several data interfaces (Figure 1.9).

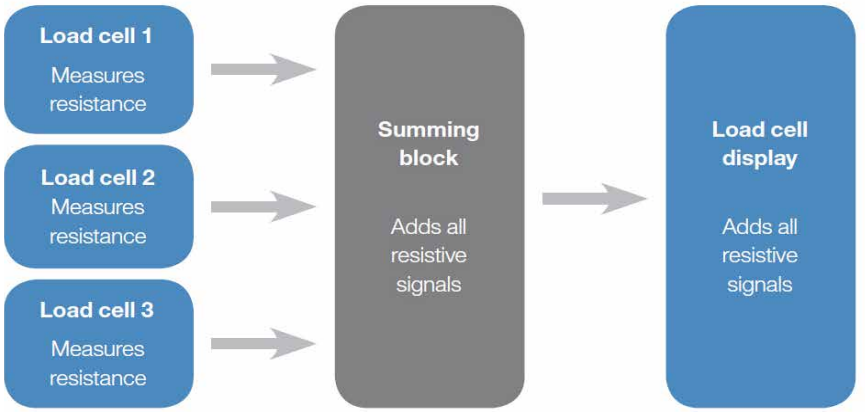


Figure 1.9. Load cell system overview.

Load cells are typically radial-mounted in sets of three. The mounting location (Figure 1.10) varies slightly for each size in order to allow easy access to the bottom drain or sparging mechanisms and tubing.

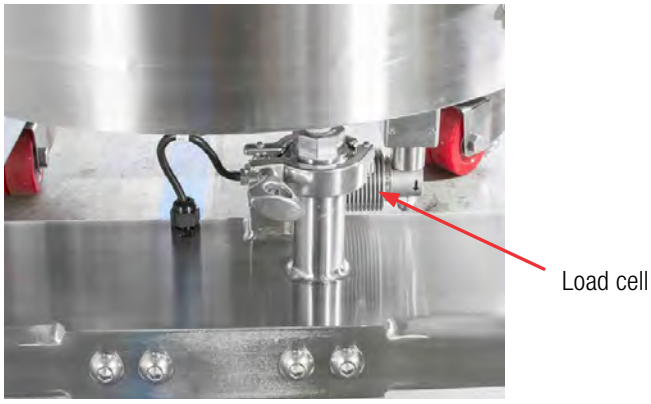


Figure 1.10. Load cell location.

Probe integration

The autoclave tray (Figure 1.11) holds the electrochemical probes and bellows in place during the autoclave sterilization process. Design elements include the following.

- Fabricated from stainless steel
- Features a plastic handle for easy transport right out of the autoclave
- Positions probes on 15% incline for greater probe/membrane longevity
- Will restrain probe bellows from collapsing during sterilization
- Accommodates two probes

Note: Figure 1.11 shows the autoclave tray used for probes with Pall™ Kleenpak™ aseptic connectors. Your system may use CPC™ AseptiQuik™ aseptic connectors instead. Consult your sales representative for more information on AseptiQuik connectors.

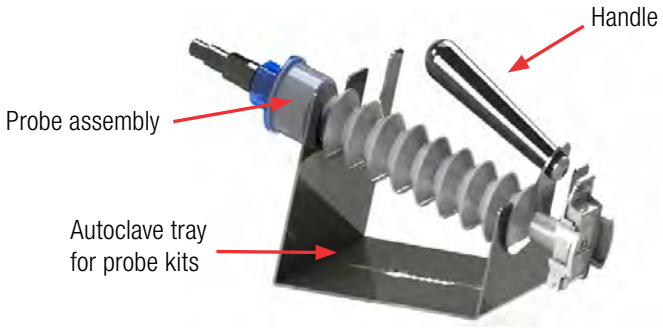


Figure 1.11. Autoclave tray and probe assembly.

The probe assembly (Figure 1.12) is an innovative design to package user-supplied pH and DO probes for sterilization, and to aseptically connect them to the BPC. The probe assembly includes a Kleenpak aseptic connector, molded bellows cover, and threaded probe adapter.

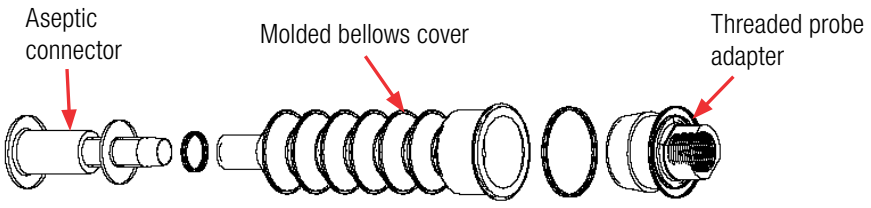


Figure 1.12. Probe assembly.

Cable management systems

The cable management system (Figure 1.13) is available as on option on 50, 100, 250, 500, and 1,000 L units. It is used to organize various lines and includes the following components.

- Internal channel for sparge lines
- External channels for feed and base addition lines
- Harvest line hook

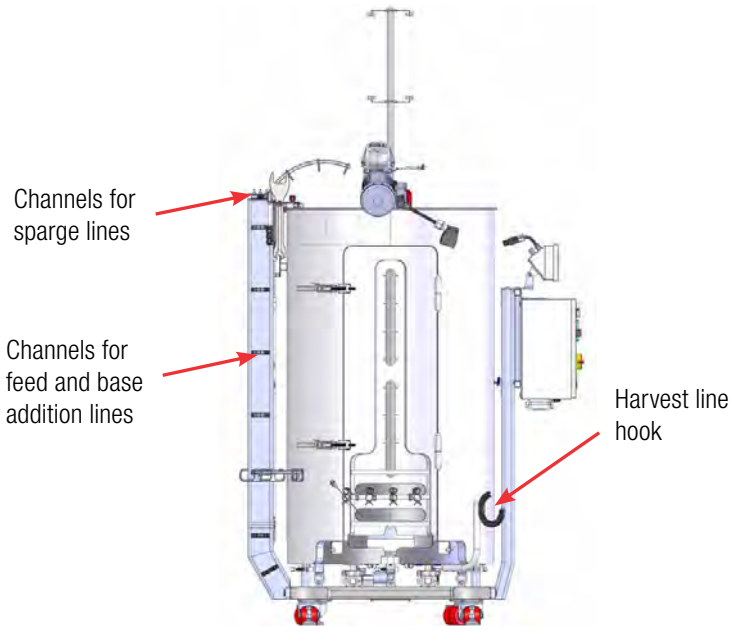


Figure 1.13. 500 L S.U.B. with cable management system.

Miscellaneous items

The miscellaneous items listed below are ancillary components that support the operation of the HyPerforma S.U.B. for cell culture production, and enhance the overall performance of the complete system.

- **Sampling manifold with luer lock**
- **S.U.B. temperature/sample port**—For resistance temperature detectors (RTD) calibration/validation
- **Sparge line support**—Keeps the drilled hole sparge line in a vertical position for optimal gas flow (Figure 1.14). For more information see section 2.2, Installation and setup.

Figure 1.14. Sparge line support.

- **Heavy-duty tubing clamps (typically four or five)**—Tubing clamps (Figure 1.15) are required for pinching off line sets that are not in use, in order to prevent process fluids from moving into the line sets. Prior to sterile probe insertion, tubing clamps must be in place to close off probe ports. For more information, see the BPC and drive shaft loading instructions in sections 3.2, 3.3, and 3.4.

Figure 1.15. Heavy-duty tubing clamps.

Note: The sparge line support is included with all standard S.U.B. units. Other items are sold separately. Please contact your sales representative for more information.

1.3 End user and third-party supplied components

1.3.1 pH and DO probes

Table 1.1 shows the length and diameter requirements for traditional sensors (probes) that can be integrated into the S.U.B. These requirements are based on the necessary insertion depth of the probe when used with the probe ports. **Note:** The presence of a properly positioned O-ring on the probe is critical for use with the S.U.B.

Table 1.1. Manufacturers and models of compatible pH/DO probes.

Probe lengths (from O-ring to tip) must not exceed 235 mm				O-ring to probe tip	
Probe manufacturer and type	Part number	Diameter	Thread type	Print/lit. length	Actual length
AppliSens DO	Z010023525	12 mm (0.47 in.)	13.5 PG	235 mm (9.25 in.)	235 mm (9.25 in.)
AppliSens pH	Z001023551	12 mm (0.47 in.)	13.5 PG	235 mm (9.25 in.)	235 mm (9.25 in.)
Mettler Toledo DO	InPRO 6800/12/220, PN 52200966	12 mm (0.47 in.)	13.5 PG	215 mm (8.46 in.)	215 mm (8.46 in.)
Mettler Toledo pH	405-DPAS-SC-K8S/225, PN 104054481IG	12 mm (0.47 in.)	13.5 PG	195 mm (7.67 in.)	219 mm (8.62 in.)
Broadley-James DO	D140-B220-PT-D9	12 mm (0.47 in.)	13.5 PG	215 mm (8.46 in.)	214 mm (8.42 in.)
Broadley-James pH	F-635-B225-DH	12 mm (0.47 in.)	13.5 PG	225 mm (8.85 in.)	219 mm (8.62 in.)
Hamilton DO	237542	12 mm (0.47 in.)	13.5 PG	225 mm (8.85 in.)	220 mm (8.66 in.)
Hamilton pH	238633-2543	12 mm (0.47 in.)	13.5 PG	225 mm (8.85 in.)	220 mm (8.66 in.)

Note: Consult the probe manufacturer's website for appropriate probe cable connection and part number.

1.3.2 Controllers

Thermo Scientific products are designed with an open-architecture approach to the integration of controls. Our industry-leading S.U.B. has been integrated with most controllers on the market, allowing customers to choose the control system they want, or to reduce expense by integrating with a controller that is already onsite. In order to facilitate integration, electrical schematics are provided in the Equipment Turnover Package (ETP) supplied with the HyPerforma S.U.B. Companies that offer control solutions in either cGMP or non-cGMP format for Thermo Scientific S.U.B. units are listed below.

- ABEC
- Bellco
- Broadley-James
- Dasgip
- Emerson
- Honeywell
- New Brunswick Scientific
- Pendotech
- Sartorius Stedim Biotech

The HyPerforma 2:1 S.U.B is also available as a complete turnkey system through Thermo Fisher Scientific. These S.U.B. units may be provided with integrated controls, pump towers, a control monitor, and advanced features such as data logging, multiple S.U.B. connections and optional 21CFR part 11 compliance for cGMP manufacturing. A variety of single-use sensors are available for pH, DO and pressure control. Thermo Fisher Scientific can provide complete, integrated solutions using the manufacturers listed below.

- Allen Bradley
- Applikon PLC eZ-controller
- Emerson Delta V
- Finesse PC controller
- Siemens

Contact your local sales representative for more information.

Note: The S.U.B. will work well with any of the various control system platforms, such as PLC, PC, DCS, or proprietary operating system based controllers.

1.4 BPC features

The cell culture itself is contained inside the BPC (Figures 1.16–1.18). The chamber is manufactured from film, which is a co-extruded structure specifically designed for biopharmaceutical process usage. All materials are qualified for a broad range of physical, mechanical, biological, and chemical compatibility requirements. Refer to data in our BPC Catalog and film validation guides; contact your sales representative for a copy. The bioreactor BPC is supplied gamma irradiated.

Operating pressure

The S.U.B. BPC does not operate as a closed system, as it has both inlet and exhaust filters that are utilized to maintain an environment for cells to grow without concern for contamination. However, conditions can be encountered when gas inlet flow rate may exceed exhaust flow rate. This may be encountered in the unlikely event of a pressure regulator failure on a gas feed, or when excessive foaming creates conditions of vent blockage. The S.U.B. BPC is not rated as a pressure vessel [gas pressure should not exceed 0.03 bar (0.5 psi) within the BPC]. Custom BPCs can be ordered with an optional single-use pressure transducer for monitoring the pressure within the S.U.B. (supplied standard with 1,000 and 2,000 L systems).

Exhaust vent filter

The exhaust vent filter used on 50–1,000 L S.U.B.s is a Pall KA3 series filter utilizing hydrophobic PVDF membranes. To maintain a sterile connection, the standard BPC is supplied with the filter arrow pointing toward the BPC. This ensures that the filter vents are outside of the sterile connection. For users with more demanding applications, an optional vent filter heater can be used.

The exhaust vent filters used on 2,000 L S.U.B.s are Meissner™ UltraCap™ series filters utilizing hydrophobic PVDF membranes. These filters are provided in normal orientation with the flow arrow on the filter housing pointing away from the BPC. The normal orientation provides maximum filter capacity. No side vents are provided. Condensate must be managed by use of the condenser system or vent filter heater.

Draining and harvest

The S.U.B. is equipped with a bottom drain line that allows for liquid harvest by means of peristaltic pump. Connection of the bottom drain line can be accomplished by using a tubing welder, the quick connect, or fitting provided. Manipulation of the BPC as the last few liters of media are removed can minimize liquid hold-up in the S.U.B.

Sparging

Gas to liquid mass transfer in cell culture bioreactors is controlled by the solubility of the gas in the liquid, its distribution, and the temperature and pressure. Direct air sparging is a method of providing for the oxygen requirements of eukaryotic cell cultures. The standard S.U.B. BPC incorporates a unique single-use dual sparging design that allows for optimal aeration of the culture process and effective carbon dioxide stripping.

Connections

Multiple aseptic connection options exist for S.U.B. users. Standard BPCs include tubing welder sections, quick connects, and Pall Kleenpak connections. **Note:** CPC AseptiQuik connectors are also available. The BPC is designed with various lengths and dimensions of thermoplastic tubing for the purpose of adding to and dispensing from the BPC.

Sampling port

The S.U.B. is equipped with a small volume sample port that is adjacent to the BPC thermowell. This small-diameter silicone dip tube of 152.4 mm length (6 in.) allows low void volume samples to be taken for cell viability and density, as well as analyte analysis. This dip tube is supplied with a luer lock connector (SmartSite™) that allows for direct sampling or attachment of various sampling manifolds by use of standard luer lock connection. Alternatively, manifolds can be welded onto the C-Flex™ sample line using a tubing welder.

Figures 1.16–1.18 on the following page, show the features of all sizes of 2:1 S.U.B. BPCs. For more information about the components labeled in the figures, see Table 1.2.

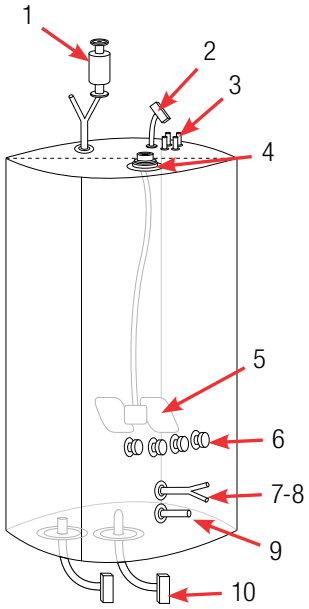


Figure 1.16. Standard BPC for 50, 100, and 250 L systems.

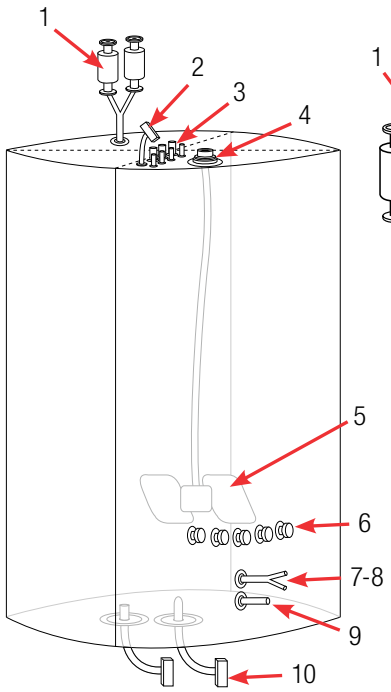


Figure 1.17. Standard BPC for 500 and 1,000 L systems.

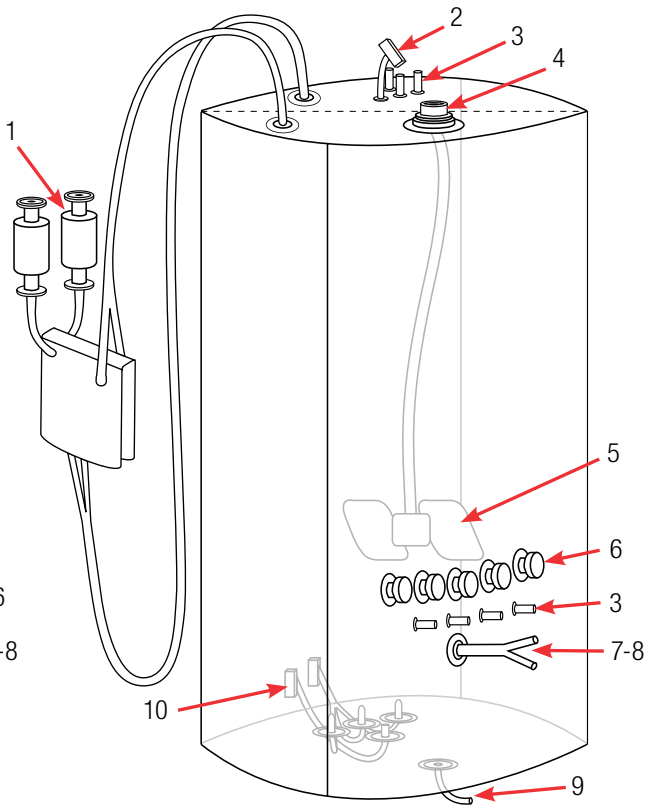


Figure 1.18. Standard BPC for 2,000 L systems.

Table 1.2. BPC information for Figures 1.16–1.18.

Item	Component	Description
1	Exhaust vent filter	Single-use capsule filter for exhaust gas exchange
2	Gas overlay port	Protected by gas filter
3	Ports	For addition of media and other liquids
4	Seal/bearing assembly	Links with motor mixer and allows impeller to turn while retaining integrity of the BPC
5	Impeller	Injection-molded plastic, linked to seal/bearing assembly by C-Flex tubing contact material of the shaft
6	Ports with Kleenpak connectors	For integration of standard 12 mm (0.47 in.) monitoring pH and DO probes
7	Temperature RTD port	For integration of temperature probe while retaining integrity of the BPC
8	Sampling port	For needleless sampling or connection to sampling manifold
9	Drain port	For draining the S.U.B.
10	Gas sparge lines	Sparger integrated into the chamber and protected by gas filters; dual micro sparger (porous frit) and macro sparger (with open pipe or drilled hole) options are available
11	Condenser system bag	Integrates with chiller plate to remove condensate from exhaust; supports effective use of 2,000 L systems (can also be used with smaller sizes) S.U.B.s that require custom gassing strategies and demand higher exhaust rates and longer durations

2

Hardware assembly and setup

Chapter contents

2.1 Initial installation preparation

2.1.1 Hardware shipment and setup

The Single-Use Bioreactor (S.U.B.) hardware will arrive crated. For unpacking instructions and detailed contents of the crate, please refer to the unpacking and assembly instructions, as well as the packaging drawings, which are included in the shipping crate. Be sure to follow the unpacking instructions provided and retain all packaging materials.

2.1.2 Hardware uncrating

The S.U.B. hardware will arrive with the following items:

- Outer support container [platform, tank, and electrical control panel (E-Box)]
- Drive shaft, resistance temperature detector (RTD), four probe brackets, and standard tool set (spanner wrench and torque wrench)
- Equipment Turnover Package (ETP), provided on a USB drive (shipped separately)

After uncrating, contact your sales representative immediately if any damage has occurred.

2.1.3 Site preparation

Electrical connections for units with AC motors and E-Boxes

S.U.B. hardware using AC motors cannot be used on circuits equipped with Ground Fault Circuit Interrupter (GFCI) circuit protection because of the potential for nuisance tripping. The electrical plug on the S.U.B. is a connector that offers a secure ground. These connectors meet the electrical safety codes for portable equipment and are International Electrical Code (IEC) rated (meet IEC standard 60309). This plug provides electrical ground prior to power connection. The supplied electrical receptacle should be hardwired into the facility by a qualified electrical technician; for U.S. installations, the receptacle will require the use of an adapter mounting plate (supplied), which will fit into a two-gang box. For additional information on the adapter mounting plate, please see the ETP. Alternatively, the system can be hardwired directly into the facility. **Note:** The yellow plug and receptacle are for 120 VAC and the blue are for 240 VAC S.U.B.s.

Electrical connections for systems with DC motors

S.U.B. units using DC motors are not supplied with E-Boxes. When using a DC motor, electrical connections must be supplied by a third-party integrator.

Outer support container preparation

Each outer support container is shipped directly from the manufacturer and arrives with various safety mechanisms in place. Follow the guidelines below to set up the S.U.B. upon arrival.

WARNING: Any procedure that requires the E-Box to be opened should be performed with the main electrical disconnect in the locked out position, and all power sources removed from the E-Box. For operator safety, secure the location of the S.U.B. outer support container by disabling the swivel casters before servicing.

Electrical preparation for 50–2,000 L systems with AC motors and E-Boxes

1. Using a flat-head screwdriver, open the E-Box and locate the breakers for the pressure sensor, continuous power outlets non E-stoppable (2), and continuous power outlets E-stoppable (2) (Figure 2.1). These breakers should be in the "on" position during operation, which will be in the "up" position or pressed in, depending on the breaker type. For electrical schematics, refer to the ETP, which is provided on a USB drive.

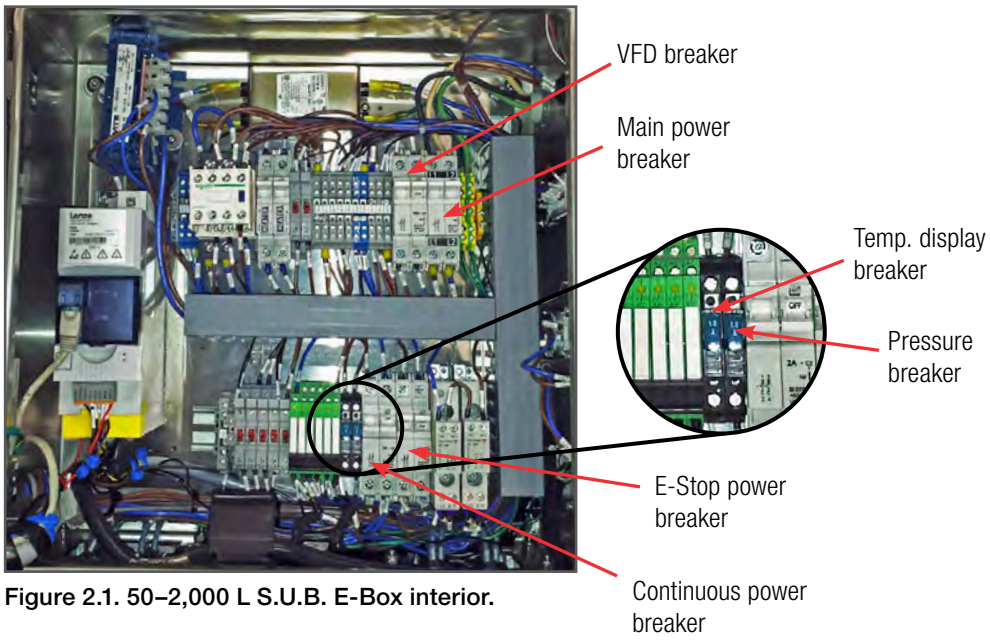


Figure 2.1. 50–2,000 L S.U.B. E-Box interior.

2. Verify that the three-way motor controller switch is in the middle position. For reference:

   - The middle position enables the speed control keypad
   - The top position is for 0–10 V controllers
   - The bottom position is for 4–20 mA controllers

   Verify that the position of the two-way temperature control switch is in the up position. This will enable the PID temperature controller.

3. Close the E-Box. Use a screwdriver to lock the E-Box before continuing.

4. For S.U.B. hardware units purchased with factory-installed load cells, the load cells are shipped in the locked position (threaded up) for equipment protection. Refer to the load cell preparation instructions later in this section for more information.

2.2 Installation and setup

2.2.1 Preparing load cells

All manual movements of mobile S.U.B. hardware should be over smooth surfaces, with the S.U.B. empty and disconnected from all power and gas/feed sources. All load cells must be fully locked down in order to move the S.U.B.

Use the following steps below to prepare load cells for use. Figure 2.2 illustrates the location and components of load cells, which will be referenced throughout the load cell preparation process.

1. For S.U.B. hardware units purchased with factory-installed load cells, the load cells are shipped in the locked position (threaded up) for equipment protection.

2. To unlock the load cells, remove and discard the delrin slip ring (if present). Remove the tri-clamp. Use the small end of the supplied tool (Figure 2.3) to loosen the lockout nut until the nut is tight against the base or leg of the S.U.B. Repeat this process for each load cell until all of the lockout nuts are disengaged from the lockout posts. Do not reinstall the tri-clamp.

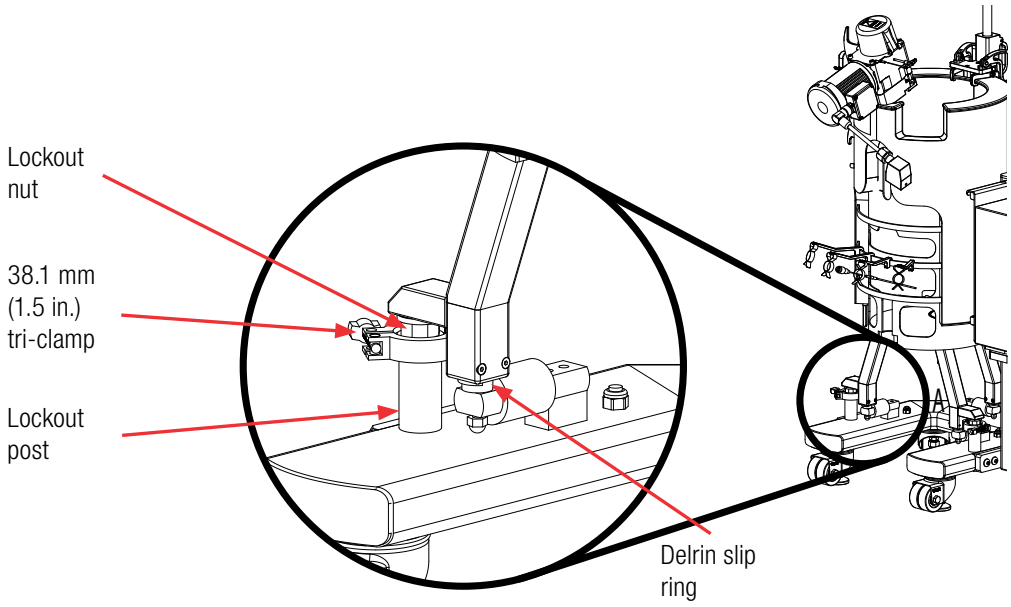


Figure 2.2. Close-up view of load cells.

Figure 2.3. Supplied wrench.

3. At this point, the S.U.B. hardware is ready to be prepared for a cell culture run.

4. For systems with load cell display screens, refer to Appendix B for information about calibrating load cells.

CAUTION: Do not move the unit (especially when filled) while load cells are unlocked, as this can damage the load cells.

5. To lock load cells that have been unlocked, hand-tighten the lockout nut onto the post. Use the supplied tool to turn the nut an extra 1/4 turn.

CAUTION: To avoid damaging the load cells, do not over-tighten the nut. Assemble a standard stainless 38.1 mm (1.5 in.) tri-clamp around the flanges. Complete this process for all load cells.

2.2.2 Leveling and connecting the system

All manual movements of mobile S.U.B. hardware should be made over smooth surfaces, with the S.U.B. empty and disconnected from all power and gas/feed sources. All load cells must be fully locked down in order to move a S.U.B. Refer to the previous subsection of this guide for illustrations.

1. Verify that the facility electrical supplies are sufficient to support the power requirements of the S.U.B. and ancillary components, such as controllers or pumps.

2. Locate the outer support container in the area for the cell culture run.

3. When monitoring the batch volume, the unit may be placed on a weight scale if load cells are not part of the system. Other methods may be used to measure all incoming and outgoing liquids.

4. Level the platform by disabling the swivel casters on the bottom of the outer support container. This is accomplished by threading the leveling feet (at the center of each caster) to the floor.

5. Verify the location of the pH/DO controllers and ensure that the cable and tubing lengths are sufficient.

WARNING: Risk of electrical shock.

6. Verify that the main power is off and the emergency stop is pulled out. **Note:** The emergency stop disconnects all power to the system. An alarm buzzer will sound when the emergency stop is activated.

7. Verify that the main motor power switch is in the "off" position.

8. Connect all electrical plugs to facility power. **Note:** 120 VAC–250 L S.U.B. should be connected to a dedicated 20 A circuit. Refer to hardware/electrical labels and schematics to ensure proper electrical voltage is connected to the S.U.B. The main power switch can now be turned on.

9. For resistive 50, 100, and 250 L systems, verify that the temperature controller is off. The display should be flashing in the stand-by position.

10. For 1,000 L units only, the water jacket ports are removed for shipping. Attach the ports to the S.U.B. using the tri-clamps provided (Figure 2.4).

Figure 2.4. Attaching water jacket port using tri-clamp.

11. Connect water inlet and outlet lines from the temperature control unit quick connects to the jacket (Figure 2.5). For 50, 100, 250, 500, and 2,000 L units, the inlet is typically on the left side if you are facing the connectors. For the 1,000 L S.U.B. unit, the inlet is the lower connection, and the outlet is the upper.

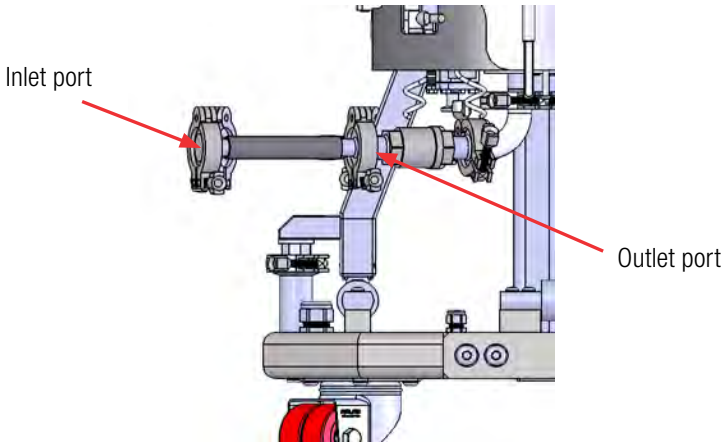


Figure 2.5. Inlet and outlet ports.

12. Insert the sparge line support (Figure 2.6) into the bottom of the S.U.B. unit, directly below where the sparger will be placed. This component holds the sparge line vertically for maximum effectiveness. The sparge line can be wound through the coil of the holder to keep the sparger properly oriented.

Figure 2.6. Sparge line support.

2.2.3 Verifying drive shaft segments for 2,000 L systems

The 2,000 L S.U.B. is supplied with a special drive shaft that differs in appearance and material when compared to the metallic shafts used in smaller S.U.B. sizes. Due to the higher mechanical stress generated in 2,000 L S.U.B.s, these systems require:

- Drive shafts made of carbon fiber composites to reduce the weight of the long shaft
- Special quick connect designs to reduce joint fatigue in multiple-segment drive shafts

Note: 2,000 L systems include two-piece drive shafts. Before starting to load a drive shaft, verify that the drive shaft serial numbers match on both shaft segments.

Always maintain a log history of the drive shaft and confirm that it has sufficient life remaining. **For warranty purposes, users must show documentation of proper drive shaft use.** A sample log for documenting drive shaft use is provided in Appendix D of this publication. If the age or history of a drive shaft is questionable, it should be discarded.

3

Operating information

Chapter contents

3.1 General system operating information

3.1.1 BPC preparation

Each outer support container is designed for a specific BioProcess Container (BPC). Confirm that the correct volume and type of BPC is being used for the corresponding volume outer support container. Sections 3.2, 3.3, and 3.4 cover the installation and setup of BPCs. Follow these instructions in the order in which they are presented.

3.1.2 BPC handling instructions

If you are using a sharp object when opening outer polybags, take care to avoid damaging the BPC. Do not drag containers over corners or sharp objects. Do not lift the container by the corners or top seams. Carefully coil the tubing on top of the BPC to prevent puncturing the container with cable ties or clamps. Use cushioning between the tubing and the container in storage and transport.

3.1.3 BPC operating information

Working volume

Each Single-Use Bioreactor (S.U.B.) is designed for a specific working volume range. The minimum working volume and the rated working volume are listed in the specification tables provided in Chapter 4 of this user's guide. The total volume listed includes the headspace needed for proper aeration and gas management. Actual working volumes should not exceed the indicated rated working volumes by more than 10%.

CAUTION: Operating 2:1 S.U.B.s at working volumes less than 50% of the rated volume without consultation from Thermo Fisher Scientific engineers can result in damage to the BPC and/or the S.U.B. hardware.

Operating pressure

The BPC does not operate as a closed system; it has both inlet and exhaust filters that are utilized to maintain a sterile environment for cell growth. However, conditions can be encountered when the gas inlet flow rate may exceed the exhaust flow rate. This may be encountered in the unlikely event of a pressure regulator failure on a gas feed, or when excessive foam within the bioreactor creates a vent blockage.

WARNING: The BPC is not rated as a pressure vessel. Gas pressure within the BPC headspace should not exceed 0.03 bar (0.5 psi) at any time. Pressure above 0.03 bar (0.5 psi) may result in BPC damage or personal injury.

- More demanding applications may warrant an optional exhaust vent heater.
- If foaming is excessive in your cell culture process, it is best to reduce the operating volume of the process to 80% of maximum rated working volume of the S.U.B. system being used to provide greater headspace volume.
- Single-use pressure transducers are available on custom S.U.B. configurations. This technology combined with high-level control systems (common with industrial applications) can regulate gas pressure within the confines of the S.U.B.

Aeration

Gas to liquid mass transfer in cell culture bioreactors is controlled by the solubility of the gas in the liquid, its distribution, and the temperature and pressure. Direct air sparging provides for the oxygen requirements of eukaryotic cell cultures. It allows optimal aeration of the culture process and effective carbon dioxide stripping.

The standard BPC is designed with special spargers that produce very efficient mass transfer of oxygen and typically will require much less gas inflow than conventional spargers. Gas inflow should only be limited to prevent foam generation and excessive pressure within the BPC. Gas flow rates supplied as overlay should also be reduced as much as possible or eliminated, which will minimize both liquid evaporation and demand on the exhaust filter. Ideally, this will reduce the likelihood that gas inflows will exceed gas outflow of the system and reduce the occurrence of foam in the headspace that may plug the exhaust filter. For more information, refer to the "Operating pressure" section on the previous page, and section 3.6.8 of this guide.

Aseptic connections

The most commonly recommended process for making connections to tubing lines is with an aseptic tubing fuser. Other connection options are available as a custom BPC assembly. By following the recommended tubing welder operating instructions, successful connections can be made for filling, supplementing, sampling, or dispensing from the BPC as needed.

Draining and harvest

The S.U.B. is equipped with a bottom drain line that allows for liquid harvest by means of peristaltic pump. Connection of the bottom drain line can be accomplished by use of a tubing welder or the fitting that is provided. The bottom drain exits the BPC at the lowest vertical position on the side of the S.U.B. This allows for easy access for the user and minimizes the accumulation of cells in the area of the drain during the cell culture run. Manipulation of the BPC as the last few liters of media drain will minimize liquid hold-up within the S.U.B. The 2,000 L S.U.B. is provided with a 25.4 mm (1 in.) bottom drain near the center line of the tank bottom.

3.1.4 Hardware operating information

Heating performance

Heating times for S.U.B. systems vary based on liquid volume and temperature, ambient or heating liquid temperature, sparging rate, and mixing rate. For heating times, see Table 3.1.

WARNING: Do not heat the system if the BPC is not at 50% liquid volume or greater. Batch temperature should not exceed 40°C.

Table 3.1. Approximate heating times for 2:1 S.U.B. systems. Ambient temperature of 25°C.

System	Liquid batch volume (half [min.]–full)	Watts	TCU	TCU watts/L (half–full)	Initial liquid temp.	Liquid target temp.	Time (half–full)
50 L electric	25–50 L	456	N/A	18.2–9.2 W/L	5°C	37°C	N/A–4.3 hr
50 L jacketed	25–50 L	2800	TF2500	112–56 W/L	5°C	37°C	0.8–1.1 hr
100 L electric	50–100 L	865	N/A	17.3–8.7 W/L	5°C	37°C	N/A–4.9 hr
100 L jacketed	50–100 L	2800	TF2500	56–28 W/L	5°C	37°C	1.2–1.9 hr
250 L electric	125–250 L	1358	N/A	10.9–5.4 W/L	5°C	37°C	N/A–7.5 hr
250 L jacketed	125–250 L	2800	TF5000	22.4–11.2 W/L	5°C	37°C	2.1–3.1 hr
500 L jacketed	250–500 L	6100	TF10000	24.4–12.2 W/L	5°C	37°C	1.5–2.6 hr
1,000 L jacketed	500–1,000 L	22500	TF24000	45–22.5 W/L	5°C	37°C	1.3–1.8 hr
2,000 L jacketed	1,000–2,000 L	22500	TF24000	22.5–11.3 W/L	5°C	37°C	2–2.8 hr

Protective earth grounding for units with AC motors

For units with AC motors, protective earth grounding for the S.U.B. hardware system and the controller is provided through the ground terminal of the power plug. Source power to the controller must provide protective earth grounding to this terminal in order to minimize the hazard of a possible shock in the occurrence of a fault condition. Please refer to Appendix A for information about electrical receptacles. A ground wire is provided underneath the S.U.B. and must be tied to the controller before operation.

Agitation control interface for units with AC motors and electrical box enclosures

The agitation control interface utilizes an LED digital display to indicate stirring speed in units of revolutions per minute (rpm). Power is supplied to the motor by a two-position power switch that is illuminated in green when turned to the on position (right position). The agitation should not be operated at volumes less than 50%. Stirring speed is adjusted using the up and down arrows on the agitation keypad interface on the control panel, or using the settings on an integrated third-party controller. **Note:** Due to the auto-restart capabilities of the S.U.B., the green start button on the keypad has been disabled; however, the red stop button on the keypad is active.

If the red stop button has been used to stop the motor, the controller can be reset and agitation restarted by using the main motor toggle switch on the left side of the control panel. For more information, see the illustrations in the control panel detail in section 4.3.

Circuit protection for units with AC motors

Electrical components of the S.U.B. are equipped with circuit protection. The variable frequency drive used to power the mixer motor is protected by the use of a 10 A double pull resettable breaker with a type C time delay (5–10 x LN). Other components, such as the temperature controller and heating element, are protected with resettable breakers.

In the case of an electrical fault condition, these safety devices are designed to protect the user from electrical shock and prevent electrical system components from being damaged. Fuses can be replaced and/or the breakers reset once the fault condition is resolved.

Electrical breaker notes:

- The normal "on" setting for these breakers is in the up position.
- A tripped breaker will be in the mid position.
- The "off" setting is in the fully down position.
- To reset a tripped breaker, it must first be moved from the mid position to the "off" setting (fully down position) before moving it to the "on" setting (fully up position).

Scales and weighing systems

Monitoring liquid volume within the S.U.B. during operation can be critical in cell culture applications that involve nutrient media feeds. This can also be a useful method for increasing the scalability of the S.U.B., by starting the process run at minimum operating volume. The ability to track operating volume by use of load cells or weigh scales allows the user the ability to control liquid volume and cell density as the bioreactor is increased to rated working volume during the process run.

A load cell kit for weight/volume measurement is available for all S.U.B. units, which can be installed at the factory or can be added later by a certified service technician. The load cell kit comes with three load cells, summing block, wiring, and display with a choice of several interfaces.

