ADINSTRUMENTS

RODENT CARDIOMYOCYTE ISOLATION

Owner's Guide

Retrograde Cardiomyocyte Isolation Perfusion Apparatus

ADINSTRUMENTS
making science easier

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Chapter 1

Safety Notes

Statement of Intended Use

All products manufactured by ADInstruments are intended for use in teaching and research applications and environments only. ADInstruments products are NOT intended to be used as medical devices or in medical environments. That is, no product supplied by ADInstruments is intended to be used to diagnose, treat or monitor a subject. Furthermore no product is intended for the prevention, curing or alleviation of disease, injury or handicap. ADInstruments products are intended to be installed, used and operated under the supervision of an appropriately qualified life-science researcher. The typical usage environment is a research or teaching lab or hospital. ADInstruments equipment is not intended for use in domestic environments.

Where a product meets IEC 60601-1 it is under the principle that:

- this is a more rigorous standard than other standards that could be chosen.
- it provides a high safety level for subjects and operators.

The choice to meet IEC 60601-1 is in no way to be interpreted to mean that a product:

- is a medical device,
- may be interpreted as a medical device, or
- is safe to be used as a medical device.

Safety and Quality Standards

In accordance with European standards PowerLab C and C Series devices also comply with the electromagnetic compatibility requirements under EN61326-1, which encompasses the EMC directive.

Quality Management System ISO 9001:2015

ADInstruments manufactures products under a quality system certified as complying with ISO 9001:2015 by an accredited certification body.

Regulatory Symbols

Devices manufactured by ADInstruments that are designed for direct connection to humans and animals are tested to IEC60601-1:1998 and IEC60601-1:2005 (including amendments 1 and 2) and EN61326-1:2006, and carry one or more of the safety symbols below.

Warning symbol. The exclamation mark inside a triangle means that the supplied documentation must be consulted for operating, cautionary or safety information before using the device.

CE Mark. All front-end amplifiers and PowerLab systems carry the CE mark and meet the appropriate EU directives.

UKCA Mark. All front-end amplifiers and PowerLab systems carry the UKCA mark and meet the appropriate UK directives.

Refer to booklet symbol. This symbol specifies that the user needs to refer to the Instruction manual or the booklet associated with the device.

Date of Manufacture/ Manufacturer's name symbol. This symbol indicates the date of manufacture of the device and the name of the manufacturer

WEEE directive symbol. Unwanted equipment bearing the Waste Electrical and Electronic Equipment (WEEE) Directive symbol requires separate waste collection. (See disposal section at the end of this chapter)

Further information is available on request.

Safety Standards

IEC Standard - International Standard - Medical Electrical Equipment

IEC 60601-1:2012 General requirements for safety

General Safety Instructions

WARNING:

This equipment is not intended to be modified or serviced by the user. No user serviceable parts inside. Refer servicing to authorized ADInstruments service center.

To achieve the optimal degree of subject and operator safety, consideration should be given to the following guidelines when setting up a PowerLab C either as stand-alone equipment or when using PowerLab equipment in conjunction with other equipment. Failure to do so may compromise the inherent safety measures designed into PowerLab equipment.

The following guidelines are based on principles outlined in the international safety standard IEC 60601-1: *General requirements for safety – Collateral standard: Safety requirements for medical systems*. Reference to this standard is required when setting up a system for human connection. The user is responsible for ensuring any particular configuration of equipment complies with IEC60601-1-1.

The PowerLab C (and many other devices) requires the connection of a personal computer for operation. This personal computer should be certified as complying with IEC 60950 and should be located outside a 1.8 m radius from the subject (so that the subject cannot touch it while connected to the system). Within this 1.8 m radius, only equipment complying with IEC 60601-1 should be present. Connecting a system in this way obviates the provision of additional safety measures and the measurement of leakage currents.

Accompanying documents for each piece of equipment in the system should be thoroughly examined prior to connection of the system.

While it is not possible to cover all arrangements of equipment in a system, some general guidelines for safe use of the equipment are presented below:

- Any electrical equipment which is located within the SUBJECT AREA should be approved to IEC 60601-1.
- Do not touch the subject to which the PowerLab (or its peripherals) is connected at the same time as making contact with parts of the PowerLab (or its peripherals) that are not intended for contact to the subject.
- Cleaning and sterilization of equipment should be performed in accordance with manufacturer's instructions. The isolation barrier may be compromised if manufacturer's cleaning instructions are not followed.
- The ambient environment (such as the temperature and relative humidity) of the system should be kept within the manufacturer's specified range or the isolation barrier may be compromised.
- The entry of liquids into equipment may also compromise the isolation barrier. If spillage occurs, ADInstruments should be contacted before using the equipment.
- The PowerLab depends on the presence of a Protective Earth for it's electrical safety requirement. This is usually provided from the power outlet through a power cord. Before connecting the equipment to mains power, ensure that the power socket has a protective earth circuit capable of carrying the fault current (see bullet point below). Note the POAG terminal on the rear of the PowerLab C is not rated as a Protective Earth.
- The Protective Earth must be cable of carrying the maximum current allowed by the circuit breaker and must be electrically insulated. It must be connected to

WARNING:

Use only with ADI supplied power cords appropriate for your region.

