

SI-MB4/SI-MB8

Four or Eight Chamber Tissue Bath System

INICTALI ATION CLUDE
INSTALLATION GUIDE
Serial No
032211
032211

SI-MB4/SI-MB8



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SI-MB4/SI-MB8



ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a **CAUTION**. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a **WARNING**. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and **TIPS** contain helpful information.



Fig. 1—Four chamber tissue bath set up. Four **SI-BAM21-LC** bridge amplifiers (included) are not shown.

INTRODUCTION

SI-MB-4 and **SI-MB-8** isolated tissue bath systems were developed for *in vitro* investigations on isolated smooth, cardiac or skeletal muscle preparations. They are specifically designed for pharmacological screening and testing that requires a high number of parallel samples.

Features

Advantages of this system include:

- Modular structure allows the bath units to be used individually
- · Easy control of the inflow and outflow of buffer
- Oxygenation and stimulation for each chamber
- Simple tissue installation
- Calibration of the chamber volume
- Temperature maintenance

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included. See **Fig. 2** for a labeled diagram of parts. See "Appendix D: Visual Parts Catalog" on page 48 for pictures of the parts listed below. Additional items needed to complete the system (like a data acquisition system, a circulating water bath and a stimulator) are available from WPI. See "Accessories" on page 40.

NOTE: The buffer reservoir and tissue chamber assemblies come with pre-cut tubing installed.

NOTE: The SI-MB8 system is comprised of two SI-MB4 systems.



	SI-MB4 Parts List		
Base Unit	(1) Base plate (4) Lower support rods		
e L	(4) Upper support rods		
sas	(1) Upper horizontal stabilizer bar		
ш	(1) Lower horizontal stabilizer bar		
Reservoirs	(4) Buffer reservoir assemblies include:		
	Buffer reservoir		
	Reservoir clamp		
	Tubing		
	(Units are labeled 1-4. Unit 1 has aeration manifold built in the clamp and a		
	water flow meter attached. Tubing length varies on each unit depending on		
	its position in they system.)		
Tissue Chambers	(4) Tissue chamber assemblies include:		
	Tissue chamber		
	Tissue chamber clamp Tissue holder with oxygenation frit		
Ŝ	Tubing		
<u>je</u>	(Units are labeled 1-4. The length of tubing attached to each assembly varies		
SSI	depending on the position of the assembly. The clamp on chamber 4 has two		
F	aeration outlets on the right side.)		
	(4) Transducer positioners		
	(4) Wire tissue mounts		
	(4) M6 screws		
₽	(4) M8 screws with washers		
Startup Kit	(1) Tool kit which includes:		
Į	18mm wrench		
Sta	10mm wrench		
	3 hex wrenches		
	(1) Silicone overflow drain system		
	(4) Stimulation cables		
	(4) SI-BAM21-LCB transducer amplifier modules in SCAS back plane		
	(4) SI-KG-20 Force transducers		
	(1) Instruction Manual		

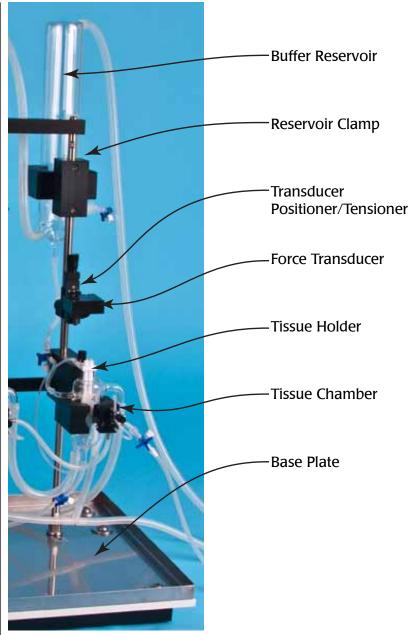


Fig. 2–The vertical tissue bath parts are labeled.





Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 52 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (4") of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 52 of this manual.

ASSEMBLING THE SI-MB4/SI-MB8

Carefully unpack the components of the tissue bath system, and place them on a large table near the location where the system will be assembled. Unpacking and assembling the tissue bath system requires more than one person, because components (like the base and the large glass vessels) are heavy.

Some of the major components of the tissue bath system, like the bath chambers and clamps, have been built into assemblies for the ease of installation.

NOTE: Please follow the installation directions carefully, because some of these assemblies need to be installed at particular locations on the tissue bath system for the system to work properly.

Assembling the System Stand

The stand holds all the major components of the tissue bath system, including:

- Tissue chambers and clamps
- Oxygenation manifolds
- Transducers and positioners
- Buffer reservoirs and clamps
- · Tubing for buffers, water jackets and oxygenation
- 1. Place the base plate on a flat, stable surface (Fig. 3).



Fig. 3-Tissue bath base



2. Locate the lower support rods. Lower support rods have flat notches near their lower (threaded) end and a threaded female socket in the top end. The upper support rods have a notch at the top and a male threaded screw at the bottom. Screw each of the lower support rods into the holes on the base plate (**Fig. 4**). Hand tighten as much as possible. There is no need to tighten them with a wrench.

NOTE: The notches are used for tightening the rods with the 10mm wrench. Upper support rods also have notches, but they are on the upper end of the rod, away from the threads.

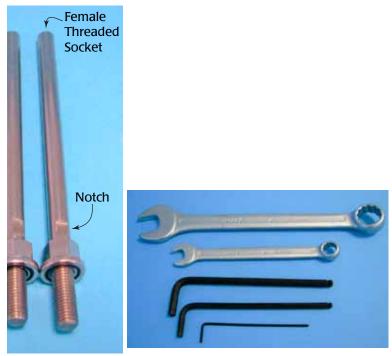


Fig. 4–(left)The lower support rod has a notch at the base. Fig. 5–(right) Tools for assembling the tissue bath system.

3. Make sure the system stand is assembled as pictured (Fig. 6).



Fig. 6-Four lower support rods installed

Mounting the Tissue Bath Assemblies

The unique double-wall construction of the tissue baths (**Fig. 7**), which is used to form a temperature-controlled water jacket around each tissue chamber, permits the baths to be held by clamps that are the same size. All the available capacities (5, 10 and 20mL) of tissue baths have the same outer diameter and the same height.

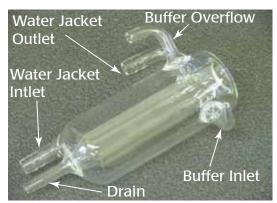


Fig. 7–All tissue chambers have the same outside diameter and height.

NOTE: Carefully follow the directions for installing the tissue bath assemblies on the system. If one or more of the components are placed in the wrong location, a major portion of the system may need to be disassembled to relocate the components to their proper positions.



The tissue bath assemblies in the system are different. Lay out the tissue bath assemblies on the work table and examine them.

- Some have a different length of tubing on the aeration inlet.
- One has a different number of aeration outlets.
- 1. Identify the tissue chamber clamp assembly with the longest piece of tubing on the aeration inlet on the bottom right of the tissue chamber clamp (**Fig. 8**). It is labeled "1." This bath chamber assembly goes on the first support rod (Far left support rod when you are looking at the front of the system).
- 2. Locate the support rod mounting hole in the back of the tissue chamber clamp (Fig. 8).