Refer to Appendix B for load cell display calibration instructions.
Ensure that load cells are locked down before any movement of the S.U.B. unit.

To lock the load cells before transporting any size S.U.B., follow the steps below and refer to Figures 2.2 and 2.3 in section 2.2.1.

1. Hand-tighten the load cell lockout nut onto the load cell lockout post. You may need to use the small end of the supplied wrench to loosen the load cell lockout nut from the bottom of the base.

2. After the nut is hand-tightened against the post, use the small end of the supplied wrench to turn it an extra 1/4 turn.

CAUTION: To avoid damaging the load cell, do not overtighten the nut.

3. Assemble a standard stainless 28.6 mm (1.5 in.) tri-clamp around the flanges.

4. Repeat steps 1 through 3 for all load cells on the S.U.B.

3.1.5 External data logging and control

Digital display weighing scales can be sourced from manufacturers such as Mettler Toledo. Bench top scales are commonly used to measure the amount of bulk source media stored in a smaller-volume BPC as it is transferred by peristaltic pump into the S.U.B.

Floor scales can be used to measure the fluid content within the S.U.B. This is accomplished by rolling the S.U.B. onto the scale platform and leveling the S.U.B. skid once in position.

The S.U.B. hardware systems are designed to allow advanced users to control all aspects of the operation of the bioreactor. Contact technical support for Thermo Scientific HyPerforma products general integration guidance.

3.2 BPC and drive shaft loading instructions for 50, 100, and 250 L systems

3.2.1 Initial BPC loading steps for 50, 100, and 250 L systems

Each outer support container is designed for a specific BPC. Verify that the correct volume and type of BPC is being used for the corresponding volume outer support container. Use the following steps to install and set up the BPC.

1. Remove the irradiated BPC from the protective double polybags (Figure 3.1). Remove the cable ties from the drain line.

2. Load the BPC from the top into the outer support container, avoiding any sharp edges that may damage the BPC (Figure 3.2).

3. Orient the BPC with the bearing port up and toward the motor drive with the aseptic connector probe ports facing the bottom access cutout.

4. Place the bearing port into the bearing port receiver (Figure 3.3), close the door, and close the clamp.

Figure 3.1. BPC removed from protective polybags.

Figure 3.2. BPC loading.

Figure 3.3. Bearing port insertion.

5. Use the back access window to route the side and bottom ports through the opening in the outer support container (Figure 3.4).

6. Route the sparge lines, bottom drain, and sampling lines through the appropriate openings (Figures 3.5 and 3.6).

7. Route the sparge lines through the bottom plate and loop them around the sparge line holder (Figure 3.5).

Figure 3.4. Bottom line access.

Figure 3.5. Sparge line setup.

Figure 3.6. Drain line and port setup.

8. If a cable management system is available (see system shown in Figure 3.7), attach the lines to the appropriate inlet ports (Figure 3.8).

Figure 3.7. Cable management system in use.

Figure 3.8. Incoming line connection to inlet ports.

9. Connect the incoming gas feed lines to both the overlay filter and the sparger filter. Ensure that the filters are located above the maximum liquid level (Figure 3.9).

Figure 3.9. Inserting lines into the cable management system channels.

10. Inflate the BPC with air through the overlay filter, but do not exceed 25 standard liters per minute (slpm) or 0.03 bar (0.5 psi) internal BPC pressure. Inflation time is approximately 10–20 minutes. Time will vary based on flow rate, inlet pressure, and container volume. As the BPC inflates, ensure that the ports, drain, and sparge lines are properly oriented in the support container.

WARNING: The BPC is not rated as a pressure vessel. The BPC should not be allowed to become tight during inflation or operation. DO NOT EXCEED 0.03 bar (0.5 psi) within the BPC or it could fail. For reference, the BPC will appear to be tight at 0.007 bar (0.1 psi). See Tables 3.7 and 3.8 in section 3.6.8 for recommended air flow rates. The operating pressures at the level of the S.U.B. are of primary importance and these values must be adhered to.

11. As the container fills with air, check to make sure the sparge lines are properly aligned.

    Sparge line note: While a sparge line check valve is provided for each sparge line, it is not uncommon for some fluid to bypass check valves during typical use. Elevating the filter will reduce the chance that the filter is exposed to liquid.

12. Use the four bottom cutouts located at the base of the support container as a reference to align the hanging tab on the BPC (Figure 3.10).

CAUTION: Do not attach cutouts to any of the bottom hanging tabs; only use them for reference in aligning the BPC within the tank. Doing so may potentially stretch and/or tear the film.

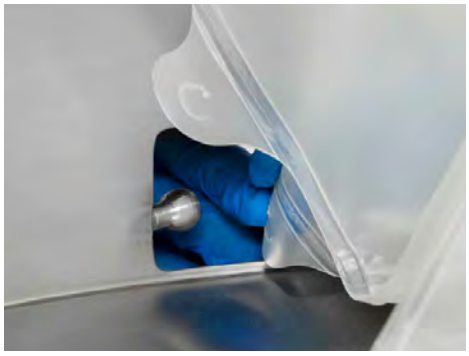


Figure 3.10. Hanging tab and hook.

13. Position the bottom side drain, pulling out and downward to position the port toward the bottom edge of the S.U.B.

14. Align the row of probe ports within the access window (Figure 3.11). **Note:** Verify all port clamps are closed and located as close as possible to the body of the BPC.

Figure 3.11. Aseptic connector alignment.

15. Connect the media ground clip to the stainless steel insert in the sample line on the BPC. This grounds the media inside the BPC and helps eliminate electrostatic charge (Figure 3.12).

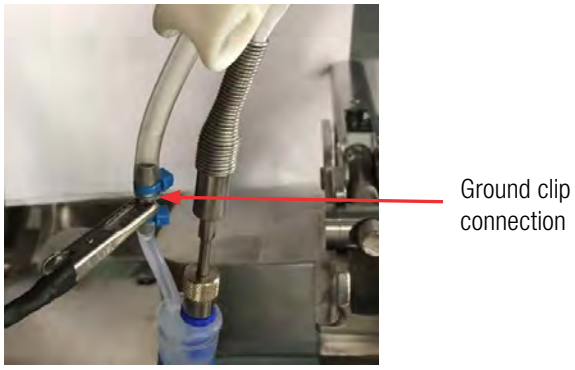


Figure 3.12. Media ground clip connection.

3.2.2 Drive shaft insertion for 50, 100, and 250 L systems

WARNING: Before you insert the drive shaft, the BPC must be adequately inflated so that it is sitting upright in the outer support container.

Figure 3.13 illustrates the components of the motor and mixing assembly. The parts labeled on the figure will be referenced throughout the drive shaft insertion process. Use the steps on the following pages to insert the drive shaft.

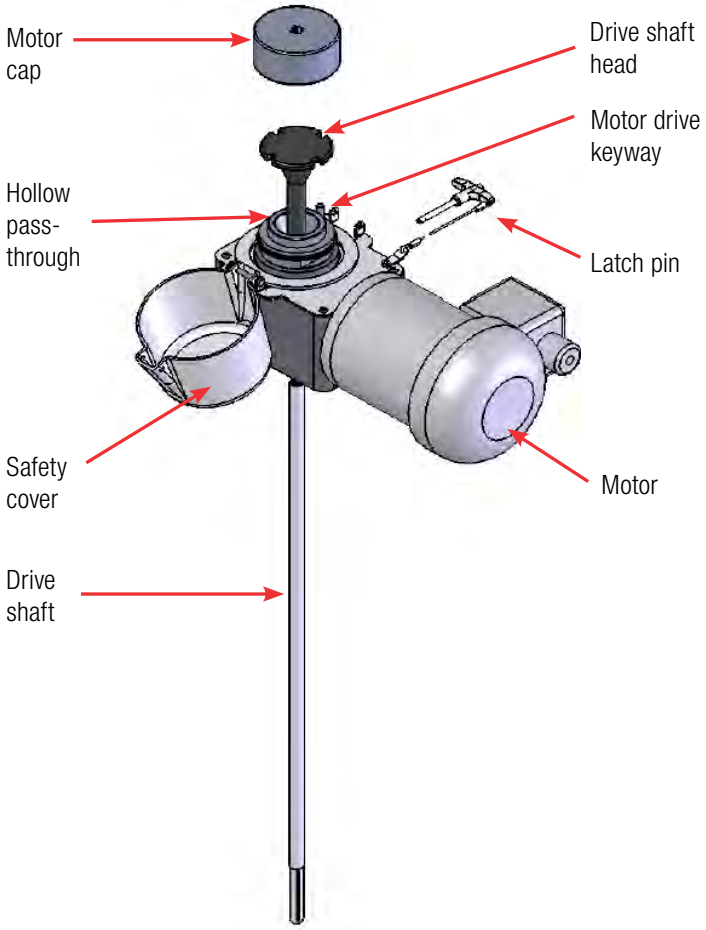


Figure 3.13. Motor and mixing assembly.

1. Remove the latch pin from the safety cover over the mixing assembly and open the cover. Unscrew the motor cap covering the hollow pass-through of the motor (Figure 3.14).

Figure 3.14. Removing motor cap.

2. Insert the drive shaft through the hollow pass-through of the motor assembly in the following manner (Figures 3.15–3.18).
   - Use two hands to load the drive shaft through the top of the motor assembly; a slight back-and-forth twisting motion will aid in insertion and avoid stretching the impeller tubing (Figures 3.15 and 3.16).

Figure 3.15. Loading drive shaft.

Figure 3.16. Twisting drive shaft to aid insertion.

- When approximately 50.8–76.2 mm (2–3 in.) of the shaft remains, twist back and forth slightly to engage the impeller (Figure 3.17).
- When approximately 25.4–50.8 mm (1–2 in.) of the shaft remains, twist back and forth slightly to engage the bearing assembly.
- When approximately 6.4 mm (0.25 in.) of the shaft remains, twist to align the motor drive keyway with one of the four outer slots on the drive shaft head (Figure 3.18).

Figure 3.17. Engaging impeller.

Figure 3.18. Drive shaft head aligned.

3. Directly couple the drive shaft to the motor drive (Figures 3.19–3.21).
   - Place the motor cap on the hollow pass-through and hand-tighten clockwise (Figure 3.19).

Figure 3.19. Replacing motor cap.

- Tighten the motor cap by placing a spanner wrench on the hollow pass-through and tighten the motor cap using the supplied torque wrench (Figure 3.20). **Wrench note:** The torque wrench is a standard 10 mm (3/8 in.) square drive, and is calibrated at the factory at 150 in-lb.

Figure 3.20. Tightening cap.

- Remove the wrenches from the system and place in the storage holders.
- Close the safety access cover and insert the latch pin (Figure 3.21).

Figure 3.21. Replacing and latching cover.

3.2.3 Final installation steps for 50, 100, and 250 L systems

1. The air supply to the overlay can be turned off once the drive shaft has been inserted.

2. Optional: Wrap and secure the vent filter heater on the exhaust filter. Connect the heater to the controller and verify that it is plugged into an appropriate 120 or 240 VAC outlet, then connect the power cord to the controller. **Note:** The controller is preset to 50°C.

3. Secure the exhaust vent filter on its holder (Figure 3.22).
   Note: Some custom BPCs are supplied with dual exhaust vents. The vent bracket can accommodate 10 in. and 4 in. filters in either single or dual configuration.

Figure 3.22. Vent filter installation.

4. Attach the overlay sparge line and any other lines to the cable management system, if available (Figure 3.23). Then, position and close a bar clamp on the bottom drain line as close as possible to the BPC port (Figure 3.24).

Figure 3.23. Optional cable management system on a S.U.B. unit.

Figure 3.24. Bar clamp installation.

5. Remove the plastic insert located in the thermowell, if present.

6. Insert the resistance temperature detector (RTD) or selected temperature sensor into the thermowell (Figures 3.25 and 3.26).
   - Place a small amount of glycerol (0.5 mL) in the well to aid in heat transfer. The glycerol also acts as a lubricant, which helps with probe insertion.
   - The sensor should be inserted until the base of the probe meets the mouth of the thermowell. Rotate the probe either clockwise or counter-clockwise to aid insertion.
   - Secure by twisting the luer lock collar, if provided. The thermowell will stretch slightly when the RTD is seated.

Figure 3.25. Sensor insertion.

Figure 3.26. Securing sensor.

7. Optional: Connect a pressure sensor to the aseptic connector at the top of the BPC. Then connect the appropriate pressure transducer cable to the third-party controller.

8. Refer to section 3.5.3 for probe insertion instructions.

3.3 BPC and drive shaft loading instructions for 500 and 1,000 L systems

3.3.1 Initial BPC loading steps for 500 and 1,000 L systems

Checkpoints prior to BPC loading

- ✓ The correct volume BPC is being used for the corresponding volume outer support container.
- ✓ The outer support container is stationary with the casters locked into place. BPC loading may require operators to step inside the bioreactor, and the unit must be stationary for the safety of both the operator and equipment.
- ✓ Two operators are available for ease in BPC loading.
- ✓ A ladder or other means of elevation is available for drive shaft insertion.

Use the following steps to install and set up the BPC.

1. Open the door on the bioreactor support container and reach inside to open the clamp on the bearing port receiver located below the motor (Figures 3.27 and 3.28).

Figure 3.27. Opening the bioreactor door.

Figure 3.28. Close-up of bearing receiver clamp.

2. Remove the irradiated BPC from the protective double polybags (Figure 3.29). Do not remove the polybags from the line sets at this stage, as the BPC may become difficult to manage. Do not allow the BPC or line sets to touch the floor.

3. Reach into or step inside the outer support container with the front face (bearing port side) of the BPC oriented toward the motor (Figure 3.30).

Figure 3.29. BPC removed from protective polybags.

Figure 3.30. Bearing port orientation.

4. Place the top line sets, still in polybags, over the top edge of the tank (Figure 3.31). This will keep the container from being restricted during the air inflation step.

Figure 3.31. Line sets on edge of tank.

5. Load the container bearing port into the receiver (Figure 3.32). Close the door and clamp it shut (Figure 3.33).

Figure 3.32. Bearing port in receiver.

Figure 3.33. Door clamped shut.

6. Remove the bubble wrap from the sparger filters. Guide the sparge inlet lines and filters through the bottom cutouts in the outer support container (Figure 3.34). The operator can reach just below the S.U.B. to further extend the sparge lines from the cutouts (Figure 3.35).

Figure 3.34. Sparge line insertion.

Figure 3.35. Sparge line extension.

7. Pass the bagged drain line set and temperature/sampling port set through the large cutout in the front of the outer support container (Figure 3.36). Extend the drain line set through the cutout (Figure 3.37).

Figure 3.36. Drain/sampling line set insertion.

Figure 3.37. Drain/sampling line extension.

8. Connect the pressure transducer to the monitor. After the display has stabilized, tare the monitor. **Note:** The monitor should be allowed to warm up for 30 minutes before taring. Verify that the monitor reads zero.

9. The BPC must be partially inflated until it is sitting upright. This allows proper insertion of the drive shaft and aids in the proper alignment of the BPC in the outer support container.
   - Attach the air supply to the overlay gas inlet line. **Note:** Air pressure to the overlay gas line on the S.U.B. BPC should be less than 25 slpm or 0.2 bar (3 psi).
   - Begin air inflation through the overlay gas line. Filling the container with air takes approximately 15–20 minutes before drive shaft insertion can begin. Times will vary based upon flow rate and inlet pressure.
   - Steps 10 through 13 can be completed while the BPC is filling with air.

WARNING: The BPC is not rated as a pressure vessel. DO NOT EXCEED 0.03 bar (0.5 psi) within the BPC or the system could fail, causing personal injury or damage to equipment. DO NOT leave the BPC unattended while inflating. See Tables 3.7 and 3.8 in section 3.6.8 for recommended air flow rates. The operating pressures at the level of the S.U.B. are of primary importance and these values must be adhered to.

10. Attach the incoming gas supply to the sparger gas inlet line. **Note:** Air pressure to the sparger on the BPC should not exceed 0.55 bar (8 psi). While a sparge line check valve is provided for each sparge line, some fluid may bypass check valves during typical use. Elevating the filter to ensure that it is not at the low point of the sparge line will reduce the chance that the filter is exposed to liquid.

11. Tare the load cell display before proceeding.

12. Attach all of the hanging tabs on the BPC to the hooks on the bottom of the outer support container to help position the ports (Figures 3.38 and 3.39).

CAUTION: For the 500 L systems, only attach the front 2 hanging tabs to the pins to assist in aligning the probe belt, drain port, and spargers.

Figure 3.38. Hanging tab and hook.

Figure 3.39. Attaching tab.

13. Verify that the sparger filter and spargers remain in the correct position. It is recommended that users secure the hanging tabs on the front BPC panel first. This way the door will not be an obstruction when connecting the last set of hooks.

14. Remove the protective packaging from the exhaust vent filters (Figure 3.40).

Figure 3.40. Removing protective packaging.

3.3.2 Drive shaft insertion for 500 and 1,000 L systems

The drive shaft is constructed in multiple segments, which must be assembled and inserted in pieces. Operators should be elevated (i.e. with the use of a ladder) to effectively assemble and insert the drive shaft.

CAUTION: Review ceiling height requirements in Chapter 4 of this user's guide before trying to insert the drive shaft.

Figure 3.41 illustrates the components of the motor and mixing assembly. The parts labeled on the figure will be referenced throughout the drive shaft insertion process. Use the steps in this section to assemble and insert the drive shaft.

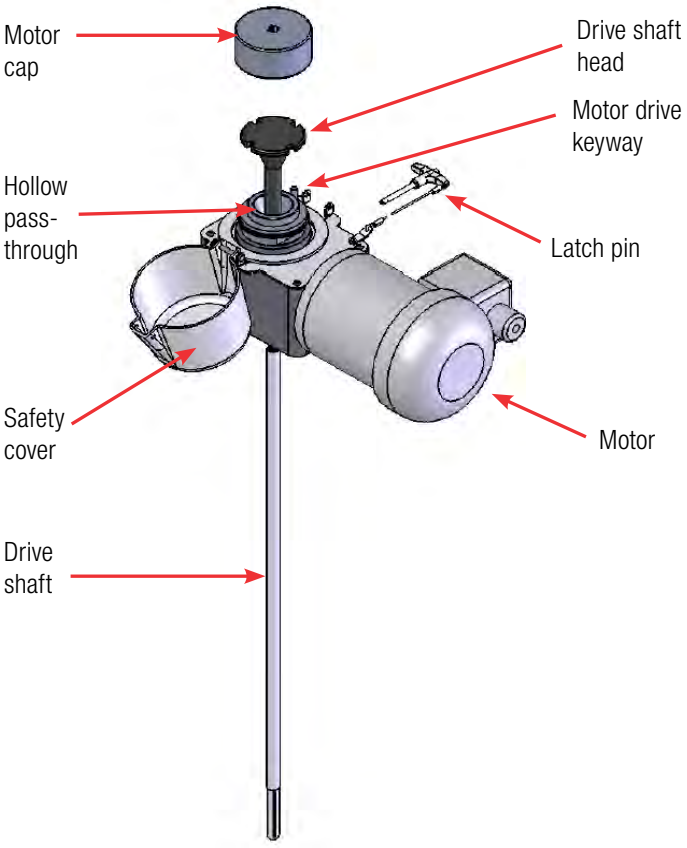


Figure 3.41. Motor and mixing assembly.

1. Prepare the hollow pass-through by first removing the latch pin on the safety cover (Figure 3.42), opening the safety cover (Figure 3.43), and removing the motor cap of the mixing assembly (Figure 3.44).

Figure 3.42. Latch pin removal.

Figure 3.43. Opening safety cover.

Figure 3.44. Cap removal.

2. Verify that the two or three segments of the drive shaft, all with matching serial numbers, are located in the drive shaft holders on the side of the outer support container. For the three-piece drive shaft loading described here, the segments will be referred to as upper (the segment with the drive shaft head), middle (the segment with the internal/external threads on each end) and lower (the segment with the square end). For 1,000 L systems, lubricate the threaded ends with a light coat of food-grade anti-seize with each use. **Each time drive shafts are assembled and used, operators must verify that the segments have matching serial numbers.**

3. First, insert the lower segment through the hollow pass-through of the mixer drive (Figure 3.45). Slide the latch pin from the motor assembly into the shaft to prevent it from falling into the tube (Figure 3.46). Assemble the middle and lower segments of the drive shaft by joining them with a twisting motion, fastening the two segments together (Figure 3.47).

Note: Segmented shafts are left-threaded (reverse-threaded) to avoid loosening during operation.

Figure 3.45. Inserting lower section.

Figure 3.46. Latch pin in shaft.

Figure 3.47. Segment assembly.

4. Place one wrench on the flat area in the middle drive shaft segment and another wrench on the lower segment, then tighten the connection using a counterclockwise rotation (Figure 3.48). After the segments are secure, return the wrenches to the tool holder. **CAUTION:** Do not over-tighten; a snug fit is sufficient. Remove the latch pin.

Figure 3.48. Tightening of shaft connections.

5. Load the partially-assembled drive shaft through the hollow pass-through and hold it in position with the latch pin. Obtain the upper segment of the drive shaft and assemble it to the middle segment in the manner described previously.

6. Using two hands, carefully guide the assembled drive shaft into the BPC using a slight back and forth twisting motion. **Note:** It may be necessary for another operator to assist with drive shaft insertion. As one operator inserts the drive shaft, another operator should carefully manipulate the impeller as the end of the drive shaft begins to couple with the impeller.

    - When 50.8–76.2 mm (2–3 in.) of the shaft remains, twist slightly to engage the impeller (Figure 3.49).

Figure 3.49. Drive shaft insertion.

- When 25.4–50.8 mm (1–2 in.) of the shaft remains, twist slightly to engage the bearing assembly.
- When 6.35–12.7 mm (0.25–0.50 in.) of the shaft remains, twist to align the motor drive keyway with one of the four outer slots on the drive shaft head (Figure 3.50).

Figure 3.50. Drive shaft head aligned.

7. Directly couple the drive shaft to the motor by placing the motor cap back on the hollow pass-through and tighten.

8. Tighten the motor cap by placing the spanner wrench counterclockwise on the hollow pass-through and tighten using the supplied torque wrench (Figure 3.51). **Wrench note:** The torque wrench is a standard 10 mm (3/8 in.) square drive, and is calibrated at the factory at 150 in-lb.

Figure 3.51. Tightening motor cap with wrenches.

9. Verify that the wrenches have been removed from the system and returned to the storage holders.

10. Close the safety access cover and insert the latch pin.

3.3.3 Final installation steps for 500 and 1,000 L systems

1. Secure the exhaust vent filters to the top-mounted holders (Figure 3.52), or if you are using elevated dual exhaust filters, use the adapter piece and extended filter bracket (Figure 3.53). **Note:** 500 L BPCs and some custom BPCs are supplied with dual exhaust vents. The vent bracket can accommodate 10 in. and 4 in. filters in either single or dual configuration.

Figure 3.52. Vent filter.

Figure 3.53. Extended dual filter bracket.

2. Fully extend the drain line set through the front cutout and attach the probe shelf.

3. Remove the polybag from the drain line set, position the line clamp as close as possible to the BPC port, and close the clamp. Use a cable tie around the clamp to ensure it does not open.

4. Align the aseptic connector ports through the front access window (Figure 3.54).

Figure 3.54. Aseptic connector port alignment.

5. Remove the plastic insert located in the thermowell, if present.

6. Insert a resistance temperature detector (RTD) or another selected temperature sensor into the thermowell (Figure 3.55).

   - Place a small amount of glycerol (0.5 mL) in the thermowell to aid in heat transfer. The glycerol also serves as a lubricant and aids in insertion.
   - The sensor should be inserted until the base of the RTD meets the mouth of the thermowell.
   - Secure by twisting the luer lock collar, if provided; the thermowell will stretch slightly when the RTD is seated (Figure 3.56).

   Note: Verify that all port clamps are closed and located as close as possible to the body of the BPC.

Figure 3.55. Inserting a temperature sensor.

Figure 3.56. Securing the temperature sensor.

7. Optional: Connect a pressure sensor to the CPC aseptic connector at the top of the BPC. Then connect the appropriate pressure transducer cable to the third-party controller.

8. Refer to section 3.5.3 for probe insertion instructions.

9. Close the bottom access door. The proper latch tension can be obtained by a combination of feel and visual inspection. When closing the latch, the handle should begin to provide resistance to closing when the leading edge of the safety pin pass-through of the latch handle aligns with the outside edge of the latch base (Figure 3.57). **Note:** When the latch is under-tensioned, the safety pin pass-through of the latch handle will be covered within the latch base and the handle will close very easily. If the latch is over-tensioned, the handle will be excessively difficult to close.

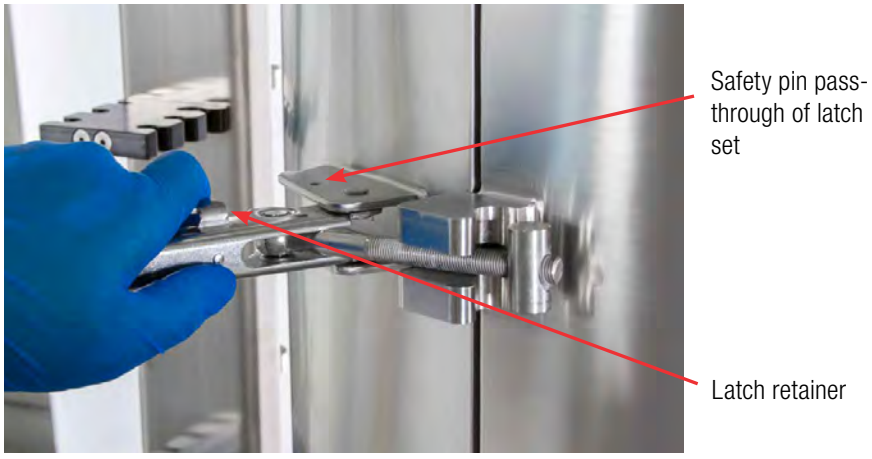


Figure 3.57. Latching the access door.

10. The access doors must be closed and fully latched prior to filling the system with liquid.

3.4 BPC and drive shaft loading, and condenser system setup instructions for 2,000 L systems

3.4.1 Initial BPC loading steps for 2,000 L systems

Checkpoints prior to BPC loading

- ✓ The correct volume BioProcess Container (BPC) is being used for the corresponding volume outer support container.
- ✓ Three operators are available for BPC loading.
- ✓ A ladder or other means of elevation is available for drive shaft insertion (see the specifications in Chapter 4 for system ceiling height requirements).
- ✓ The equipment has been evaluated against your confined space safety standards and procedures.

Use the following steps to install and set up the BPC.

1. Switch on the main power to the control panel. Ensure that the drive motor is not running. Open both the front and rear doors on the outer support container.

2. Use an elevated platform to open the clamp on the bearing port receiver located below the motor. Lower the hoist lifting frame to a position just above the top of the rear door by using the pneumatic control lever (near the rear door).

3. Two operators should carefully remove the irradiated BPC from the protective double polybags (Figure 3.58). Do not remove the polybags from the line sets at this stage, as the BPC may become difficult to manage. Do not allow the BPC or line sets to touch or drag on the floor.

Figure 3.58. Removing the BPC from polybags.

4. Load the BPC through the rear access door (Figure 3.59), orienting the bottom of the container into the door first with the bearing port facing upward. Keep the container folded as supplied in the packaging to allow the BPC to unfold naturally when it is lifted by the hoist.

Figure 3.59. Loading the BPC.

5. Using the rear or front door for access, connect the retainer hooks on the hoist to the top of the BPC via the hanging tabs, starting with the furthermost two tabs (Figure 3.60). Finish with the closest two tabs (Figure 3.61).

Figure 3.60. Connecting furthermost retainer hooks.

Figure 3.61. Connecting closest retainer hooks.

6. Raise the BPC using the pneumatic lift. One operator should observe from above while another operator controls the lifting valve at ground level (Figure 3.62).

CAUTION: While operating the 2,000 L bag hoist watch for excess stress on the BPC during lifting. Reposition the BPC as necessary to avoid tearing the BPC hanging tabs.

Figure 3.62. Operating the pnuematic lift control to raise the bag hoist.

7. Raise the hoist until the lift reaches full stroke. Once lifting stops, the top-level operator should hold the hoist frame and apply minimal lifting force. This will assist the lifting device to pull in any remaining slack in the cable, and ensure the lift device has been fully raised. Place the valve in the "stop" position.

8. Use an elevated platform to open the clamp on the bearing port receiver located underneath the motor. Remove the black protective cap from the bearing port (Figure 3.63), load the BPC bearing port into the receiver (Figure 3.64), close the bearing assembly door, and latch it (Figure 3.65).

Figure 3.63. Removing the cap.

Figure 3.64. Bearing port loading.

Figure 3.65. Closing/latching door.

9. Place the top line sets (still in polybags) over the side of the outer support container. This will help support the weight of the BPC and also keep the BPC from being restricted during the air inflation step.

10. Open the tubing set polybag and connect the pressure transducer to the monitor. After the display has stabilized, tare the monitor.
Note: Allow the monitor to warm up for 30 minutes and connect the sensor 10 minutes before taring. Verify that the monitor reads zero.

11. If you are using the exhaust condenser system, follow the setup instructions in section 3.4.2 of this guide. If you are using elevated exhaust vent filters, use the corresponding extended dual vent filter bracket and filter heaters.

    To load the optional exhaust vent filters, follow the steps below.

    - Clip each filter one at a time into the elevated vent filter holder system (Figure 3.66). Carefully center the filter housing, allowing the clip to secure it near the hose barb connections.

Figure 3.66. Clipping filter to holder.

    - Ensure that the routing of the exhaust tubing is not likely to become kinked.
    - Place the vent heaters around each filter (Figure 3.67), verifying that the snap retainers are secured. Position the power leads to avoid interfering with the vent holder brackets.

Figure 3.67. Installing heaters.

- Raise and rotate the vent holder bracket as needed (Figure 3.68). Make a final inspection to ensure that no kinks or low spots will occur in the tubing between the BPC and the filter, even if the BPC becomes pressurized.

Figure 3.68. Raising the vent holder bracket.

- Connect the power to the vent heaters and verify operation of the controllers.
- Inspect the controller setpoints (recommended 60°C). After two to five minutes of operation, verify that the vent heaters are warm and are near the desired temperature setpoints. Verify that no alarm indicators are active.

12. Fill the BPC with air via the DHS and overlay gas inlet line.

13. Clamp the drilled hole sparge and exhaust lines so that the air supplied by the overlay gas inlet line flows directly into the BPC (Figure 3.69). **Note:** Remove the clamp prior to sparging.

Figure 3.69. Clamping the exhaust lines prior to filling the BPC with air.

14. The BPC must be partially inflated to aid in the proper alignment of the BPC in the outer support container, and proper insertion of the drive shaft.
    - Attach the air supply to the overlay gas inlet line at the top of the BPC.
    - Begin filling the BPC with air. Allow the container to fill to greater than half volume. This typically takes less than 20 minutes.
    - Steps 15 through 18 can be completed while the BPC is filling with air.

    Note: Air pressure to the overlay gas line on the BPC should be less than 100 slpm or 0.34 bar (5 psi).

WARNING: The BPC is not rated as a pressure vessel. **DO NOT EXCEED 0.03 bar (0.5 psi) within the BPC** or the system could fail, causing personal injury or damage to equipment. Do not leave the BPC unattended while inflating. See Table 3.7 in section 3.6.8 for recommended air flow rates. The operating pressures at the level of the S.U.B. are of primary importance, and these values must be adhered to.

15. Feed the probe belt, sample line, and the subsurface addition lines through the front access door (Figure 3.70).

Figure 3.70. Feeding lines through the front access door.

16. Remove the sparge lines from the polybags and the bubble wrap from the sparge filters. Use the rear door to gain inside access to the floor of the hardware. Place a clamp on the bottom drain line at this time (Figure 3.71).

Figure 3.71. Clamping bottom drain line.

17. The center insert on the tank floor provides the port locations for both the bottom drain (Figure 3.72) and the gas lines for the open pipe or drilled hole sparger. Guide the sparger inlet line and filter through the bottom cutout in the tank (Figure 3.73) to provide access for loading the porous frit sparger gas line (Figure 3.74). To remove the bottom cutout, lift and rotate it in a counterclockwise direction.

Figure 3.72. Drain line support.

Figure 3.73. Macro sparger line support.

Figure 3.74. Porous frit sparger line loading.

18. Three cutout holes are provided in the tank for porous frit spargers. These holes are located furthest from the tank center line and align with the inside edge of the access cover door (Figure 3.75).

Figure 3.75. Cutouts for porous frit spargers.

Sparger notes:

- Air pressure to the spargers on the BPC should not exceed 0.55 bar (8 psi).
- While a sparge line check valve is provided for each sparge line, it is not uncommon for some fluid to bypass check valves during typical use. We recommend elevating the sparge line filter as is feasible to help reduce this tendency.

19. Attach all of the hanging tabs to help position the ports. Secure the BPC by attaching the tabs on the bottom of the BPC onto the position tab pins (Figures 3.76 and 3.77). Verify that the sparger filter and spargers remain in position while attaching the tabs. It is recommended that users secure the tabs on the front BPC panel first. This way, the larger rear door will allow access when connecting the last set of tab pins.

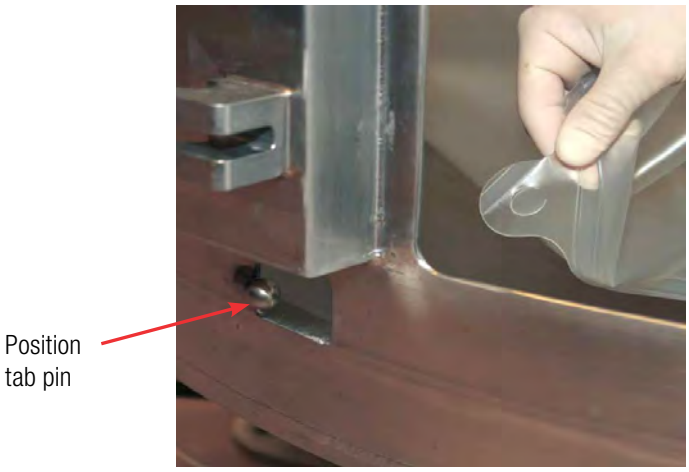


Figure 3.76. Pulling the container tab toward the pin.

Figure 3.77. Securing the container tab on the pin.

20. After the BPC has filled to greater than half volume, unclamp the drilled hole and exhaust lines.

3.4.2 Condenser system setup for 2,000 L systems

Condenser system functional overview

The condenser system is intended to be used as an accessory for large S.U.B.s as an alternative to vent filter heaters. Condenser systems are recommended for use with 2,000 L S.U.B.s. The condenser prevents liquids and solids from condensing and collecting inside of the vent filters of the S.U.B. The condenser system cools the exhaust gases leaving the S.U.B. chamber, condensing the moisture out of the saturated gases coming from the S.U.B. The liquid condensate that is stripped from the exhaust gases is then pumped back into the BPC chamber, creating a sterile loop and significantly reducing liquid loss due to evaporation. The condenser plate on condenser systems with a cart assembly is chilled by a closed bath recirculating chiller, which has sufficient capacity to cool two condenser plates simultaneously. The condenser plate on side-mounted condenser systems is chilled by a house recirculating chilling loop. Figures 3.78 and 3.79 show both the cart assembly and side-mounted (2,000 L systems only) condenser system options.

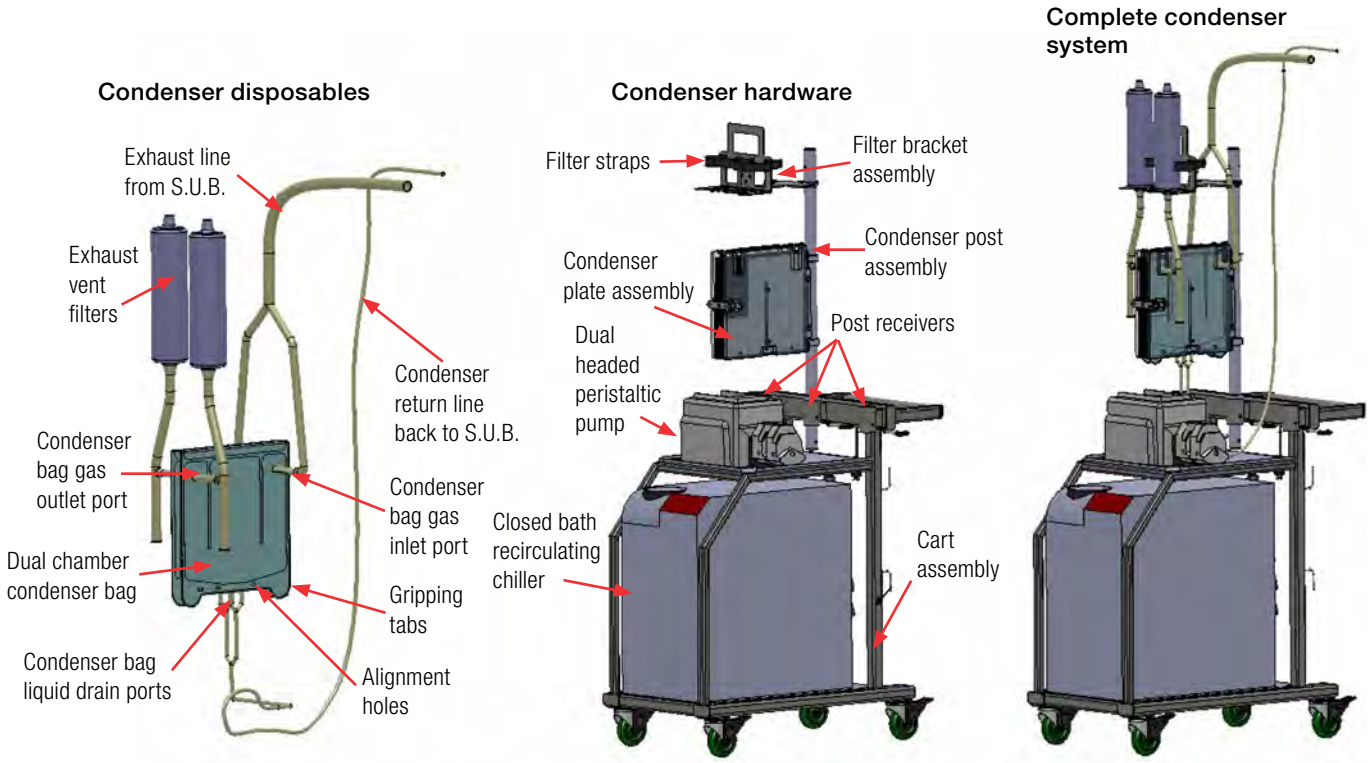


Figure 3.78. Overview of condenser system cart assembly option for 2,000 L S.U.B.s.

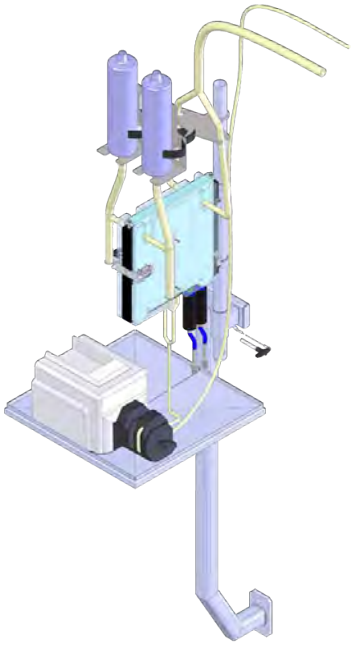


Figure 3.79. Side-mounted condenser system option for 2,000 L S.U.B.s.