WARNING:
To avoid risk of electric shock, this equipment must only be connected to a supply mains with protective earth

an equi-potential source (a metal stake drive into the soil is a typical situation). A licensed electrician must perform this installation.

- Power cords should never be modified so as to remove the earth connection. The integrity of the Protective Earth connection between each piece of equipment and the Protective Earth should be verified regularly by qualified personnel.
- PowerLabs are compatible with electrical safety devices (sometimes known as Safety Switches, Ground fault circuit interruptor, Residual Current Devices or Earth-leakage Circuit Breaker). ADInstruments recommends the use of such devices supplied in fixed wiring installations.
- Avoid using multiple portable socket-outlets (such as power boards) where possible as they provide an inherently less safe environment with respect to electrical hazards. Individual connection of each piece of equipment to fixed mains socket-outlets is the preferred means of connection.
- When used in ambient temperatures of 38 degrees Celcius and above, do not touch PowerLab enclosure or the USB cable continuously for more than a minute.
- To safely shut down the PowerLab, press the stop button and then close LabChart. Turn the PowerLab off at the inlet switch.

If multiple portable socket outlets are used, they are subject to the following constraints:

- They shall not be placed on the floor.
- Additional multiple portable socket outlets or extension cords shall not be connected to the system.
- They shall only be used for supplying power to equipment which is intended to form part of the system.

Cleaning and Sterilization

ADInstruments products may be wiped down with a lint free cloth moistened with industrial methylated spirit.

Inspection and Maintenance

PowerLab systems and ADInstruments front-ends are all maintenance-free and do not require periodic calibration or adjustment to ensure safe operation. Internal diagnostic software performs system checks during power up and will report errors if a significant problem is found. There is no need to open the instrument for inspection or maintenance, and doing so within the warranty period will void the warranty.

The PowerLab system can be periodically checked for basic safety by using an appropriate safety testing device. Tests such as earth leakage, earth bond, insulation resistance, subject leakage and auxiliary currents and power cable integrity can all be performed on the PowerLab system without having to remove the covers. Follow the instructions for the testing device if performing such tests. If the PowerLab system is found not to comply with such testing you should contact your ADInstruments representative to arrange for the equipment to be checked and serviced.

Environment

Electronic components are susceptible to corrosive substances and atmospheres, and must be kept away from laboratory chemicals.

Transport and Storage Conditions

- Temperature in the range 0–40 °C
- Non-condensing humidity in the range 0–95%.

Operating Conditions

- Temperature in the range 5–35 °C
- Non-condensing humidity in the range 0–90%.

WEEE Directive symbol

Disposal

- Forward to recycling center or return to manufacturer.
- Unwanted equipment bearing the Waste Electrical and Electronic Equipment (WEEE) Directive symbol requires separate waste collection. For a product labeled with this symbol, either forward to a recycling center or contact your nearest ADInstruments representative for methods of disposal at the end of its working life.

Chapter 2

Introduction

Isolated Langendorff heart preparations have been used for over a hundred years, providing researchers with convenient physiological models that can be studied without the systemic influences of the intact animal. Additionally, these preparations allow control over factors such as temperature, perfusate, oxygen, and drug concentrations. Hence, associated studies have provided valuable insights and significantly contributed to the understanding of the inherent nature of the heart. Therefore, for many years, ADInstruments have partnered with gold-standard manufacturers of isolated heart apparatuses to provide researchers with comprehensive solutions.

Continuing our mission to ***Make Science Easier***, we have worked with Radnoti LLC to provide a high-quality apparatus with the greatest flexibility, while being presented in a relatively compact design. This design leverages over 30 years of combined experience in interacting with, supporting, and listening to feedback from researchers. It features high-quality borosilicate glassware components, corrosion-resistant stainless-steel supports, fine gassing control, recirculating buffer overflow options, comprehensive water jacketing for temperature control and maintenance.

The ADInstruments Rodent Cardiomyocyte Isolation Apparatus is designed to use the Langendorff technique to enable the isolation of functional adult rodent cardiomyocytes, which includes mice and rats.

It is designed to provide:

- a clear visual pathway of its constant perfusion flow,
- the option of having up to two enzyme buffer reservoirs,
- the option to recirculate one of the enzymatic solution,
- several insertion ports for interfacing with other compatible hardware to measure temperature, pH and pressure if required.

Furthermore, its modular design offers much flexibility, such as modifying it to operate in constant perfusion pressure with the additonal purchase of few accessories and spare parts.

Chapter 3

Apparatus

This chapter describes in detail how to set up the cardiomyocyte isolation apparatus, including the components of the heating and perfusion subsystems.

PLEASE NOTE: Several components of the apparatus are made of glass. Please ensure you read the special guidelines for the care of this system, included in Chapter 5. Please read them. Failure to follow these guidelines could invalidate any warranty claims.

WARNING:

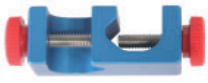
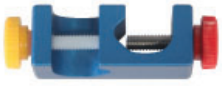
Several apparatus components are made of glass and require care during handling.

Apparatus Components

The cardiomyocyte isolation apparatus has many components, as shown in Table 3-1. Familiarizing yourself with the names used for these components will help you to follow the instructions in this manual.