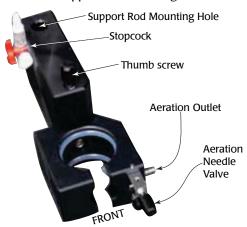


Fig. 8-Tissue chamber clamp

3. Position the bottom of the support rod hole of the clamp over the tip of the lower support rod (**Fig. 9**).



Fig. 9-Mount the tissue chamber clamp on the lower support rod. (Viewed from the rear.)

SI-MB4/SI-MB8

- 4. Lower the first tissue chamber clamp onto the support rod.
- 5. Identify the tissue chamber assembly that has two aeration outlets on the right side of the bath clamp (**Fig. 10**). It is labeled "4." One of the outlets has a piece of tubing with a stopcock in the middle and an aeration frit on the other end. If you are looking at the stand from the front, this bath chamber assembly belongs on the support rod on the far right side of the stand.

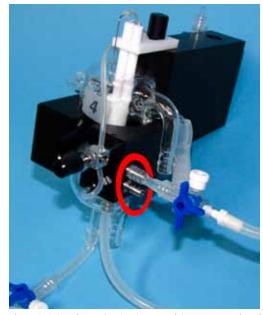


Fig. 10—The tissue bath clamp with two aeration inlets belongs on the fourth support rod, the one on the far right.

- 6. Examine the two remaining tissue chamber assemblies. Identify the one that has the longer piece of tubing attached to buffer overflow outlet of the chamber. It is labeled "3." This tissue chamber assembly goes on the third support rod (center right).
- 7. Place the remaining tissue bath assembly (labeled "2") on the last open support rod (center left).
- 8. Attach the lower horizontal stabilizer bar (**Fig. 11**) to the backs of the chamber clamps (**Fig. 12**). Use the four large screws and the hex wrench to attach the bar to the chamber clamps on the ends first. The screws should be installed loosely until all four screws are in place.



Fig. 11-Lower stabilizer bar



Fig. 12–Attach the lower stabilizer bar to the back of the tissue chamber clamps. The M8 screws go in hand tight until they are all installed.

9. When all four screws are in place, finish tightening the screws that hold the lower stabilizer bar to the chamber clamps.

Mounting the Upper Support Rod on the Stand

1. Lower the threaded end of the upper support rod into the hole in the back of the chamber clamp. Screw the upper support rod into the threaded hole in the top of the lower support rod (**Fig. 13**).

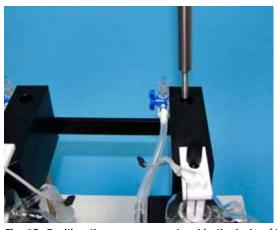


Fig. 13—Position the upper support rod in the hole of the tissue chamber support and screw it into the threaded top of the lower support rod.

2. Attach the other rods to the stand using the same process (Fig. 14).

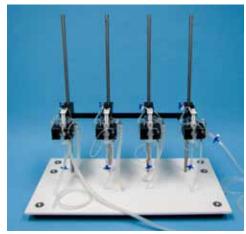


Fig. 14–The upper support rods are installed.

3. Tighten the upper support rods using the 10mm wrench provided (Fig. 15).



Fig. 15–Position the wrench in the notches of the upper support rod to tighten it.

4. Tighten the set screws on the back of the tissue chamber clamps. (Fig. 16).



Fig. 16–Tighten the set screw on the back of the tissue chamber clamp. (Rear view.)



Mounting the Force Transducer Positioner to the Stand

The force transducer positioners are identical and can be mounted on any of the upper support rods. The force transducers are installed in their brackets after the positioners are secured to the upright posts.



Fig. 17–The force transducer is mounted in the holder and installed on the positioner.

1. Position the hole on the bottom of the positioner over the tip of the upper support rod (**Fig. 18**).





Fig. 18–(left) Align the manipulator with the upper support rod. Fig. 19–(right) Position manipulator on the support rod and tighten the thumb screw.

- **TIP**:If the positioner doesn't slide easily over the top of the upper support rod, you may need to unscrew the set screw and support rod, slide the positioner up from the bottom and reinstall the upper support rod.
- Lower the manipulator to the desired height (just above the tissue chamber. Tighten
 the thumb screw on the back of the manipulator (Fig. 19). Orient the manipulator so
 that the support rod thumbscrew is on the back side and the force transducer mounting screw is directly above the tissue bath chamber (Fig. 27).

3. Remove (from the positioner) the screw and nut used to mount the force transducer and its bracket (Fig. 17).



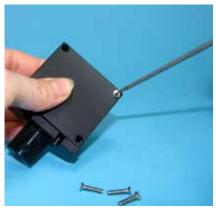


Fig. 20–(left) Remove the force transducer bracket from the positioner. Fig. 21–(right) Remove the screws for the force transducer bracket.

- 4. Use a small flat head screwdriver and remove the four small screws from the bottom of the force transducer bracket (**Fig. 21**).
- 5. Carefully unpack the force transducer. Save the box for future storage of the force transducer. Then, use a small Phillips screwdriver to remove the two screws on the back side of the force transducer (**Fig. 22**).





Fig. 22–(left) Remove the two screws on the back of the force transducer. Fig. 23–(right) Install the force transducer in the bracket with the bottom side up.

6. Place the force transducer in its bracket. The bottom side of the force transducer should be exposed so that the mounting hook is pointed down(**Fig. 23**).



7. Reinstall the two force transducer screws (Fig. 24).

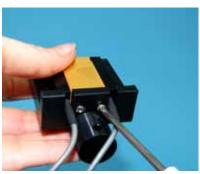




Fig. 24–(left) Reinstall the two force transducer screws. Fig. 25–(right) Reinstall the four bracket screws.

- 8. Reinstall the four bracket screws (Fig. 25).
- 9. Reinstall the bracket on the positioner. Place the transducer and its bracket on the mounting platform of the positioner. Put the mounting screw through the hole in the force transducer bracket and tighten the nut on the screw. Make sure the force transducer is positioned under the mount as shown in **Fig. 26**.



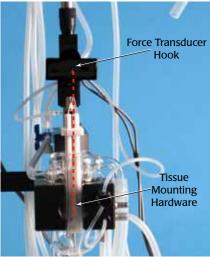


Fig. 26–(left) Position the force transducer so that the mounting hook is aligned properly with the tissue mount. Then, secure the force transducer with the nut. Fig. 27–(Right) The force transducer mounting hook should be aligned so that it is directly above the tissue mounting hardware.

- 10. Repeat this process for the other positioners and transducers.
- 11. To align the hook on the transducer over the chamber, adjust the position of the transducer on the manipulator either forward or backward. Or, adjust the position of the positioner (**Fig. 27**).