When to use the condenser system

2,000 L S.U.B. BPC with single-use condenser system

Large 254 mm (10 in.) hydrophobic PVDF filters with a nominal 0.2 micron pore size were specified in order to increase the available surface area for off-gassing. In conjunction, the standard 2,000 L S.U.B. is designed for use with a single-use condenser system. This allows the S.U.B. to utilize a powerful phase-change type system which provides improved exhaust vent protection and reliability due to the ability to strip condensate and atomized materials that may be present from the off-gas stream of the S.U.B. This system has been shown to significantly reduce the “fouling” load on the vent filters that inherently increases operating back pressure as the cell culture run batch progresses. See the HyPerforma 2:1 Single-Use Bioreactor Validation Guide (DOC0016) for details.

2,000 L S.U.B. BPC with vent and heaters only

Some end users may prefer to omit the condenser system on the 2,000 L S.U.B. with the expectation that this will allow for a more uniform installation (similar to smaller S.U.B. systems used in the upstream seed train), or will perhaps reduce system complexity and cost. The use of exhaust vent heaters and 254 mm (10 in.) filters will provide impressive flow capacity over short periods (less than 5 days). However, the high sparge rates required during the scale-up of the S.U.B. to the 2,000 L working volume may eventually create conditions of increased operating back pressure, usually due in part to blocking of the filter media. Depending upon the application, the user has the option of using both filters in parallel or initiating the run with a single filter, temporarily clamping off the line to the other filter (it being reserved as redundant back-up).

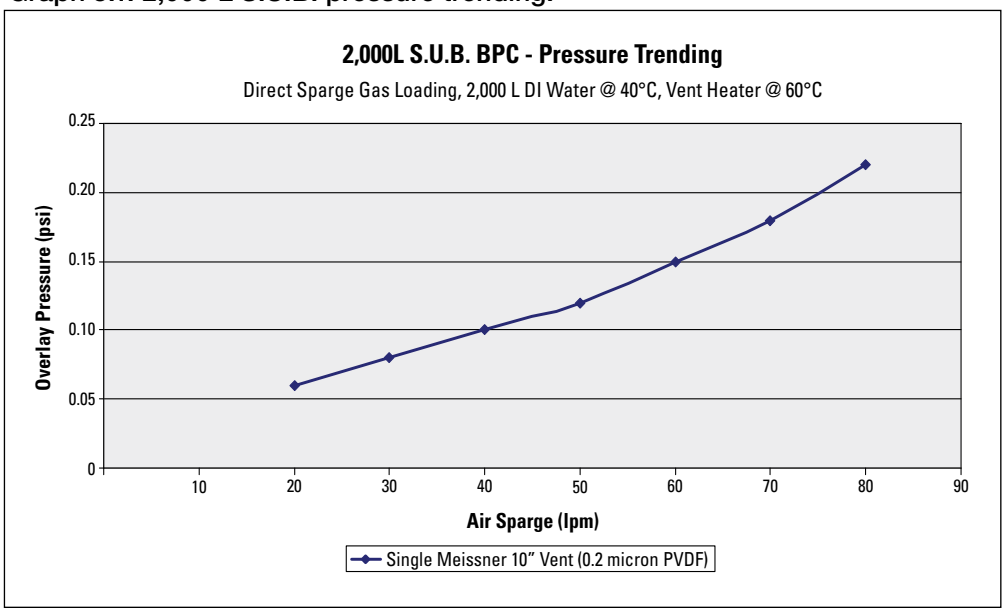
Table 3.2 may help end users specify the BPC configuration and operating parameters for custom 2,000 L S.U.B. applications when not utilizing the exhaust condenser. Because the operating parameters of different cell cultures vary widely, a safety factor should be used to temper the data. Accordingly, the data we used to generate a control base line are for reference only (filter fouling will vary and must be considered to ensure reliable performance). It is assumed that no foam is present in the exhaust stream.

Table 3.2. Condenser system overview.

S.U.B. system 2 each 254 mm (10 in.) vents	Run duration	Maximum combined flow rate recommended	Resulting safety factor
2,000 L S.U.B.	7 days	40 slpm	2x
2,000 L S.U.B.	10 days	32 slpm	2.5x
2,000 L S.U.B.	14 days	27 slpm	3x
2,000 L S.U.B.	21 days	Single-use condenser strongly recommended	

The above recommendations were generated using the test conditions shown in Graph 3.1. In this case, a 2,000 L S.U.B. was filled with 2,000 liters of DI water with a batch temperature of 40°C using a MKS vent filter heater at 60°C. Safety factor estimates are based on a maximum continuous internal S.U.B. BPC pressure not to exceed 0.006 bar (0.1 psi), which corresponds to 40 slpm with a single 254 mm (10 in.) vent. **Note:** These results do not take into consideration a “fouling” safety factor.

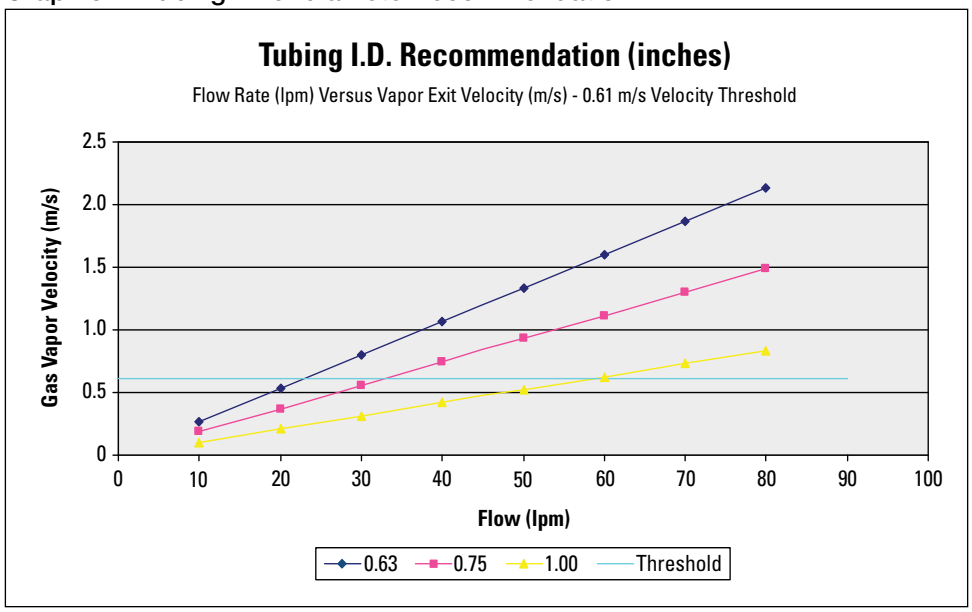
Graph 3.1. 2,000 L S.U.B. pressure trending.



Also consider the size and type of tubing used to connect the exhaust vents to the S.U.B. BPC (when not using an exhaust condenser). Braid reinforced tubing provides the best protection against kinking or accidental pinching of the exhaust line. The 254 mm (10 in.) vents are supplied with 19.1 mm (0.75 in.) hose barbs. This tubing diameter will allow condensate to return to the S.U.B. at total off-gas flow rates up to 30 slpm, assuming that the tubing is near a vertical orientation. Testing has shown that large-diameter tubing will allow for lower exhaust gas velocities, and if the vapor velocity is below 0.6 m/s, gravity will allow the condensate formed in the tubing to return to the batch process (Graph 3.2).

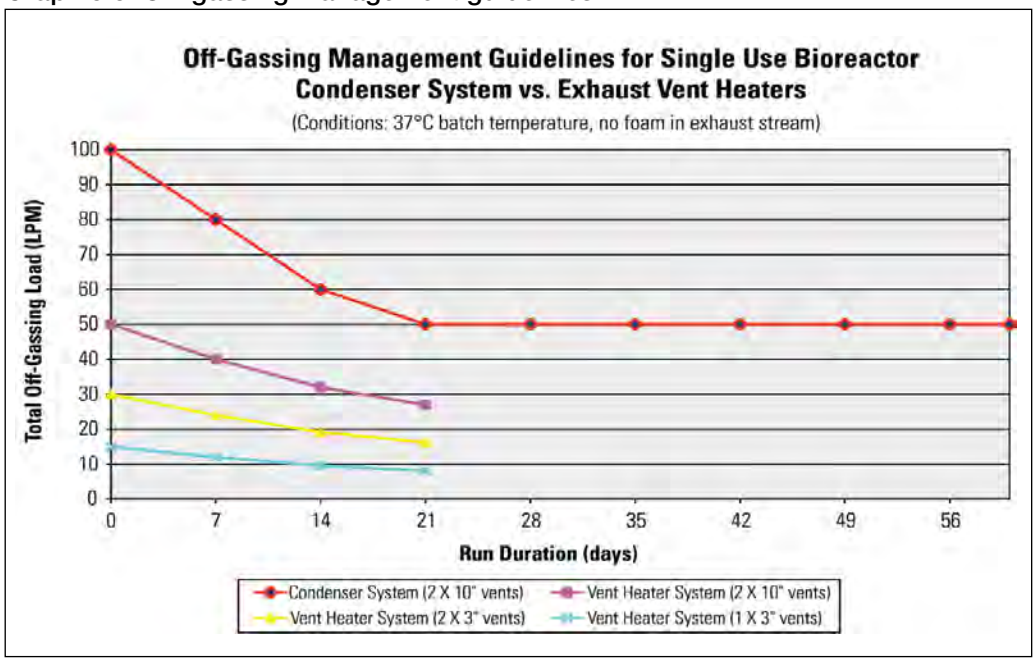
Note: Restrictive tubing connectors can create flow bottle necks; 12.7 mm (0.5 in.) inner diameter (ID) tubing is typically deemed too small for the 2,000 L S.U.B.

Graph 3.2. Tubing inner diameter recommendation.



Various vent filter configurations are available on the S.U.B. depending upon the process scale and intended application. Graph 3.3 provides a reference for determining the relative capacity of different filters, depending on the amount of gas flow anticipated and the length of the run. In all cases, using a vent filter heater will reduce the chance of condensate blocking the filter, but over time, suspended solids carried in the exhaust stream will impede the flow of exhaust gas (resulting in increased back pressure). In addition, it is good practice to monitor the amount of foam present in the head space. In all cases, a vent filter heater has very little tolerance for handling the presence of foam in the exhaust stream. A small feed of antifoam (e.g., FoamAway™ Irradiated AOF Antifoaming Agent, catalog number A1036901) added directly to the liquid surface of the culture head space typically provides excellent foam control. 1,000 and 2,000 L systems can benefit from the use of a condenser system. It has been shown to increase system reliability at high flow rates (beyond 50 slpm) and should warrant strong consideration when performing batch runs beyond 10 days. Results will vary; however, it is strongly recommended that end users select a vent filter configuration providing reserve capacity where possible. For example, dual vent configurations can be used independently, with the second filter serving as a redundant backup (providing a quick reserve in the event that issues arise in process).

Graph 3.3. Off-gassing management guidelines.



Condenser system setup

1. Remove the reservoir cap of the chiller and add the appropriate type and volume of fluid per the chiller user's guide.

2. Verify that the peristaltic pump and chiller power cords are connected to a power source.

3. If you are using a condenser system with a cart assembly, plug in the system.

4. Turn on the power to the chiller. This will allow the chiller to prime.

5. If you are using a temperature control unit (TCU), ensure that the TCU coolant is filled to the maximum level.

   CAUTION: Low TCU coolant levels can increase the temperature of the plates, and cause excessive pressure and/or residue buildup in the condenser bag. Please note that the chiller plates may run warmer than the TCU setpoint.

6. Purge the chill plate by loosening the bleed plug on top of the plate. This is accessed using a hex wrench passing through the top tensioning plate of the chill plate assembly. Loosen the plug only enough to allow trapped air to escape, then re-tighten.

7. The settings for the chiller and peristaltic pump are preset at the factory. These settings allow for the system to resume setpoint if the power is temporarily disrupted. Verify that the chiller and pump setpoints are at the recommended levels (5°C and 12 rpm).

8. If you are using a condenser system with a cart assembly, verify that the peristaltic pump is in place on the cart beneath the chill plate. Side-mounted condenser systems have an attached tray for the peristaltic pump.

Condenser system loading

Two operators are required to safely set up the exhaust system. Setup time is typically 2–3 minutes.

Note: The figures in this section show a condenser system with a cart assembly. Side-mounted condenser systems have the same chiller plate, and use the same loading instructions.

1. One operator, located at an elevated position, should remove the condenser BPC carefully from the polybag packaging. Lower the assembly (directed in a vents-first orientation) to the second operator located at ground level, standing to the rear of the S.U.B. For systems with a cart assembly, the second operator should stand between the condenser cart and the S.U.B. (Figure 3.80).

Figure 3.80. Removing condenser from packaging.

2. The operator at the upper position should move to ground level, open both doors on the chiller plate (Figure 3.81), and load the condenser BPC from the front, keeping the BPC in a saddle bag shape. Allow the vents to hang freely.

Figure 3.81. Opening the chiller plate doors.

3. Route the gas inlet lines around and behind the vent holders. Inspect both lines to ensure they are connected to the S.U.B. and are not twisted or kinked (Figure 3.82). Adjust as needed.

Figure 3.82. Routing gas inlet lines.

4. The second operator should hold the vent filters and place them into the vent filter holders above the chill plate (Figure 3.83).

Figure 3.83. Placing vent filters.

5. The first operator should use the Velcro straps to secure the filters in position (Figure 3.84). Then use the grasping tabs to position the container using the two lower button pins on each side of the chiller plate (Figure 3.85).

Figure 3.84. Securing vent filters.

Figure 3.85. Positioning container.

6. Close the clear side doors while carefully manipulating first the gas inlet line and then the gas outlet line (Figure 3.86) to clear the doors as each is closed and latched (Figure 3.87).

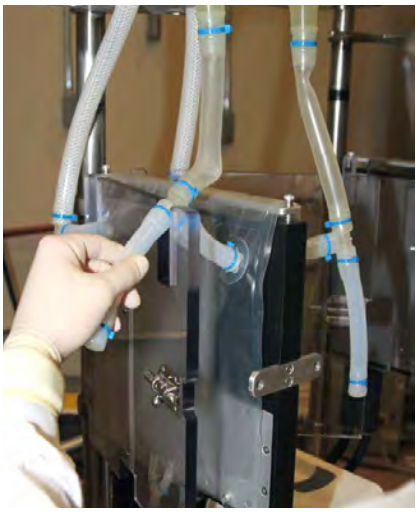


Figure 3.86. Moving gas lines.

Figure 3.87. Latching door.

7. As the doors are closed and latched, the second operator should route the gas inlet lines into the clips behind the vents (Figure 3.88).

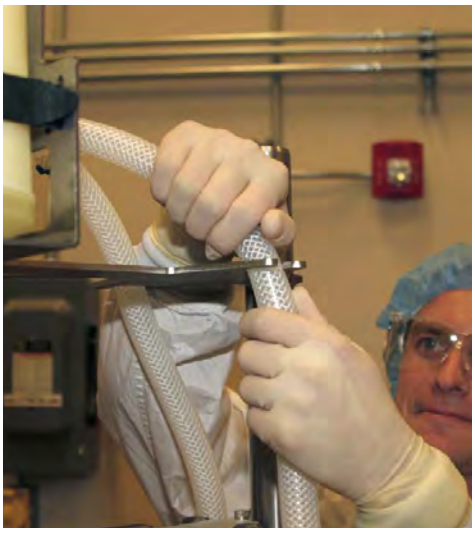


Figure 3.88. Clipping gas lines into place.

8. The first operator should load the peristaltic tubing into the pump (located on the cart for condenser systems with a cart assembly, or on the tray for side-mounted condenser systems), verifying that there is sufficient slack at each end of the pump tubing. Then align the tubing in the pump channel and close the pump ramp (Figure 3.89).

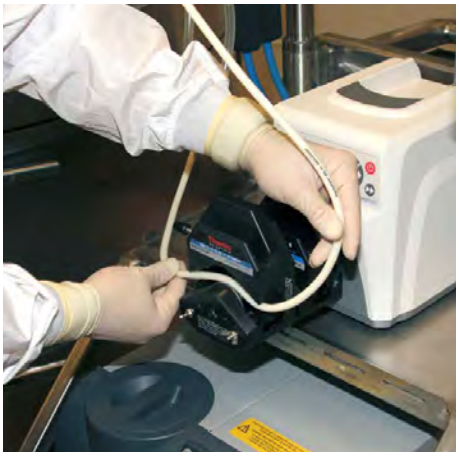


Figure 3.89. Loading pump tubing.

9. Start the pump by pushing the red button (Figure 3.90). Verify that both the pump and chiller are enabled and running at the proper settings. We recommend setting the pump at 12–30 rpm and the chiller at 5°C. The specified pumping system is qualified to run continuously (wet or dry) beyond 21 days.

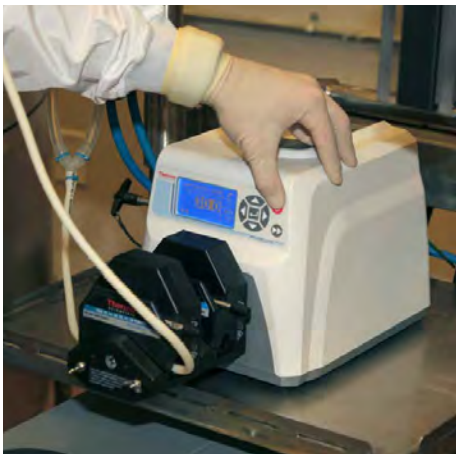


Figure 3.90. Starting the pump.

10. After setup, verify the following:
    - ✓ The elbow fittings on the inlet and outlet of the condenser saddle bag are straight and level.
    - ✓ The gas inlet line and the condensate line are not twisted, pinched, or obstructed.
    - ✓ There are no low spots in the gas inlet line. Adjust the lines to avoid condensation pooling.
    - ✓ The pump union is loose on both ends of the pump and running smoothly in the peristaltic rollers.

Contact technical support for specific condenser system performance questions.

3.4.3 Drive shaft insertion for 2,000 L systems

The drive shaft is constructed by assembling two quick-connect segments. These segments must be assembled and inserted in sections. Operators should be elevated (i.e. with the use of a ladder) to assemble and insert the drive shaft.

CAUTION: Review ceiling height requirements in Chapter 4 of this user's guide before trying to insert the drive shaft.

Figure 3.91 illustrates the components of the motor and mixing assembly. The parts labeled on the figure will be referenced throughout the drive shaft insertion process.

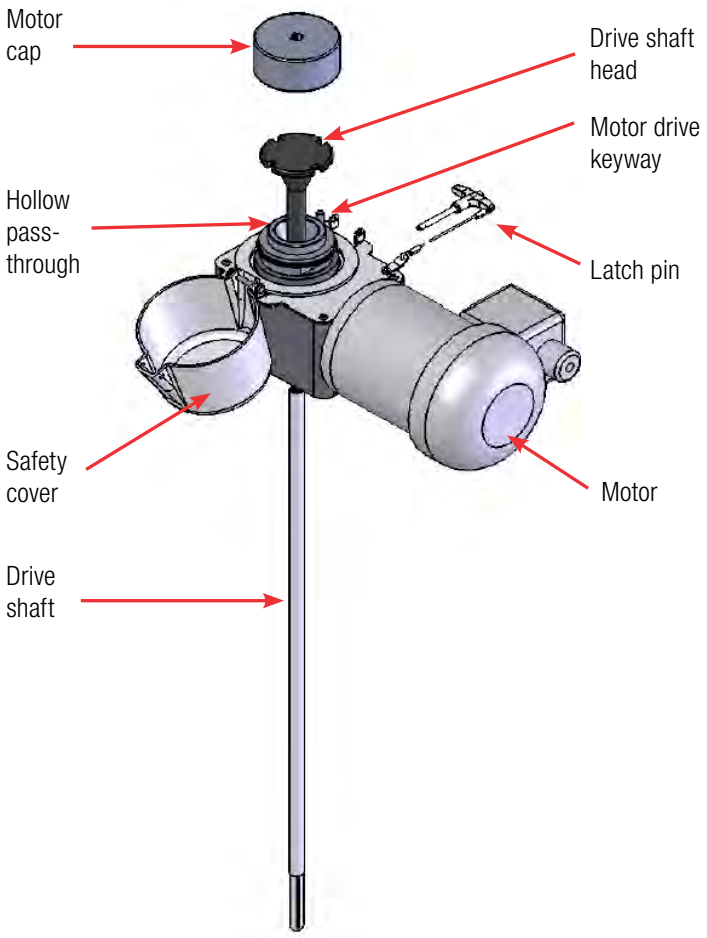


Figure 3.91. Motor and mixing assembly.

Use the following steps to insert the drive shaft.

1. The BPC must be filled with air to greater than approximately 50% volume to allow for unrestricted loading of the angled drive shaft. **Note:** After inflation, the impeller tubing should be hanging straight down inside the BPC, with the impeller near the bottom.

2. Verify that the proper drive shaft segments and tools are available.

3. Prepare the hollow pass-through by first removing the latch pin on the safety cover (Figure 3.92), opening the safety cover (Figure 3.93), and removing the threaded cap by turning it counterclockwise. Use the wrench and spanner provided, if necessary.

Figure 3.92. Removing latch pin.

Figure 3.93. Opening safety cover.

4. Verify that both segments of the drive shaft have matching serial numbers, and are located in the drive shaft holders on the side of the outer support container. The segments will be referred to as the upper (segment with the drive shaft head) and lower (segment with the square end). **Important notes:** Each time drive shafts are assembled and used in the S.U.B., operators must verify that the segments have matching serial numbers. No lubrication is required with the quick connect assembly design.

5. First, insert the lower segment through the hollow pass-through of the mixer drive (Figure 3.94). Once inserted, slide the latch pin from the mixing assembly into the shaft to prevent it from falling into the impeller sleeve (tube) (Figure 3.95).

Figure 3.94. Insertion of the lower section of the drive shaft.

Figure 3.95. Use of the latch pin.

6. To connect two shafts together, depress the button on the female side (Figure 3.96) and slide the sleeve back. This will expose a red ring underneath the sleeve (Figure 3.97). This is a visual indicator that the sleeve is not in a locked position.

Figure 3.96. Button used for connection.

Figure 3.97. Sliding sleeve exposing red "not locked" indicator.

7. Obtain the upper section of the drive shaft. Place the female side of the quick connect over the male end of the lower section (Figure 3.98). The connection is fully seated when the red indicator ring (Figure 3.99) on the male end is no longer exposed.

Figure 3.98. Quick connection.

Figure 3.99. Red indicator ring exposed.

8. Slide the sleeve toward the connection, allowing the push button to lock into position. This will engage the locking mechanism and also cover the red indicator ring (Figure 3.100).

   Note: When fully connected, no red coloring should be visible.

Figure 3.100. Sliding the sleeve into place.

9. Once the sections are secure, remove the latch pin and return the wrenches to the tool holder.

10. Using two hands, carefully guide the completed drive shaft into the BPC using a slight back and forth twisting motion, or a counter-clockwise rotation (Figure 3.101). **Do not push the drive shaft straight in.**

    Note: Figures 3.101 and 3.102 show a drive shaft with a white shaft head. All 2,000 L S.U.B.s use a new, longer drive shaft with a black drive shaft head.

Figure 3.101. Insertion of the drive shaft.

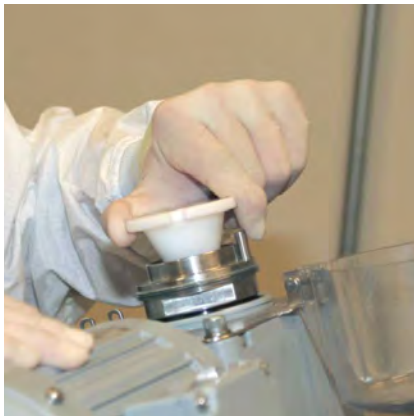


Figure 3.102. Engaging the bearing port.

- When 50.8–76.2 mm (2–3 in.) of the shaft remains, twist slightly to engage the impeller.
- When 25.4–50.8 mm (1–2 in.) of the shaft remains, twist slightly to engage the bearing assembly (Figure 3.102, above).
- When 6.4 mm (0.25 in.) of the shaft remains, twist to align the motor drive keyway with one of the four outer slots on the drive shaft head.

11. Ensure that the head is fully seated before directly coupling the drive shaft to the motor. Any spring-back indicates that the drive shaft is not properly seated in the impeller. Figure 3.103 illustrates a drive shaft that is completely inserted into the impeller.

    Note: The cap should be easy to install when the drive shaft head is fully engaged in the hollow pass-through. Otherwise, repeat steps 1 through 10 before replacing the cap.

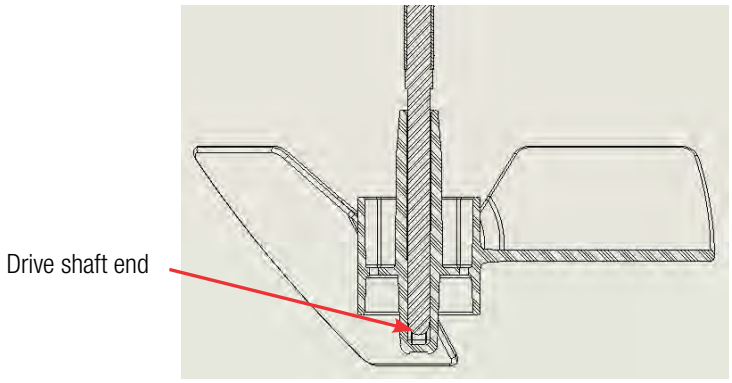


Figure 3.103. Drive shaft fully inserted into the impeller.

12. Place the threaded cap back on the hollow pass-through. Secure the cap by placing a spanner wrench on the hollow pass-through and tightening, using the supplied torque wrench (Figure 3.104).

    Note: The torque wrench is a standard 10 mm (3/8 in.) square drive, and is calibrated at the factory at 150 in-lb.

Figure 3.104. Tightening cap.

13. Verify that the wrenches have been removed from the system and returned to the storage holders.

14. Close the safety access cover and insert the latch pin.

3.4.4 Final installation steps for 2,000 L systems

1. Verify the proper position of the exhaust filters. The exhaust flow path must be unobstructed. Connect the gas supply lines. Verify the intended flow paths for overlay, porous frit, and drilled hole spargers.

2. Verify that the overlay and direct sparger lines are correctly positioned and free of kinks. Verify that the rear access door is closed with proper latch tension.

3. Remove the polybag from the drain line set and verify that the redundant line clamps are in position. Use a cable tie around the clamp to ensure the clamp cannot be accidentally opened.

4. Align the aseptic ports through the front access window (Figure 3.105). This will be the lower cutout if your system is supplied with two horizontal slots. **Note:** The latest style of hardware has a third opening.

Figure 3.105. Aseptic port alignment.

5. Secure the access doors with the latches. Proper tension is obtained by adjusting the threaded latch pin. Tension of the latch is adjusted by varying the position of the pin on the threaded shank. The proper latch tension can be obtained by a combination of feel and visual inspection. When closing the latch, the handle should begin to provide resistance to closing when the leading edge of the safety pin pass-through of the latch handle aligns with the outside edge of the latch base (Figure 3.106).

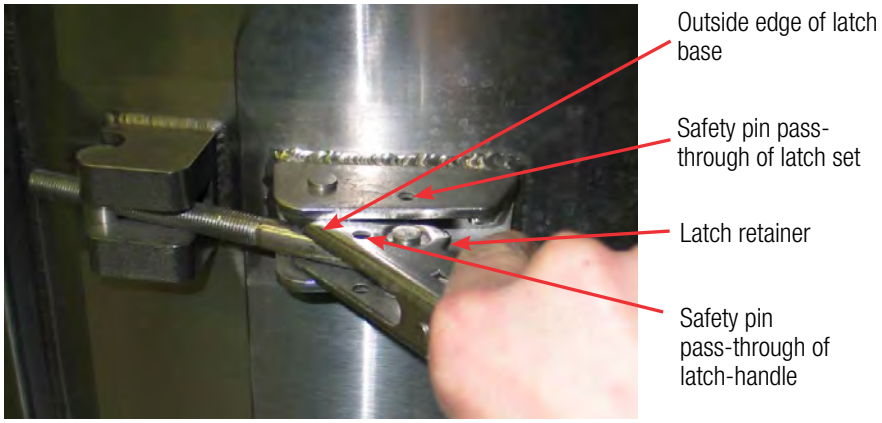


Figure 3.106. Latch access door.

Note: When the latch is under-tensioned, the safety pin pass-through of the latch handle will be covered within the latch base and the handle will close very easily. If the latch is over-tensioned, the handle will be excessively difficult to close.

6. For maximum security, insert pins (not included) into the respective latches.

7. Turn off the air supply to the overlay line.

8. Position clamps as close as possible to the BPC, and close them on all tube ports (Figure 3.107). Place clamps on subsurface lines as close to the port as possible. This will eliminate media from filling these lines prior to use.

Figure 3.107. Clamp installation.

9. Remove the plastic insert located in the thermowell, if present.

10. Insert the resistance temperature detector (RTD) or selected temperature sensor into the thermowell (Figure 3.108). **Note:** In the latest hardware, there is a new thermowell.
    - Place a small amount of glycerol (0.5 mL) in the thermowell to aid in heat transfer (Glycerol—Sigma G6279).
    - The sensor should be inserted until the base of the RTD meets the mouth of the thermowell.
    - If provided, secure by twisting the luer lock collar. The thermowell will stretch slightly when the RTD is seated.

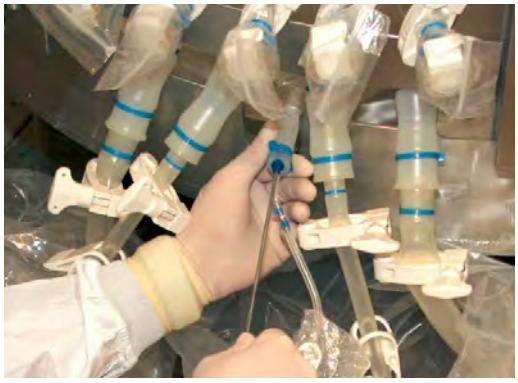


Figure 3.108. Insertion of the RTD into the thermowell.

11. Connect the batch-to-tank grounding cable to the stainless steel connector of the sample line (Figure 3.109).

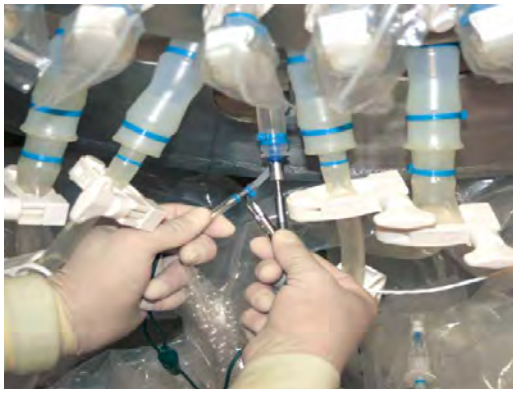


Figure 3.109. Grounding cable connected.

Note: Verify that all of the port clamps are closed and located as close as possible to the body of the BPC.

12. **IMPORTANT:** During media fill, verify the position of all critical ports (drain, spargers, line sets, and probes) before the container is filled with more than 50 liters of liquid. This will still allow time for adjustments, if required, after the fill is initiated.

13. Typically, two fill lines (12.7 mm (0.5 in.) x 19.1 mm (0.75 in.)) and peristaltic pumps are recommended in order to fill the 2,000 L S.U.B. in a timely manner.

3.5 Probe preparation and insertion

3.5.1 Preparation and sterilization

1. Select the appropriate probe (see section 1.3.1). Verify the presence of a Teflon™ support ring and O-ring on the probe and visually inspect the probe for damage.

2. Perform any required probe maintenance and calibrate the pH probe (see section 3.5.4 for probe calibration information).

3. Insert the probe into the probe assembly through the threaded adapter.

4. Verify that the probe tip is not touching [more than 6.35 mm (0.25 in.) gap] the membrane of the aseptic connector before threading into the probe adapter.

5. Hand-tighten the adapter and verify that the probe tip is not touching the membrane.

6. Place the probe assembly with probe into the autoclave tray for probe kits (Figure 3.110).

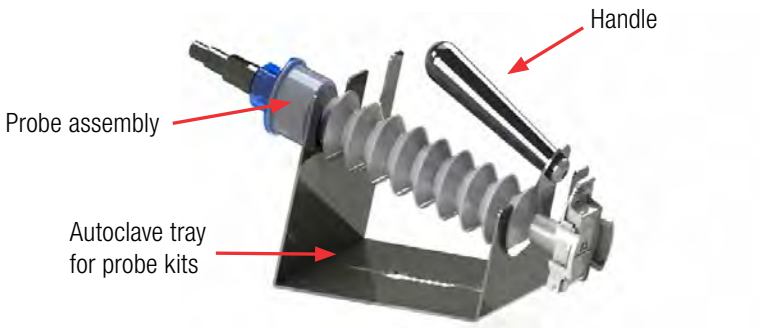


Figure 3.110. Probe assembly and autoclave tray.

7. Autoclave the probe assembly using a validated sterilization cycle (approximately 30 minutes at 122°C). A 30-minute sterilization cycle is generally sufficient. Options of wet or dry cycle parameters can be used. Slow exhaust cycles are preferred, as this minimizes stress on the probes during the temperature and pressure changes of autoclaving.

8. Allow sufficient time for the probe assembly to cool completely before connecting to the BPC for probe insertion.

9. When stored properly, autoclaved probe assemblies can be stored dry for short periods of time (less than 24 hours) without loss of sensor longevity, performance, or sterility.

3.5.2 Making Kleenpak connections

Kleenpak connector specifications

The Pall™ Kleenpak™ connector has a maximum working pressure of 3 bar (43.5 psi) at 40°C in compatible fluids.

CAUTION: Operation outside the above specifications and/or with fluids incompatible with construction materials may cause personal injury and result in damage to the device.

Receipt of equipment

The male and female Kleenpak connectors are supplied in separate packages. There are several types of end fittings to accommodate different tubing size requirements, and to allow for different attachment possibilities to flexible tubing. To access full part number availability, visit the Pall website.

- Store both male and female Kleenpak connectors in a clean, dry environment, and kept in the external packaging wherever practical.
- DO NOT remove the connector from the inner device bag packaging until just before installation.
- Male and female Kleenpak connectors are supplied protected by an inner and outer bag. Ensure that the packaging is undamaged.
- The assembly aid is provided non-sterile, and can be reused multiple times. It must be stored in a clean, dry environment between each use. The assembly aid is supplied separately, and is available for purchase from your Pall representative.

Installation

Before installation, it is essential to verify that the Kleenpak connector is suitable for the liquid with which it will be in contact for the application. Use the following applicable guidelines.

- Install the male and female Kleenpak connectors using compatible connections.
- Ensure that the tubing is firmly attached to the hose barb to prevent leakage during operation using cable ties or another method.
- During tubing assembly, premature actuation of the male plunger is prevented by the anti-actuation ring. The anti-actuation ring must remain in place until the actual connection is made.
- The presence of valves on the tubing before the connector is recommended to prevent liquid contact with the Kleenpak connectors prior to use.
- If the connectors are to be autoclaved, orient them with the peel strips facing upward to prevent peel strip blockage by condensate.

CAUTION: The device must remain dry prior to connection of the male and female Kleenpak connectors. Do not use if there is fluid present in the line or around the devices, or if the protective cap has been removed.

CAUTION: These disposable Kleenpak connectors must not be in-line steam sterilized. Material design limitations will be exceeded when these devices are exposed to pressurized steam, and they will rupture.

Gamma irradiation

- Connect the male or female Kleenpak connector to the single-use system. A valve or clamp must be installed close to the connector to prevent accidental wetting after the system is filled with liquid.
- Ensure that the protective cap is firmly in place. Autoclave paper or another radiation-resistant material can be used to ensure that the cap does not become dislodged during handling.
- It is recommended that the entire assembly be placed in an inner and outer bag for protection prior to gamma irradiation.
- Treat with gamma radiation. The maximum allowable radiation dose is 50 kGy (5 Mrad).

Note: Pall recommends that the efficiency of the gamma irradiation cycle is validated using an appropriate method. These connectors have not been validated for repeated gamma irradiation exposure.

Autoclave instructions

- Install the male or female Kleenpak connector to the equipment to be autoclaved. If the Kleenpak connector is attached to a tank, the tank should be appropriately vented with a vent filter.
- Ensure that the protective cap of the Kleenpak connector is firmly in place. Autoclave paper or another autoclavable and air/steam-permeable material can be used to cover the cap loosely to ensure that the cap does not become dislodged during handling.
- The Kleenpak connectors should be allowed to vent during autoclaving. The venting strip should be oriented upward to prevent blockage by condensates.

CAUTION: To avoid the collection of condensate within the connectors, do not place the venting strip downward during autoclaving. The connector should not be covered with heavy objects during the autoclave cycle.

Notes:

- The maximum temperature for ACD part numbers is 121°C, and the maximum temperature for KPCHT part numbers is 130°C. The maximum exposure time is 75 minutes. Do not autoclave at a higher temperature or for a longer period of time. A slow exhaust cycle is recommended.
- Pall recommends that the efficiency of the autoclave cycle is validated using an appropriate method.

Making the connection

CAUTION: Do not use if fluid is in contact with the connector, or if the protective cap is loose or displaced.

See Figure 3.111 for a schematic of both the male and female parts of the Kleenpak connector.

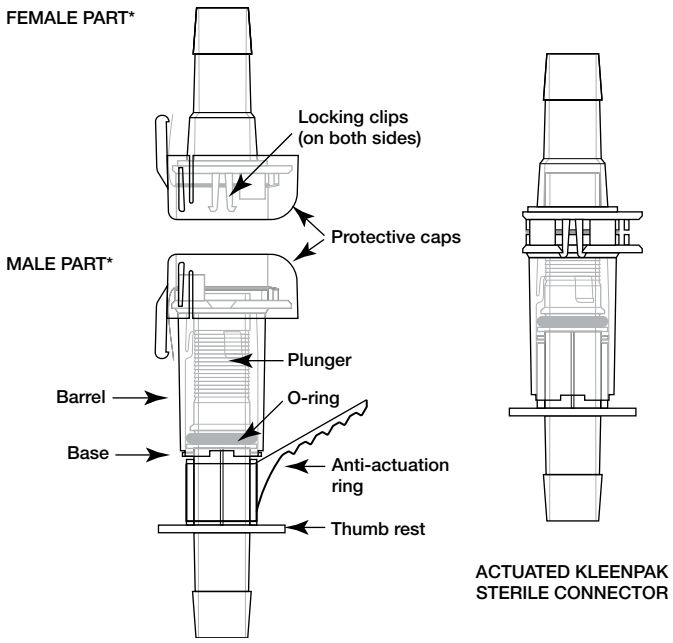


Figure 3.111. Kleenpak connector schematic.

Making the connection using the assembly aid

1. Lift and pull the tab off of the protective caps on the Kleenpak connectors (Figure 3.112).

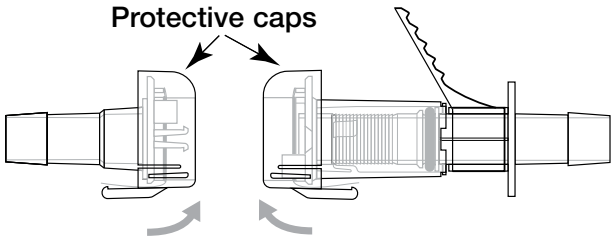


Figure 3.112. Removing tabs from protective caps.

2. Hold the barrel of the larger (male) Kleenpak connector above the base. Align the smaller (female) Kleenpak connector with the male connector. The flat sides should be aligned. Both peel-away strips must remain folded (Figure 3.113).