Table 3–1

The components required to complete the basic assembly of the apparatus

Part No.	Description	Qty	Image
159950-B2	Steel Base for 2-Rod Lab Stand	1	
159950-24	Stainless Steel Support Rod (24”)	2	
159949-45	Stabilizer Support Bar (5.5”)	1	
159949-08	Aluminium Rod (8”)	1	
130153-2	Cannula Shaft (Short, Stainless Steel)	1	
159952	Radnoti Universal Stand Clamp (SSteel)	6	
159952-G	Radnoti Universal Stand Clamp (Nylon)	2	
130163-14	Aortic Cannula Tip (14 Gauge)	1	
130163-16	Aortic Cannula Tip (16 Gauge)	1	
130163-20	Aortic Cannula Tip (20 Gauge)	1	
130163-22	Aortic Cannula Tip (22 Gauge)	1	

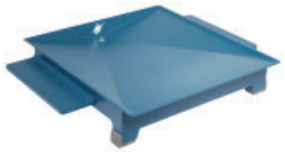
NOTE:

All the Quick Disconnect (Q.D.) inlets and outlets of the glassware are protected by black drilled caps in their packaging, which are removed during installation.

Part No.	Description	Qty	Image
120142-1	Radnoti 1.0 L Water Jacketed Reservoir	1	
1583-101-LL	Cardiomyocyte Isolation Heart Chamber (100 mL) - with Teflon Needle Valve (unused)	1	
158326-LL	Enzyme Buffer Reservoir (25 mL) - with Teflon Needle Valve	2	
158840	Radnoti Heating Coil w/ Bubble Trap (10 mL)	1	
140143-1	Oxygenating Bubbler with Inlet for 1 L Reservoir	1	
120168	Radnoti 2-Way TNV “Y” Manifold	1	
120140-A	Radnoti Reservoir Filling Funnel	1	

Part No.	Description	Qty	Image
120141-1	Double Ring Clamp for 1 L Reservoir	1	
159953-10	Single Ring Clamp for Heating Coil (Diameter 38-45 mm)	1	
159953-25	Single Ring Clamp for Enzyme Buffer Reservoir (Diameter 40-50 mm)	2	
159953-100	Single Ring Clamp for Heart Chamber (Diameter 55-80 mm)	1	
120169-2	Hose Barb Adapter (Pump to Q.D. Water Jacketed Tubing, 2pk)	1	
120159-15	Radnoti Q.D. Water Jacketed Tubing Assembly (15”)	2	
120159-25	Radnoti Q.D. Water Jacketed Tubing Assembly (25”)	2	
120159-40	Radnoti Q.D. Water Jacketed Tubing Assembly (40”)	1	
120159-60	Radnoti Q.D. Water Jacketed Tubing Assembly (60”)	1	

Part No.	Description	Qty	Image
120157-05	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 5”)	1	
120157-10	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 10”)	2	
120157-15	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 15”)	1	
120157-25	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 25”)	4	
120157-30	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 30”)	1	
120157-36	Radnoti Tygon Perfusate/Gas Tubing (0.093” x 0.156”, 36”)	4	
120155-188	Radnoti Silicone Drain/Overflow Tubing (0.188” x 0.375”, 50”)	1	
120140-B	Radnoti Tubing Adapter Kit	1	

Part No.	Description	Qty	Image
MLA2820	Stand Base Spill Tray (3 Rod)	1	
	20 mL Damping Syringe	1	
	In-line Filter Holders for 25 mm Filter Membranes	2	
SP7300	In-line A/C Glass Fiber Filter Membrane (1.0µm, 25mm, 100pk)	1	

NOTE:

In-line Filter Holder replacements can be purchased as SP7301 In-line Filter Holders for 25 mm Membranes (Polypropylene, 10pk) if required.

Electronic Components

The cardiomyocyte isolation apparatus requires several electronic hardware to operate. The following components may be supplied depending on the purchased configuration.

Table 3–2

The components required to complete the basic assembly of the apparatus

Part No.	Description	Qty	Image
MLA216-V	Thermal Water Circulator (5L)	1	
ML172B-V	Minipuls 3 Peristaltic Pump	1	
SP2848	Pump Tubing - Flow Rated Yellow / Yellow (12 pk)	1	
SP2849	Pump Tubing - Flow Rated Black / White (12 pk)	1	

Apparatus Assembly

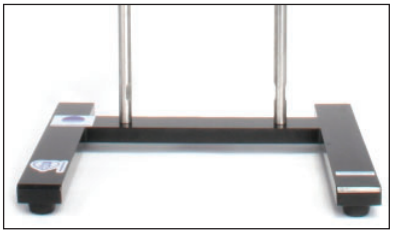
This section describes and illustrates how to assemble the apparatus.

Glassware Assembly

1. Place the Steel Base for 2 Rod Lab Stand on the intended bench space. If possible, select an area that will allow for accidental spills. Attach the threaded ends of each Stainless Steel Support Rod (24”) to the outer threaded holes of the Steel Base for 2 Rod Lab Stand.