Mounting the Buffer Reservoir Assemblies

Each tissue chamber has its own buffer reservoir. This allows each tissue bath to be used independently, if needed. Each buffer reservoir has an integrated water jacket that keeps the buffer at the same temperature as the tissue in the chamber (**Fig. 28**). Also, each reservoir has its own aeration frit for oxygenation of the buffer before it enters the tissue bath.

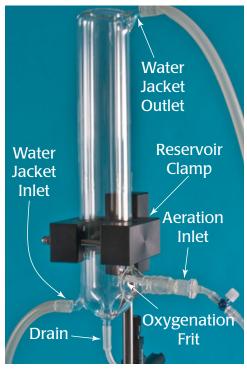




Fig. 28–(left) Buffer reservoir (Rear view)

Fig. 29–(right) The manifold is built into the clamp of one of the buffer reservoirs.

NOTE: Carefully follow the directions for installing the buffer reservoir assemblies. If one or more of the components are placed in the wrong location, a major portion of the system may need to be disassembled to relocate the components to their proper position.

All buffer reservoir assemblies in the system are different. Lay out the reservoir assemblies on the work table and examine them. Some have a different length of tubing on their water jacket outlets, and one has an aeration manifold built into its clamp.

Identify the reservoir assembly with the aeration manifold built into its clamp (Fig. 29). It is labeled "1." This buffer reservoir assembly belongs on the first support rod (far left).

2. Locate the hole in the back of the reservoir clamp. Position the bottom hole of the clamp over the tip of the first upper support rod (**Fig. 30**). When you are looking at the system, this buffer reservoir assembly should be on the far left support rod.





Fig. 30–(left) Align the hole in the bottom of the reservoir clamp with the upper support rod. Fig. 31–(right) Tighten the upper set screws before tightening the lower ones.

- 4. Lower the reservoir clamp onto the support rod until the top of the reservoir clamp is about 6-7cm below the top of the upper support rod.
- 5. Use the hex wrench and tighten the upper set screw on the back of the reservoir clamp. Then, tighten the lower set screw on the reservoir clamp (**Fig. 31**).
- 6. Identify the reservoir assembly with the longest piece of tubing on the water jacket outlet of the reservoir. It is labeled "4." This reservoir assembly goes on the last support rod (far right). The water jacket outlet of a reservoir is on the upper right of the unit.
- 7. Repeat Steps 2–5 to mount this reservoir assembly on the far right support rod.
- 8. The two remaining reservoir assemblies are identical. Mount them on the two center support rods using the directions in Steps 2–5.
- 9. Attach the upper horizontal stabilizer bar (**Fig. 32**) to the tops of the support rods. Align the four holes on the bottom of the bar with the tops of the four support rods, and lower the bar onto the support rods until the bar stops (**Fig. 33**).



Fig. 32-Upper horizontal stabilizer bar



Fig. 33–Align the holes of the upper horizontal stabilizer bar with the upper support rods.

10. Insert the small screws through the four holes in the top of the stabilizer bar and into the tops of the support rods and hand tighten (**Fig. 34**).





Fig. 34–(left) Insert the M6 screw into the upper support rod and finger tighten. Fig. 35–(right) Tighten the screw with the hex wrench.

11. Tighten the screws with the hex wrench provided (Fig. 35).



Fig. 36—The reservoirs are mounted and the horizontal stabilizer is installed (Front view).

Installing the Tissue Holders

1. Remove the thumb screw in the top of the tissue bath clamp (Fig. 37).





Fig. 37–(left) The tissue bath thumb screw is located on the top side of the tissue chamber clamp.

Fig. 38–(right) The top view of the tissue bath chamber shows the black thumb screw.

2. To gauge the proper positioning of the tissue holder, gently slide the Teflon tissue holder into the bath chamber (**Fig. 39**).



CAUTION: Make sure that no parts of the tissue holder touch the bottom of the tissue chamber.

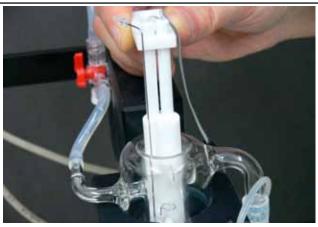


Fig. 39–The tissue holder is adjustable so that it does not contact the bottom of the chamber.

3. Remove the tissue holder and slide the mounting plate on the two vertical rods of the tissue holder to a position that supports the holder above the bottom of the tissue chamber and allows the mounting plate to rest on the top of the tissue chamber clamp (Fig. 40).

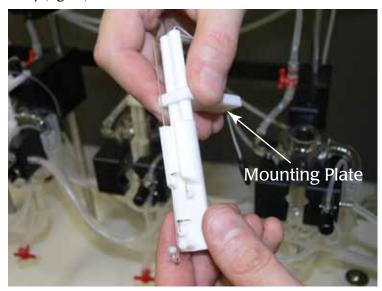


Fig. 40–Slide the mounting plate up or down to a suitable position.



4. Thread the thumb screw through the hole in the mounting plate of the tissue holder and into its hole in the tissue chamber clamp. Tighten the thumb screw with your fingers (**Fig. 41**).



Fig. 41–Reinstall the thumb screw to mount the tissue holder securely.

В1

В2

ВЗ

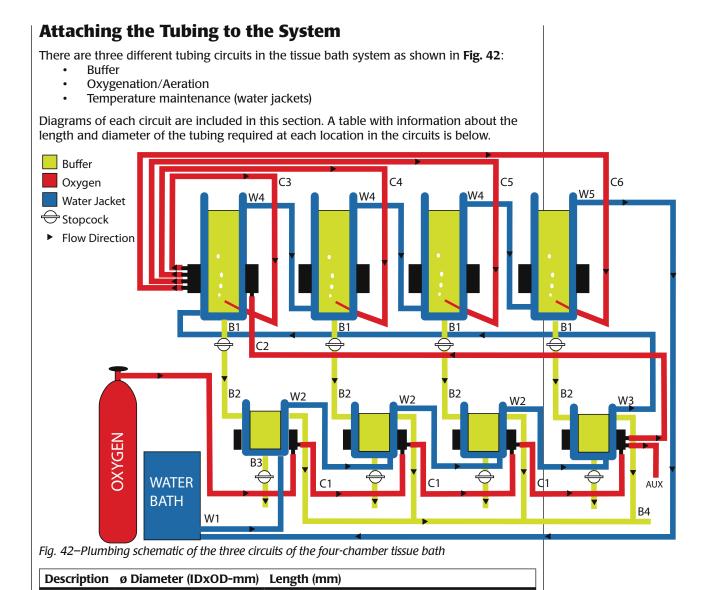
B4

3 x 6

3 x 6

3 x 6

6 x 10



22 SI-HEIDELBERG

connectors: 30, 60, 80, 110

Lengths of the vertical pieces on the quick

Lengths of the horizontal pieces between the L and T connectors: 135, 155, 155, 1100

185 (7.3")

75 (3")

65 (2.6")



Description	ø Diameter (IDxOD-mm)	Length (mm)
W1	8 x 12	2000 (78.75")
W2	8 x 12	280 (11")
W3	8 x 12	1120 (44")
W4	8 x 12	470 (18.5")
W5	8 x 12	2500 (98.4")
C1	3 x 6	200 (7.875")
C2	3 x 6	560 (22")
C3	3 x 6	310 (12.2")
C4	3 x 6	500 (19.7")
C5	3 x 6	640 (25.2")
C6	3 x 6	800 (31.5")

Table 1-Length and diameter of tubing that corresponds with the markings on Fig. 42.