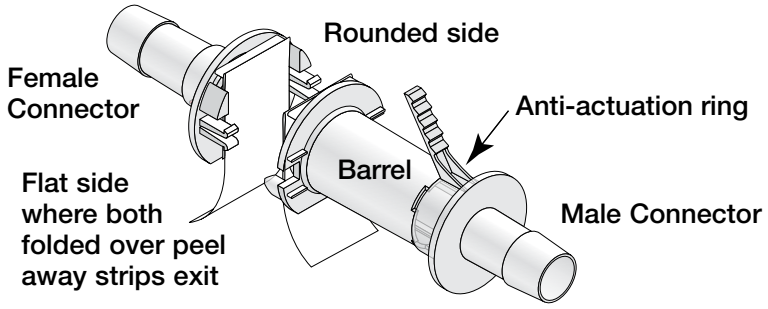


Figure 3.113. Connectors aligned.

Note: If the Kleenpak connectors are not aligned properly, the connection cannot be made.

3. After the connectors have been correctly aligned, firmly press them together until both locking clips snap together tightly (Figure 3.114).

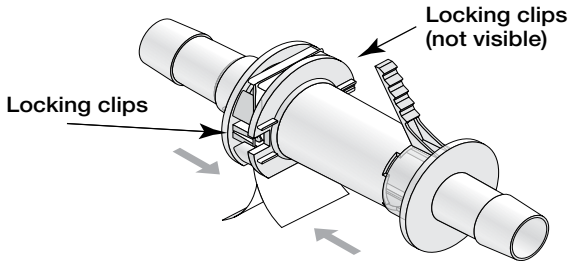


Figure 3.114. Locking clips snapped together.

4. Support both the male and female Kleenpak connectors, and remove the anti-actuation ring from the male connector by pulling the tab toward the barbed end of the male Kleenpak connector (Figure 3.115). A cross-section view is shown in Figure 3.116.

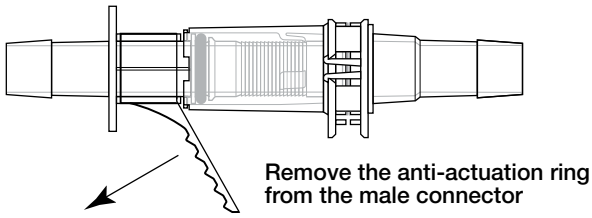


Figure 3.115. Removing anti-actuation ring.

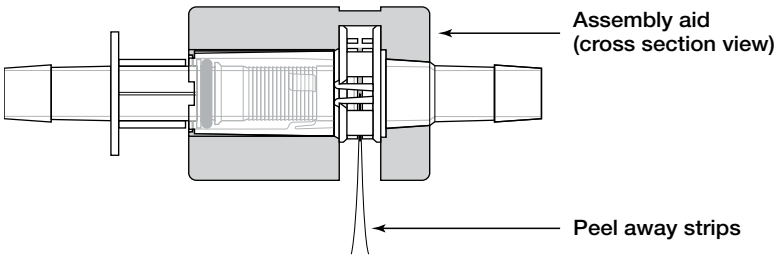


Figure 3.116. Cross-section view showing assembly aid.

Note: The Kleenpak connector should be securely seated in the assembly aid when properly installed.

5. Hold the assembly aid in the palm of your hand with the Kleenpak connector facing outward, and with your thumb supporting the Kleenpak connector in the assembly aid. Using your other hand, firmly grasp both of the peel-away strips as close as possible to the body of the assembly aid (to ensure a secure grip), and pull both peel-away strips simultaneously in one continuous movement. Ensure that the Kleenpak connector is perpendicular to the peel-away strips (Figures 3.117 and 3.118).

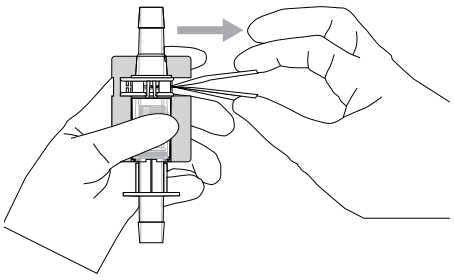


Figure 3.117. Firmly grasping both peel-away strips.

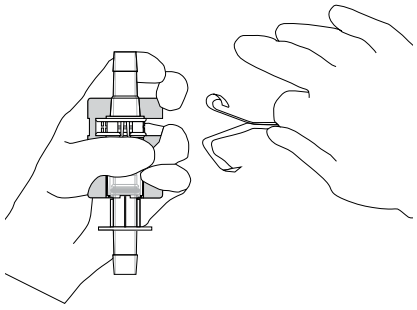


Figure 3.118. Pulling peel-away strips simultaneously.

CAUTION: Do not use if only one of the peel-away strips is removed.

6. With the Kleenpak connector still secured in the assembly aid, push the thumb rest of the male connector down toward the base of the barrel (Figure 3.119).

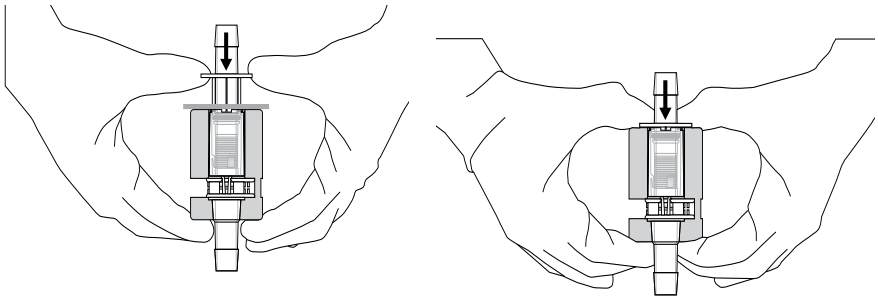


Figure 3.119. Pushing the thumb rest down toward the barrel.

Note: In order to establish a proper connection, the plunger inside the male connector must be fully inserted into the female connector. For verification, repeat actuation until a hard stop is reached. If necessary, the connector may be removed from the assembly aid to complete the plunger movement.

7. After the Kleenpak connector assembly is complete, the assembly aid may be removed. When the assembly aid is removed, verify actuation until a hard stop is reached. Then, begin fluid transfer (Figure 3.120).

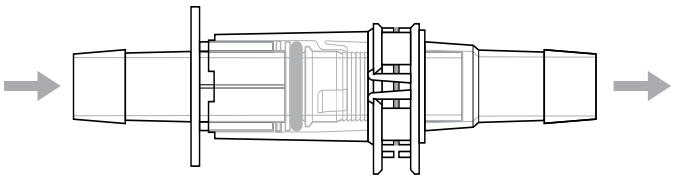


Figure 3.120. Beginning fluid transfer.

Making the connection without the assembly aid

1. Lift and pull the tabs off of the protective caps on the Kleenpak connector (Figure 3.121).

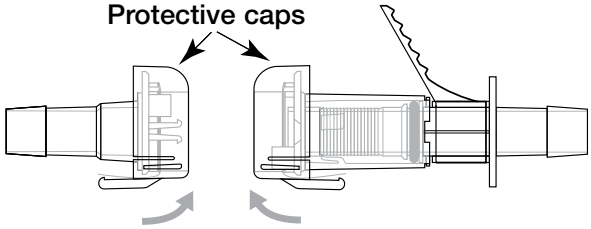


Figure 3.121. Pulling tabs from protective cap.

2. Hold the barrel of the larger (male) Kleenpak connector above the base. Align the smaller (female) connector with the larger connector. The flat sides should be aligned. Both peel-away strips must remain folded (Figure 3.122).

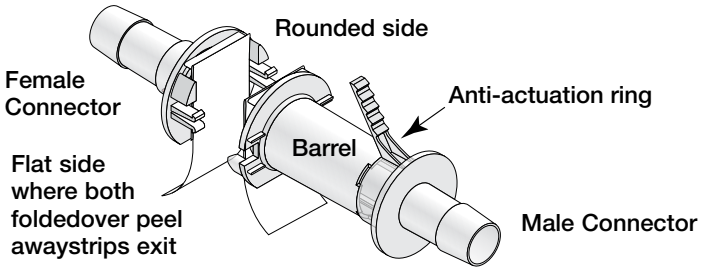


Figure 3.122. Aligning male and female connectors.

Note: If the Kleenpak connectors are not properly aligned, the connection cannot be made.

3. After the connectors have been properly aligned, firmly press them together until both of the locking clips snap together tightly (Figure 3.123).

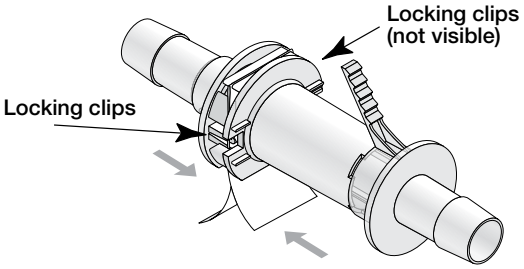


Figure 3.123. Locking clips snapping together.

4. Support both the male and female Kleenpak connectors, and remove the anti-actuation ring from the male connector by pulling the tab toward the barbed end of the male connector (Figure 3.124).

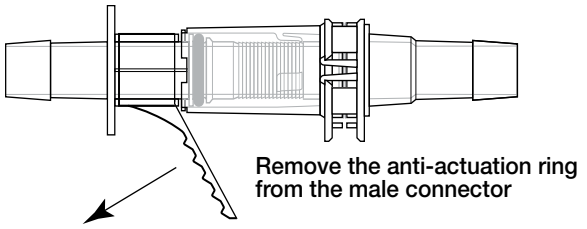


Figure 3.124. Removing anti-actuation ring.

5. With one hand, support the male and female sides of the Kleenpak connector by wrapping your finders around both sides of the connector, next to the flange. Using your other hand, grab both while peel-away strips as close as possible to the flat side of the connector to ensure a good grip, and pull them out simultaneously in one continuous movement (Figures 3.125 and 3.126). Ensure that the connector is perpendicular to the peel-away strips shown in Figure 3.125. The perpendicular orientation must be maintained while the two strips are pulled simultaneously. **CAUTION:** Do not use if only one of the peel-away strips is removed instead of both.

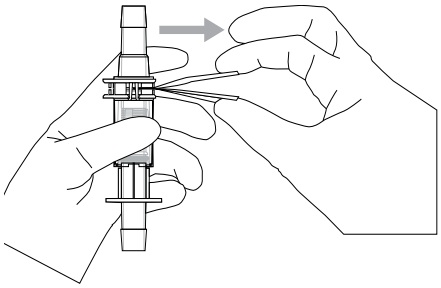


Figure 3.125. Firmly grasping both peel-away strips.

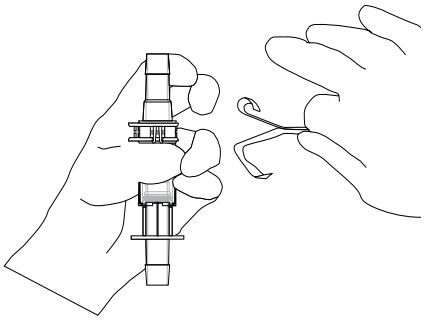


Figure 3.126. Pulling peel-away strips simultaneously.

6. Push the thumb rest of the male Kleenpak connector down toward the base of the barrel until they meet (Figure 3.127).

Figure 3.127. Pushing the thumb rest down toward the barrel.

Note: In order to establish a proper connection, the plunger inside the male connector must be fully inserted into the female connector. For verification, repeat actuation until a hard stop is reached. Then, begin fluid transfer (Figure 3.128).

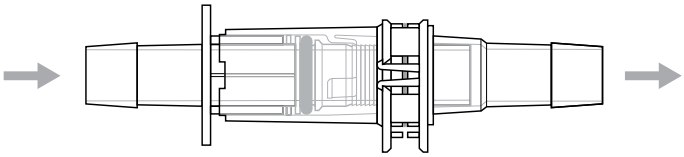


Figure 3.128. Beginning fluid transfer.

3.5.3 Probe insertion

Before beginning probe insertion, please become familiar with the Kleenpak connector procedures outlined in section 3.5.2. If you are using CPC AseptiQuik aseptic connectors instead, consult your sales representative for these procedures.

1. Attach probe clips onto the outer support container above the probe assembly (Figure 3.129). Plastic probe clips slide on with firm pressure.

Figure 3.129. Attaching probe clip.

2. Install the pre-sterilized sensor and probe kit using the aseptic connection methods described in section 3.5.2. The aseptic connection is completed prior to the bellows being collapsed.

3. Insert the probe by collapsing the bellows (Figures 3.130 and 3.131). **Note:** If the BPC is already filled with liquid, the best practice is to squeeze the bellows to expel air prior to collapsing it. Then insert the probe fully, as described.

Figure 3.130. Probe insertion.

Figure 3.131. Collapsed bellows.

4. Position the probe clip in the desired horizontal location. Lift the probe and set it into the probe clip.

5. Rest the probe in the bellows hook (Figure 3.132). Release the probe assembly and verify that the probe remains at the proper insertion depth and angle when the bellows expand to rest freely in the probe clip.

Figure 3.132. Probe resting in the hook.

3.5.4 Probe calibration

Probe calibration is controller-specific; however, the following general rules apply:

- If you are using a liquid batch-to-tank grounding cable with the stainless steel connector of the sample line, the sample line should be purged of air prior to probe calibration.
- pH probes must be calibrated prior to steam sterilization; the calibration of the probe can be standardized by comparison of an off-line sample once the pH probe has been connected to the S.U.B.

- Dissolved oxygen (DO) probes are generally calibrated after steam sterilization. They can be calibrated once the probe is connected to the S.U.B. and is given time to polarize (six to eight hours of continuous connection to the power supply provided by a controller or polarization module).

3.6 Cell culture operating instructions

3.6.1 Operating conditions for cell culture applications

Optimal operating parameters for cell culture vary greatly between cell lines and media formulations. Table 3.7 in section 3.6.8 is provided as a reference for establishing safe upper operating control limits with the standard BPC design. Exceeding these operating limits may result in premature exhaust filter failure, excessive foaming, and excessive pressure build-up in the gas delivery line sets or the BPC.

In many cell culture operations, the limits listed in Table 3.7 are excessive, and should be further reduced when possible. When reducing gas flow rate limits, the following trade-offs should be expected:

- Reducing drilled hole sparger maximum operating limits will reduce system foaming, but will increase oxygen reliance. A suggested gas operating control strategy is to run the drilled hole sparger on air initially, and after total flow rate limits are reached, substitute oxygen as shown in Graph 3.6.
- Reducing overlay maximum operating limits will reduce the exhaust load (increasing filter lifespan), but will sacrifice sparger performance if reduced far enough to allow carbon dioxide buildup in the headspace.
- Reducing macro sparge maximum operating limits will reduce system foaming and exhaust load, but will also reduce system carbon dioxide stripping potential, leading to carbon dioxide buildup in the culture solution. This can result in large amounts of base addition to maintain the desired pH.

If cell culture density is not increasing at expected rates, this may be due to carbon dioxide buildup in the headspace. Increasing air flow in the headspace may resolve this problem.

3.6.2 Checkpoints prior to media fill

Verify the following before proceeding to liquid fill.

- ✓ The BPC has been loaded into the hardware by following the instructions provided in sections 3.2, 3.3, or 3.4.
- ✓ All aseptic connector port heavy-duty clamps are closed and located as close as possible to the BPC.
- ✓ The exhaust filter is upright and secured using the holder.
- ✓ The clamp on the drain tube is closed and located as close as possible to the BPC.
- ✓ The RTD/temperature sensor is completely seated and secured in the thermowell.
- ✓ The air-filled BPC is properly oriented in the outer support container and the BPC bottom tabs are secured.
- ✓ The gas line sets are connected to the drilled hole sparger and the overlay sparger.
- ✓ All gas filters are placed above the maximum liquid level.
- ✓ The load cell display has been tared.
- ✓ All sensors are inserted and connected to their respective transmitters. Sensors must be properly oriented to ensure that they are below the liquid level after media fill.

3.6.3 Media fill

1. Select the desired line set from the BPC for fluid introduction.

2. Make an aseptic connection (tubing welder, quick connect, or tri-clamp), and begin liquid fill.

3. When approximately 20–30 liters have been added, verify the position of the BPC in the outer support container, particularly the sparger and the drain line. Adjust positioning if necessary for proper fit. **Note:** Do not add more than 30 liters at this stage, as the excess weight will make the BPC difficult to adjust.

4. Pull the top corners of the BPC upward to reduce wrinkles during filling. **Note:** If the BPC wrinkles are not eliminated during liquid fill, excessive film tension below the bearing port will result.

5. Fill to the desired liquid volume; 50–100% of the rated volume is recommended.

6. Ensure that all sensors are below the liquid level after the BPC has been filled.

3.6.4 Agitation for units with E-Boxes

1. **After the media has reached half of the S.U.B. volume**, use the motor controller power switch to start agitation using the E-Box (Figure 3.133).

Figure 3.133. Front view of E-Box for 50–2,000 L S.U.B.s.

Table 3.3. Recommended agitation rates. The values given are based on a standard scale-up criteria of power input to working volume (P/V) using an estimated power impeller number of 2.1, not the parameters of the motor. For rated maximum and minimum operating speeds for the motor, see the hardware specifications in Chapter 4 of this user's guide. For information on calculating agitation rates using power input to volume ratios, see section 3.6.5.

Power ratio	10 Watts per meters^3 (rates in rpm)		Nominal agitation 20 Watts per meters^3 (rates in rpm)		40 Watts per meters^3 (rates in rpm)		Impeller diameter (cm)
	50% working volume	100% working volume	50% working volume	100% working volume	50% working volume	100% working volume	
50 L	115	145	145	183	183	230*	11.1
100 L	92	116	116	146	146	184	14.6
250 L	74	93	93	117	117	148	20.0
500 L	64	80	80	101	101	127	25.1
1,000 L	53	67	68	86	86	109	32.1
2,000 L	47	59	60	75	**	**	39.8

* This value is outside the recommended operating motor range of 30–200 rpm (variable frequency drive (VFD) settings). See Table 4.1 in Chapter 4.

** Consultation with Thermo Scientific engineers is required.

2. Using the arrow keys on the motor speed control keypad, adjust the setpoint speed to the desired level. The adjustment of the stirring speed rpm is done using Hz. The display reverts back to displaying rpm after 2–3 seconds of inactivity. Adjust desired agitation rate within the recommended range as described in Table 3.3.

3. Allow the speed to stabilize, then make fine adjustments if necessary.

WARNING: Agitation must be stopped or slowed to the minimum mixing speed when the volume falls below 50% of the rated working volume, otherwise damage to the hardware or BPC may result. To prevent damage to your system, use safety interlocks to prevent agitation from running below the minimum volume. See the Warnings, safety, and warranty information section in the front of this publication and section 3.6.5 for more information about using safety interlocks and agitation speed governors on the controller.

3.6.5 Agitation rate calculations

Using power input to volume ratios for agitation speed

The power input to volume (P/V) ratio allows for scale-up or scale-down of bioreactor platforms by equating mixing power between differently-sized systems. P/V is the most broadly accepted method for determining a practical scale-up approach for agitation speed in stirred tank reactors used for animal cell culture. This relationship is dependent on the density of the liquid in the system (ρ), the inherent power number of the impeller (Np), the diameter of the impeller (Di), the volume of liquid in the vessel (V), the speed of the drive shaft in rpm (n), and the power input to the liquid (P).

$$P/V = \frac{N_p * \left(\frac{n}{60}\right)^3 * \rho * {D_i}^5}{V}$$

This relationship can be rearranged to solve for mixing speed as a function of the other variables.

$$n = 60 * \left(\frac{P/V * V}{N_p * \rho * {D_i}^5}\right)^{\frac{1}{3}}$$

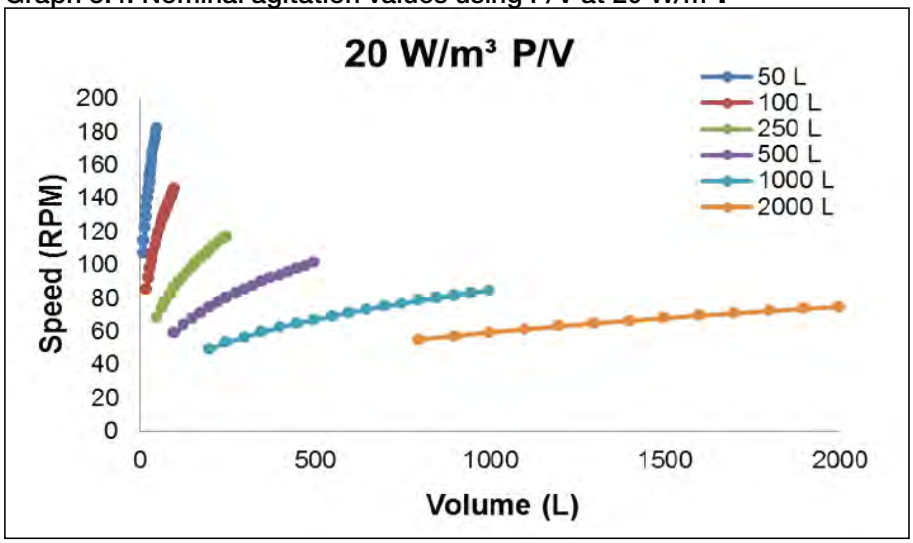
For known values of P/V, impeller power number, density, and impeller diameter, this equation can be simplified by consolidating all other values into a single coefficient (A).

$$n = A * V^{\frac{1}{3}}$$

Values for appropriate agitator drive shaft speed were calculated for different sizes of S.U.B.s using 20–100% of the rated working volumes. These values assume an impeller power number of 2.1, viscosity similar to water, and a constant density of 993 kg/m^3.

Values of 20 watts/meter^3 (W/m^3) provide the nominal rating (the suggested default parameter for CHO cultivation), and values of 40 W/m^3 provide the maximum recommended parameter that has been qualified for use in the system. Graph 3.4 is provided as a reference, showing the 20 W/m^3 P/V curve. While some discrete values are shown, in practice it is acceptable to operate at lower speeds to suit special needs.

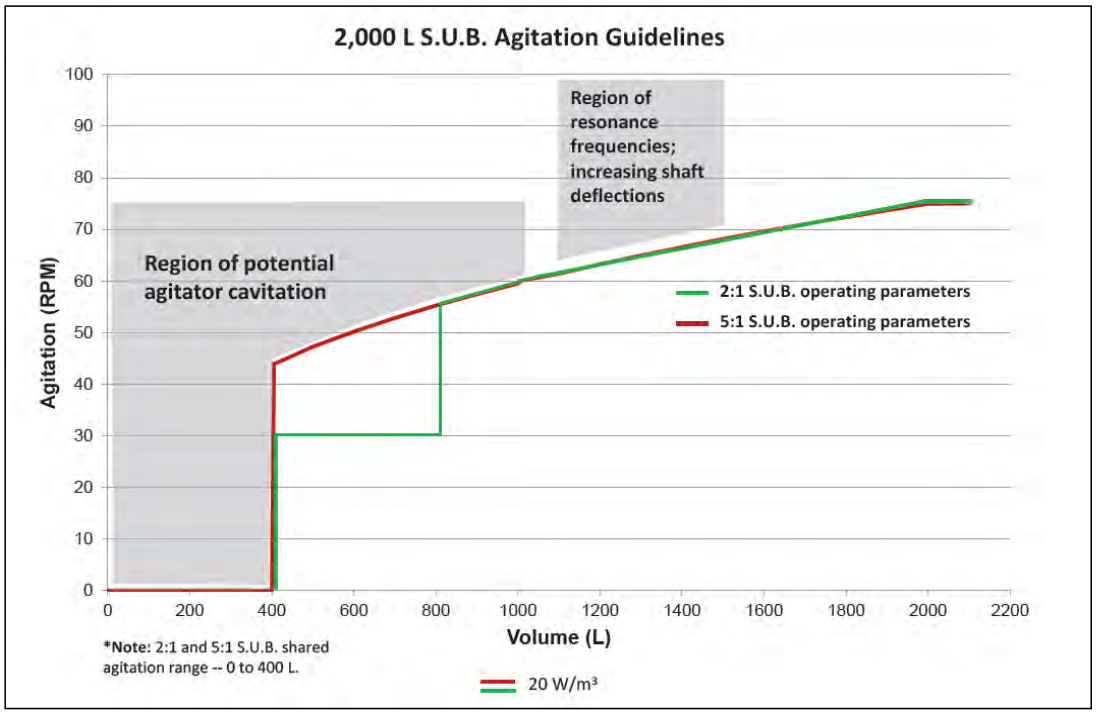
Graph 3.4. Nominal agitation values using P/V at 20 W/m^3.



Fed batch cultures present unique challenges to the operation of the 2,000 L S.U.B., because the fluid height can change dramatically through the duration of a fed batch cell culture run. This drastic change in operating fluid height requires a very long agitator shaft supporting the impeller, requiring it to sustain significant forces of rotating torque and deflection of stress. The 2,000 L S.U.B. was originally qualified to operate reliably when using discrete working volumes of either 1,000 or 2,000 L with a maximum recommended operating speed of 75 rpm. This represented a worst case P/V of 40 W/m^3 (1,000 L) and best case of 20 W/m^3 (2,000 L) as the extra volume and column height at 100% working volume offer significant protection against drive shaft instability.

An in-depth study of the 2,000 L S.U.B. has shown that harmonics can begin to propagate fatigue into the drive shaft when the system is operated between 1,100 and 1,500 L when the agitator P/V is above 20 W/m^3. In order to maximize shaft life and reliability, it is important to scale P/V with this constraint in mind. See Graph 3.5 for values of potential cavitation and harmonics. The green curve represents a 20 W/m^3 P/V. It is recommended to operate your S.U.B. at agitation values below the green curve.

Graph 3.5. Regions of potential agitator harmonics and cavitation for various liquid working volumes of the 2,000 L S.U.B.



P/V agitation values for all S.U.B. sizes

Equation coefficient values for every size of S.U.B. for P/V values of 20 and 40 W/m^3 are provided in Table 3.4. Agitation speeds for P/V values of 20 and 40 W/m^3 are provided in Tables 3.5 and 3.6.

Table 3.4. Equation coefficient values (A) for different S.U.B.s.

$n = A \times V^{1/3}$ (for values of n in rpm)						
	50 L S.U.B.	100 L S.U.B.	250 L S.U.B.	500 L S.U.B.	1,000 L S.U.B.	2,000 L S.U.B.
P/V						
20 W/m^3	49.650	31.491	18.634	12.764	8.471	5.950*
40 W/m^3	62.554	39.676	23.477	16.081	10.673	7.031**

* Coefficient value is used to determine operating speeds for drive shafts implemented for a 180-day working duration at 20 W/m^3.

** Consult Thermo Scientific engineers for the drive shaft working duration at > 20 W/m^3.

Table 3.5. Agitation rates (in rpm) for S.U.B.s at a P/V of 20 W/m^3.

	50 L S.U.B.	100 L S.U.B.	250 L S.U.B.	500 L S.U.B.	1,000 L S.U.B.	2,000 L S.U.B.*
Fill %						
100	183	146	117	101	86	75
95	180	144	115	100	83	74
90	177	141	113	98	82	72
85	173	138	111	96	80	71
80	170	136	109	94	79	70
75	166	133	107	92	77	68
70	162	130	104	90	75	67
65	158	127	102	88	73	65
60	154	123	99	85	71	63
55	150	120	96	83	69	61
50**	145	116	93	80	68	60
40	135	108	86	75	62	30
35	129	103	83	71	60	30
30	122	98	79	68	57	30
25	115	92	74	64	53	30
20	107	85	69	59	50	30

* Using these rpm values requires replacement of the drive shaft after 180 days of use.

Note: System motor speed/volume control to be controlled by a speed governor system (limit operation within P/V guidance). Single run requires that < 80% of batch agitation time occur between 1,100 and 1,700 L liquid volume. Operational parameters have been qualified for reliability according to good engineering practices by using water and air sparge to simulate bioreactor cell culture operating conditions. They will not take into account defects related to improperly maintained equipment, lack of proper operator training, or use outside of the qualified operating parameters.

** Values (in rpm) assume the system is operating at no less than 50% volume. In order to ensure proper volume measurements, end users are responsible for ensuring proper load cell calibration prior to system use.

Table 3.6. Agitation rates (in rpm) for S.U.B.s at a P/V of 40 W/m^3.

	50 L S.U.B.	100 L S.U.B.	250 L S.U.B.	500 L S.U.B.	1,000 L S.U.B.	2,000 L S.U.B.
Fill %						
100	230	184	148	127	109	*
95	227	181	145	125	105	*
90	222	178	143	123	103	*
85	218	174	140	121	101	*
80	214	171	137	118	99	*
75	209	167	134	116	97	*
70	205	164	131	113	95	*
65	200	160	128	111	92	*
60	194	155	125	108	90	*
55	189	151	121	105	87	*
50**	183	146	117	101	86	*

* Consultation with Thermo Scientific engineers is required when operating 2,000 L S.U.B.s at a P/V of > 20 W/m^3.

Note: System motor speed/volume control to be controlled by a speed governor system (limit operation within P/V guidance). Single run requires that < 80% of batch agitation time occur between 1,100 and 1,700 L liquid volume. Operational parameters have been qualified for reliability according to good engineering practices by using water and air sparge to simulate bioreactor cell culture operating conditions. They will not take into account defects related to improperly maintained equipment, lack of proper operator training, or use outside of the qualified operating parameters.

** Values (in rpm) assume the system is operating at no less than 50% volume. In order to ensure proper volume measurements, end users are responsible for ensuring proper load cell calibration prior to system use.

Note: The impeller power number assumption of Np=2.1 is only an approximation, based on an academic exercise. Recommended best practice for determination and comparison of Np between bioreactors should be carried out by the end user and should be based on conditions matching the specific application. End users should also anticipate and accept some variability that may be inherent in the analysis technique or modeling method chosen. If a different or alternative Np value is determined by the end user, the safety interlocks must be based solely on the maximum prescribed rpm for a working volume as found in the reference tables in this publication. **Never rely strictly on an estimated magnitude of P/V when setting the agitator speed interlock levels.**

3.6.6 Drive shaft rotation

Verify that the drive shaft is rotating counterclockwise when viewed from the top looking down. The S.U.B. is designed to mix in this direction only.

3.6.7 Temperature control

Temperature control for 50–250 L resistive systems with E-Boxes

1. After agitation has been set, use the left button on the electric heater control keypad to turn on the temperature controller (see Figure 3.133 in section 3.6.4).

2. Adjust the temperature set value using the up and down arrows located to the right of the keypad. The process temperature value will be shown in red.

Temperature control for all jacketed systems

Temperature setpoints are controlled by the TCU or controller. Refer to the TCU/controller manufacturer's guidelines for setup and operating instructions.

1. Connect to an external TCU using the large couplings located on the vessel jacket. Ensure the inlet/outlet ports are connected properly; improper installation may result in poor heating/cooling performance.

2. Open the valves after connecting the TCU (Figure 3.134).

Figure 3.134. Opening valves.

Note: The water jacket should be purged of air any time the vessel jacket lines are reconnected. To purge the water jacket, open the bleed valve located near the bottom of the S.U.B. A container may be needed to catch any glycol that is released. Close the valve as soon as glycol begins flowing.

3.6.8 Sparging strategy

Optimal operating parameters

Optimal operating parameters for cell culture vary greatly between cell lines and media formulations. Tables 3.7 and 3.8 (later in this section) are provided as a reference for establishing safe upper operating control limits with the standard BPC design. Exceeding these operating limits may result in premature exhaust filter failure, excessive foaming, and excessive pressure build-up in the gas delivery line sets or the BPC.

In many cell culture operations, the limits listed in Tables 3.7 and 3.8 are excessive, and should be further reduced when possible. When reducing gas flow rate limits, the following trade-offs should be expected:

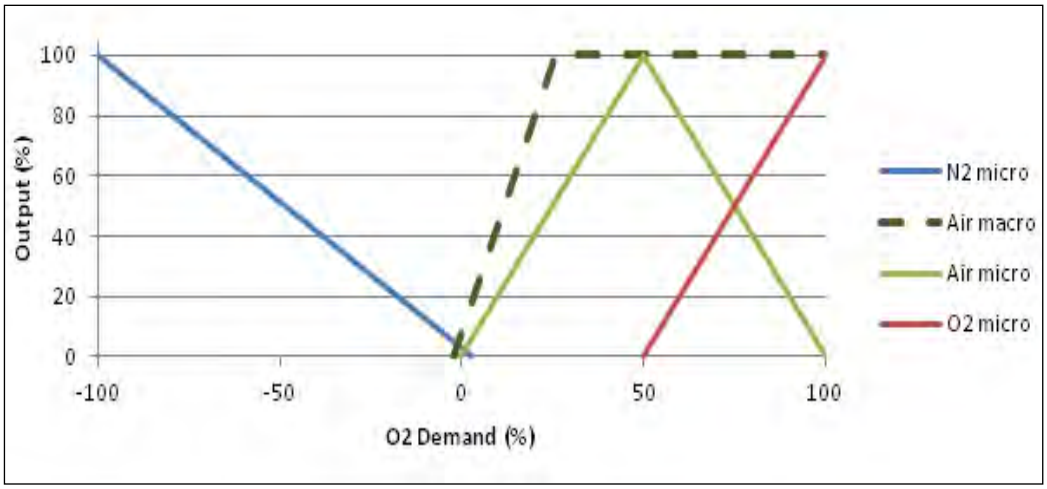
- Reducing porous frit maximum operating limits will reduce system foaming but increase reliance on oxygen and/or the macro sparge (open pipe or drilled hole sparge) if reduced too far below system oxygen demand. Generally speaking, it is best to run the porous frit on air initially, and after the porous frit total flow rate limits are reached, substitute oxygen as shown in Graph 3.6 in the following section.
- Reducing overlay maximum operating limits will reduce exhaust load (increase filter lifespan), but if it is reduced far enough to allow carbon dioxide buildup in the headspace, which will sacrifice sparger performance.
- Reducing macro sparge maximum operating limits will reduce system foaming and exhaust load but also reduce system carbon dioxide stripping potential, leading to carbon dioxide buildup in the culture solution, which could result in large amounts of base addition to maintain the desired pH.

If sparging seems to lose efficiency disproportionate to the cell culture density increase, and if the efficiency loss is due to carbon dioxide buildup in the headspace, increasing the headspace flow rate may help.

Gas supply setup for dual sparger systems with micro and macro delivery systems

Graph 3.6 depicts a DO management strategy. Refer to Tables 3.7 and 3.8 for gas flow rate recommended maximum values. In developing a gassing strategy for a S.U.B. using a dual sparger configuration, it is optional to have a crossover from nitrogen (N_2) to air when progressing from negative to positive DO control action (Graph 3.6 depicts a minor crossover).

Graph 3.6. DO control strategy for 2:1 S.U.B. systems.



Gas flow rates for BPCs with open pipe and porous frit spargers

Table 3.7 (on the following page) contains a listing of gas flow rates for all sizes of S.U.B.s with open pipe and porous frit spargers. This data should be used in specifying maximum volumes for mass flow controllers or rotameters. In optimal conditions (no condensation or fouling), the exhaust filters have a flow capacity of at least 20 slpm at 0.007 bar (0.1 psi). The total flow rate of gas into the system must be less than 20 slpm per active exhaust filter. The values listed take into account the number of exhaust filters that are standard on each size of S.U.B. (one 6 in. filter for 50, 100, and 250 L systems, and two 6 in. filters for the 500 and 1,000 L systems). The components of the 2,000 L S.U.B. are scaled up to allow flow ratings of 50 slpm at 0.007 bar (0.1 psi). These values are a good rule of thumb, but are not absolute requirements. They are also not intended to be process gas flow settings. The process gas flow settings should be adjusted as discussed below, with starting conditions not exceeding 50% of the listed maximum values in order to help avoid accidental foam-out of the exhaust filter.

Table 3.7. Range of operating parameters with open pipe and porous frit spargers.

	50 L			100 L			250 L			500 L			1,000 L			2,000 L		
Temperature (°C)	2.0–40.0 ± 0.1																	
Operating volume (L)	25–50			50–100			125–250			250–500			500–1,000			1,000–2,000		
Recommended max. gas flow rates (slpm)	Open pipe	Porous frit	Overlay	Open pipe	Porous frit	Overlay	Open pipe	Porous frit	Overlay	Open pipe	Porous frit	Overlay	Open pipe	Porous frit	Overlay	Open pipe	Porous frit	Overlay
Air	1	0.5	5	2	1	10	5	2.5	10	10	5	15	10	8	15	12	16	15
O_2	-	0.25	-	-	0.5	-	-	1.25	-	-	2.5	-	-	4	-	-	8	-
CO_2	-	0.1	-	-	0.2	-	-	0.5	-	-	1	-	-	1	-	-	1	-
N_2	-	0.25	-	-	0.5	-	-	1.25	-	-	2.5	-	-	2.5	-	-	2.5	-
Total	1	0.85	5	2	1.7	10	5	4.25	10	10	8.5	15	10	13	15	12	25	15
Exhaust load	20			20			20			40			40			90		

Gas flow rates for BPCs with drilled hole and porous frit spargers

If foaming or exhaust filter load/lifespan is of primary concern, priority should be placed on tuning the system to operate primarily by adding oxygen through the porous frit. If carbon dioxide stripping is of primary concern, priority should be placed on running the macro sparge at flow rates sufficient to reduce or eliminate the need for base addition.

Table 3.8 on the following page contains a listing of values for all sizes of S.U.B.s. This data may be used in specifying maximum gas flow rates for mass flow controllers or rotameters, when using drilled hole and porous frit spargers. In optimal conditions (no condensation or fouling), the exhaust filters have a flow capacity of at least 20 and 90 slpm at 0.007 bar (0.1 psi) for the small and large standard equipped filter types, respectively. The total flow rate of gas into the system must be less than the sum flow rate capacity of active exhaust filters. The values listed take into account the number and type of exhaust filters that are standard on each size of the S.U.B. (one small filter installed on the 50 and 100 L systems, one large filter on the 250 and 500 L systems, two large filters on the 1,000 L system, and three large filters on the 2,000 L system). These values are not absolute requirements. They are also not intended to be process gas flow settings. The process gas flow settings should be adjusted as discussed below with starting conditions not exceeding 25% of the listed maximum values to prevent unnecessary reduction of exhaust filter life span and foam generation.

Table 3.8. Range of operating parameters with drilled hole and porous frit spargers.

	50 L			100 L			250 L			500 L			1,000 L			2,000 L		
Temperature (°C)	2.0–40.0 ± 0.1																	
Operating volume (L)	25–50			50–100			125–250			250–500			500–1,000			1,000–2,000		
Recommended max. gas flow rates (slpm)	Drilled hole	Porous frit	Overlay	Drilled hole	Porous frit	Overlay	Drilled hole	Porous frit	Overlay	Drilled hole	Porous frit	Overlay	Drilled hole	Porous frit	Overlay	Drilled hole	Porous frit	Overlay
Air	2.5	1	5	5	2	10	12	4	14	25	6	35	100	8	60	200	16	1292
O_2	-	1	-	-	2	-	-	4	-	-	6	-	-	8	-	-	16	-
CO_2	-	0.25	-	-	0.5	-	-	1	-	-	1.5	-	-	2	-	-	4	-
N_2	-	1	-	-	2	-	-	4	-	-	6	-	-	8	-	-	16	-
Total	2.5	1.25	5	5	2.5	10	12	5	14	25	7.5	35	100	10	60	200	20	129
Exhaust load	20			20			90			90			180			270		

Note: 2,000 L S.U.B.s may require additional exhaust filters if running drilled hole and porous frit spargers at maximum capacity and overlay is run over 50 slpm. The standard drilled hole plus porous frit dual sparge 2,000 L BPC is designed to safely handle an exhaust load of up to 270 slpm.

Manual operation of drilled hole spargers

Table 3.9 (on the following page) provides guidelines for operating a BPC equipped with standard drilled hole and porous frit spargers when the user must manually set flow rates for the drilled hole sparger. Recommendations assume three manual flow rate adjustments of the drilled hole sparger during the course of a batch run, and are balanced with priority to maintain even to excessive carbon dioxide stripping throughout the culture operation.