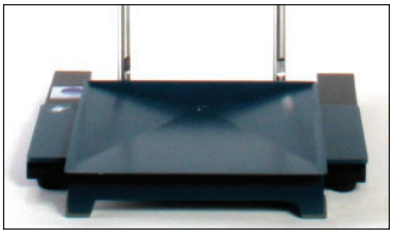
Threaded end of a Stainless Steel Support Rod

2. Carefully connect the drain tubing to the plastic hose adapter, which is then inserted into its slot at the bottom of the Spill Tray. Slip the drain tubing to one side under the steel base and slide the spill tray so that its sides rest on the base.
3. Familiarize yourself with the attaching/detaching mechanism of the Double Ring Clamp for 1 L Reservoir, which is effectively a hose-clamp. Rotate both tightening/loosening knob screws to loosen both perforated bands until they are completely disconnected from their respective slots.



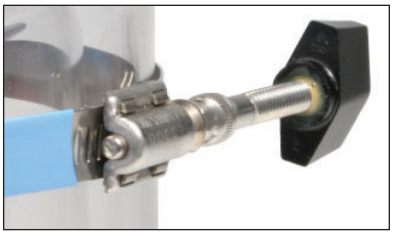
Attaching Stainless Steel Support Rods to Steel Base

4. Orientate the Radnoti 1.0 L Water Jacketed Reservoir so that its outflow port is positioned near the front. This is highly recommended as it provides easier access from the front. Correspondingly, first orientate the Double Ring Clamp for 1 L Reservoir so the tightening/loosening knob screws are facing the back. Fit the bands of the double ring clamp around the reservoir. Push each band against its slot and rotate the knob screw to catch and tighten the band.

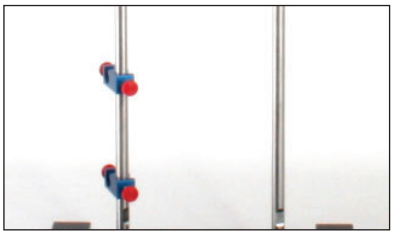


Placing the Spill Tray on the Steel Base

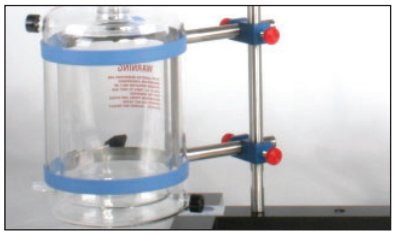
5. Place two Radnoti Universal Stand Clamp (SSteel) on the left Stainless Steel Support Rod (24”) with their distance matching the distance between the support rods of a Double Ring Clamp for 1 L Reservoir.
6. Attach the double ring clamp with reservoir to the stainless steel support rod on the left.
7. Remove the large black cap from the top of the Water Jacketed Reservoir. Insert the Oxygenating Bubbler with Inlet for 1 L Reservoir and secure it by screwing on its threaded cap.



Assembling Double Ring Clamp around Reservoir



Placing Universal Stand Clamp (SSteel) on Support Rod

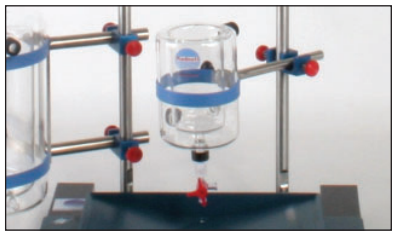


Attaching assembled Water Jacketed Reservoir to the left Support Rod

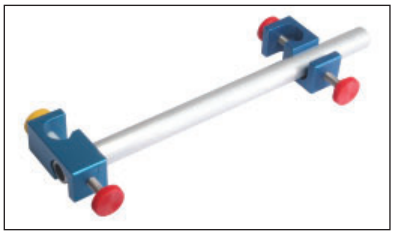
NOTE:

The supplied Teflon Needle Valve for the Heart Chamber will not be used unless required by the experiment.

8. Similarly, attach the Single Ring Clamp for Heart Chamber to the Cardiomyocyte Isolation Heart Chamber (100 mL). Close off its side gassing port with a full black cap (13 mm), which can be found in the Radnoti Tubing Adapter Kit.
9. Place a Radnoti Universal Stand Clamp (SSteel) on right stainless steel support rod. Attach the single ring clamp with the heart chamber to it.
10. Attach a Radnoti Universal Stand Clamp (Nylon) to one end of the Aluminium Rod via the stainless steel screw. Attach the other end of the Aluminium Rod with a Radnoti Universal Stand Clamp (SSteel). Attach the Cannula Shaft to the Aluminium Rod via the nylon screw of the Radnoti Universal Stand Clamp (Nylon).
11. Four aortic cannula tips of different sizes are supplied. Select the appropriate sized cannula that suitably matches the aortic diameter of the heart that will be isolated.
12. Attach the aortic cannula either directly to the Cannula Shaft or with a 3-Way Tap with a Lock Ring in between to provide additional directional control of the perfusion pathway or an additional access port closer to an isolated heart (see Figure 3-1).
13. Attach the entire cannula holder assembly to the right stainless steel support rod, above the heart chamber, using the Aluminium Rod's Radnoti Universal Stand Clamp (SSteel). Adjust it as required so that the aortic cannula will be positioned in the center of the heart chamber (viewing from the top).