The Buffer Circuit

This circuit delivers buffer to the tissue baths from the buffer reservoirs and carries any buffer overflow from the bath chambers to a suitable waste collection container (like carboy). The waste container is not included. A valve attached to the bottom of each chamber allows buffer to be drained from the chamber at any time.

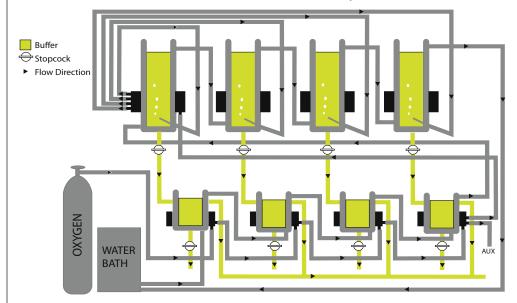


Fig. 43-Schematic diagram of the buffer circuit

1. On each tissue bath unit, connect the tubing on the buffer outlet of the buffer reservoir to the stopcock on the left side of the tissue chamber clamp. (**Fig. 44**).

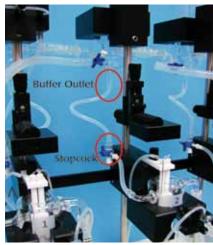




Fig. 44–(left) Connect the tubing from the buffer outlet to the stopcock. Fig. 45–(right) Attach the tube to the stopcock.

2. Connect the drainage system that prevents buffer from overflowing from the tissue baths. The overflow drainage system is assembled at the factory (**Fig. 46**). Since the overflow drainage system is driven by gravity, the main drainage tube must be sloped. A suitable waste collection container (not included) is needed to collect spent buffer.



Fig. 46–(right) Overflow drain tubing

To install the drainage system properly:

a. Place the overflow drainage system behind the tissue baths to keep the work-space in front of the tissue baths open (Fig. 47). Make sure the end of the drainage tube with the L-shaped tubing connector and the shortest piece of vertical tubing is on the left side of the system when you are looking at the front of the system.





Fig. 47–The overflow drain tubing is positioned behind the rest of the system.

b. Join the quick connector on the end of the main drainage tube to the matching quick connector on the buffer overflow outlet of the tissue bath on the far left side of the system (**Fig. 48**).



Fig. 48–Install the L-shaped connector to the first tissue chamber drain.

- c. Join the next quick connector on the drain tube to the matching connector on the overflow outlet of the second tissue bath (center left).
- d. Join the third and fourth connectors on the drainage tube to the overflow outlets of the last two tissue baths.
- 3. Place the open end of the overflow drainage tube into the waste collection container (not included). The waste collection container must be located below the base of the tissue bath system.

The Oxygenation Circuit

This circuit delivers a mixture of oxygen and carbon dioxide to the buffer reservoirs and the tissue bath chambers for aeration of the tissue buffer. Beside providing oxygen to the tissue being examined, the gas mixture maintains the pH of the buffer being stored in the reservoirs or used in the chambers.

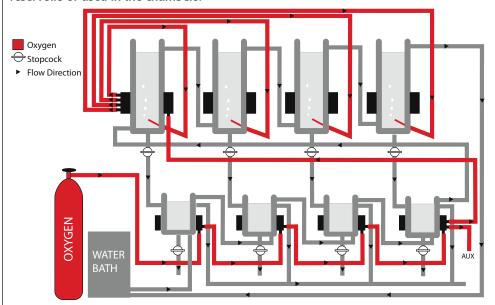


Fig. 49-Schematic diagram of the oxygen circuit

- 1. Secure the gas cylinder (not included) containing the prescribed mixture of oxygen and carbon dioxide conveniently near the tissue bath system, preferably on the left side. Carefully attach the proper regulator (not included) to the cylinder.
- 2. Each tissue bath clamp has its own aeration controller built into the right side of the tissue bath clamp (**Fig. 50**). This feature allows the bubbling rate in each bath chamber to be controlled independently. Each aeration controller has the following:
 - At least one outlet located on the right side of the tissue bath clamp
 NOTE: One of the tissue bath clamps has two outlets (Fig. 51). This tissue bath assembly should be positioned on the fourth support rod (far right, when you are looking at the front of the system). A long tube with a stopcock and aeration frit are already attached to the two outlets on the right side of the tissue bath clamp.
 - Pressure equalizer that is built into the tissue bath clamp.
 - Inlet on the bottom right side of the tissue bath clamp. The aeration inlets all have a piece of tubing attached to them. The tubing will be attached to the outlet of the aeration controller in the adjacent tissue bath assembly to the left.
 - Smaller outlet on the right front of the tissue bath clamp. It delivers the gas mixture to the bubbling pipe in the bath chamber.
 - Needle valve for regulating the flow of the gas mixture between the aeration controller and the bubbling pipe.



3. Connect a piece of silicone tube between the outlet of the needle valve on the regulator of the gas cylinder and the inlet on the right bottom of the tissue bath clamp on the far left support rod. The tubing is already be attached to the tissue bath clamp.



Aeration Outlet

Aeration Inlet



Fig. 50–(left) The aeration inlet and outlet are on the right side of the tissue chamber clamp. Fig. 51–(right) One tissue chamber clamp has two aeration outlets on the right side.



Fig. 52–Attach the tubing to aeration inlet of tissue chamber 2 to the aeration outlet of tissue chamber 1.

4. Connect the tubing from the inlet of the aeration controller of the adjacent tissue bath clamp (on the right) to the outlet of the aeration controller on the first tissue bath clamp (**Fig. 53**).

NOTE: The outlets are on the right side of the tissue bath clamps.

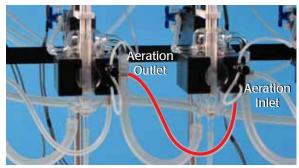


Fig. 53–The four aeration ports are daisy chained together.

5. Repeat step 4 to connect the Inlet of the aeration controller of the tissue bath clamp on the right to the outlet of the aeration controller of the tissue bath clamp on the left (**Fig. 54**).



Fig. 54—Connect the aeration outlet of each chamber to the inlet of the next. The last chamber outlet connects with the aeration inlet on the reservoir manifold.

6. Connect a tube on the outlet of the aeration controller on the final tissue bath clamp (4) in the sequence (the one on the far right) to the inlet of the aeration manifold for the reservoirs that is located on the reservoir clamp 1 on the far left.

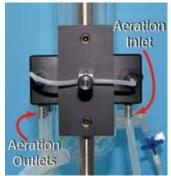


NOTE: This manifold provides the gas mixture to the four buffer reservoirs.

NOTE: The tubing on the aeration outlet of this bath clamp assembly is longer than the tubing on the other bath clamps. It is long enough to reach the inlet of the aeration manifold that is on the other side of the tissue bath system.