WARNING: Operating caution must be taken when manually setting drilled hole sparger gas flow rates if the drilled hole sparger is left inactive for any length of time. It is possible for liquid to flow between the drilled hole sparger and the flow check valve if it is left idle. Never set an idle drilled hole sparger directly to values higher than 10% of their maximum recommended flow rate. Pushing the water through the drilled hole sparger pores at higher flow rates may result in a damaging pressure spike. After the drilled hole sparger has been purged, it can be safely set to the target gas flow rate.

For more information on growth of specific cell lines in the S.U.B. system, please contact technical support.

Table 3.9. Recommended operation in standard dual sparger BPC configuration using manual drilled hole sparger operation. Assumes a 5 day log growth phase.

	50 L S.U.B.	100 L S.U.B.	250 L S.U.B.	500 L S.U.B.	1,000 L S.U.B.	2,000 L S.U.B.
Stage 1: Seed to 1/2 log growth (seed to day 2.5)	0.015 vvm	0.0138 vvm	0.0125 vvm	0.09132 vvm	0.0126 vvm	0.0175 vvm
Stage 2: 1/2 log growth to 3rd quarter log growth (day 2.5 to day 4)	0.0288 mid vvm	0.027 mid vvm	0.025 mid vvm	0.0268 mid vvm	0.0304 mid vvm	0.375 mid vvm
Stage 3: 3rd quarter log growth through stationary phase (day 4 and beyond)	0.0538 final vvm	0.052 final vvm	0.05 final vvm	0.0539 final vvm	0.0618 final vvm	0.0775 final vvm

Note: Recommendation is based on a porous frit operated by a controller and CHO Gpex culture run using CDM4CHO with 6 g/L Cell Boost 2 in a 250 L S.U.B. at 250 L liquid volume, 37°C, 50% DO, pH 7, and 124 rpm agitation (approximately 19.7 W/m3). Recommendation also assumes culture is capable of reaching near 10+E6 cells/mL density and has a log growth phase of approximately five days. Values for 100 , 500, and 1,000 L drilled hole sparger parts are estimates based on drilled hole sparger parameter scaling. End users should always verify performance in their specific operating environment.

3.6.9 pH probe calibration

If employing a liquid batch-to-tank grounding cable with the stainless steel connector of the sample line, the sample line should be purged of air prior to probe calibration.

In general, the pH probe calibration (post-autoclave) can be verified by pulling a sample and analyzing the pH on another calibrated pH meter.

3.6.10 DO probe calibration

After polarizing for six to eight hours, the DO probe can be calibrated in the S.U.B. using standard protocols. Turn on full air sparging. Do not exceed the maximum gassing rates listed in Tables 3.7 and 3.8 in section 3.6.8. Sparge until the DO reading stabilizes. Use the stabilized value as the 100% DO setpoint for the controller. Set the zero percent DO setpoint by sparging with nitrogen or by unplugging the DO probe momentarily.

3.6.11 Checkpoints prior to inoculation

Before inoculation, verify that:

- ✓ After the S.U.B. is filled with media (to 50% or more volume), BPC tabs have been disconnected from the S.U.B. hardware.
- ✓ The pH probe is calibrated, autoclaved, and connected via an aseptic connector port. Perform 1-point offset on your controller as necessary.
- ✓ The DO probe is autoclaved, connected via an aseptic connector port, polarized (six to eight hours), and calibrated.
- ✓ The RTD/temperature sensor is completely seated and secured in the thermowell.
- ✓ Ensure that all sensors are below the liquid level.
- ✓ Operating parameters (temperature, agitation, pH, and DO) are at the desired setpoints.
- ✓ A method for making aseptic tubing connections is available.

3.6.12 Cell inoculation

Once the S.U.B. is operating at the targeted steady equilibrated state and has achieved the proper temperature, the S.U.B. is ready for inoculation. Connect the inoculum addition line set to the seed culture vessel (equipped with the proper connectors/tubing) and transfer the inoculum into the S.U.B. Typically this is done with the tubing connection process (aseptic luer lock connection or tube welding) and peristaltic pump. Pump the desired volume of seed cells into the S.U.B.

Note: For shear sensitive cultures, cells can be introduced by manipulating the addition port to direct the inoculum down the interior wall of the BPC and into the bulk fluid, reducing the shear on the cells. Custom line sets can be supplied with dip tubes, which shorten the distance between the point of inoculum introduction and the bulk fluid level.

3.6.13 Volume scale up

1. Using a sterile process, connect media to the BPC with the media fill port.

2. Begin pumping media into the BPC at the desired flow rate. Ensure that the vessel temperature does not drop below culture limits.

3. Increase the volume to the desired level.

3.6.14 In-process checkpoints

Verify the following once or twice daily during the culture run.

- ✓ Rising bubbles are visible through the access window.
- ✓ Process parameters, such as temperature and agitation, are at the setpoint.
- ✓ The BPC is not operating under pressure.
- ✓ The cap is tight on the drive shaft.
- ✓ The RTD/temperature sensor is completely seated and secured.
- ✓ No condensate accumulates in exhaust filter housing. Accumulated condensate indicates that the use of a filter heater is required.

Noise note: Noise may be emitted from the mixing assembly during operation. This noise may vary in intensity and frequency, but generally has no significant effect on performance or overall durability of the BPC during the intended life of the product.

3.6.15 BPC sampling

During operation of the S.U.B., samples may need to be taken for monitoring of various parameters established by the user. The following sections describe two techniques for sampling: aseptic sampling with a sterile syringe, and sampling with a sterile manifold.

Aseptic sampling

Using a standard luer lock on a 60 mL syringe or manifold:

1. Remove the dust cover from the SmartSite™ needle-free valve, which is connected to the end of the sample port (Figure 3.135).

Figure 3.135. Removal of dust cover from SmartSite.

2. Clean the SmartSite with a sanitary wipe.

3. Connect the sanitary luer lock type syringe (Figure 3.136).

Figure 3.136. Connecting the syringe.

4. To purge the sample line, apply a small amount of vacuum pressure by pulling out the syringe plunger slightly.

5. Open the pinch clamp and pull sample (approximately 20 mL), using care not to allow any back flow.

6. Close the pinch clamp and remove the syringe. This will be a purge sample.

7. Clean the SmartSite with a sanitary wipe.

8. Connect the sanitary luer lock type syringe.

9. Pull the sample by applying a small amount of vacuum pressure using the syringe.

10. Open the pinch clamp and pull the desired sample volume (approximately 10–20 mL), using care not to allow any back flow.

11. Close the pinch clamp and remove the syringe. This will be a representative sample.

12. Clean the SmartSite with a sanitary wipe and replace the dust cap.

Sampling with a sterile manifold

Use the following steps to attach a sample manifold (if purchased).

1. Remove the manifold from its protective polybag package.
2. Close all of the clamps on the manifold lines.
3. Use a sterile tubing welder to connect the manifold to the sample line (Figure 3.137).

Figure 3.137. Tubing welder.

4. Inspect the welds and open flow path by pinching the welds.
5. Open two clamps at the inlet and the clamp at the purge container (100 mL container).
6. Purge the sample line by filling the purge container (30–60 mL is recommended).
7. Close the clamp nearest to the purge container.
8. Open the clamp to the sample container (50 mL container) (Figure 3.138).

Figure 3.138. Manifold—50 mL.

9. Allow the container to fill with liquid by the force of gravity (10–20 mL is recommended).

10. Close the clamps at the sample manifold inlet.

11. Close the clamps nearest the sample container.

12. Remove the filled manifold from the S.U.B. by welding a new manifold onto the sample line, which will be used for taking the next sample.

3.6.16 Dispense and harvest

1. Connect the bottom drain tubing set to the intended transfer line.

2. Open the clamp positioned at the bottom drain port.

3. Begin to drain, using a peristaltic pump.

4. Stop the impeller motor when volume reaches 20% maximum volume.

5. Remove and store the drive shaft by reversing the steps used during assembly (provided in sections 3.2, 3.3, and 3.4).

6. Disable the temperature control to ensure that the S.U.B. does not overheat.

7. When approximately three to five liters remain in the BPC, lift the BPC at the top hanging tabs located opposite of the bottom drain (this will pool media toward the drain).

8. Hold the bottom drain line near the floor while lifting the exhaust filter side of the BPC to facilitate draining the final liter of harvest media.

3.6.17 BPC disposal

After the drive shaft has been removed and the BPC has been drained, the BPC can be removed from the outer support container. Filters can be removed and integrity tested as needed according to the user's standard procedures. All product contact materials related to the S.U.B. can be disposed of in an appropriate waste container or incinerator.

3.6.18 S.U.B. shutdown

1. After the run is complete, verify that the motor agitation is off and turn off the power to the outer support container by switching off the main power disconnect.

2. If the S.U.B. hardware has come in contact with caustic materials during the course of a run, rinse the affected areas with a light water rinse, followed by normal routine cleaning (see section 3.6.19).

3. Loose items such as the drive shaft, tools, and RTD probes should be returned to their storage locations to prevent accidental damage.

3.6.19 Preparation for the next run

Between runs, the S.U.B. hardware (outer support container, probe shelf, drive shaft, mixer drive, etc.) can be wiped down with a sanitary wipe. The outer support container can be cleaned with standard stainless steel cleaner. Store the drive shaft in the storage holder located near the handle of the outer support container.

The S.U.B. hardware system can be cleaned to the extent of standard laboratory cleaning procedures. Care should be taken to ensure electrical connections have been disconnected and electrical enclosures are closed tightly. It is also recommended that excess water is not introduced under the heat shield or over the control panel. A wipe-down with normal disinfectant solutions is sufficient. Avoid using excessive amounts of liquid. The unit must be allowed to fully dry prior to being brought back into operation.

3.7 Verification procedures

3.7.1 Mixing speed verification

To verify the mixing speed, use a calibrated tachometer. Expect accuracy of ± 1.5 rpm or 1% of the setpoint, whichever is greater. Speed scaling can be modified if the calibration needs to be adjusted.

3.7.2 Temperature controller verification

To verify the temperature controller/RTD, use a S.U.B. silicone thermowell, the existing 3.175 mm (1/8 in.) outer diameter (OD) RTD, and a user-supplied calibrated temperature bath.

3.7.3 Pressure monitor verification (when present)

To verify the calibration of the pressure monitor, use a calibrated pressure standard. Pressures can be verified by clamping the BPC inlet line and supplying gas through the overlay gas filter. Expect accuracy of ± 0.1 psi. The monitor can be calibrated manually by referencing the monitor user's guide supplied in the Equipment Turnover Package (ETP).

3.7.4 Load cell verification

It is recommended that the load cell manufacturer or a qualified technician verify the load cells onsite. Expect an accuracy of ± 0.5 kg. Basic load cell default parameters are listed in the electrical schematic included with the ETP.

4

System features and specifications

Chapter contents

4.1 Hardware features

4.1.1 Design features for 50–250 L systems

Figures 4.1 and 4.2 (below) illustrate the hardware features of 50–250 L jacketed S.U.B. systems. Electrical control panel (E-Box) features for both jacketed and resistive systems are illustrated in section 4.3.

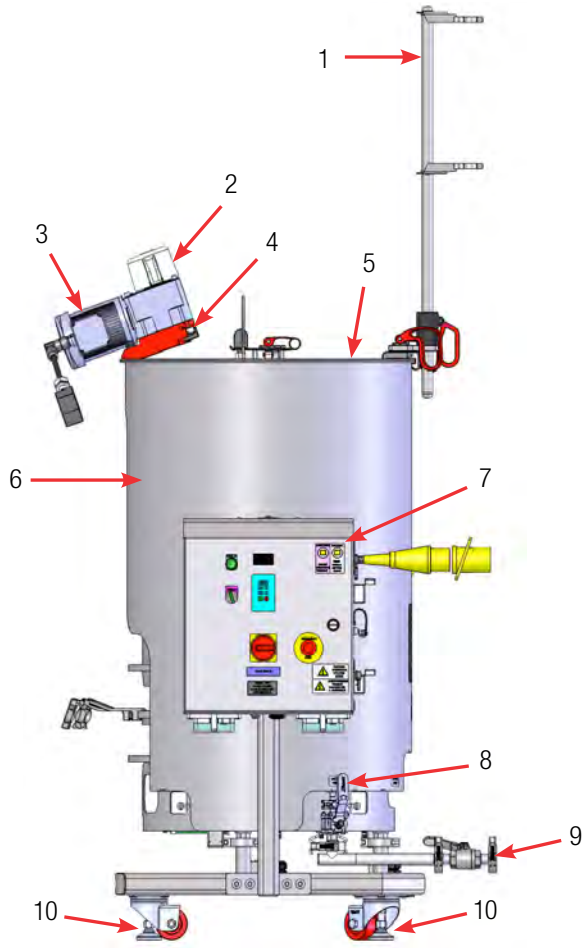


Figure 4.1. Front/side view of 50 L jacketed S.U.B.

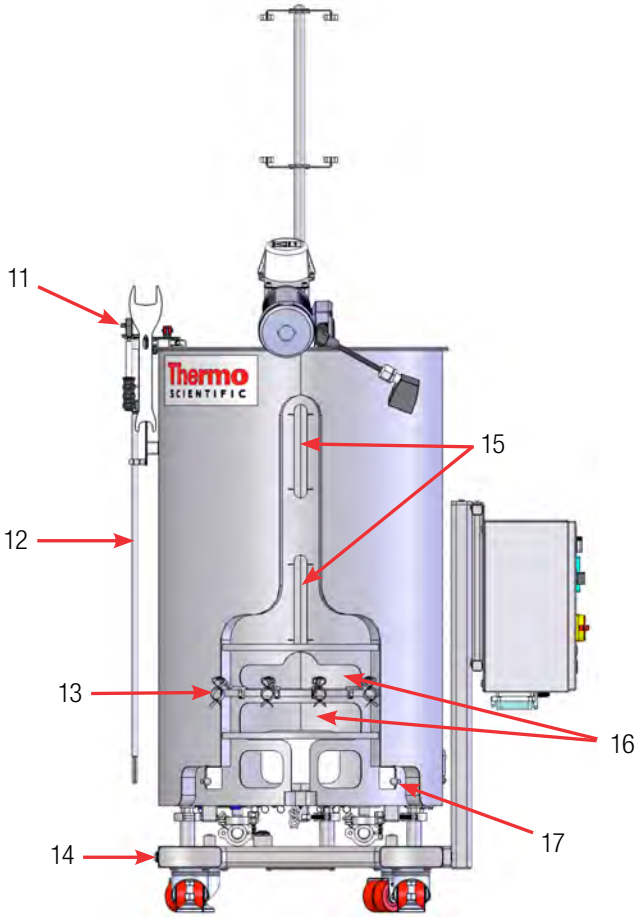


Figure 4.2. Back view of 50 L jacketed S.U.B.

1. Exhaust vent filter holder (optional)
2. Mixing assembly with shield
3. Mixer motor
4. Bearing port receiver with clamp
5. 0.95 cm (3/8 in.) Dimpled water jacket
6. Stainless steel outer support container
7. E-Box (optional)
8. Bleed valve
9. Quick connect water inlet/outlet ports (jacketed models only)
10. Leveling casters
11. Standard tool set: 10 mm (3/8 in.) x 16.9 Nm (150 in-lb.) square torque wrench, load cell and motor cap lockout wrench
12. Drive shaft, stored
13. Probe hanger bracket
14. Cart assembly
15. Liquid sight windows
16. Probe access windows
17. Bottom cutouts/pins for BPC attachment and alignment

4.1.2 Design features for 500–1,000 L systems

Figures 4.3 and 4.4 (below) illustrate the features of 500–1,000 L S.U.B. systems. E-Box features are illustrated in section 4.3.

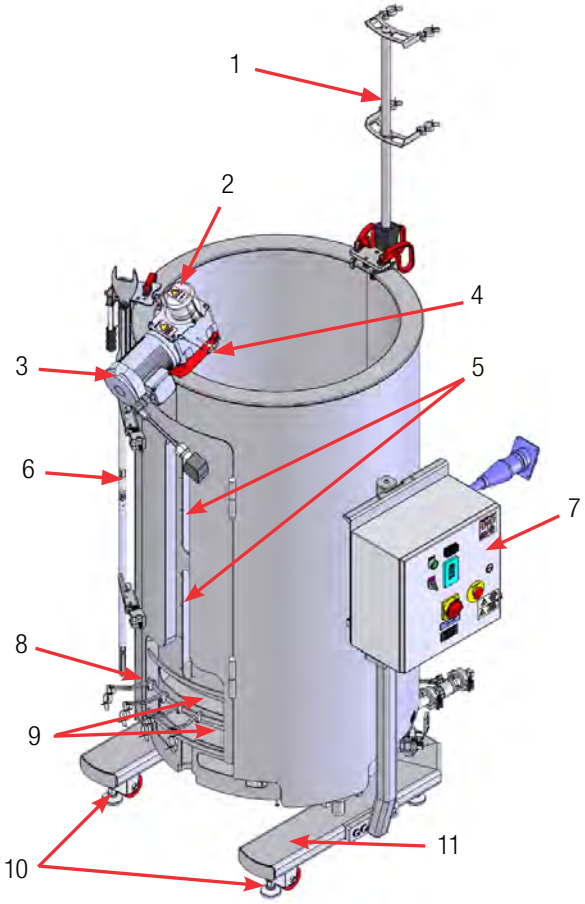


Figure 4.3. Front/side view of 500 L S.U.B.

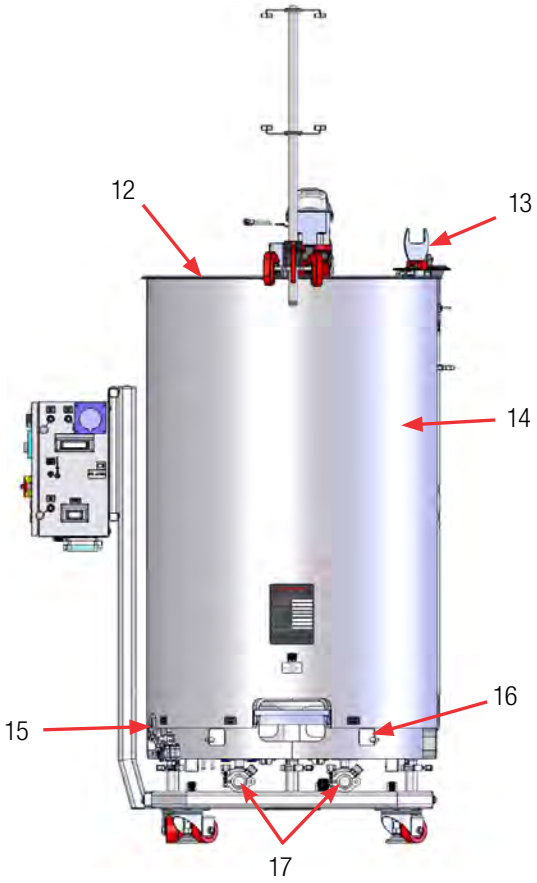


Figure 4.4. Back view of 500 L S.U.B.

1. Exhaust vent filter holder
2. Mixing assembly with shield
3. Mixer motor
4. Bearing port receiver with clamp
5. Liquid sight windows
6. Drive shaft, stored
7. E-Box (optional)
8. Probe hanger bracket
9. Probe access windows
10. Leveling casters
11. Cart assembly
12. 0.95 cm (3/8 in.) Dimpled water jacket
13. Standard tool set: 10 mm (3/8 in.) x 16.9 Nm (150 in-lb.) square torque wrench, load cell and motor cap lockout wrench
14. Stainless steel outer support container
15. Bleed valve
16. Bottom cutouts/pins for BPC attachment/ alignment

4.1.3 Design features for 2,000 L systems

Figures 4.5 and 4.6 (below) illustrate the features of 2,000 L S.U.B. systems. E-Box features are illustrated in section 4.3.

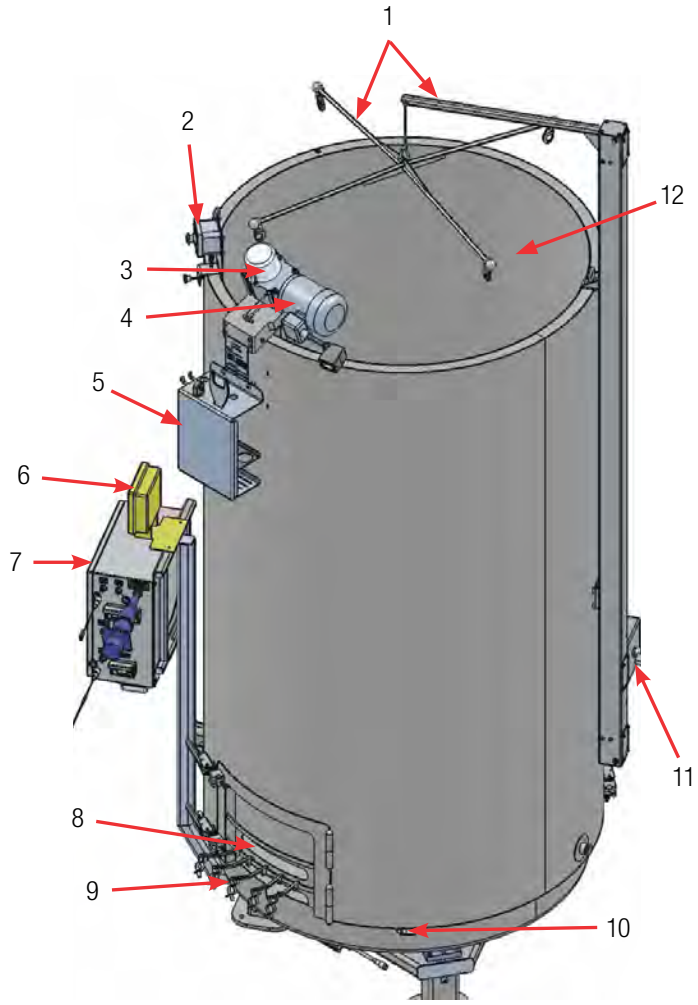


Figure 4.5. Front/side view of 2,000 L S.U.B.

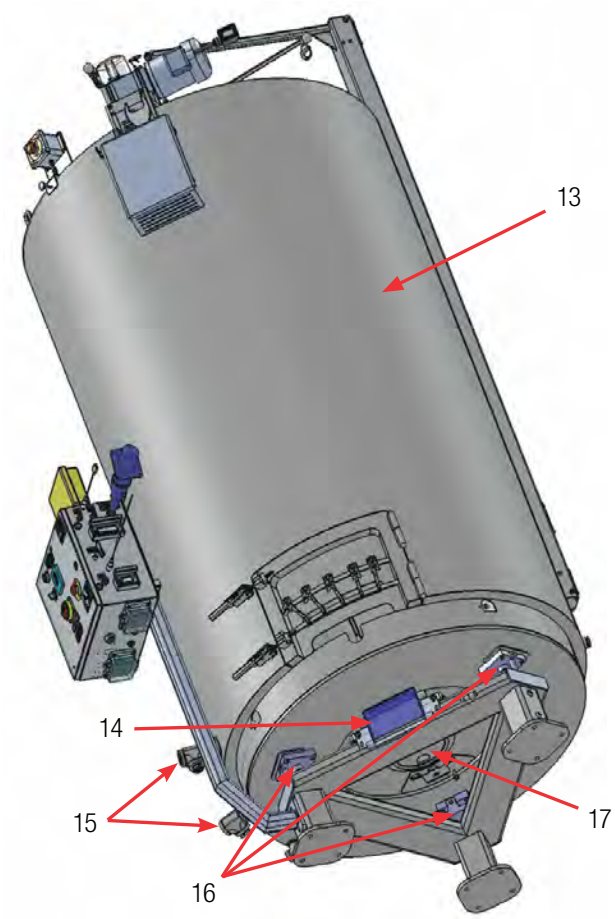


Figure 4.6. Side/bottom view of 2,000 L S.U.B.

1. Quick connect water inlet/outlet ports
2. Bag lift assembly
3. Auxiliary E-Stop
4. Mixing assembly with shield
5. Mixer motor
6. Standard tool set: 10 mm (3/8 in.) x 16.9 Nm (150 in-lb.) square torque wrench, load cell and motor cap lockout wrench
7. Load cell display
8. E-Box
9. Probe access window
10. Probe clips
11. Bottom cutouts/pins for BPC alignment
12. Pneumatic bag lift control
13. Water jacket
14. Stainless steel outer support container
15. Load cell summing block
16. Quick connect water inlet/outlet ports
17. Load cells (3)
18. Sparge plate access

4.2 Hardware specifications

The following tables and figures provide specifications for 50, 100, 250, 500, 1,000, and 2,000 L S.U.B.s. See section 1.2.3 for drive shaft specifications.

Table 4.1. 50 L S.U.B. specifications.

		AC and DC motors	
		Resistive	Jacketed
Bioreactor geometry	Rated liquid working volume	50 L	
	Minimum liquid working volume	25 L	
	Total reactor volume (liquid & gas)	65.5 L	
	BPC chamber diameter	34.9 cm (13.75 in.)	
	BPC chamber shoulder height	80 cm (31.5 in.)	
	Liquid height at rated working volume	52.1 cm (20.5 in.)	
	Fluid geometry at working volume (height/diameter) ratio	1.5:1	
	Overall reactor geometry (height/diameter ratio)	1.9:1	
	Tank baffles	No	
Impeller	Impeller (quantity x blade count)	1 x 3	
	Impeller scaling (impeller diameter/tank diameter)	1/3	
	Impeller blade pitch (angle)	45°	
	Impeller diameter	11.11 cm (4.375 in.)	
	Impeller—calculated power number (N)	2.1	
Agitation	Agitation speed range	30–200 ± 1.5 rpm or 1% of setpoint, whichever is greater	
	Nominal agitation rating—(P/V ratio)	20 W/m^3	
	Nominal agitation—50% working volume	145 rpm	
	Nominal agitation—100% working volume	183 rpm	
	Nominal tip speed	103.9 cm/s (204.6 ft./min.)	
	Counterclockwise mixing flow direction	Down-pumping	
	Agitation shaft resolved angle	19.6°	
	Agitation shaft centerline offset	1.9 cm (0.75 in.)	
	Overall drive shaft length	76.2 cm (30 in.)	
	Drive shaft diameter	1.27 cm (0.5 in.)	
	Drive shaft poly-sheath outside diameter	2.54 cm (1 in.)	
	Impeller clearance from tank bottom	11.75 cm (4.63 in.)	
Rec. operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	30–200 rpm	
	Volume range	25–50 L	
	Maximum bag pressure	0.03 bar (0.5 psi)	

Table 4.2. 50 L S.U.B. specifications (continued).

		AC motor		DC motor	
		Resistive	Jacketed	Resistive	Jacketed
Motor	Agitation motor drive (type, voltage, phase) AC motor only	Induction, 208 VAC, 3		N/A	
	Agitation motor drive (type, voltage) DC motor only	N/A		Brushless, 48 VDC	
	Motor power rating (AC motor)	186.4 W (0.25 hp)		N/A	
	Motor power rating (DC motor)	N/A		200 W (0.268 hp)	
	Motor torque rating	9.5 Nm (82 in-lb.)		N/A	
	Gear reduction	10:1			
	Programmable VFD, remote panel interface, power fault auto restart	Standard		N/A	
	Motor communication methods (for external controller)	0–10 V, 4–20 mA, Modbus		N/A	
Temperature control	Jacket area (full/half volume)	N/A	0.41 m^2 (4.4 $ft.^2$) / 0.19 m^2 (2 $ft.^2$)	N/A	0.41 m^2 (4.4 $ft.^2$) / 0.19 m^2 (2 $ft.^2$)
	Jacket volume	N/A	2.4 L	N/A	2.4 L
	Jacket flow rate at 3.4 bar (50 psi)	N/A	136 L/min.	N/A	136 L/min.
	Jacket process connection	N/A	1.5 in. sanitary tri-clamp	N/A	1.5 in. sanitary tri-clamp
	Jacket nominal heating/cooling load (W)	N/A	500 W	N/A	500 W
	Approximate liquid heat-up time (5°C to 37°C)—100% volume	4.32 hr	1.7 hr	4.32 hr	1.7 hr
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)			
	Programmable PID temperature controller	Standard	N/A	Standard	N/A
	Solid state relay (discrete voltage signal)	24–240 VAC	N/A	24–240 VAC	N/A
	Heater power rating—total	455.7 W	N/A	455.7 W	N/A
	Heater power rating—sides	360 W	N/A	360 W	N/A
	Heater power rating—bottom	95.7 W	N/A	95.7 W	N/A
Support container	Overall width	116.4 cm (45.8 in.) with E-Box		64.21 cm (25.3 in.)	
	Overall length	95.52 cm (38.0 in.) with E-Box		85.85 cm (33.8 in.)	
	Overall height	198.5 cm (78.2 in.) with filter bracket			
	Dry skid weight (mass)	134.3 kg (296 lb.)	169.0 kg (372.5 lb.)	134.3 kg (296 lb.)	115.7 kg (255 lb.)
	Wet skid weight—rated working volume (mass)	184.2 kg (406.2 lb.)	218.9 kg (482.7 lb.)	184.2 kg (406.2 lb.)	165.7 kg (365.2 lb.)
General	Ceiling height required for drive shaft loading	213.4 cm (84 in.)			
	Electrical power supply requirement (voltage, phase, amp)	120/240 VAC, single, 20/10 A		Dependent on controller	
	Tested system reliability (minimum)	0.9 at 90%			
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread			
	Noise level	< 70 dB at 1.5 m			

50 L Dimensional drawings

The dimensions provided below are displayed in (centimeters [inches]). See your sales representative for ordering information.

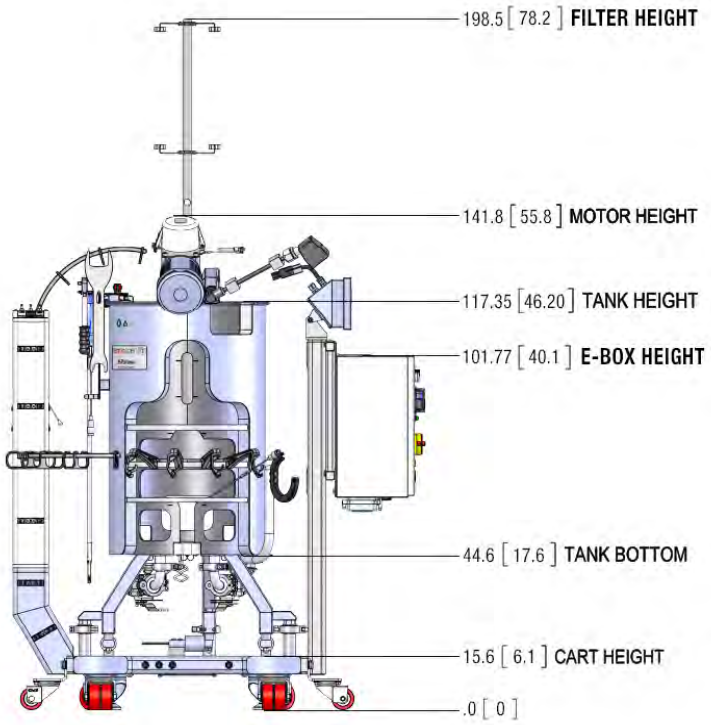


Figure 4.7. Dimensions of 50 L S.U.B. (front view).

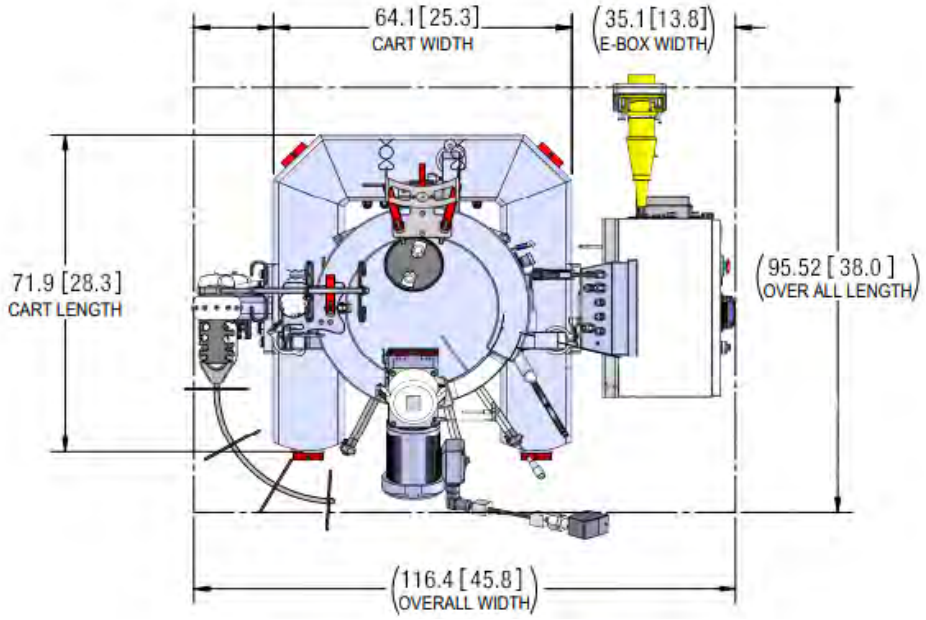


Figure 4.8. Dimensions of 50 L S.U.B. (top view).

Table 4.3. 100 L S.U.B. specifications.

		AC and DC motors	
		Resistive	Jacketed
Bioreactor geometry	Rated liquid working volume	100 L	
	Minimum liquid working volume	50 L	
	Total reactor volume (liquid & gas)	120 L	
	BPC chamber diameter	43.8 cm (17.25 in.)	
	BPC chamber shoulder height	95.3 cm (37.5 in.)	
	Liquid height at rated working volume	66 cm (26 in.)	
	Fluid geometry at working volume (height/diameter) ratio	1.5:1	
	Overall reactor geometry (height/diameter ratio)	1.9:1	
	Tank baffles	No	
Impeller	Impeller (quantity x blade count)	1 x 3	
	Impeller scaling (impeller diameter/tank diameter)	1/3	
	Impeller blade pitch (angle)	45°	
	Impeller diameter	14.6 cm (5.75 in.)	
	Impeller—calculated power number (N)	2.1	
Agitation	Agitation speed range	30–200 ± 1.5 rpm or 1% of setpoint, whichever is greater	
	Nominal agitation rating—(P/V ratio)	20 W/m^3	
	Nominal agitation—50% working volume	116 rpm	
	Nominal agitation—100% working volume	146 rpm	
	Nominal tip speed	110.7 cm/s (218 ft./min.)	
	Counterclockwise mixing flow direction	Down-pumping	
	Agitation shaft resolved angle	19.6°	
	Agitation shaft centerline offset	2.54 cm (1 in.)	
	Overall drive shaft length	88.9 cm (35 in.)	
	Drive shaft diameter	1.27 cm (0.5 in.)	
	Drive shaft poly-sheath outside diameter	2.54 cm (1 in.)	
	Impeller clearance from tank bottom	14.6 cm (5.75 in.)	
Rec. operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	30–200 rpm	
	Volume range	50–100 L	
	Maximum bag pressure	0.03 bar (0.5 psi)	

Table 4.4. 100 L S.U.B. specifications (continued).

		AC motor		DC motor	
		Resistive	Jacketed	Resistive	Jacketed
Motor	Agitation motor drive (type, voltage, phase) AC motor only	Induction, 208 VAC, 3		N/A	
	Agitation motor drive (type, voltage) DC motor only	N/A		Brushless, 48 VDC	
	Motor power rating (AC motor)	186.4 W (0.25 hp)		N/A	
	Motor power rating (DC motor)	N/A		200 W (0.268 hp)	
	Motor torque rating	9.5 Nm (82 in-lb.)		N/A	
	Gear reduction	10:1			
	Programmable VFD, remote panel interface, power fault auto restart	Standard		N/A	
	Motor communication methods (for external controller)	0–10 V, 4–20 mA, Modbus		N/A	
Temperature control	Jacket area (full/half volume)	N/A	0.60 m^2 (6.5 $ft.^2$) / 0.21 m^2 (2.3 $ft.^2$)	N/A	0.60 m^2 (6.5 $ft.^2$) / 0.21 m^2 (2.3 $ft.^2$)
	Jacket volume	N/A	4.5 L	N/A	4.5 L
	Jacket flow rate at 3.4 bar (50 psi)	N/A	136 L/min.	N/A	136 L/min.
	Jacketed process connection	N/A	1 in. sanitary tri-clamp	N/A	1 in. sanitary tri-clamp
	Jacketed nominal heating/cooling load	N/A	1,000 W	N/A	1,000 W
	Approximate liquid heat-up time (5–37°C)—100% volume	4.9 hr	1.9 hr	4.9 hr	1.9 hr
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)			
	Programmable PID temperature controller	Standard	N/A	Standard	N/A
	Solid state relay (discrete voltage signal)	24–240 V	N/A	24–240 V	N/A
	Heater power rating—total	866 W	N/A	866 W	N/A
	Heater power rating—sides	676 W	N/A	676 W	N/A
	Heater power rating—bottom	190 W	N/A	190 W	N/A
Support container	Overall width	109.2 cm (43.0 in.) with E-Box		61.6 cm (24.3 in.)	
	Overall length	100.2 cm (39.4 in.) with E-Box		93.24 cm (36.71 in.)	
	Overall height	201 cm (79.1 in.) with filter bracket			
	Dry skid weight (mass)	138.3 kg (305 lb.)	199.8 kg (440.5 lb.)	138.3 kg (305 lb.)	160.6 kg (354 lb.)
	Wet skid weight—rated working volume (mass)	238.3 kg (525.5 lb.)	299.8 kg (661.0 lb.)	238.3 kg (525.5 lb.)	260.6 kg (574.5 lb.)
General	Ceiling height required for drive shaft loading	228.6 cm (90 in.)			
	Electrical power supply requirement (voltage, phase, amp)	120/240 VAC, Single, 20/10 A		Dependent on controller	
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread			
	Noise level	< 70 dB at 1.5 m			

100 L Dimensional drawings

The dimensions provided below are displayed in (centimeters [inches]). See your sales representative for ordering information.

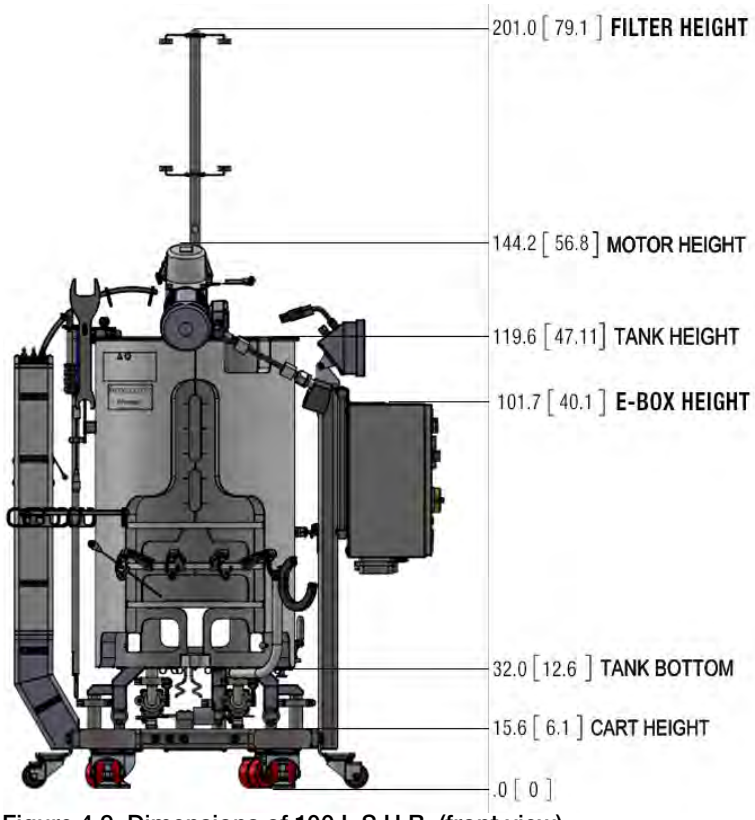


Figure 4.9. Dimensions of 100 L S.U.B. (front view).