Attaching Cardiomyocyte Isolation Heart Chamber to right Support Rod



Attaching both Universal Stand Clamps (SSteel and Nylon) to the Aluminium Rod

Attaching the Cannula Holder Assembly above the Heart Chamber

Figure 3–1

Options for attaching the Aortic Cannula to the Cannula Shaft

Aortic Cannula's direct attachment

Aortic Cannula's attachment via 3-Way Tap with a Lock Ring

NOTE:

If required, the 5” tubing can be cut to further shorten the distance between Heating Coil and Cannula Shaft to minimize heat loss, especially during low flow rates.

NOTE:

A 1-Way Tap may be also placed in between the syringe and the Heating Coil to provide additional control during experiments.

14. Similarly, attach the Single Ring Clamp for Heating Coil (Diameter 38-45 mm) to the Radnoti Heating Coil w/ Bubble Trap (10 mL).

15. Attach to the Heating Coil w/ Bubble Trap (10 mL) to the right stainless steel support rod via another Radnoti Universal Stand Clamp (SSteel). Position it so that the Radnoti Tygon Perfusate/ Gas Tubing (0.093” x 0.156”, 5”) can easily connect it and the Cannula Shaft. Ensure that there are no kinks in the tubing.

16. Attach a 1-Way Tap to the overflow port (top right) of the Heating coil and another 1-Way Tap to its side injection/measurement port (bottom left).

17. Fit the supplied 20 mL Damping Syringe to the top of the Heating Coil w/ Bubble Trap (10 mL) to further reduce the pressure oscillations in the perfusion circuit that are caused by the rollers of the pump. Adjust the syringe plunger and align the holes in the syringe barrel and plunger, so as to contain about 10 mL of air in the syringe. Push the supply metal rod through the holes in the barrel and plunger to prevent the plunger from moving when the system becomes pressurized.

18. Prepare the Enzyme Buffer Reservoir (25 mL).

- Attach the Single Ring Clamp for Enzyme Buffer Reservoir (Diameter 40-50 mm) to the Enzyme Buffer Reservoir (25 mL).
- Fit its teflon needle valve to its gas port at its side and a 3-Way Tap with Lock Ring to its outflow port at the bottom.
- Orientate the reservoir so that it can be attached to the stainless steel support rod without any interfering protrusions.

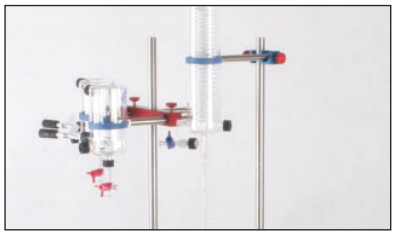
Attaching Heating Coil w/ Bubble Trap (10 mL) to right Support Rod

Attaching Single Ring Clamp, Teflon Needle Valve and 3-Way Tap with Lock Ring to Enzyme Buffer Reservoir

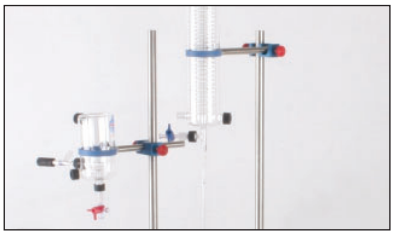
19. The apparatus provides the option of having up to two Enzyme Buffer Reservoirs. It can be modified to only have one Enzyme Buffer Reservoir if experimentally required (see Figure 3-2).

Figure 3–2

Enzyme Buffer Reservoir options.



Apparatus with two Enzyme Buffer Reservoirs



Apparatus with one Enzyme Buffer Reservoir

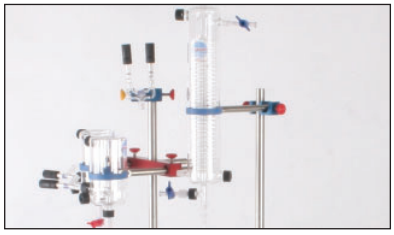
NOTE:

Attachment depth of both Enzyme Buffer Reservoirs to the Stabilizer Support Bar can be staggered to prevent them from clashing against each other .

20. For the **dual Enzyme Buffer Reservoirs option**, firstly insert the Stabilizer Support Bar on the left stainless steel support rod via its center hole. Orientate the Stabilizer Support Bar to be facing at an angle away from the front. Attached both fitted Enzyme Buffer Reservoirs to the Stabilizer Support Bar, one on either side.

21. For the **single Enzyme Buffer Reservoir option**, directly attached the fitted Enzyme Buffer Reservoir to the left stainless steel support rod via a Radnoti Universal Stand Clamp (SSteel).

22. Lastly, attach the Radnoti 2-Way TNV "Y" Manifold to left stainless steel support rod via a Radnoti Universal Stand Clamp (Nylon). Ensure the nylon screw is used to attach the manifold.