NOTE The inlet of the aeration manifold is on the bottom right side of the reservoir clamp on the first support rod (far left). Attach the open end of the long tubing from the bath clamp assembly to the stopcock and tubing on the inlet of the aeration manifold.

7. The outlets of the aeration manifold that supply oxygen to the buffer reservoirs are on the bottom left side of the reservoir clamp on the first support rod (far left when you are looking at the system from the front). Tubing of varying lengths is already attached to these outlets (**Fig. 55**).



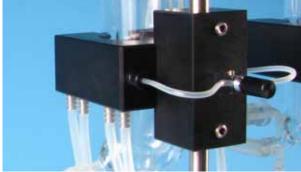


Fig. 55–The aeration inlet for the reservoirs is on the bottom right side of reservoir clamp 1, and the aeration manifold with four outlets is on the bottom left side of the clamp. Fig. 56–Four outlet ports can be seen from the side.

a. Find the aeration manifold outlet with the shortest piece of tubing. Connect the open end of that tubing to the stopcock and aeration assembly of reservoir 1, the closest buffer reservoir (**Fig. 57**).



Fig. 57-Push the quick connect into the stopcock found at the base of the buffer column.

b. Connect the outlet with the next shortest piece of tubing to the stopcock and aeration assembly of reservoir 2, the next closest buffer reservoir.

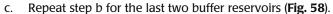




Fig. 58–The manifold outlets are connected to the four buffer reservoir inlets (rear view).

8. Finish connecting the aeration controller on each bath clamp assembly to the tissue bath (**Fig. 59**).

NOTE: Tissue bath assemblies are pre-assembled at the factory. This step is only necessary when you are replacing tubing or glassware.

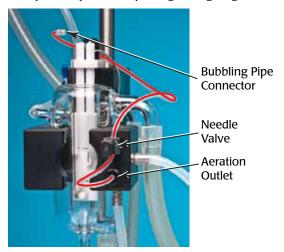


Fig. 59–Connect the aeration to the bubbling pipe.

a. Connect a piece of small diameter tubing to the aeration outlet on the right front of the tissue bath clamp. Put the end of the tube through the cap of the small outlet. Tighten the cap (finger tight) to secure the tube.



- b. Feed the other end of the tube through the bottom of the vertical hole in the needle valve on the front of the tissue bath clamp.
- c. Attach the other end of this small diameter aeration tube to the connector of the upper end of the bubbling pipe on the tissue holder that is in the bath chamber (**Fig. 60**).



Fig. 60- Connect the bubbling pipe.

d. Repeat this process for the other tissue baths in the system.

The Water Jacket Circuit

This circuit delivers water from the circulating water bath (not included) to the water jackets that surround the tissue baths and the buffer reservoirs. The water in the circuit, which is heated or cooled by the circulating water bath, maintains the temperature of the buffer and the tissue during the course of the experiment.

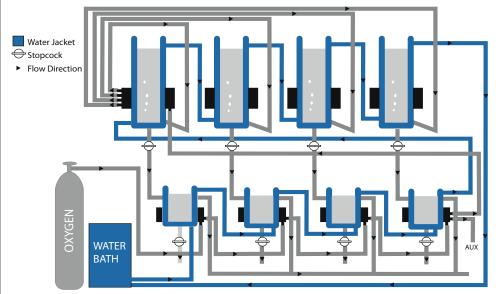


Fig. 61-Schematic diagram of the water jacket circuit

- 1. Place the circulating water bath as close as possible to the tissue bath system to reduce the length of tubing needed to deliver water to the system.
- 2. Connect the long piece of tubing on the water jacket inlet of the tissue bath chamber labeled "1" (far left when you are looking at the front of the system) to the outlet of the circulating water bath (**Fig. 62**).

NOTE: The inlet of the water jacket is on the lower left side of the chamber.



Fig. 62–The inlet of the tissue chamber water jacket is located on the bottom.

Connect the water jacket outlet of the first tissue chamber to the water jacket inlet of the adjacent tissue bath chamber. A short piece of tubing is already attached to the water jacket outlet of the first tissue bath chamber(Fig. 63).

NOTE: The water jacket outlet is on the upper right front of the tissue bath chamber.

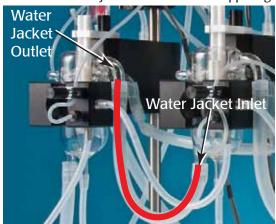


Fig. 63-The outlet of the tissue chamber water jacket is connects to the water jacket inlet of the next tissue chamber.

4. Repeat step 3 until the water jacket outlets of all the tissue baths are connected to the water jacket inlets of the adjacent tissue baths, in sequence from left to right.



5. Connect the long piece of tubing on the water jacket outlet of the fourth tissue bath chamber (far right) to the water jacket inlet of the first buffer reservoir (far left). The tubing is long enough to pass behind the support rods at a level below the tissue bath assemblies (Fig. 64). This tubing is connected to the water jacket inlet of the first reservoir through an in-line rotary flow meter that is already attached to the reservoir with a short piece of tubing.





Fig. 64–(left) The inlet of the reservoir water jacket is located on the bottom of the reservoir. Fig. 65–(right) The inline water flow meter is attached to the buffer reservoir.

6. Connect the tubing on the water jacket outlet of the first reservoir (far left) to the water jacket inlet of the adjacent reservoir (**Fig. 66**).

NOTE: Water jacket outlets are located on the upper right side of the buffer reservoirs, and water jacket inlets are located on the lower left side of the buffer reservoirs.



Fig. 66—The reservoir water jackets are also daisy chained together. This image is shown from the rear.

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- 7. Repeat the process to connect the outlets and inlets of the water jackets on the remaining reservoirs.
- 8. Connect the long piece of tubing on the water jacket outlet of the fourth buffer reservoir (far right) to the inlet of the circulating water bath.



Fig. 67–Add a circulating bath on the left and a waste container on the right for a complete water jacket circuit.



OPERATING INSTRUCTIONS

Filling the Tissue Bath System with Buffer and Water

Filling the Water Jackets

- 1. Fill the circulating water bath (not included) with deionized water.
- 2. Turn on the water bath and set the desired temperature. It takes about 30-40 minutes to equilibrate the water in the jackets, and the buffer in the chambers and reservoirs.
- 2. Fill the circulating water bath with more deionized water when the complete water jacket circuit is filled with water.
- 3. Every day, check the level of water in the circulating water bath before the bath is turned on and after all the water jackets are filled.

Filling the Buffer Vessels

- 1. Make at least three (3) liters of the buffer that will be used in the chamber and the reservoirs.
- 2. Fill all the reservoirs with buffer.
- 3. Rinse the tissue chamber with buffer by opening the stopcock between the outlet of the buffer reservoir and the buffer inlet of the tissue chamber. Fill the chamber until the buffer is just below the overflow outlet of the chamber.
- 4. Use an empty beaker that is three to five times the capacity of the tissue chamber. Place the empty beaker under the drain of the chamber. Open the stopcock on the drain to empty the buffer from the chamber.
- 5. Rinse the tissue chamber two more times with buffer. After the last rinse, refill tissue chamber, and refill the buffer reservoir.
- 6. Repeat the process for the other reservoirs and tissue baths.
- 7. Turn on the aeration system and bubble the gas mixture through the buffer in the reservoirs and chambers. It takes approximately 30 minutes for the buffer to become saturated with oxygen and carbon dioxide.