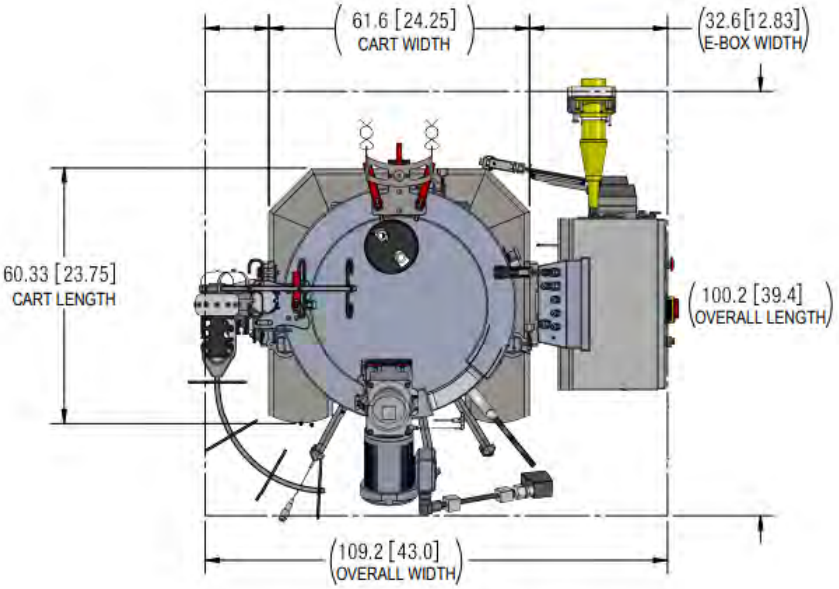


Figure 4.10. Dimensions of 100 L S.U.B. (top view).

Table 4.5. 250 L S.U.B. specifications.

		AC and DC motors	
		Resistive	Jacketed
Bioreactor geometry	Rated liquid working volume	250 L	
	Minimum liquid working volume	125 L	
	Total reactor volume (liquid & gas)	316 L	
	BPC chamber diameter	59.7 cm (23.5 in.)	
	BPC chamber shoulder height	115.6 cm (45.5 in.)	
	Liquid height at rated working volume	91.4 cm (36 in.)	
	Fluid geometry at working volume (height/diameter) ratio	1.5:1	
	Overall reactor geometry (height/diameter ratio)	1.9:1	
	Tank baffles	No	
Impeller	Impeller (quantity x blade count)	1 x 3	
	Impeller scaling (impeller diameter/tank diameter)	1/3	
	Impeller blade pitch (angle)	45°	
	Impeller diameter	20 cm (7.875 in.)	
	Impeller—calculated power number (N)	2.1	
Agitation	Agitation speed range	30–150 ± 1.5 rpm or 1% of setpoint, whichever is greater	
	Nominal agitation rating—(P/V ratio)	20 W/m^3	
	Nominal agitation—50% working volume	93 rpm	
	Nominal agitation—100% working volume	117 rpm	
	Nominal tip speed	123.6 cm/s (243.3 ft./min.)	
	Counterclockwise mixing flow direction	Down-pumping	
	Agitation shaft resolved angle	19.6°	
	Agitation shaft centerline offset	3.3 cm (1.3 in.)	
	Overall drive shaft length	106.7 cm (42 in.)	
	Drive shaft diameter	1.27 cm (0.5 in.)	
	Drive shaft poly-sheath outside diameter	2.54 cm (1 in.)	
	Impeller clearance from tank bottom	20 cm (7.87 in.)	
Rec. operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	30–150 rpm	
	Volume range	125–250 L	
	Maximum bag pressure	0.03 bar (0.5 psi)	

Table 4.6. 250 L S.U.B. specifications (continued).

		AC motor		DC motor	
		Resistive	Jacketed	Resistive	Jacketed
Motor	Agitation motor drive (type, voltage, phase) AC motor only	Induction, 208 VAC, 3		N/A	
	Agitation motor drive (type, voltage) DC motor only	N/A		Brushless, 48 VDC	
	Motor power rating (AC motor)	186.4 W (0.25 hp)		N/A	
	Motor power rating (DC motor)	N/A		400 W (0.536 hp)	
	Motor torque rating	11.5 Nm (102 in-lb.)		N/A	
	Gear reduction	12.5:1			
	Programmable VFD, remote panel interface, power faults auto restart	Standard		N/A	
	Motor communication methods (for external controller)	0–10 V, 4–20 mA, Modbus		N/A	
Temperature control	Jacket area (full/half volume)	N/A	1.26 m^2 (13.6 $ft.^2$) / 0.54 m^2 (5.8 $ft.^2$)	N/A	1.26 m^2 (13.6 $ft.^2$) / 0.54 m^2 (5.8 $ft.^2$)
	Jacket volume	N/A	8.6 L	N/A	8.6 L
	Jacket flow rate at 3.4 bar (50 psi)	N/A	136 L/min.	N/A	136 L/min.
	Jacketed process connection	N/A	1.5 in. sanitary tri-clamp	N/A	1.5 in. sanitary tri-clamp
	Jacketed nominal heating/cooling load	N/A	2,500 W	N/A	2,500 W
	Approximate liquid heat-up time (5°C to 37°C)—100% volume	7.5 hr	1.9 hr	7.5 hr	1.9 hr
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)			
	Programmable PID temperature controller	Standard	N/A	Standard	N/A
	Solid state relay (discrete voltage signal)	24–240 V	N/A	24–240 V	N/A
	Heater power rating—total	1,566.8 W	N/A	1,566.8 W	N/A
	Heater power rating—sides	1,168.8 W	N/A	1,168.8 W	N/A
	Heater power rating—bottom	398 W	N/A	398 W	N/A
Support container	Overall width	113 cm (44.4 in.) (with E-box)		78 cm (30.6 in.)	
	Overall length	106 cm (41.7 in.) (with E-Box)			
	Overall height	215 cm (84.8 in.) with filter bracket			
	Dry skid weight (mass)	192.8 kg (425 lb.)	223.6 kg (493 lb.)	192.8 kg (425 lb.)	223.6 kg (493 lb.)
	Wet skid weight—rated working volume (mass)	442.8 kg (976.2 lb.)	473.6 kg (1044 lb.)	442.8 kg (976.2 lb.)	473.6 kg (1044 lb.)
General	Ceiling height required for drive shaft loading	256.5 cm (101 in.)			
	Electrical power supply requirement (voltage, phase, amp)	120/240 VAC, single, 20/10 A		Dependent on controller	
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG thread			
	Noise level	< 70 dB at 1.5 m			

250 L Dimensional drawings

The dimensions provided below are displayed in (inches [centimeters]). See your sales representative for ordering information.

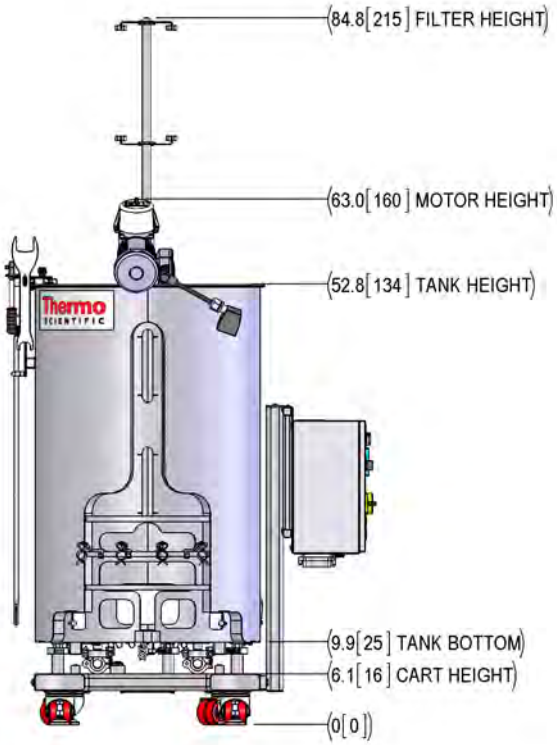


Figure 4.11. Dimensions of 250 L S.U.B. (front view).

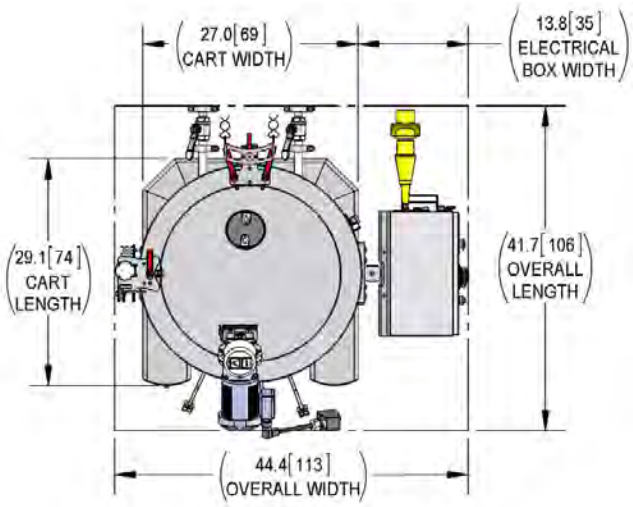


Figure 4.12. Dimensions of 250 L S.U.B. (top view).

Table 4.7. 500 L S.U.B. specifications.

		AC and DC motors
Bioreactor geometry	Rated liquid working volume	500 L
	Minimum liquid working volume	250 L
	Total bioreactor volume (liquid & gas)	660 L
	BPC chamber diameter	75.56 cm (29.75 in.)
	BPC chamber shoulder height	152.4 cm (60 in.)
	Liquid height at rated working volume	113.36 cm (44.63 in.)
	Fluid geometry at working volume (height/diameter) ratio	1.5:1
	Overall bioreactor geometry (height/diameter) ratio	1.9:1
	Tank baffles	No
Impeller	Impeller (quantity x blade count)	1 x 3
	Impeller scaling (impeller diameter/tank diameter)	1/3
	Impeller blade pitch	45°
	Impeller diameter	25.1 cm (9.875 in.)
	Impeller—calculated power number (N)	2.1
Agitation	Agitation speed range	30–150 ± 1.5 rpm or 1% of setpoint, whichever is greater
	Nominal agitation rating (P/V ratio)	20 W/m^3
	Nominal agitation—50% working volume	80 rpm
	Nominal agitation—100% working volume	101 rpm
	Nominal tip speed	137.2 cm/s (270 ft./min.)
	Counterclockwise mixing flow direction	Down-pumping
	Agitation shaft resolved angle	19.6°
	Agitation shaft centerline offset	5.08 cm (2 in.)
	Overall drive shaft length	127 cm (50 in.)
	Drive shaft diameter	1.90 cm (0.75 in.)
	Drive shaft poly-sheath outside diameter	3.49 cm (1.37 in.)
	Impeller clearance from tank bottom	25.1 cm (9.87 in.)

Table 4.8. 500 L S.U.B. specifications (continued).

		AC motor	DC motor
Motor	Agitation motor drive (type, voltage, phase) AC motor only	Induction, 208 VAC, 3	N/A
	Agitation motor drive (type, voltage) DC motor only	N/A	Brushless, 48 VDC
	Motor power rating (AC motor)	372.8 W (0.5 hp)	N/A
	Motor power rating (DC motor)	N/A	400 W (0.536 hp)
	Motor torque rating	9.5 Nm (82 in-lb.)	N/A
	Gear reduction	10:1	
	Programmable VFD, remote panel interface, power fault auto restart	Standard	N/A
	Motor communication methods (for external controller)	0–10 V; 4–20 mA; Modbus	N/A
Temperature control	Jacket area (full/half volume)	1.99 m^2 (21.4 $ft.^2$) / 0.78 m^2 (8.4 $ft.^2$)	
	Jacket volume	15.2 L	
	Jacket flow rate at 3.4 bar (50 psi)	136 L/min.	
	Process connection	1 in. Sanitary tri-clamp	
	Nominal heating/cooling load (W)	5,000 W	
	Approximate liquid heat-up time (5°C to 37°C)—100% volume	2.67 hr	
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)	
Support container	Overall width	125.2 cm (49.3 in.) with E-Box	86.4 cm (34 in.)
	Overall length	124.4 cm (47.8 in.) with E-Box	116 cm (45.7 in.)
	Overall height	251.1 cm (98.9 in.) with filter bracket	
	Dry skid weight (mass)	353.8 kg (780 lb.)	
	Wet skid weight—rated working volume (mass)	853.8 kg (1882.3 lb.)	
General	Ceiling height required for drive shaft loading	266.7 cm (105 in.)	
	Electrical power supply requirement (voltage, phase, amp)	208–240 VAC, single, 10 A	Dependent on controller
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread	
	Noise level	< 70 dB at 1.5 m	
Recommended operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	30–150 rpm	
	Volume range	250–500 L	
	Maximum BPC pressure	0.03 bar (0.5 psi)	

500 L Dimensional drawings

The dimensions provided below are displayed in (inches [centimeters]). See your sales representative for ordering information.

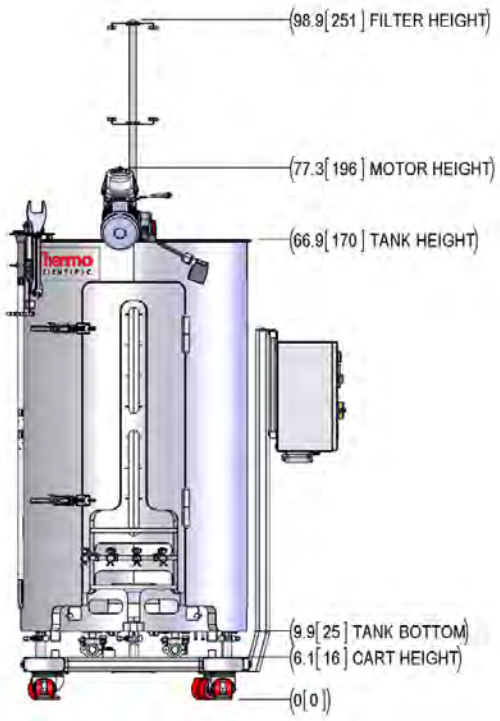


Figure 4.13. Dimensions of 500 L S.U.B. (front view).

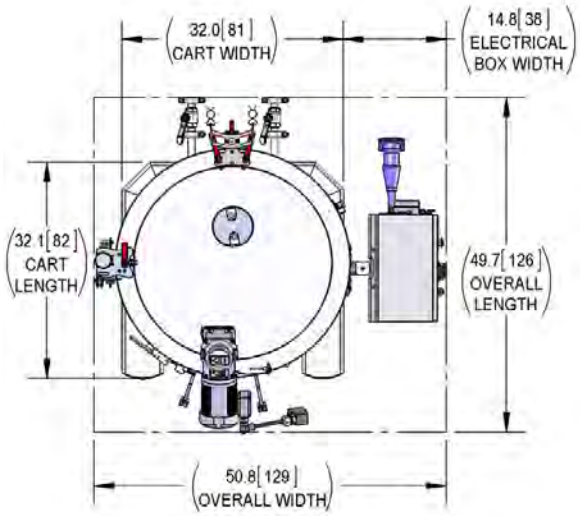


Figure 4.14. Dimensions of 500 L S.U.B. (top view).

Table 4.9. 1,000 L S.U.B. specifications.

		AC motor	DC motor
Bioreactor geometry	Rated liquid working volume	1,000 L	
	Minimum liquid working volume	500 L	
	Total bioreactor volume (liquid & gas)	1,320 L	
	BPC chamber diameter	95.9 cm (37.75 in.)	
	BPC chamber shoulder height	200.7 cm (79 in.)	
	Liquid height at rated working volume	142.2 cm (56 in.)	
	Fluid geometry at working volume (height/diameter) ratio	1.5:1	
	Overall bioreactor geometry (height/diameter) ratio	1.9:1	
	Tank baffles	No	
Impeller	Impeller (quantity x blade count)	1 x 3	
	Impeller scaling (impeller diameter/tank diameter)	1/3	
	Impeller blade pitch	45°	
	Impeller diameter	32.1 cm (12.62 in.)	
	Impeller—calculated power number (N)	2.1	
Agitation	Agitation speed range	20–110 ± 1.5 rpm or 1% of setpoint, whichever is greater	
	Nominal agitation rating (P/V ratio)	20 W/m^3	
	Nominal agitation—50% working volume	68 rpm	
	Nominal agitation—100% working volume	86 rpm	
	Nominal tip speed	146.1 cm/s (287.6 ft./min.)	
	Counterclockwise mixing flow direction	Down-pumping	
	Agitation shaft resolved angle	19.6°	
	Agitation shaft centerline offset	5.08 cm (2.0 in.)	
	Overall drive shaft length	167.6 cm (66 in.)	
	Drive shaft diameter	1.90 cm (0.75 in.)	
	Drive shaft poly-sheath outside diameter	3.49 cm (1.37 in.)	
	Impeller clearance from tank bottom	32.1 cm (12.62 in.)	

Table 4.10. 1,000 L S.U.B. specifications (continued).

		AC motor	DC motor
Motor	Agitation motor drive (type, voltage, phase)	Induction, 208 VAC, 3	Brushless, 48 VDC
	Motor power rating	372.8 W (0.5 hp)	400 W (0.53 hp)
	Motor torque rating	27.7 Nm (245 in-lb.)	N/A
	Gear reduction	15:1	
	Programmable VFD, remote panel interface, power fault auto restart	Standard	N/A
	Motor communication methods (for external controller)	0-10 V; 4-20 mA; Modbus	N/A
Temperature control	Jacket area (full/half volume)	3.31 m^2 (35.6 $ft.^2$) / 1.38 m^2 (14.9 $ft.^2$)	
	Jacket volume	23.5 L	
	Jacket flow rate at 3.4 bar (50 psi)	136 L/min.	
	Process connection	1 in. sanitary tri-clamp	
	Nominal heating/cooling load (W)	10,000 W	
	Approximate liquid heat-up time (5°C to 37°C)—100% volume	6.4 hr	
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)	
Support container	Overall width	143.8 cm (56.6 in.) with E-Box	109.7 cm (43.2 in.)
	Overall length	139.2 cm (54.8 in.) with E-Box	133.9 cm (52.7 in.)
	Overall height	284 cm (111.8 in.) with filter bracket	
	Dry skid weight (mass)	539.8 kg (1,190 lb.)	
	Wet skid weight—rated working volume (mass)	1,540 kg (3,395 lb.)	
General	Ceiling height required for drive shaft loading	287 cm (113 in.)	
	Electrical power supply requirement (voltage, phase, amp)	208–240 VAC, single, 30 A	Dependent on controller
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread	
	Noise level	< 70 dB at 1.5 m	
Recommended operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	20–110 rpm	
	Volume range	500–1,000 L	
	Maximum BPC pressure	0.03 bar (0.5 psi)	

1,000 L Dimensional drawings

The dimensions provided below are displayed in (inches [centimeters]). See your sales representative for ordering information.

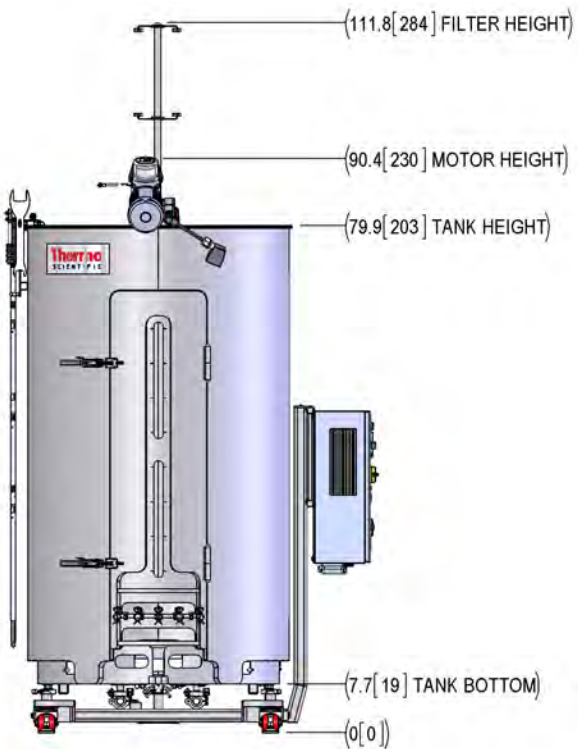


Figure 4.15. Dimensions of 1,000 L S.U.B. (front view).

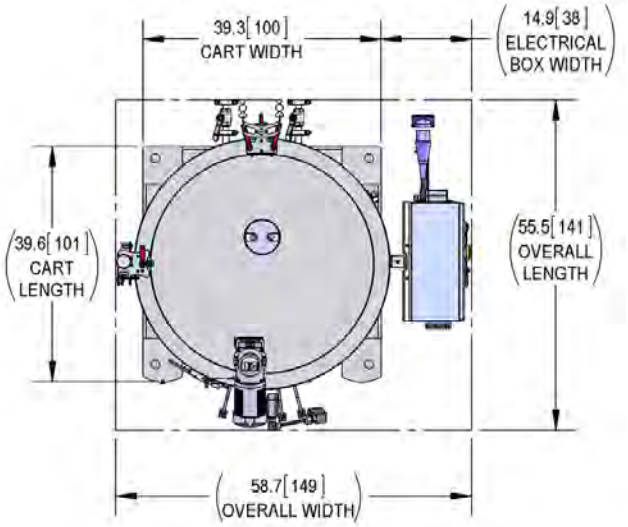


Figure 4.16. Dimensions of 1,000 L S.U.B. (top view).

Table 4.11. 2,000 L S.U.B. specifications.

		AC motor	DC motor
Bioreactor geometry	Rated liquid working volume	2,000 L	
	Minimum liquid working volume	1,000 L	
	Total bioreactor volume (liquid & gas)	2,575 L	
	BPC chamber diameter	119.4 cm (47 in.)	
	BPC chamber shoulder height	229.9 cm (90.5 in.)	
	Liquid height at rated working volume	178.7 cm (70.35 in.)	
	Fluid geometry at working volume (height/diameter) ratio	1.5:1	
	Overall bioreactor geometry (height/diameter) ratio	1.9:1	
	Tank baffles	No	
Impeller	Impeller (quantity x blade count)	1 x 3	
	Impeller scaling (impeller diameter/tank diameter)	1/3	
	Impeller blade pitch	45°	
	Impeller diameter	39.8 cm (15.67 in.)	
	Impeller—calculated power number (N)	2.1	
Agitation	Agitation speed range	Standard operation: 20–75* rpm ± 1.5 rpm or 1% of setpoint, whichever is greater	
	Nominal agitation rating (P/V ratio)	20 W/m^3	
	Nominal agitation—50% working volume	60 rpm	
	Nominal agitation—100% working volume	75 rpm	
	Minimum acceleration and deceleration rate	60 seconds	
	Nominal tip speed	154.9 cm/s (305 ft./min.)	
	Counterclockwise mixing flow direction	Down-pumping	
	Agitation shaft resolved angle	19.6°	
	Agitation shaft centerline offset	6.7 cm (2.63 in.)	
	Overall drive shaft length	210.6 cm (82.9 in.)	
	Drive shaft diameter	1.91 cm (0.75 in.)	
	Drive shaft poly-sheath outside diameter	3.51 cm (1.38 in.)	
	Impeller clearance from tank bottom	39.8 cm (15.67 in.)	
General	Ceiling height required for drive shaft loading	381 cm (150 in.)	
	Electrical power supply requirement (voltage, phase, amp)	208–240 VAC, single, 10 A	N/A
	pH & DO probe—autoclavable type (Applisens, Broadley James, Mettler Toledo)	12 mm diameter x 215–235 mm insertion length x 13.5 PG (pipe) thread	
	Noise level	< 70 dB at 1.5 m	

Table 4.12. 2,000 L S.U.B. specifications (continued).

		AC motor	DC motor
Motor	Agitation motor drive (type, voltage, phase)	Induction, 208 AC, 3	Brushless, 48 VDC
	Motor power rating	372.8 W (0.5 hp)	400 W (0.53 hp)
	Motor torque rating	27.7 Nm (245 in-lb.)	N/A
	Gear reduction	15:1	20:1
	Programmable VFD, remote panel interface, power fault auto restart	Standard	N/A
	Motor communication methods (for external controller)	0–10 V; 4–20 mA; Modbus	N/A
Temperature control	Jacket area (full/half volume)	6.23 m^2 (67.1 $ft.^2$) / 5 m^2 (53.9 $ft.^2$)	
	Jacket volume	44 L	
	Jacket flow rate at 3.4 bar (50 psi)	75 L/min.	
	Process connection	1 in. Male national pipe thread (NPT) nipple with Hansen quick connect check valves	
	Nominal heating/cooling load (W)	18,000 W	
	Approximate liquid heat-up time (5°C to 37°C)—100% volume	4 hr	
	RTD or thermocouple, 3.18 mm (1/8 in.) OD	RTD: Pt-100 (standard)	
Support container	Overall width	179.1 cm (70.5 in.) with E-Box	148.5 cm (58.5 in.)
	Overall length	171.4 cm (67.5 in.)	
	Overall height	321.1 cm (126.4 in.) with filter bracket	
	Dry skid weight (mass)	962.1 kg (2,121 lb.)	
	Wet skid weight—rated working volume (mass)	2,962.1 kg (6,530 lb.)	
Recommended operating parameters	Operating temperature range	Ambient to 40°C ± 0.5°C (104°F ± 0.9°F)	
	Motor speed	Standard operation: 20–75 rpm, working volumes must stay above 50% during agitation*	
	Volume range	1,000–2,000 L	
	Maximum BPC pressure	0.03 bar (0.5 psi)	

*** WARNING:** Mixing speeds must stay within the recommended operating parameters. Higher speed operation compromises system reliability and will void standard Thermo Fisher warranties. Your control strategy should include governors that regulate rpm based on liquid volume as well as safety interlocks that disable mixing when the liquid drops below the recommended volume. See section 3.6.5 for more information about operating parameters, agitation speed governors, and safety interlocks.

2,000 L Dimensional drawings

The dimensions provided below are displayed in (inches [centimeters]). See your sales representative for ordering information.

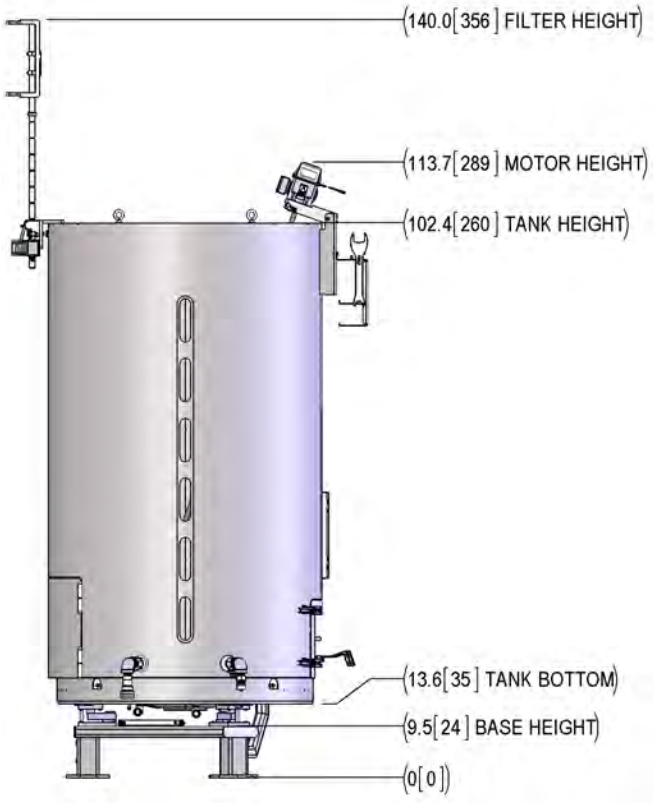


Figure 4.17. Dimensions of 2,000 L S.U.B. (front view).

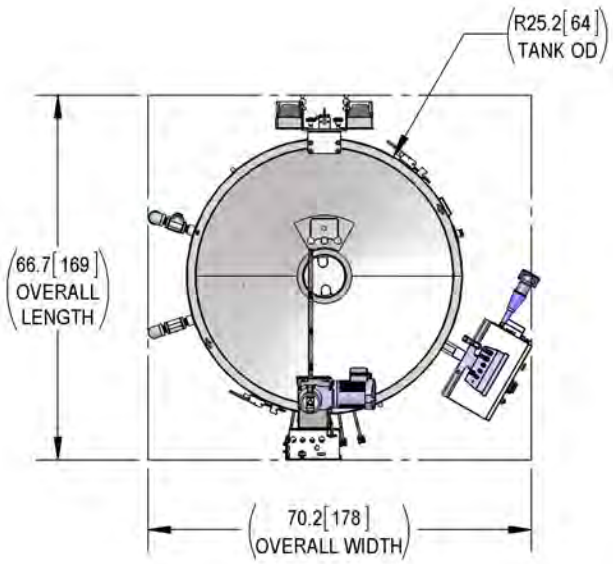


Figure 4.18. Dimensions of 2,000 L S.U.B. (top view).

4.3 E-Box features

Figure 4.19 illustrates the features of the E-Box available for 50, 100, 250, 500, 1,000, and 2,000 L S.U.B. units. Figure 4.20 illustrates the bottom view.

Figure 4.19. Front view of E-Box for 50–2,000 L S.U.B.s.

Continuous power: For devices that should not be turned off during an E-Stop. Examples are recording devices, sensors, and controllers. The maximum for each receptacle is 1/2 amp.

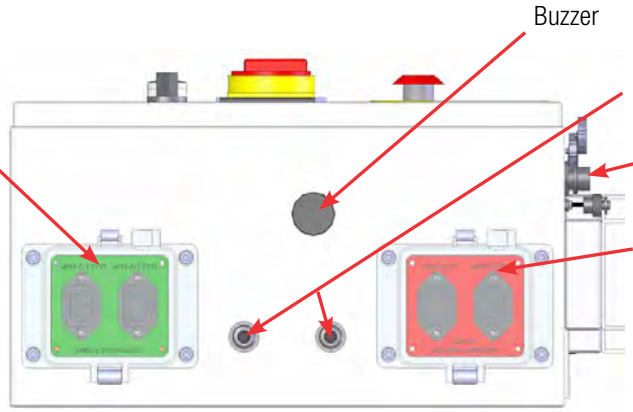


Figure 4.20. Bottom view of all E-Boxes.

Emergency shut-off power: For devices that should be turned off when an operator activates an E-Stop. Examples are pumps, motors, and any device that could harm an operator or ruin a batch during an E-Stop. The maximum for each receptacle is 2 amps.

4.4 BPC specifications

The following figures and tables provide specification information for 2:1 BPCs in 50, 100, 250, 500, 1,000, and 2,000 L sizes.

2:1 S.U.B. 50 and 100 L BPC with porous frit and open pipe spargers

Specification information for the numbered items in Figure 4.21 is located in Table 4.13 on the following page.

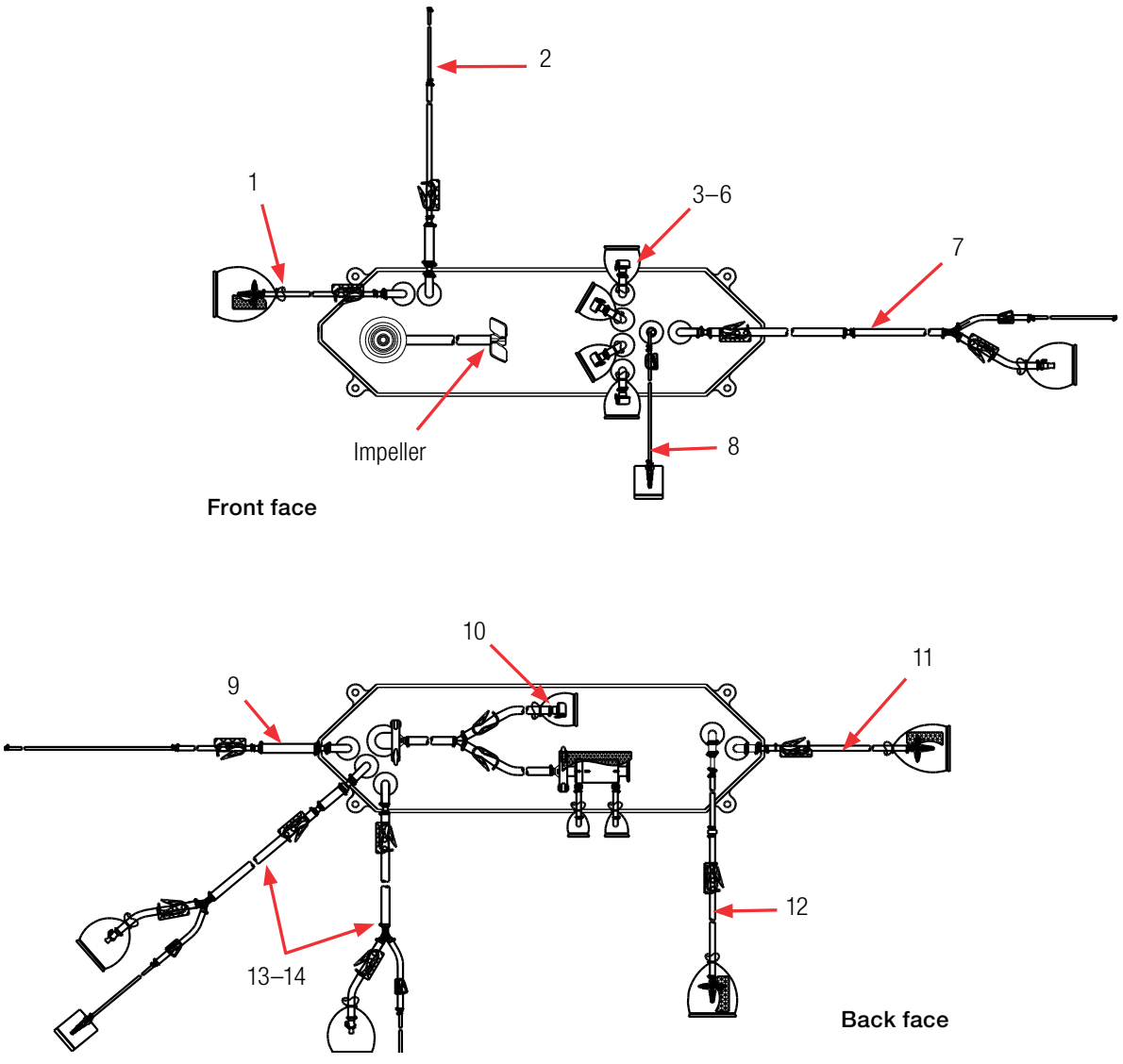


Figure 4.21. Front and back faces of 50 and 100 L S.U.B. BPC with porous frit and open pipe.

Table 4.13. Specification information for 50 and 100 L BPCs with porous frit and open pipe spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	0.2 micron hydrophobic vent filter with Emflon II
2	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7.16 in.) C-Flex tubing x 152 cm (60 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged
3–6	Probe ports (4)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
7	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex Tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged 9.5 mm (3/8 in.) MPC insert
8	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter RTD and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 46 cm (18 in.)	SterilEnz pouch with injection site assembly
9	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 152 cm (60 in.)	Plugged
10	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 30 cm (12 in.)	Pall Kleenpak aseptic connector—KPCHT series (female), Pall Kleenpak 0.2 micron exhaust vent filter
11	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) connected to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 122 cm (48 in.) for 50 L size, or 122 cm (48 in.) for 100 L size	Hydrophobic vent filter with Emflon II
12	Open pipe macro sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 122 cm (48 in.)	0.2 micron hydrophobic vent filter with Emflon II
13–14	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 152 cm (60 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm 12 in.) and 9.5 mm (3.8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly, 9.5 mm (3/8 in.) MPC body

2:1 S.U.B. 50 and 100 L BPC with porous frit and drilled hole spargers

Specification information for the numbered items in Figure 4.22 is located in Table 4.14 on the following page.

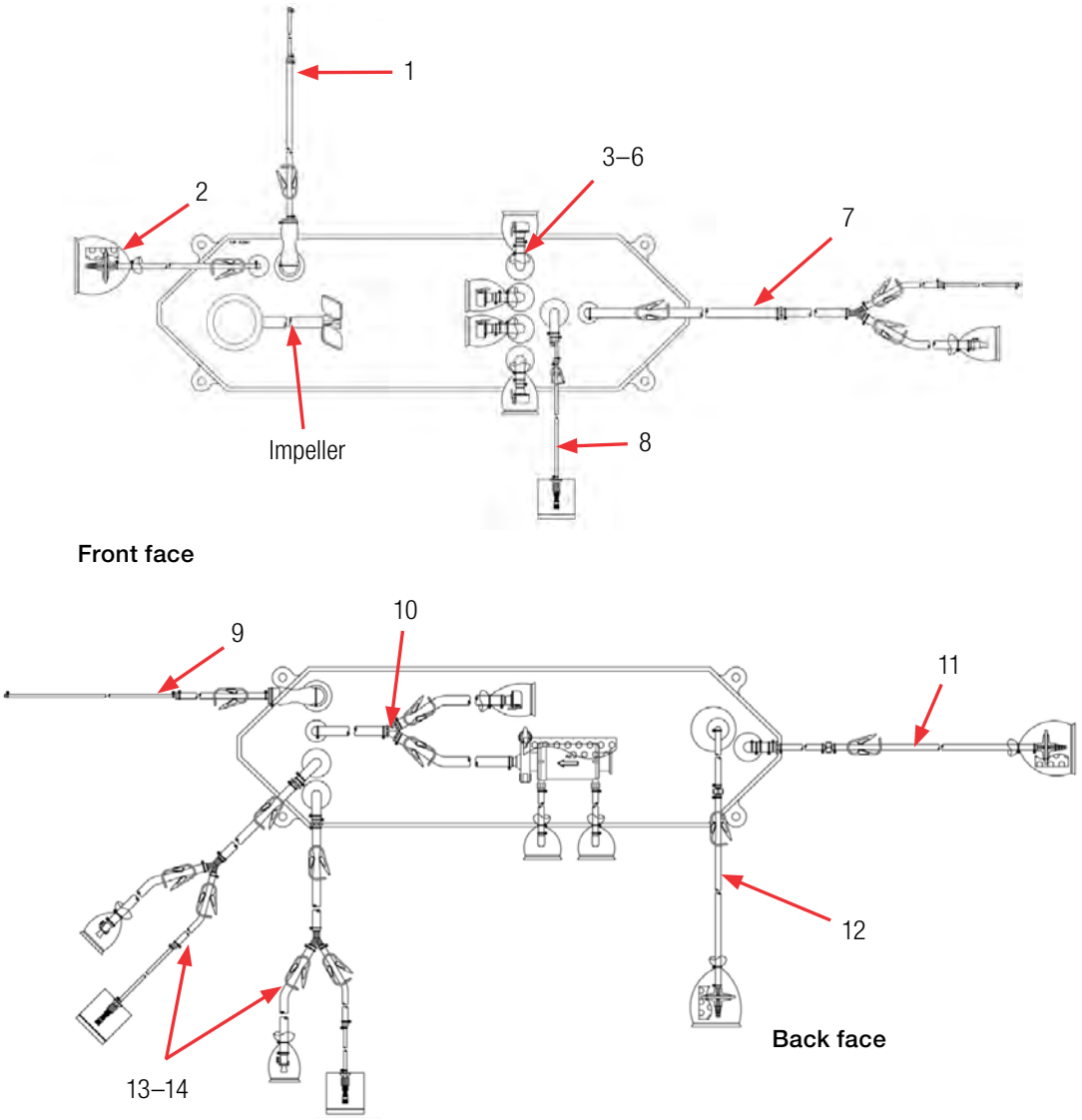


Figure 4.22. Front and back faces of 50 and 100 L S.U.B. BPC with porous frit and drilled hole spargers.