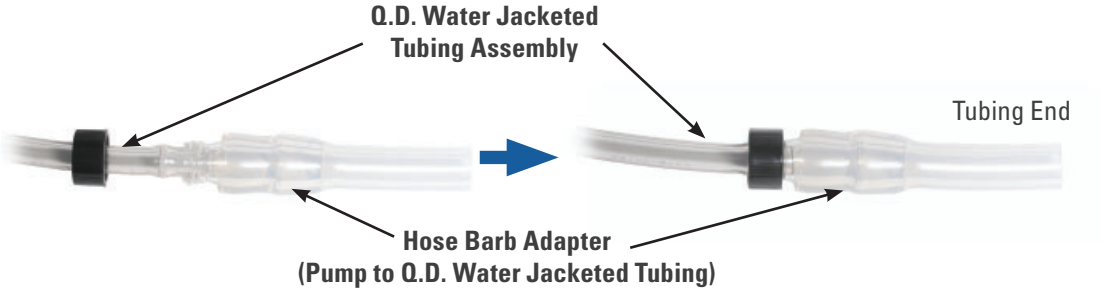
Attaching Radnoti 2-Way TNV "Y" Manifold to left Support Rod

Water Jacketing Assembly

1. First remove the protective caps from the threaded glass end of each Hose Barb Adapter (Pump to Q.D. Water Jacketed Tubing). Using one Hose Barb Adapter (Pump to Q.D. Water Jacketed Tubing), carefully insert one end of a Radnoti Q.D. Water Jacketed Tubing Assembly (40”) and carefully fasten it by screwing the drilled cap onto the threaded glass end.

Figure 3–3

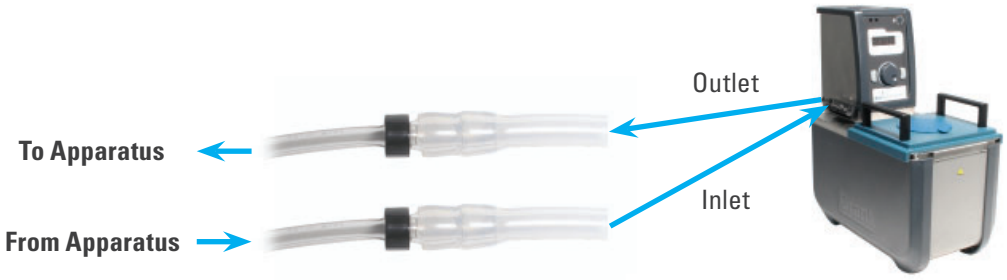
Connecting a Quick Disconnect Water Jacketing Tubing Assembly to a Hose Barb Adapter



2. Identify the inlet and outlet of the Thermal Water Circulator. Connect the Hose Barb Adapter with the connected Water Jacketed Tubing Assembly (40”) to the outlet of the circulator via the tubing end of the Hose Barb Adapter.

Figure 3–4

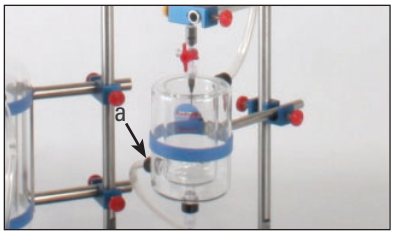
Connecting apparatus to Thermal Water Circulator



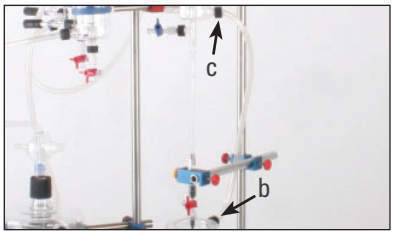
NOTE:

The apparatus is designed to be compatible with a range of water bath recirculators, including the Radnoti Thermal Bath/Circulator.

3. Remove any protective drilled caps on the input and output of the Cardiomyocyte Isolation Heart Chamber (100 mL).
   - Insert and attach the other free end of the Water Jacketed Tubing Assembly (40”) to the threaded water jacketing inlet (a) of the heart chamber.
   - Insert and attach one end of the Radnoti Q.D. Water Jacketed Tubing Assembly (15”) to the threaded water jacketing outlet (b) of the heart chamber.
4. Similarly, ensure that any protective drilled caps are removed from the Heating Coil w/ Bubble Trap (10 mL). Connect the other free end of the Water Jacketed Tubing Assembly (15”) to the threaded water jacketing inlet (c) of the Heating Coil.



Connecting to Heart Chamber’s inlet (a)

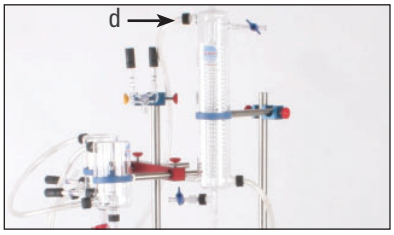


Connecting Heart Chamber’s outlet (b) to Heating Coil’s inlet (c)

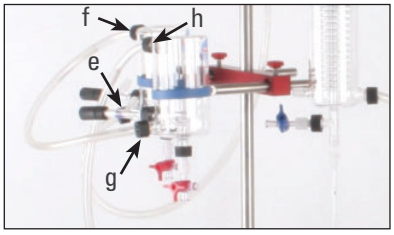
NOTE:

The ends of a Q.D. Water Jacketed Tubing Assembly can be wetted with clean water to aid insertion.