Using the Tissue Holders

A variety of tissue holders are available for use on whole muscles, tissue strips and vascular rings in each size of tissue chamber. Features of these holders include:

- Each tissue holder has either field or point stimulation electrodes built in.
- Each has an aeration pipe that can be positioned for optimal oxygenation of the tissue.
- Tissue holders can be removed from the tissue chambers to facilitate the installation of the tissue samples.
- They can be positioned in the tissue chambers for accurate measurement of the chamber volume.
- They are made of Teflon to prevent contamination of the buffer with trace metals.





Fig. 68–(left) The SI-OHO2F tissue holders have field stimulation electrodes. Fig. 69–(right) The SI-OHO2P tissue holders have point stimulation electrodes.

Selecting the Right Tissue Holder

Each size of tissue chamber has a variety of tissue holders that all match the inside diameter of the chamber. Tissue holders are built with either standard point or standard field stimulation electrodes (**Fig. 68**, **Fig. 69**) that are made of platinum-iridium. Tissue holders can also be designed to the customer's specifications with electrodes in a variety of shapes that are in different positions and distances apart (up to 20mm or 0.75").



Muscle and tissue strips are usually secured to the attachment at the bottom of the tissue holder and the arm of the transducer using suture thread. The recommended suture material is 4-0 braided silk for large strips of tissue, and 6-0 braided silk for smaller tissue strips. In the case of vascular tissue, vessel rings are suspended on the tissue holder using specialized hooks designed to reduce damage to vascular tissue.





Fig. 70–(left) The vascular tissue is mounted using the specialized hooks. Fig. 71–(right) Close up of the specialized hooks used for mounting vascular tissue

Attaching the Tissue to the Holder

- 1. Remove the small aeration tube from the top of the bubbling pipe on the tissue holder. (See "Installing the Tissue Holders" on page 19 for more information.)
- 2. Loosen the thumb screw that holds the tissue holder in place and remove the holder from the chamber. Place the tissue holder in a clean beaker or on a clean petri dish to avoid its contamination.
- 3. Obtain a piece of the tissue to be tested. Keep the tissue in a beaker of aerated buffer at the desired temperature until it is ready to be attached to the tissue holder.
- 4. Remove the tissue from the beaker of buffer and place it on a clean work surface near the tissue holder. Work carefully when mounting the tissue on the holder to avoid damaging the tissue. Moisten the tissue frequently with buffer to prevent its desiccation.

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- 5. Tie one end of a 15cm piece of suture thread to lower (distal) end of the tissue strip. Tie one end of a 30cm piece of suture thread to the upper (proximal) end of the tissue strip.
- 6. Attach the tissue to the tissue holder using one of the three methods below:
 - If you are using a tissue holder with field stimulation electrodes:
 - a. Feed the free end of the suture on the lower end of the tissue strip through the loop of the lower stimulation electrode. Tie the suture to the attachment post at the bottom of the tissue holder so that tissue does not touch the loop of the electrode when it is suspended in the chamber.
 - b. Feed the free end of the suture on the upper end of the tissue strip through the loop of the upper stimulation electrode. The end of this suture will be attached to the arm of the transducer after the tissue holder is placed back in the chamber.
 - If you are using a tissue holder with point stimulation electrodes, tie the suture
 on the lower end of the tissue strip to the attachment post at the bottom of the
 tissue holder so that end of the tissue strip touches both of the point stimulating
 electrodes when it is suspended in the chamber.
 - If you are using a tissue holder with vascular hooks for supporting cylindrical sections of vascular, intestinal, uterine or excretory tissue:
 - Locate a set of vascular hooks that were included with your system (Fig. 71
 on page 37). The upper hook has a triangular shape, and the lower hook has
 a square shape.
 - **NOTE**: Vascular hooks can only be used with tissue holders equipped with field stimulation electrodes.
 - b. Open the clasps of both the upper and lower vascular hooks. Carefully slip the cross bar of both hooks into the lumen of the tissue cylinder. Close the clasps on both hooks.



CAUTION: Keep the tissue moist with buffer while performing this step to keep the tissue viable.

- c. Place the wire loop on the bottom of the lower (square) vascular hook over the plastic post on the bottom of the tissue holder (Fig. 70 on page 37). The large wire square of this vascular hook fits around the lower stimulation electrode when the tissue hooks, and suture thread are aligned.
- d. Feed a 30cm piece of suture thread through loop of the upper field stimulation electrode, and tie it to the small wire loop on the top of the upper (triangular) vascular hook.
- Replace the tissue holder in the chamber. Install and tighten the thumb screw that secures the tissue holder in place. Reattach the aeration line to the top of the bubbling pipe. Verify that the tissue is properly aerated.



8. Attach the suture thread on the upper end of the tissue strip to the arm on the transducer. There should be no slack in the suture when the tissue is stretched out to its *in situ* length.

Removing or Replacing the Bubbling Pipe

The tissue in the chamber is oxygenated through a bubbling pipe held in a groove on the tissue holder. The pipe easily slides and rotates in the groove, which allows the aeration frit at the bottom of the pipe to be positioned properly near the lower end of the tissue. The bubbling pipe is made with a highly purified metal alloy, which prevents contamination of the buffer in the tissue chamber. The top of the pipe is connected to aeration system with a silicone tube. The bubbling pipe can be removed easily from the tissue holder for cleaning or replacement. To remove and replace the bubbling pipe:

- 1. Remove the tissue holder from the tissue bath chamber.
- 2. Carefully pull the top of the bubbling pipe from the groove that retains the pipe of the tissue holder.
- 3. When a small portion of the bubbling pipe is out of the retaining groove, pull the pipe down until the whole pipe is out of the groove.
- 4. To replace the bubbling pipe in the groove, place the top of the pipe in the bottom of the groove, then push up on the pipe to put the remainder of the tube into the groove.

Calibrating the Buffer Volume in the Tissue Chamber

If the volume of buffer used in the tissue bath needs to be a precise amount, the tissue holder can be positioned in the chamber in a way that raises the buffer to a level that covers the tissue and reaches the buffer overflow port of the chamber.

- Before performing the experiment, remove the tissue holder from the tissue chamber. Drain any buffer from the tissue bath chamber. Close the stopcocks that deliver or drain buffer from the chamber.
- Pipette the exact amount of buffer needed for the experiment into tissue chamber.
 Use 5mL of buffer for the 5mL chambers, 10mL of buffer for the 10mL chambers or 20mL of buffer for the 20mL chambers.
- 3. Slide the mounting plate on the support rods of the tissue holder to the position that raises the level of the buffer to the edge of the buffer overflow port.
- 4. Note or mark the position of the mounting plate on the support rods of the tissue holder. Proceed with the experiment by attaching the tissue to the tissue holder.