Table 4.14. Specification information for 50 and 100 L BPCs with porous frit and drilled hole spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 152 cm (60 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged
2	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Hydrophobic vent filter with Emflon II, connected to 15 cm (6 in.) C-Flex tubing
3–6	Probe ports (4)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
7	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex Tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged 9.5 mm (3/8 in.) MPC insert
8	Thermowell/small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter RTD and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 46 cm (18 in.)	SterilEnz pouch with injection site assembly
9	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 152 cm (60 in.)	Plugged
10	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 20 cm (8 in.) connected to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	Pall Kleenpak aseptic connector—KPCHT series (female) Pall Kleenpak 0.2 micron exhaust vent filter
11	Porous frit micro sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) connected to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 89 cm (35 in.) for 50 L size, or 107 cm (42 in.) for 100 L size	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
12	Drilled hole macro sparger 8.9 cm (3/5 in.) disk with 360 x 0.178 mm (0.007 in.) holes for 50 L size or 570 holes for 100 L size	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 97 cm (38 in.) for 50 L size, or 114 cm (45 in.) for 100 L size	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
13–14	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 152 cm (60 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly, 9.5 mm (3/8 in.) MPC body

2:1 S.U.B. 250 L BPC with porous frit and open pipe spargers

Specification information for the numbered items in Figure 4.23 is located in Table 4.15 on the following page.

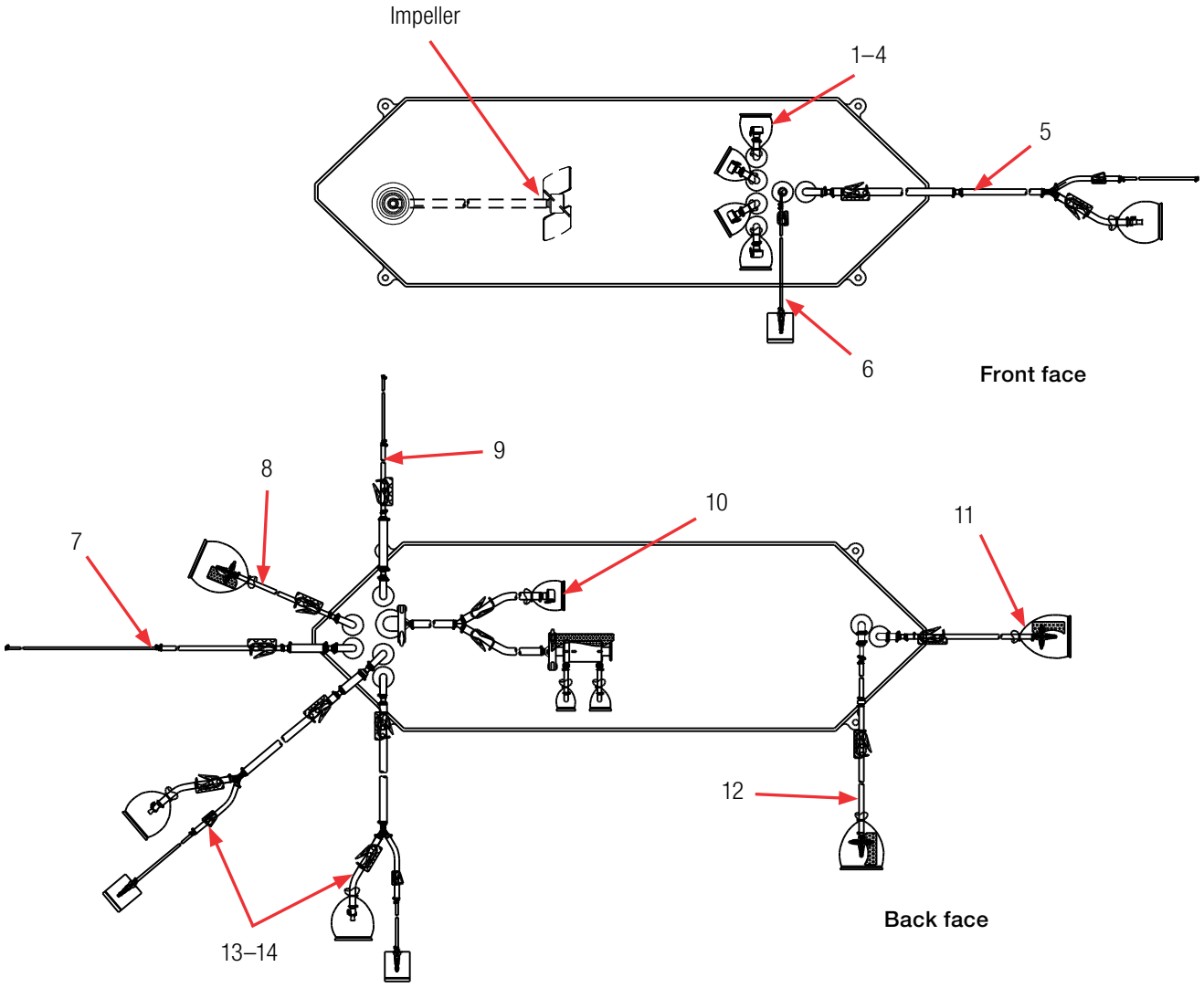


Figure 4.23. Front and back faces of 250 L S.U.B. BPC with porous frit and open pipe spargers.

Table 4.15. Specification information for 250 L BPCs with porous frit and open pipe spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–4	Probe ports (4)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
5	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex Tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged 9.5 mm (3/8 in.) MPC insert
6	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter RTD and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 46 cm (18 in.)	SterilEnz pouch with injection site assembly
7	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 152 cm (60 in.)	Plugged
8	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Hydrophobic vent filter with Emflon II
9	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 152 cm (60 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm 1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged
10	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	Pall Kleenpak connector—KPCHT series (female), Pall Kleenpak 0.2 micron exhaust vent filter
11	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 122 cm (48 in.)	Hydrophobic vent filter with Emflon II
12	Open pipe macro sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 (7/16 in.) C-Flex tubing x 122 cm (48 in.)	Hydrophobic vent filter with Emflon II
13–14	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 152 cm (60 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SteriEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body

2:1 S.U.B. 250 L BPC with porous frit and drilled hole spargers

Specification information for the numbered items in Figure 4.24 is located in Table 4.16 on the following page.

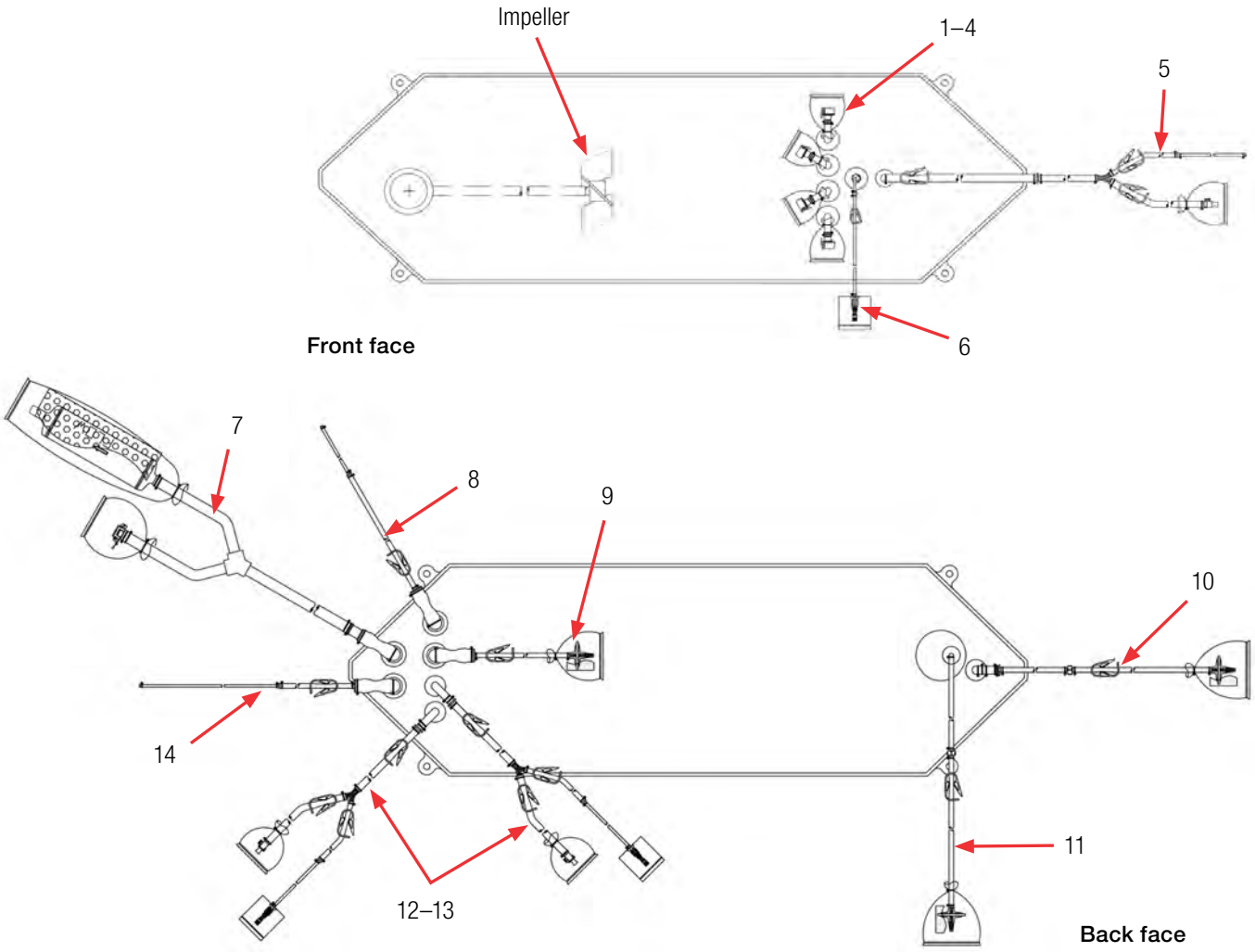


Figure 4.24. Front and back faces of 250 L S.U.B. BPC with porous frit and drilled hole spargers.

Table 4.16. Specification information for 250 L BPCs with porous frit and drilled hole spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–4	Probe ports (4)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
5	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex Tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged and 9.5 mm (3/8 in.) MPC insert
6	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter RTD and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 46 cm (18 in.)	SterilEnz pouch with injection site assembly
7	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
8	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 152 cm (60 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) x 30 cm (12 in.)	Plugged
9	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 30 cm (12 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.)	CPC AseptiQuik G (genderless) connector, 2 Meissner Ultracap 0.2 micron hydrophobic filters connected to 15 cm (6 in.) C-Flex tubing
10	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 142 cm (56 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
11	Drilled hole macro sparger 12.2 cm (4.8 in.) disk with 760 x 0.233 mm (0.009 in.) holes	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 150 cm (59 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
12–13	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 152 cm (60 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SteriEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
14	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 152 cm (60 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing

2:1 S.U.B. 500 L BPC with porous frit and open pipe spargers

Specification information for the numbered items in Figure 4.25 is located in Table 4.17 on the following page.

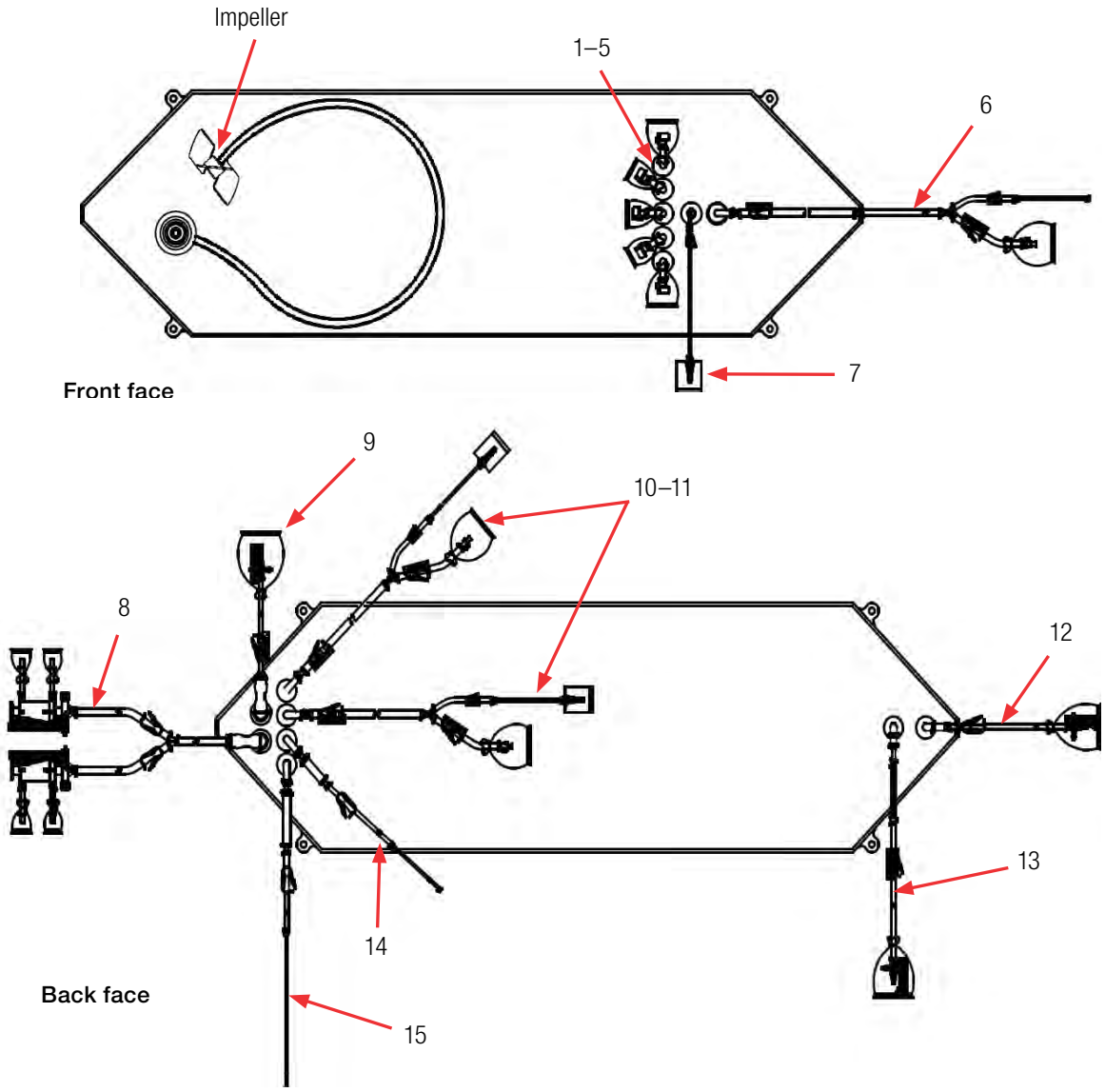


Figure 4.25. Front and back faces of 500 L S.U.B. BPC with porous frit and open pipe spargers.

Table 4.17. Specification information for 500 L BPCs with porous frit and open pipe spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–5	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
6	Bottom drain line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged, 9.5 mm (3/8 in.) MPC insert
7	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 60 cm (24 in.)	SterilEnz pouch with injection site assembly
8	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	2 Pall Kleenpak 0.2 micron exhaust vent filters
9	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Hydrophobic vent filter with Emflon II
10–11	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
12	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
13	Open pipe macro sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
14	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
15	Inoculum addition	6.4 mm (1/4 in) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged

2:1 S.U.B. 500 L BPC with porous frit and drilled hole spargers

Specification information for the numbered items in Figure 4.26 is located in Table 4.18 on the following page.

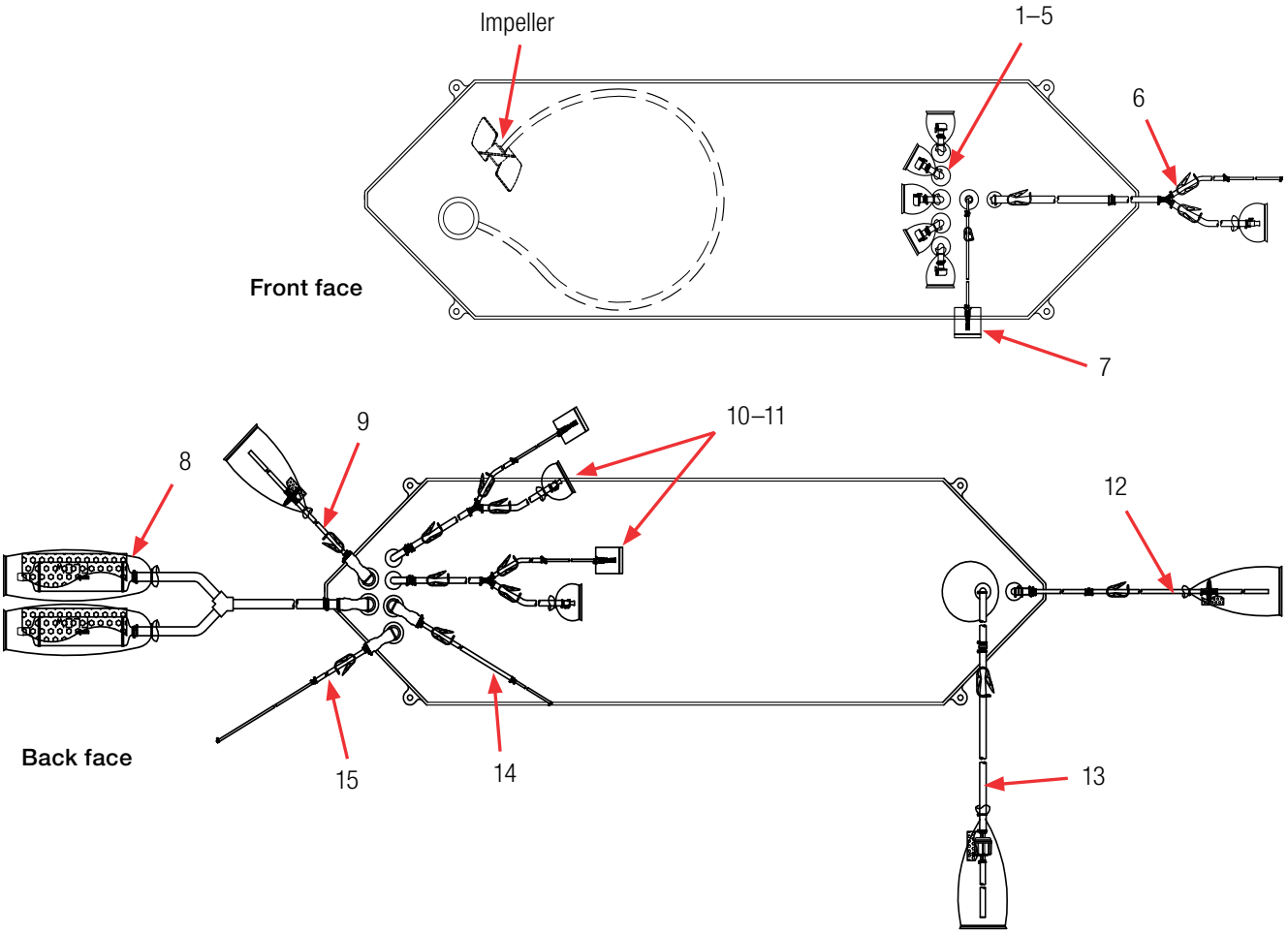


Figure 4.26. Front and back faces of 500 L S.U.B. BPC with porous frit and drilled hole spargers.

Table 4.18. Specification information for 500 L BPCs with porous frit and drilled hole spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–5	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
6	Bottom drain line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged, 9.5 mm (3/8 in.) MPC insert
7	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 60 cm (24 in.)	SterilEnz pouch with injection site assembly
8	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 30 cm (12 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.)	(2) Meissner Ultracap 0.2 micron hydrophobic filters connected to 15 cm (6 in.) C-Flex tubing
9	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 30 cm (12 in.) C-Flex tubing
10–11	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
12	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
13	Drilled hole macro sparger 17.1 cm (6.75 in.) disk with 1,180 x 0.445 mm (0.018 in.) holes	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 190 cm (75 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
14	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
15	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged

2:1 S.U.B. 1,000 L BPC with porous frit and open pipe spargers

Specification information for the numbered items in Figure 4.27 is located in Table 4.19 on the following page.

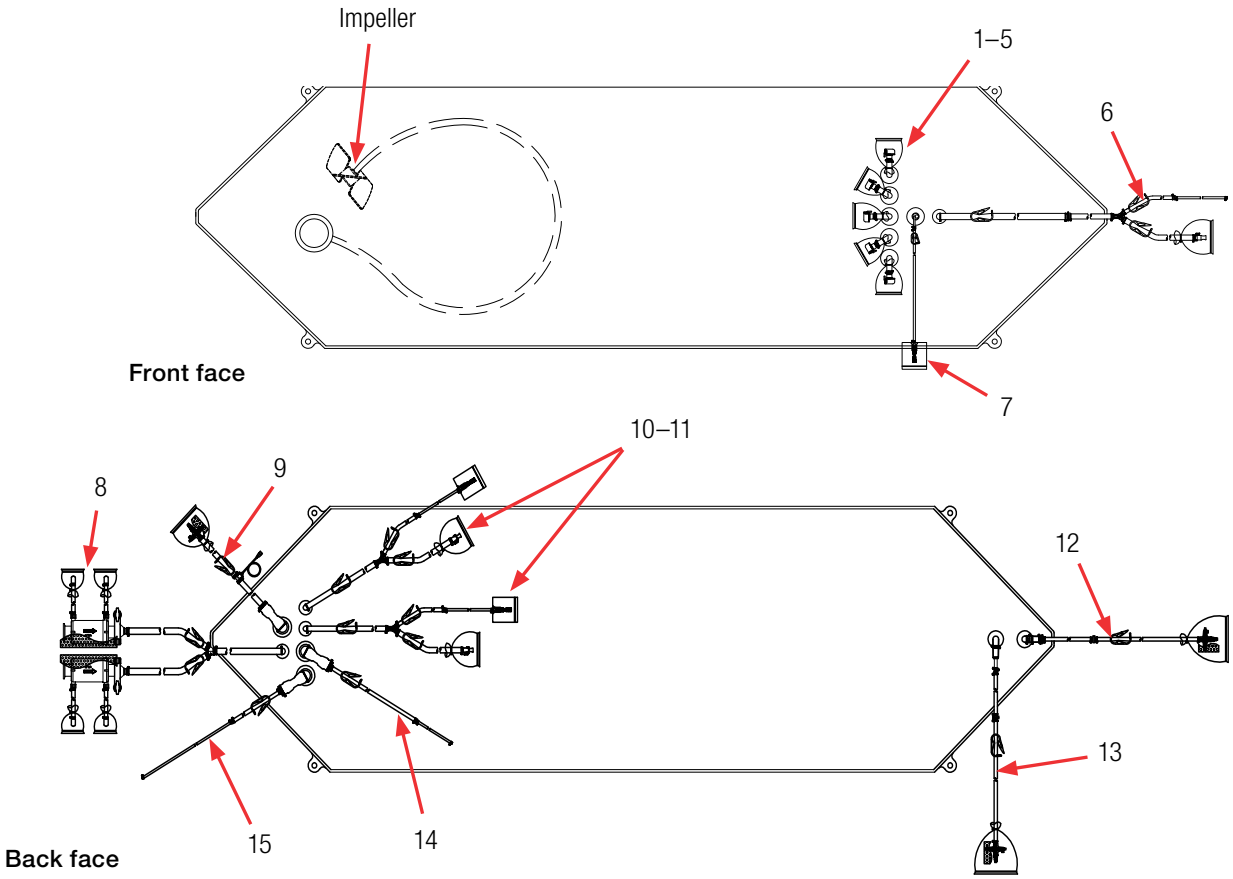


Figure 4.27. Front and back faces of 1,000 L S.U.B. BPC with porous frit and open pipe spargers.

Table 4.19. Specification information for 1,000 L BPCs with porous frit and open pipe spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–5	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
6	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged 9.5 mm (3/8 in.) MPC insert
7	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 60 cm (24 in.)	SterilEnz pouch with injection site assembly
8	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	2 Pall Kleenpak 0.2 micron exhaust vent filters
9	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Hydrophobic vent filter with Emflon II, pressure transducer
10–11	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
12	Open pipe macro sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 8 cm (3 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
13	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
14	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
15	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged

2:1 S.U.B. 1,000 L BPC with porous frit and drilled hole spargers

Specification information for the numbered items in Figure 4.28 is located in Table 4.20 on the following page.

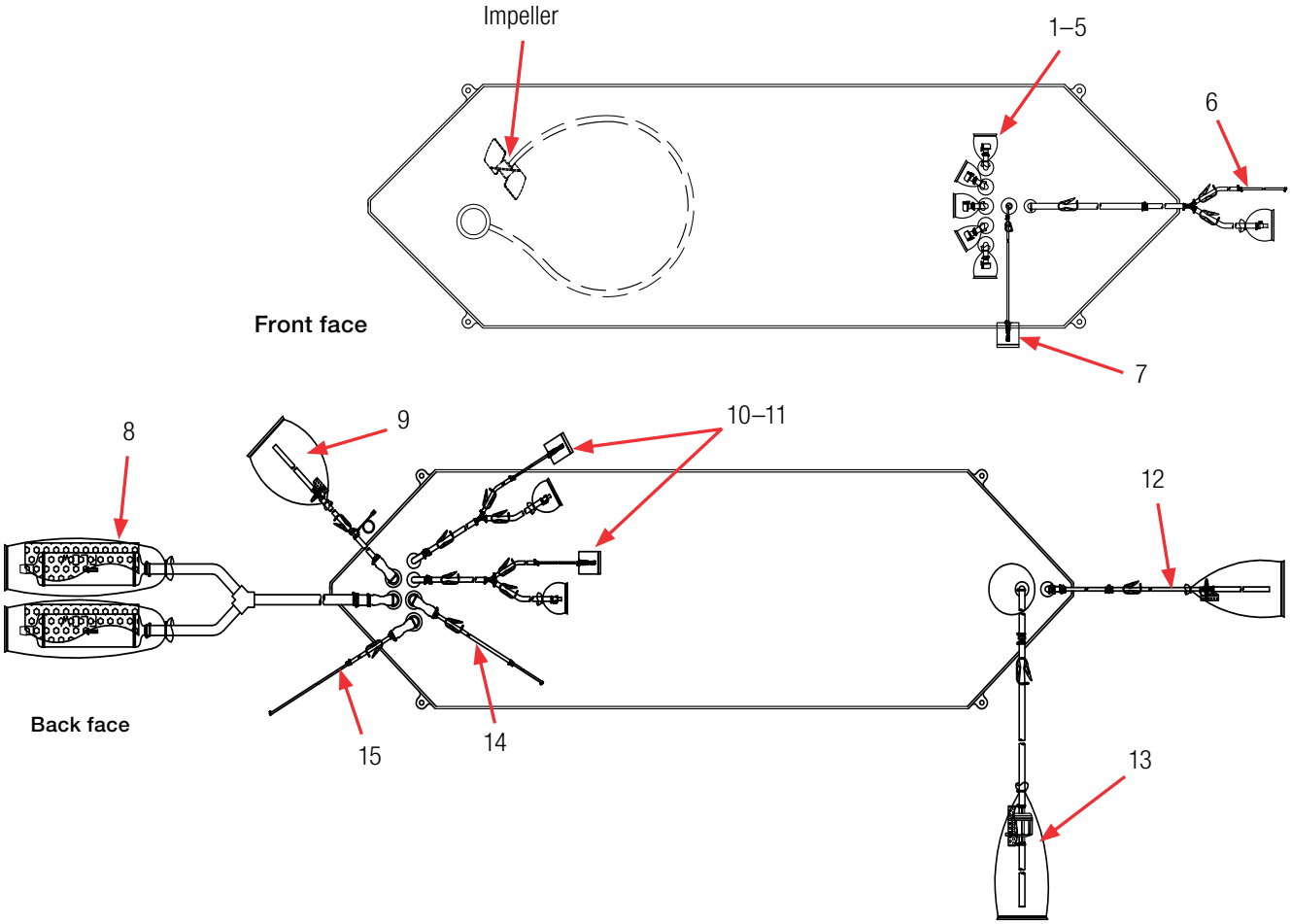


Figure 4.28. Front and back faces of 1,000 L S.U.B. BPC with porous frit and drilled hole spargers.

Table 4.20. Specification information for 1,000 L BPCs with porous frit and drilled hole spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–5	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors—KPCHT series (female)
6	Bottom drain harvest	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 152 cm (60 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	Plugged 9.5 mm (3/8 in.) MPC insert
7	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 60 cm (24 in.)	SterilEnz pouch with injection site assembly
8	Exhaust line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 30 cm (12 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 15 cm (6 in.)	(2) Meissner Ultracap 0.2 micron hydrophobic filters connected to 15 cm (6 in.) C-Flex tubing
9	Overlay gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 30 cm (12 in.) C-Flex tubing
10–11	Feed lines	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.) splits to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 30 cm (12 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.) and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 30 cm (12 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
12	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Hydrophobic vent filter with Emflon II
13	Drilled hole macro sparger 17.1 cm (6.75 in.) disk with 1,180 x 0.445 mm (0.018 in.) holes	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 246 cm (97 in.)	Meissner Steridyne 0.2 micron hydrophobic filter connected to 15 cm (6 in.) C-Flex tubing
14	Base addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
15	Inoculum addition	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged

2:1 S.U.B. 2,000 L BPC with porous frit and open pipe spargers

Specification information for the numbered items in Figure 4.29. is located in Table 4.21 on the following page.

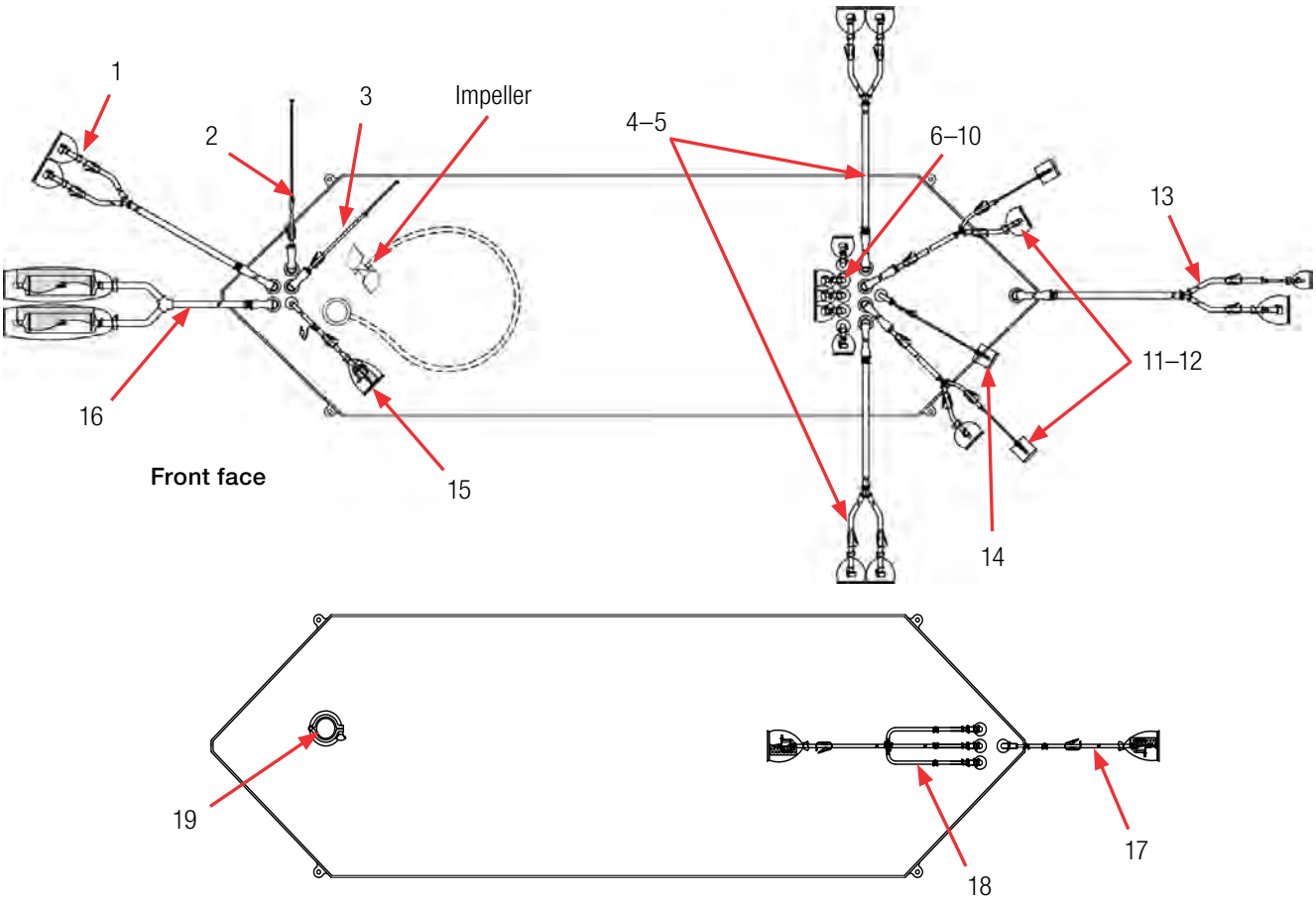


Figure 4.29. Front and back faces of 2,000 L S.U.B. BPC with porous frit and open pipe spargers.

Table 4.21. Specification information for 2,000 L BPCs with porous frit and open pipe spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1	Media fill	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex tubing x 213 cm (84 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.)	Pall Kleenpak aseptic connectors (female)
2	Inoculum addition/fill line	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 8 cm (3 in.) reduced to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
3	Base addition	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 8 cm (3 in.) reduced to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged
4–5	Media fill/ auxiliary drain	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex tubing x 213 cm (84 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.)	Pall Kleenpak aseptic connectors (female)
6–10	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors (female)
11–12	Feed lines	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
13	Bottom drain harvest	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex tubing x 122 cm (48 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.) reduced to 6.4 mm (1/4 in.) x 9.5 mm (3/8 in.) C-Flex tubing x 30 cm (12 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.)	6.4 mm (1/4 in.) MPC insert and Pall Kleenpak (male)
14	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 61 cm (24 in.)	SterilEnz pouch with injection site assembly
15	Overlay gas sparger	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.)	Pall Kleenpak Emflon II capsule and pressure transducer
16	Exhaust line		
17	Open pipe macro gas sparger	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Pall Kleenpak Emflon II capsule
18	Porous frit micro gas sparger (12 mm diameter, 25 micron pores)	(3) 12 mm PDVF porous sparger inserts connected to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) converge to one 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	Pall Kleenpak Emflon II capsule
19	7.6 cm (3 in.) Tri-clamp port	N/A	Gasket end cap and clamp

2:1 S.U.B. 2,000 L BPC with porous frit and drilled hole spargers

Specification information for the numbered items in Figure 4.30 is located in Table 4.22 on the following page.

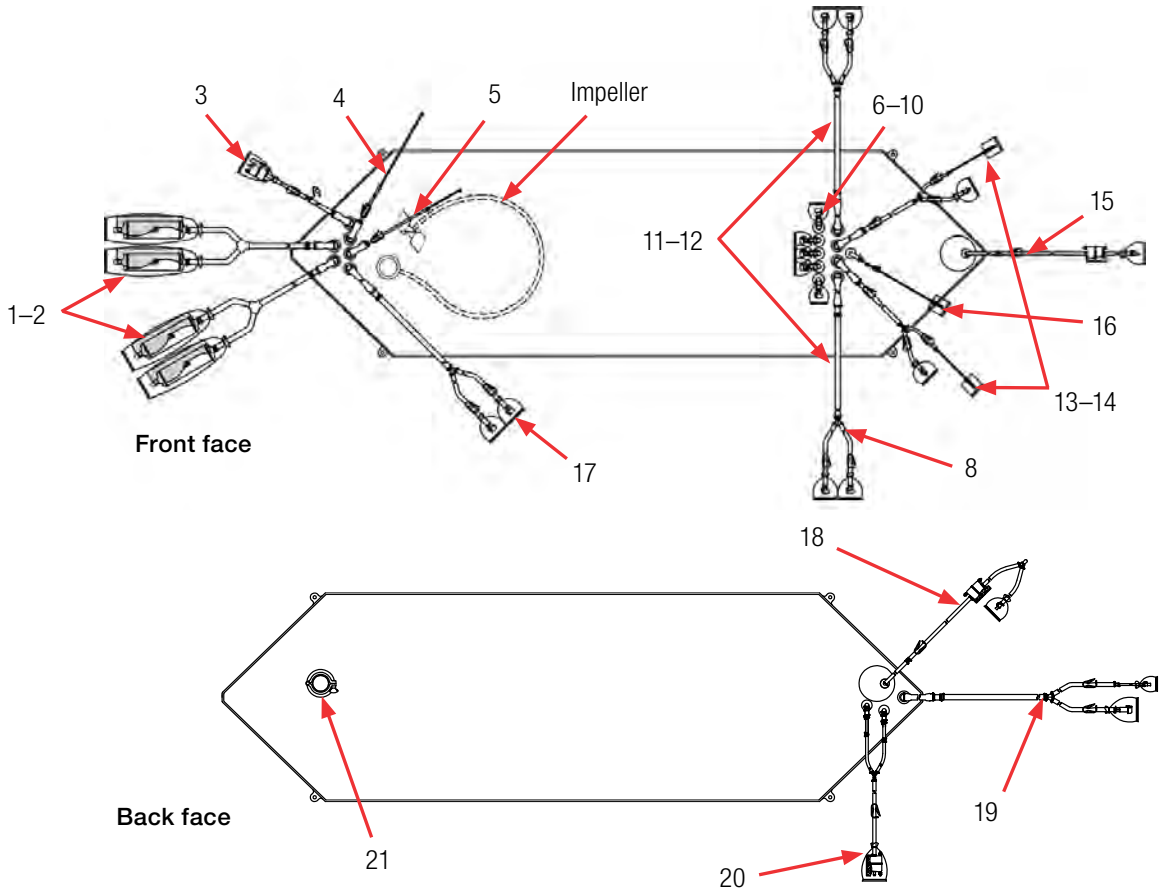


Figure 4.30. Front and back faces of 2,000 L S.U.B. BPC with porous frit and drilled hole spargers.

Table 4.22. Specification information for 2,000 L BPCs with porous frit and drilled hole spargers.