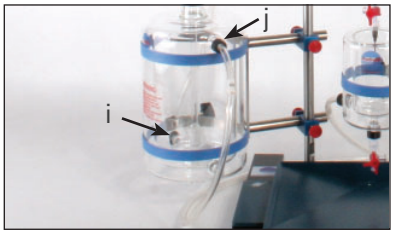
5. Insert and attach one end of a Radnoti Q.D. Water Jacketed Tubing Assembly (25”) to the threaded water jacketing outlet (d) of the heating coil.
6. Remove any protective drilled caps from both Enzyme Buffer Reservoirs.
7. Connect the other free end of the Water Jacketed Tubing Assembly (25”) to the threaded water jacketing inlet (e) of one of the Enzyme Buffer Reservoir. Insert and attach one end of a Radnoti Q.D. Water Jacketed Tubing Assembly (15”) to the threaded water jacketing outlet (f) of this Enzyme Buffer Reservoir.
8. Connect the other free end of the Water Jacketed Tubing Assembly (15”) to the threaded water jacketing inlet (g) of the other Enzyme Buffer Reservoir. Insert and attach one end of a Radnoti Q.D. Water Jacketed Tubing Assembly (25”) to the threaded water jacketing outlet (h) of this second Enzyme Buffer Reservoir.
9. Connect the other free end of the Water Jacketed Tubing Assembly (25”) to the inlet (i) of the Water Jacketing Reservoir.
10. Connect the threaded water jacketing outlet (j) of the Water Jacketed Reservoir to the free end of a Water Jacketed Tubing Assembly (60”).
11. Connect the other free end of this Water Jacketed Tubing Assembly (60”) to the other available Hose Barb Adapter (Pump to Q.D. Water Jacketed Tubing). Attach the tubing end of this Hose Barb Adapter to the inlet of the Thermal Water Circulator.
12. The overall water jacketing circuit is illustrated in Figure 3-5.



Connecting from Heating Coil’s outlet (d)



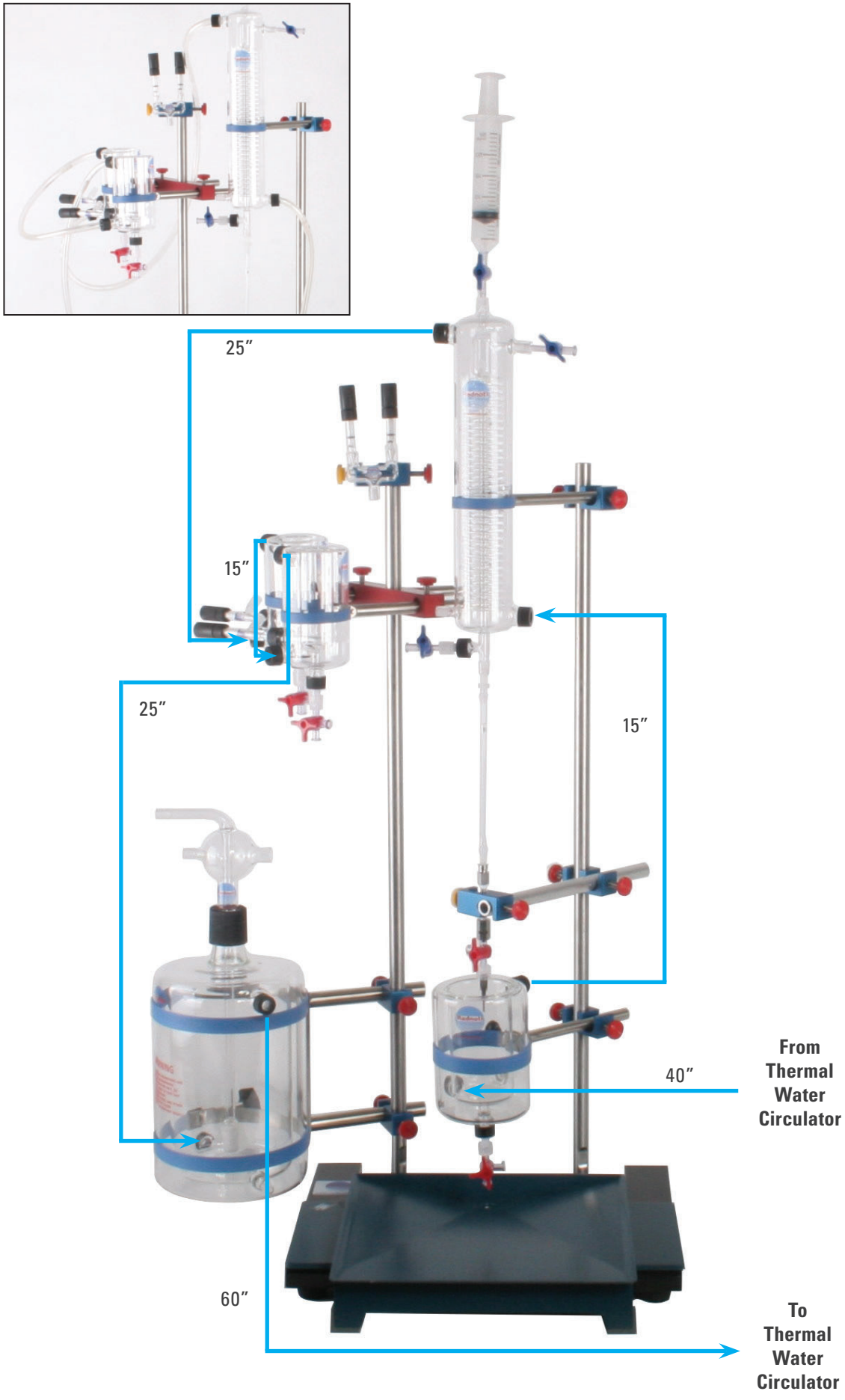
Connecting the inlets and outlets of both Enzyme Buffer Reservoirs



Connecting Reservoir’s inlet and outlet

Figure 3–5

Water Jacket Tubing Connections



Gas Tubing Connections

NOTE:

Additional gas accessories might be required, which can include gas regulators, inlet and outlet fittings (i.e. 3/8" NPT to Gas Hose Barb Fitting, Tubing Reducer etc.). They should be separately and locally sourced according to the laboratory requirements.