NOTE: Once a tissue holder is used to calibrate the volume of liquid in a chamber, the tissue holder and the chamber should always be used together as a set. To use a tissue holder with a different chamber requires re-calibration of the new set.

ACCESSORIES

Table 2: Accessories	
Part Number	Description
SI-ISO0802-5	5mL Calibrated Tissue Bath
SI-ISO0802-10	10mL Calibrated Tissue Bath
SI-ISO0802-20	20mL Calibrated Tissue Bath
SI-OHO2F-5	Tissue Holder, Field Stimulation/Oxygenation for 5mL bath
SI-OHO2F-10	Tissue Holder, Field Stimulation/Oxygenation for 10mL bath
SI-OHO2F-20	Tissue Holder, Field Stimulation/Oxygenation for 20mL bath
SI-OHO2P-5	Tissue Holder, Point Stimulation/Oxygenation for 5mL bath
SI-OHO2P-10	Tissue Holder, Point Stimulation/Oxygenation for 10mL
	bath
SI-OHO2P-20	Tissue Holder, Point Stimulation/Oxygenation for 20mL bath
SI-KG20	Force Transducer
LABTRAX4-24T	Lab Trax Data Recording, 4 channel
503843	Circulating Tissue Bath (110V)
503844	Circulating Tissue Bath (220V)
WPI-118	Digital Data Recorder, 8 channel

SPECIFICATIONS

This instrument conforms to the following specifications:

System Dimensions

	SI-MB4	SI-MB8
Width	500mm (19.7")	500mm (19.7")
Length	1000mm (39.4")	2000mm (78.7")
Height	1050mm (41.3")	1050mm (41.3")
Weight	30Kg (13.6#)	60Kg (27.2#)

Tissue Bath Dimensions

	Height*	ID	
5mL	65mm (2.6")	14mm (0.55")	
10mL	65mm (2.6")	18mm (0.71")	
20mL	65mm (2.6")	20mm (0.79")	

^{*}The inside height is measured between the outflow port at the bottom and the overflow port at the top.



APPENDIX A: BUILDING A TISSUE BATH ASSEMBLY

If the tissue bath chambers, clamps and tubing are not already built into assemblies, use this procedure to construct your own.

1. Find the chamber clamps in the collection of components of the tissue bath system (Fig. 72).

NOTE: One of the bath chamber clamps has two tubing inlets on the right side.

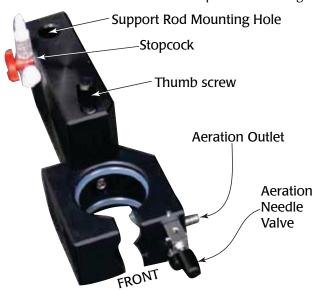


Fig. 72-Tissue chamber clamp

- 2. Inspect each chamber clamp. Make sure each clamp has the following:
 - Pair of silicone gaskets inside the large ring that holds the chamber
 - Aeration valve on its right front
 - Screw knob on its top
 - Stopcock on its left side
 - Set screw on the back
- Carefully slide the bottom of the chamber into the top of the ring so that the buffer inlet is on the left and the water jacket outlet is on the right side of the chamber clamp.

4. Slide the chamber into the clamp until the base of the buffer inlet is just above the top of the chamber clamp (**Fig. 73**).



Fig. 73-Tissue chamber properly positioned and setup

- Place a Luer-Lock tubing connectors on the upper inlet and the front outlet of the stopcock mounted on the left side of the tissue bath chamber clamp. Repeat this procedure for all the tissue bath chamber and clamp assemblies.
- 6. Connect a 75mm (3") piece of 3mm ID x 6mm OD tubing between the front outlet of the stopcock and the buffer inlet of the tissue bath chamber. The inlet of the tissue bath chamber is on its upper right side. Repeat this procedure for all the chamber and clamp assemblies.
- 7. Connect a 65mm (2.5") piece of 3mm ID x 6mm OD tubing on the buffer drain in the center of the bottom of the tissue bath chamber. Attach a stopcock to the open end of this tube using a Luer-Lock tubing connector. Repeat this procedure for all the chamber and clamp assemblies.
- 8. Find the chamber and clamp assembly that has two aeration outlets on the right side of its bath clamp.
 - a. Connect a 1130mm (44.5") piece of 8mm ID x 12mm OD tubing on the water jacket outlet on the right upper front of the bath chamber.
 - b. Attach a 120mm (4.75") piece of 6mm ID x 10mm OD tubing to the buffer overflow outlet on the upper right back of the tissue bath chamber.



c. Attach a 200mm (7.875") long piece of 3mm ID x 6mm OD tubing to the inlet of the aeration controller on the right bottom of the bath clamp.

NOTE: This assembly can only be installed on the fourth (far right) support rod.

- On the three remaining chamber and clamp assemblies that only have one aeration outlet on the right side of the bath clamp connect a 280mm (11") piece of 8mm ID x 12mm OD tubing to the water jacket outlet on the right upper front of each bath chamber.
- 10. Select one of the three remaining tissue bath chamber and clamp assemblies from Step 9.
 - a. Connect a 2000mm (78.75") piece of tubing to the water jacket inlet on the lower left side of the tissue bath chamber.
 - b. Attach a 30mm (1.25") piece of 6mm ID x 10mm OD tubing to the buffer overflow outlet on the upper right back of the tissue bath chamber.
 - c. Attach a 560mm (22") piece of 3mm ID x 6mm OD tubing to the inlet of the aeration controller on the right bottom of the tissue bath clamp.

NOTE: This assembly can only be installed on first (far left) support rod of the tissue bath system.

- 11. Select one of the two remaining tissue bath chamber and clamp assemblies from Step 9.
 - a. Attach a 60mm (2.375") piece of 6mm ID x 10mm OD tubing to the buffer over-flow outlet on the upper right back of the tissue bath chamber.
 - b. Attach a 200mm (7.875") piece of 3mm ID x 6mm OD tubing to the inlet of the aeration controller on the right bottom of the tissue bath clamp.

NOTE: This assembly can only be installed on the second support rod (left center) of the tissue bath system.

- 12. Find the remaining chamber and clamp assembly from Step 9
 - a. Attach a 90mm (3.5") piece of 6mm ID x 10mm OD tubing to the buffer overflow outlet on the upper right back of the tissue bath chamber.
 - b. Attach a 200mm (7.875") piece of 3mm ID x 6mm OD tubing to the inlet of the aeration controller on the right bottom of the tissue bath clamp.

NOTE: This assembly can only be installed on the third support rod (right center) of the tissue bath system.

APPENDIX B: BUILDING A BUFFER RESERVOIR ASSEMBLY

If the buffer reservoirs, their clamps and tubing are not already assembled as units, use this procedure to construct your own.

1. Find the reservoir clamps (**Fig. 74**, **Fig. 75**) in the collection of components of the tissue bath system.

NOTE: One of the reservoir clamps has an aeration manifold built into it.