Item	Description	Tubing set (ID x OD x length)	End treatment
1–2	Exhaust lines		
3	Overlay gas sparger	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) reduced to 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 213 cm (84 in.)	Pall Kleenpak Emflon II capsule and pressure transducer
4	Inoculum addition	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 8 cm (3 in.) reduced to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 213 cm (84 in.)	Plugged
5	Base addition	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 8 cm (3 in.) reduced to 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 213 cm (84 in.) reduced to 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 30 cm (12 in.)	Plugged
6–10	Probe ports (5)	12.7 mm (1/2 in.) tube ports	Pall Kleenpak aseptic connectors (female)
11–12	Media fill/ auxiliary drain (2)	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex tubing x 213 cm (84 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.)	Pall Kleenpak aseptic connectors (female)
13–14	Feed lines (2)	12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 10 cm (4 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 25 cm (10 in.)	SterilEnz pouch with injection site assembly and 9.5 mm (3/8 in.) MPC body
15	Drilled hole macro sparger 17.1 cm (6.75 in.) disk with 690 x 0.582 mm (0.023 in.) holes	9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 8 cm (3 in.) connected to check valve and 9.5 mm (3/8 in.) x 15.9 mm (5/8 in.) C-Flex tubing x 183 cm (72 in.)	(2) Meissner Steridyne 0.2 micron hydrophobic filter with 9.5 mm (3/8 in.) C-Flex tubing (Y-connector and quick connects provided for joining lines)
16	Thermowell/ small volume sample	Thermowell adapter for 6.4 mm (1/4 in.) diameter and 3.2 mm (1/8 in.) x 6.4 mm (1/4 in.) C-Flex tubing x 61 cm (24 in.)	SterilEnz pouch with injection site assembly
17	Media fill	(See items 11–12)	(See items 11–12)
18	Drilled hole macro sparger	(See item 15)	(See item 15)
19	Bottom drain harvest	19.1 mm (3/4 in.) x 25.4 mm (1 in.) C-Flex tubing x 122 cm (48 in.) splits to 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.) reduced to 6.4 mm (1/4 in.) x 9.5 mm (3/8 in.) C-Flex tubing x 30 cm (12 in.) and 12.7 mm (1/2 in.) x 19.1 mm (3/4 in.) C-Flex tubing x 61 cm (24 in.)	6.4 mm (1/4 in.) MPC insert and Pall Kleenpak (male)
20	Porous frit micro sparger 12 mm diameter (25 micron pores)	6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 15 cm (6 in.) reduced to check valve and 6.4 mm (1/4 in.) x 11.1 mm (7/16 in.) C-Flex tubing x 183 cm (72 in.)	(2) Meissner Steridyne 0.2 micron hydrophobic filter
21	7.6 cm (3 in.) tri-clamp port	N/A	Gasket end cap and clamp

Table 4.23. Custom BPC options.

Category	Options/capability	Notes
Tubing type	C-Flex, platinum cured silicone, PVC, PharMed, PharmaPure	More information is available in the Tubing Selection Guide
Tubing size	Ranges from 1/8 in. ID to 1 in. ID in various lengths	More information is available in the Tubing Selection Guide
Connectors	Luers, CPC quick connects, SIP connectors, tri-clamp, Kleenpak, Lynx, SmartSite, Clave, Lynx steam thru, CPC steam thru, Gore steam valve, Gore Mini TC, BioQuate, SterilEnz, end plug, etc.	More information is available in the Connection System Selection Guide
Probe ports	Additional ports: second row of four for 50 to 250 L S.U.B.; second row of five for 500 and 1,000 L S.U.B.s	N/A
Disposable sensors	Pressure sensor: PendoTECH (PendoTECH comes standard on 500 and 1,000 L) DO and pH: Hamilton pH: Mettler Toledo	Choice of qualified sensors available
Addition of ports/lines (other than 2nd row of probe ports)	Limited engineer-to-order customization only	Dependent on location in bag and compatibility with hardware
Port sizes	Limited engineer-to-order customization only	Dependent on location in bag and fit with hardware (e.g. 1 in. ID port on harvest line)
Rearrangement of lines on existing ports	Limited customization possible, e.g. moving sample/thermowell port to a probe tube port, or swapping overlay inlet line with supplement line.	Dependent on location in bag and fit with hardware
Sparger	Dual sparger (open pipe or drilled hole plus porous frit) standard	N/A
Dip tube lines	Limited customization possible	Length cannot interfere with impeller and shaft
Overlay and sparge line filters	Filter options available from standard component library	N/A
Vent filters	Standard is Pall Kleenpak or Meissner SterilEnz 0.2 micron exhaust vent filter	Filters must be compatible with available vent filter heater configurations
Vent filter tubing length	Extended filter height above the S.U.B. bag is make-to-order	Must be compatible with a vent filter bracket option
Filters on media and supplement inlets	Limited engineer-to-order customization only; choice of filters used to sterilize incoming media or supplements are available	N/A

Note: Not all options are available for all ports. No customization of port type and location, chamber dimensions, or mixing assembly is possible. For additional information, please see the Selection Guides in the S.U.B. BPC Catalog.

2:1 S.U.B. BPC packing information

Standard 2:1 S.U.B. BPC packaging is listed in Table 4.24, below.

Table 4.24. Standard 2:1 BPC packaging.

Component	Description
Outer packaging	Supplied "flat-packed"; two polyethylene outer layers
Label	Description, product code, lot number, expiry date on outer packaging, and shipping container
Sterilization	Irradiation (25 to 40 kGy) inside outer packaging
Shipping container	Durable cardboard carton
Documentation	Certificate of Analysis provided with each lot for delivery

4.5 Additional system component part numbers

The following tables list part numbers for additional 2:1 S.U.B. system components, such as drive shafts, load cell kits, and accessories.

Table 4.25. Drive shaft part numbers.

Description	Cat. no.
50 L 1-piece aluminum drive shaft	SV50177.34
100 L 1-piece aluminum drive shaft	SV50177.14
250 L 1-piece aluminum drive shaft	SV50177.35
250 L 1-piece stainless steel drive shaft	SV50177.40
250 L 2-piece stainless steel drive shaft	SV50177.41
500 L 2-piece stainless steel drive shaft	SV50177.36
500 L 3-piece stainless steel drive shaft	SV50959.05
1,000 L 3-piece stainless steel drive shaft	SV50177.38
1,000 L 4-piece stainless steel drive shaft	SV50177.39
2,000 L 2-piece carbon fiber drive shaft	SV50959.21

Table 4.26. Load cell part numbers. 1,000 and 2,000 L S.U.B.s include load cells as standard equipment. The following kits are for retro-fitting to 50, 100, 250, and 500 L S.U.B. systems.

Description	Cat. no.
50–100 L S.U.B. load cell kit with summing block, no display	SV50988.01
250 L S.U.B. load cell kit with summing block, no display	SV50988.02
500 L S.U.B. load cell kit with summing block, no display	SV50988.03

Table 4.27. Harsh mount load cell display part numbers for 50–500 L and 2,000 L systems.

Description	Cat. no.
Mettler Toledo IND331 display, harsh mount style with analog interface (STD), 120 VAC US line cord/plug	SV50177.306
Mettler Toledo IND331 display, harsh mount style with Allen-Bradley RIO interface, 120 VAC US line cord/plug	SV50177.307
Mettler Toledo IND331 display, harsh mount style with Device Net interface, 120 VAC US line cord/plug	SV50177.308
Mettler Toledo IND331 display, harsh mount style with Ethernet/IP and Modbus TCP interface, 120 VAC US line cord/plug	SV50177.309
Mettler Toledo IND331 display, harsh mount style with Profibus interface, 120 VAC US line cord/plug	SV50177.310

Table 4.28. Panel mount load cell display part numbers for 1,000 L systems only.

Description	Cat. no.
Mettler Toledo IND331 display, panel mount style with analog interface (STD), 120 VAC US line cord/plug	SV50177.291
Mettler Toledo IND331 display, panel mount style with Allen-Bradley RIO interface, 120 VAC US line cord/plug	SV50177.292
Mettler Toledo IND331 display, panel mount style with Device Net interface, 120 VAC US line cord/plug	SV50177.293
Mettler Toledo IND331 display, panel mount style with Ethernet/IP and Modbus TCP interface, 120 VAC US line cord/plug	SV50177.294
Mettler Toledo IND331 display, panel mount style with Profibus interface, 120 VAC US line cord/plug	SV50177.295

Table 4.29. Cable management system part numbers.

Description	Cat. no.
Cable management system (50 and 100 L S.U.B.s)	SV50992.01
Cable management system (250 L S.U.B.s)	SV50992.02
Cable management system (500 L S.U.B.s)	SV50992.03
Cable management system (1,000 L S.U.B.s)	SV50992.04

Table 4.30. Vent filter heater kit part numbers for use with Pall KA3 vent filters. Includes vent filter heater, controller with water-tight closure, quick connects, and installation power cord.

Description	Cat. no.
NEMA rated vent heater with programmable controller (100–120 VAC), power cord. Includes low-temp. alarm, preset temp. 50°C, and power cord with flying leads.	SV50191.11
NEMA rated vent heater with programmable controller (200–240 VAC), power cord. Includes low temp. alarm, preset temp. 50°C, and power cord with flying leads.	SV50191.13

Table 4.31. Vent filter heater kit part numbers for use with Meissner Ultracap 10 inch vent filters. Includes vent filter heater, controller with water-tight closure, quick connects, and installation power cord.

Description	Cat. no.
NEMA rated vent heater with programmable controller (100–120 VAC). Includes low-temp. alarm, preset temp. 50°C, and 20 ft. NEMA 5–15 power cord for US/Japan.	SV50191.16
NEMA rated vent heater with programmable controller (200–240 VAC). Includes low temp. alarm, preset temp. 50°C, and 20 ft. BS1363 power cord for UK.	SV50191.17
NEMA rated vent heater with programmable controller (200–240 VAC). Includes low temp. alarm, preset temp. 50°C, and 20 ft. CEE7/7 power cord for Europe.	SV50191.18
NEMA rated vent heater with programmable controller (200–240 VAC). Includes low temp. alarm, preset temp. 50°C, and 12 ft. IEC320 power cord for 2,000 L S.U.B. control box.	SV50191.19

Table 4.32. AC and DC motor part numbers.

Description	Cat. no.
50, 100, and 250 L S.U.B. DC motor	SV50237.07
50, 100, and 250 L S.U.B. AC motor	SV50237.16
500, 1,000, and 2,000 L S.U.B. DC motor	SV50237.22
500 L S.U.B. AC motor	SV50237.18
1,000 and 2,000 L S.U.B. AC motor	SV50237.19

Table 4.33. Condenser system part numbers (for 2,000 L systems only).

Description	Cat. no.
Condenser system (120 V) including cart, chill plate, and mounting post with filter brackets, TCU, and pump	SV50232.01
Condenser system (240 V) including cart, chill plate, and mounting post with filter brackets, TCU, and pump	SV50232.02

Table 4.34. Miscellaneous and accessory part numbers.

Component	Cat. no.
Probe assembly with CPC AseptiQuik connector (non-sterile, for use in autoclave)	SH30720.02
Probe assembly with Pall Kleenpak connector (non-sterile, for use in autoclave)	SH30720.01
Heavy-duty tubing clamp	SV20664.01
Stainless steel autoclave tray, for autoclaving probe assemblies	SV50177.01
Probe clips	SV50177.23
Sterile sampling manifold with luer lock	SH30845.01
Temperature/sample port	SV20750.01
Sparge line support	SV50177.19
Mobile stairs (for 2,000 L systems only)	SV50935.01

5

Maintenance and troubleshooting

Chapter contents

5.1 Maintenance

5.1.1 Routine maintenance

Environmental conditions, operating parameters, and adhering to standard operating procedures as outlined in this user's guide have significant impact upon the useful life of your S.U.B. hardware system. The following guidelines are based upon the standard operating conditions outlined in this user's guide.

High-wear items such as bearings, seals, O-rings, and sterilization valves common to conventional bioreactor systems have been purposefully considered in the design of the construction of the S.U.B. The S.U.B. system is inherently robust and requires low levels of routine maintenance. Taking time between bioreactor runs to clean the exterior of the hardware will improve the appearance and overall longevity of the system. The drive motor is an industrial grade induction motor with a permanently sealed and lubricated gear box. The drive shaft is constructed to wear slightly with use and should be visually inspected after each run. Visual inspection of wear components and following the guidelines listed below will be sufficient to ensure dependable service. Replacement parts are available.

5.1.2 Preventive maintenance

- Lightly coat the drive cap threads with food-grade anti-seize if the motor cap becomes difficult to turn.
- For multiple-segment drive shafts without quick connects, lightly coat the threads with food-grade anti-seize during assembly.
- Replacement of the mixing motor is recommended every five years, or as needed.
- Refer to the following section of this user's guide for expected drive shaft longevity based on usage.

Replace worn drive shaft head assembly when the hex diameter at its widest location measures equal to or less across the points (Figure 5.1). Diameters are measured at the widest location across the points.

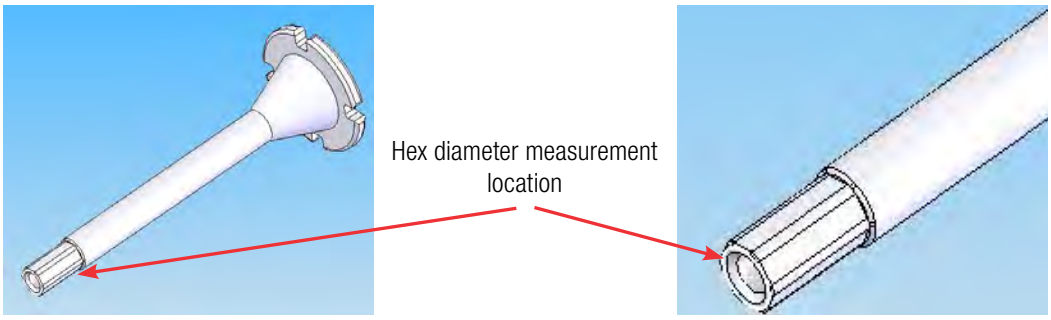


Figure 5.1. Shaft head assembly.

Under normal operating conditions, replace the drive shaft assembly for 50–1,000 L systems after 360 days of service, or refer to the drive shaft head wear specifications in Table 5.1, below.

Table 5.1. Drive shaft head hex diameters.

S.U.B. system	New hex diameter	Minimum hex diameter
50, 100, and 250 L	14.9 mm (0.587 in.) ± 0.127 mm	14.4 mm (0.566 in.)
500 and 1,000 L	21.3 mm (0.839 in.) ± 0.127 mm	20.8 mm (0.820 in.)

Operating parameters for the 2,000 L two-piece drive shaft are provided in Table 5.2, below.

Table 5.2. 2,000 L S.U.B. drive shaft operating parameters.

Max P/V Np = 2.1	Impeller/motor speed (rpm)	Motor speed ramp rate (rpm/sec)*	Volume (L)	Max. operation time
N/A	30	1	400–800	180 days (4,320 hrs)
≤ 20 W/m^3	55–75	1	800–2,100	180 days (4,320 hrs)
> 20 W/m^3 and ≤ 40 W/m^3	63–95	1	800–2,100	Single run, 21 days (456 hrs)

***Note**: System motor speed/volume control to be controlled by a speed governor system (limit operation within P/V guidance). Single run requires that < 80% of batch agitation time occur between 1,100 and 1,700 L liquid volume. Operational parameters have been qualified for reliability according to good engineering practices by using water and air sparge to simulate bioreactor cell culture operating conditions. They will not take into account defects related to improperly maintained equipment, lack of proper operator training, or use outside of the qualified operating parameters.

Drive shaft longevity and replacement

For 50, 100, 250, 500, and 1,000 L S.U.B. systems, we recommend replacing your drive shaft every 360 days of cumulative use.

2,000 L S.U.B. systems experience a greater amount of fatigue-related stress than smaller systems due to the use of a longer drive shaft. If you are operating a 2,000 L S.U.B. at a P/V ratio of 20 W/m^3, replace your drive shaft every 180 days of cumulative use. If you are operating a 2,000 L S.U.B. at > 20 W/m^3, consultation with Thermo Scientific engineers is required. For more information on the use of P/V ratios, see section 3.6.5 of this publication. **Note:** Operating 2,000 L systems below 50% working volume requires the use of safety interlocks and speed governors. See the Warning, safety,and warranty information section in the front of this publication for more information.

Note: For warranty purposes, drive shaft use must be documented. A drive shaft use log is provided in Appendix D of this publication.

5.2 Troubleshooting and frequently asked questions

5.2.1 Hardware operation issues

Issue: **The S.U.B. will not operate.**

Solution: **Check the power supply.**

- Verify the main electrical plug connection at the wall outlet, the position of the main power disconnect, and the position of the emergency stop switch.
- Verify the condition of the main electrical circuit breaker of your facility. If the protection breaker has been tripped, determine the fault condition. The condition may exist where other electrical systems are requiring current loads beyond those allowed by the breaker. The S.U.B. system should be placed on its own electrical circuit.
- Disconnect the main power cord. Inspect the electrical circuit breakers and fuses inside the electrical box of the S.U.B. controller. Determine the fault condition by visual inspection. If the fault condition cannot be determined by visual inspection, contact the manufacturer.

Issue: **The S.U.B. temperature is below target or slow to respond.**

Solution: **Check the temperature controller and sensor.**

- Verify that the temperature probe (RTD) is not loose and has been fully inserted into the BPC thermowell.
- Verify that the thermowell has been filled with sufficient glycerol to aid in heat transfer.
- Verify that the temperature control unit is operating and all of the ball valves are open.
- Verify that the system is filled with a sufficient volume of fluid. There must be enough volume of media (minimum volume) in the BPC to provide contact with the container. Add more media if the BPC is not touching the heater area.

Issue: **Noise is being emitted from the mixer assembly.**

Solution: **No action is required.**
The bearing port assembly supplied with the S.U.B. is an important component in maintaining a sterile environment during cell growth. The special seals used in the S.U.B. may generate some noise during operation, particularly after the first day of operation. This noise may vary in intensity and frequency, but generally has no significant effect upon performance or overall durability of the BPC during the intended life of the product.

Issue: **The mixer controller does not respond to user inputs.**

Solution: **Allow the speed to stabilize before using the keypad interface.**

- Adjusting the speed control too rapidly may require several seconds for speed stabilization.
- Wait ten seconds, then attempt to adjust the speed at the keypad interface.
- Verify the position of the input select switch of the variable frequency drive (VFD). If the toggle switch is not in the middle position, the controller will not be able to receive control inputs from the control keypad on the front panel.

Issue: **I typically use level sensors to control the volume and feed rate or supplement during a bioreactor run; how would I do this with the S.U.B.?**

Solution: **Use load cells or a scale to control volumes based upon weight.**
The S.U.B. is not equipped with level sensors. However, the S.U.B. can be set up to allow supplement feeds and volumes to be managed by weight.

5.2.2 Cell culture operation issues

Issue: **Dissolved oxygen (DO) readings are low or slow to respond.**

Solution: **Check the physical condition of the DO probe, calibration of the DO probe and flow rate of gas into the S.U.B.**

- DO probes require routine maintenance; replace the damaged probe or membrane when necessary.
- Verify the DO probe calibration relative to setpoints of zero and span.
- Inspect the line sets connected to direct spargers for restriction (closed tubing clamp, pinched line, low supply pressure).

Issue: **DO readings are erratic or unstable.**

Solution: **Adjust the bioreactor controller to suit the volume of your S.U.B. system.**

- Many different parameters can affect the ability of a bioreactor controller to maintain a target setpoint during process control. Modern controllers utilize computer algorithms to adjust targeted parameters; the most common technique is that of a tunable controller that uses variables of proportional integral derivative (PID). Tuning these PID values to the specific characteristics of the system dynamics will, in most cases, stabilize process parameters to an acceptable level. We recommend that you consult the user guide of the particular bioreactor controller you are using.
- A grounding reference to the media can be created by using a grounding lead between the tank and the body of the stainless steel DO probe or to the stainless steel connector (if present) on the sample line of the BPC.

Issue: **pH levels are questionable or out of range.**

Solution: **Verify the calibration of the probe and utilize either media or gas buffers.**

- pH levels can be managed in a similar manner to conventional bioreactors once calibration of the probe is verified by use of an off-line sample. Carbon dioxide gas sparged through the media or headspace, biocarbonate levels in the media and the addition of liquid titrant solutions all serve to manage the pH balance of the bioreactor environment. See section 3.5.4 for more information on probe calibration.

- A grounding reference to the media can be created by using a grounding lead between the tank and the body of the stainless steel DO probe or to the stainless steel connector (if present) on the sample line of the BPC.

Issue: **We are not achieving the cell growth we expected in the S.U.B. while running under our normal bioreactor agitation and sparging rates. What should we do?**

Solution: **Reduce agitation and sparging rates.**

- Often low cell viability and cell growth can be attributed to excessive sparging or agitation. We recommend that you reduce the sparge rate compared to what you might use in a conventional bioreactor. Gas flow rates supplied as overlay should also be reduced as much as possible. Too much gas creates excess foam and higher shear conditions. Provide only the level of agitation needed (low viability and lysed cells), reduce agitation speed (cell aggregation and settling), and increase agitation.
- Media formulation can also have a significant effect on cell culture growth in the S.U.B. Surfactants such as Pluronic decrease shear and increase kLa, but at a cost of increased foaming. Thermo Scientific can offer custom media especially for the S.U.B. and your specific cell line(s).

5.2.3 Sparging issues

Issue: **There is excessive foam in the bioreactor headspace.**

Solution: **Alter the liquid surface tension related to the culture media and/or sparge gas.**

- A media supplement of antifoam can be used in the S.U.B. These serve to lower the surface tension of the media and will reduce the presence of foam.
- High sparge rates of air can result in the presence of excessive foam. Testing has shown that sparging with oxygen will typically result in a dramatic reduction of foam in the headspace.

Issue: **The sparger does not seem to be working, although gas is present.**

Solution: **Allow sparger membrane to purge.**

- If the S.U.B. is filled with liquid and allowed to sit idle for extended periods of time without gas being supplied to the sparger, liquid can accumulate between the membrane and check valve. Various media additives may restrict the membrane temporarily. Several minutes of gas pressure being supplied to the sparger should purge the membrane, allowing it to function properly.
- Certain operating conditions can create situations when the sparger membrane may become restricted due to insufficient line pressure from the bioreactor controller gas feed line. Increasing the flow rate to one liter per minute, or momentarily raising the pressure regulator outlet pressure to 0.3 bar (5 psi) may alleviate the problem. Alternatively, several seconds at this higher pressure will allow the membrane to purge pores that may be blocked due to the presence of accumulated liquid.

5.2.4 Probe and connector issues

Issue: **We forgot to introduce the pH and DO probes prior to media fill; can we still make a sterile connection under these conditions?**

Solution: **Yes, as long as the clamps were closed on the aseptic connector probe ports before liquid fill.**

- The aseptic connectors must be dry to make the connection of the probe assemblies. When media is already present in the S.U.B., follow the probe insertion procedures as outlined in section 3.5.3.
- Some fluid may enter the bellows when the probe is inserted into a BPC already filled with media. This is normal and will not affect the sterility of the system.

5.2.5 Other issues

Issue: **The BPC seems overly tight.**

Solution: **Verify that the container is venting, and inspect it for the cause of overpressure.**

- Reduce the inlet gas flow rate of the overlay and direct sparger.
- Inspect the exhaust filter for restriction or blockages.

WARNING: Burst hazard.
Avoid excessive foaming in the BPC. If foam levels are allowed to reach the exhaust filter, the filter will become restricted, resulting in excessive internal pressure within the S.U.B. This may cause product failure and bursting of the BPC.

Issue: **There is excessive pressure in the condenser bag for my 2,000 L system.**

Solutions: **Check for kinks in the exhaust tubing between the S.U.B. and the condenser bag.**

- Ensure that the condenser components are properly installed on the condenser hardware.
- Regularly inspect the tubing for kinks, and monitor the pressure.

Check for liquid buildup in the condenser bag outlet tubing and/or the vent filter.

- Ensure that the chiller power is on, the auto-restart option on the chiller is activated, and the chiller setpoint and actual temperature are correct.
- Ensure that the TCU coolant level in the chiller is at the maximum capacity before use, as low levels of coolant will increase the chiller plate temperature.
- Check that the batch flow rates do not exceed recommendations.
- Check coolant lines between the chiller and condenser plate for abnormalities.
- Temporarily plug off vent filters (one at a time) while manipulating the tubing to drain liquids back into the condenser bag.
- Continuously monitor the pressure in the S.U.B.

Check the exhaust tubing between the S.U.B. and the condenser bag to ensure it is positioned correctly.
The braided exhaust hose tubing should not allow condensate to collect, but should be able to drain into the vessel or condenser.

Check for liquid buildup in the condenser bag.

- Ensure that the pump power is on, the auto-restart option on the pump is activated, the pump head is turning at the set speed, and the pump is set to the recommended speed (12–30 rpm).
- Ensure that the pump tubing is properly installed in the pump head, and that there are no kinks or blockages in the liquid drain line tubing.
- Ensure that foam has not reached the condenser bag. If foam has reached the bag, reduce gas flow rates and add an anti-foam agent. After the foam has been controlled, it will naturally dissipate and drain out of the condenser bag.

Issue: **There is excessive residue buildup in the condenser bag for my 2,000 L system.**

Solution: **Ensure that the TCU coolant level in the chiller is always at maximum capacity before use.**

Low levels of coolant will increase the chiller plate temperature, which can result in excessive pressure and/or residue buildup in the bag.

6

General ordering information

Chapter contents

6.1 Ordering instructions

BPCs and hardware components for the 2:1 S.U.B. can be ordered directly from Thermo Fisher Scientific. These items include all components that have part numbers beginning with the following digits:

- SH
- SV
- SUB

6.2 Ordering/support contact information

In the Americas and Asia
1726 Hyclone Drive
Logan, Utah 84321
United States
Tel: +1 435 792 8500
Email: customerservice.bioprocessing@thermofisher.com

In Europe
Unit 9 Atley Way
Cramlington, NE 23 1WA
Great Britain
Tel: +44 (1) 670 734 093
Fax: +44 (1) 670 732 537
Email: customerservice.bioprocessing@thermofisher.com

6.3 Technical support

Technical support for the 2:1 S.U.B. is available in a variety of formats. Some or all of the following may be appropriate, depending on individual experience and circumstances.

Technical service hotline and email

Contact your Thermo Scientific sales representative for general product pricing, availability, delivery, order information, and product complaints.

Call +1 435 792 8500 (United States) or +44 (0) 670 734 093 (Europe, U.K.) for direct and immediate response to overall product questions, and product technical information (Technical Support). You can also contact Tech Support by emailing: techsupport.bioprocessing@thermofisher.com

Initial setup and operation

Appropriate technical support is available to assist in the initial setup and operation of each S.U.B. system. If you require assistance in setting up and operating your S.U.B. system, please inquire at the time of purchase.

Training

Training can be provided for startup and operation of the S.U.B. Contact your Thermo Scientific sales representative.

Appendix A—Installation of female electrical receptacle for units with AC motors and electrical boxes

1. In order to complete the installation for units with AC motors, the facility must be equipped with an electrical housing of sufficient size.
   - Typically in the U.S. the plug will require a two-gang box when using the adapter plate (supplied).
   - For installations outside the U.S. (where an adapter plate is not supplied), we recommend that an electrical panel be modified to accommodate the cutout dimensions as shown in Figure A.1 below.

2. Verify that electrical power has been disconnected and locked out for safety.

3. Verify that the holes for mounting the receptacle housing are positioned properly. Center to center measurement of respective mounting holes is 85 mm (3.35 in.) tall and 77 mm (3 in.) wide.

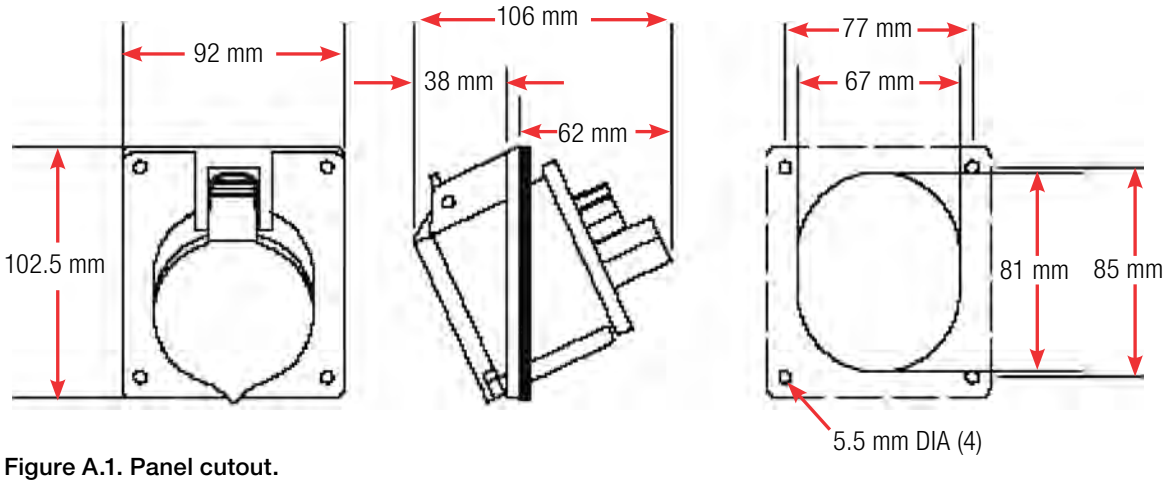


Figure A.1. Panel cutout.

4. Verify the condition of the three exposed wire leads and strip back to expose new wire if needed.

5. Connect the wire leads on the receptacle (shown in Figure A.2 below) using the screw terminals, paying strict attention to obtain the correct wiring position as it is labeled on the receptacle.
   - Green (ground)
   - White (common)
   - Black in the U.S., Blue in the E.U. (hot)

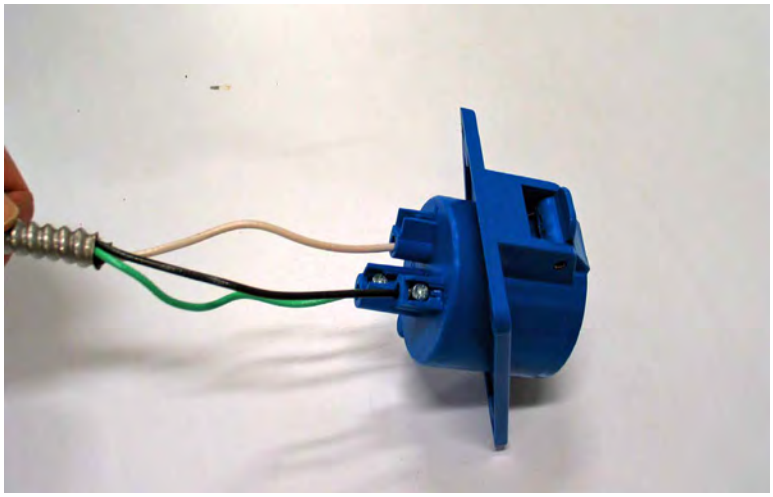


Figure A.2. Female receptacle (blue for 240 VAC; yellow for 110 VAC).

6. If using the adapter mounting plate, secure it to the selected facility electrical housing as per drawing (Figure A.3 below), otherwise proceed to Step 7.

7. Secure the electrical receptacle using four supplied screw fasteners.

8. Connect power back to the electrical circuit.

9. Test the circuit with multi-meter prior to making any connections to the electrical receptacle.

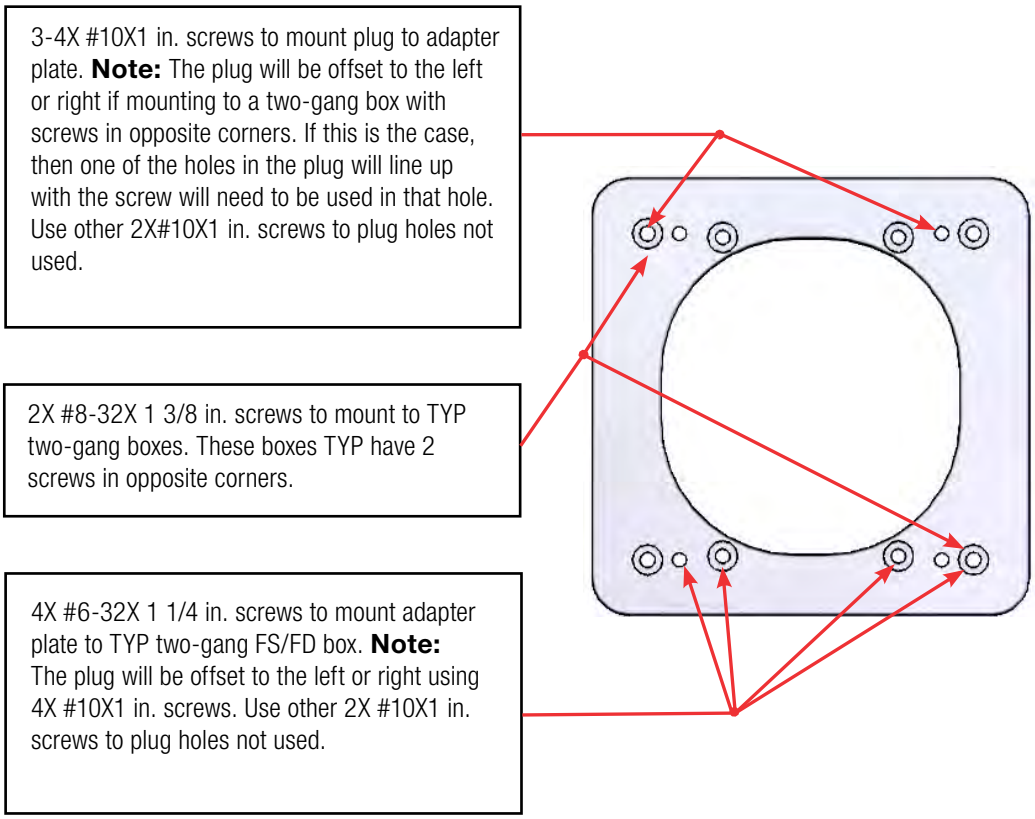


Figure A.3. Adapter mounting plate.

Appendix B—Mettler Toledo IND331 display load cell calibration instructions

Please refer to the instructions and reference material found in the Mettler Toledo IND Terminal Technical Manual for specific procedures and troubleshooting methods.

Verify the following before beginning load cell calibration:

- The Mettler Toledo IND Display, load cell summing block, and load cell transducers have been specified, installed, and configured properly.
- The load cell transducers do not have the transport lockout nuts in place (the load cells must be ready for use prior to calibration).

The calibration accuracy achieved cannot exceed the precision of the reference used for calibration.

- Field calibration is most often performed using calibrated reference weights or flow meters for volumetric mass reference.
- Factory-trained technicians have the experience and tools necessary to provide the best system performance and reliability. **If in doubt, contact your factory service representative.**

Introduction

- Setup mode is accessed by pressing and holding the **Print** key for approximately three seconds. See Chapter 2 of the Mettler Toledo IND Terminal Technical Manual for further detail.
- Pressing **Print** is equivalent to pressing **Enter**. Use this key to proceed through the sub-block numbers until you find your desired choice.
- Press **Select** to toggle the values of the selected sub-block.
- The S.U.B. electrical schematic contains a table showing the sub-blocks that have changed from the default settings.

Span calibration

The scale's span calibration can be determined with or without a linearity adjustment. With linearity disabled, a single reference point is used to calibrate the scale. This is the normal method of span calibration. If linearity is enabled, an additional mid-range weight reference point is added to the adjustment procedure. Linearity can be enabled or disabled in the setup mode.

For further information, refer to the Mettler Toledo IND331 manual at: http://mt.com

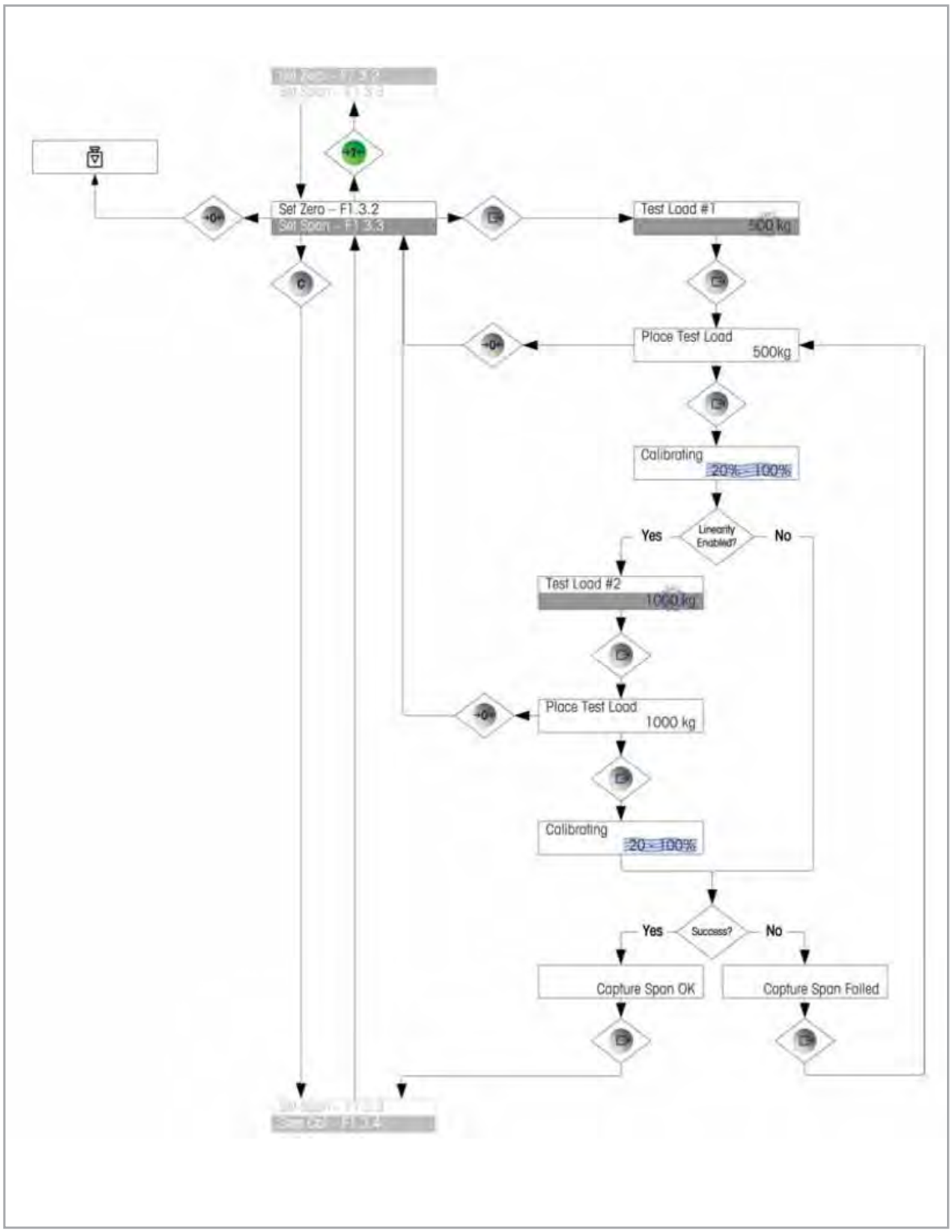


Figure B.1. Span calibration

Appendix C—2,000 L S.U.B. agitator operation and maintenance guidelines

2,000 L Critical operating recommendations

WARNING: To prevent drive shaft breakage and maintain the equipment warranty, follow the operating recommendations listed below.

- ✓ Verify that the motor variable frequency drive (VFD) is programmed to accelerate and decelerate in a minimum of 60 seconds.
- ✓ Use a controller agitation speed governor and safety interlocks to prevent the system from running outside of recommended limits.
- ✓ Verify that the drive shaft serial numbers match on both segments; do not interchange shaft segments.
- ✓ Always maintain a log history of drive shaft usage (Appendix D) and confirm that it has sufficient life remaining. If the age or history of a shaft is questionable, it should be replaced. For more information, see Maintenance in section 5.1 of this publication.
- ✓ Only activate agitation after the BPC has been filled with media.
- ✓ Use at least three operators to load the BPC into the 2,000 L S.U.B.
- ✓ Fully inflate the BPC prior to insertion of the drive shaft. This will keep the impeller tubing from stretching and being damaged.

Agitation speed recommendations

Table 3.3 in section 3.6.4 of this guide provides agitation speed recommendations for all system sizes.

The nominal agitation recommendations in Table 3.3 are based on P/V values of 20 W/m^3. This is the suggested default parameter for CHO cultivation. For more information on P/V calculation, see section 3.6.5 of this user's guide.

Appendix D—Drive shaft use log

A sample log is provided below for tracking and documenting drive shaft usage. **Important note: For warranty purposes, users must document proper drive shaft use.**

Drive shaft serial number:						
Vessel serial number:						
Start date	**Agitation setting (start)**	**Agitation setting (stop)**	**Starting volume**	**Finishing volume**	**End date**	**Number of days**
					Cumulative run time:	

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