Fig. 74–(left) Reservoir clamp with the manifold, front view Fig. 75–(right) Standard reservoir clamp, front view

- Inspect each reservoir clamp. Make sure each clamp has the following:
 - Pair of silicone gaskets inside the large ring that holds the reservoir
 - Bolt and nut on the back
 - Two set screws on the front
- 3. Remove the nut and bolt on the back of the reservoir clamp.
- Align the water jacket outlet on the top of the buffer reservoir with the gap in the back of the reservoir clamp (Fig. 76).



Fig. 76-Buffer reservoir, rear view.



- 5. Carefully slide the top of the reservoir into the ring until the base of the aeration inlet is just below the bottom of the clamp (**Fig. 76**).
- 6. Rotate the reservoir 90° to the left so that (when you look at the system from the front) the water jacket outlet and aeration inlet point toward the left, and the water jacket inlet points to the right.
- 7. Replace the nut and bolt in the clamp and gently tighten them to prevent the reservoir from sliding out of the clamp.



CAUTION: Do not over-tighten the bolt! Excessive tightening could crack the reservoir.

- 8. Repeat Steps 3–7 for the other three reservoirs and clamps.
- 9. Attach a 185mm (7.25") piece of 3mm ID x 6mm OD tubing to the buffer drain in the center of the bottom of the reservoir. Repeat this procedure for all the reservoir and clamp assemblies.
- 10. Find the aeration tubes and seals for the reservoirs in the collection of components.
 - Insert an aeration tube through the access port on the lower right side of the reservoir until the bubbling frit on the aeration tube is even with the inner wall of the reservoir.
 - b. Push the seal on the aeration tube over the outside of the aeration port.
 - c. Attach a stopcock to the open end of the aeration tube using a Luer-Lock tubing connector.

Repeat this procedure for all the reservoir and clamp assemblies.

- 11. Select one of the three reservoir and clamp assemblies that does not have the aeration manifold. Connect a 2500mm (98.5") piece of 8mm ID x 12mm OD tubing to the water jacket outlet on the right upper side of the reservoir in the assembly. This assembly is now the only unit that can be installed on the fourth (far right) support rod of the tissue bath system.
- 12. On the three remaining reservoir and clamp assemblies without pieces of tubing on their water jacket outlets, connect a 470mm (18.5") piece of 8mm ID x 12mm OD tubing to the outlet on the upper right side of each reservoir.
- 13. Install the aeration control tube on the front of the reservoir clamp with the aeration manifold.

a. Connect a piece of small diameter tubing to the aeration outlet on the right front of this reservoir clamp. Put the end of the tube through the cap of the small outlet. Tighten the cap (finger tight) to secure the tube.

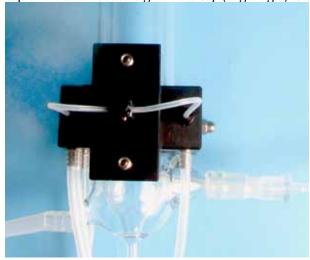


Fig. 77–Connect the aeration to the bubbling pipe.

- b. Feed the open end of the tube through the right side of the horizontal hole in the needle valve on the front of this reservoir clamp.
- c. Connect the end of this tube to the inlet on the left front of this reservoir clamp. Put the end of the tube through the cap of the small inlet. Tighten the cap (finger tight) to secure the tube.
- 14. Attach a piece of tubing to each of the four outlets of the aeration manifold on the reservoir clamp.
 - a. Connect a 310mm (12.25") piece of 3mm ID x 6mm OD tubing to the first (front) outlet on the bottom of the manifold.
 - b. Connect a 500mm (19.75") piece of 3mm ID x 6mm OD tubing to the second outlet.
 - c. Connect a 640mm (25.25") piece of 3mm ID x 6mm OD tubing to the third outlet.
 - d. Connect a 800mm (31.5") piece of 3mm ID x 6mm OD tubing to the fourth (back) outlet.



APPENDIX C: BUILDING THE BUFFER OVERFLOW DRAINAGE TUBE

If the drainage tube that is attached to the buffer overflow outlets of the tissue baths has not been assembled, use this procedure to build one.

- 1. Find a 135mm (5.3") piece of 6mm ID x 10mm OD tubing, an L-shaped tubing connector and a T-shaped tubing connector in the collection of components.
 - a. Attach the short stem of a L-connector to one end of the tubing. The other (long) stem of this connector will be attached to the buffer overflow outlet of the tissue bath that is on the far left of the tissue bath stand.
 - b. Attach a T-connector to the other end of the 135mm (5.3") piece of tubing. Use one of the two stems of the T-connector that permits the next piece of tubing in the drainage system to line up with the first piece of tubing.
 - c. The stem of the T-connector, which is at a right angle to the drain tube, should point in the same direction as the open stem of the L-connector on the other end of the tubing. If these stems are not aligned, the drain tube cannot be properly attached to the overflow outlets.
- 2. Find two pieces of tubing that are both 155mm (6") long x 6mm ID x 10mm OD, and two more T-connectors.
 - a. Attach the end of a 155mm (6") piece of tubing to the T-connector on the first piece of tubing. Put this piece of tubing on the stem of the T-connector that aligns the two pieces of tubing. If the first and second pieces of tubing form a right angle, move the second piece of tubing to other open stem of the T-connector.
 - b. Attach a new T-connector to the open end of the second (155mm/6") piece of tubing. Use a stem on the connector that aligns the third and fourth pieces of tubing with the first two pieces of tubing. Also, the side stem of this new connector needs to point in the same direction as the open stems on the first two tubing connectors to allow proper installation of the drainage system.
 - c. Attach the other 155mm (6") piece of tubing to the T-connector on the end of the second piece of tubing. Again, use the stem on the connector that aligns all the sections of the drainage tube in the same direction.
 - d. Attach the second new T-connector to the open end of the third piece of tubing. Use a stem on the connector that aligns the fourth piece of tubing with the first three pieces of tubing. Also, the side stem of this new connector needs to point in the same direction as the open stems on the first three tubing connectors to allow proper installation of the drainage system.
- 3. Find a 1100mm (43.3") x 6mm ID x 10mm OD piece of tubing in the collection of components. Attach the end of the 1100mm (43.3") piece of tubing to the T-connector on the third piece of tubing. Again, use the stem on the connector that aligns all the sections of the drainage tube in the same direction.







Tool kit



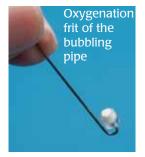














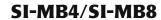
Wire tissue mounts



SI-BAM21-LCB transducer amplifiers

SI-MB4/SI-MB8

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WARRANTY

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Claims and Returns

- Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.
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- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
- Goods returned for repair must be reasonably clean and free of hazardous materials.
- A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.
- Equipment which was built as a special order cannot be returned.
- Always refer to the RMA# when contacting WPI to obtain a status of your returned item.
- For any other issues regarding a claim or return, please contact the RMA department

Warning: This equipment is not designed or intended for use on humans.

* Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items

World Precision Instruments, Inc.

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