NOTE:

It is recommended to gas the buffer for at least 20-30 minutes before use.

1. First connect one end of the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 36") to a gas supply (not supplied). Further adaptation or adaptors may be required to fit it to the gas supply.
2. Connect the other free end of this Radnoti Tygon Perfusate/Gas Tubing to the central bottom port (a) of the Radnoti 2-Way TNV "Y" Manifold (see Figure 3-6).
3. Join one end of a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 15") to one end of a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 25") via the female luers of a 3-Way Tap. Insert the male luer of the 3-Way Tap into the manifold port (b) on the right side that is controlled by a teflon needle valve.
4. Connect the free end of the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 15") to the teflon needle valve port (c) of the nearest Enzyme Buffer Reservoir (25 mL). Then connect the free end of the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 25") to the teflon needle valve port (d) of the other Enzyme Buffer Reservoir (25 mL).
5. Connect one end of the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 30") to the other port (e) of the manifold that is controlled by a teflon needle valve. Then connect its other free end to the port (f) of the Oxygenating Bubbler with Inlet for 1 L Reservoir that connects to its sintered glass gas dispersion tube.
6. Fill the reservoir with the desired buffer (please see Buffer Filling) and Enzyme Buffer Reservoirs with desired enzyme solutions (please see Priming).
7. Turn on the gas supply to gas the solutions. Minor gas flow adjustments can be made by rotating each teflon needle valves within the gas circuit.

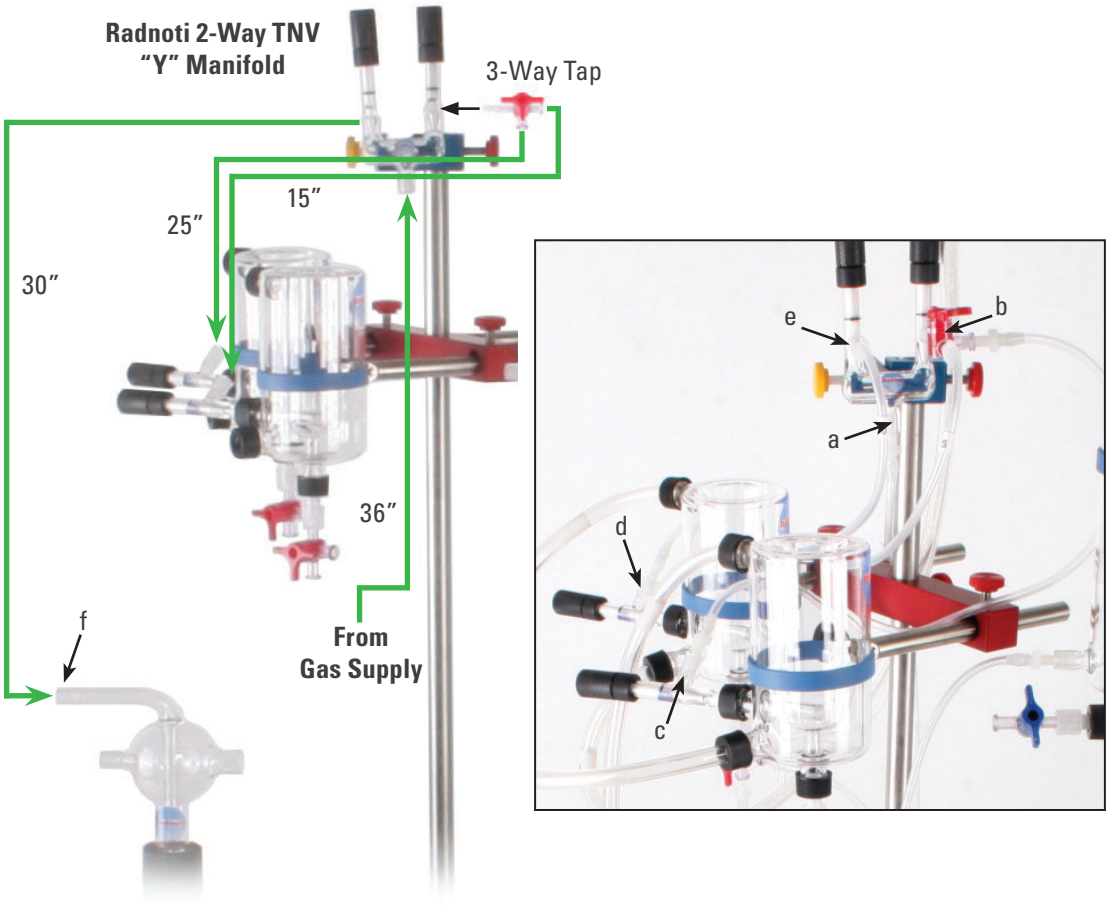


Figure 3–6

Connecting gas tubings

Perfusion Connections

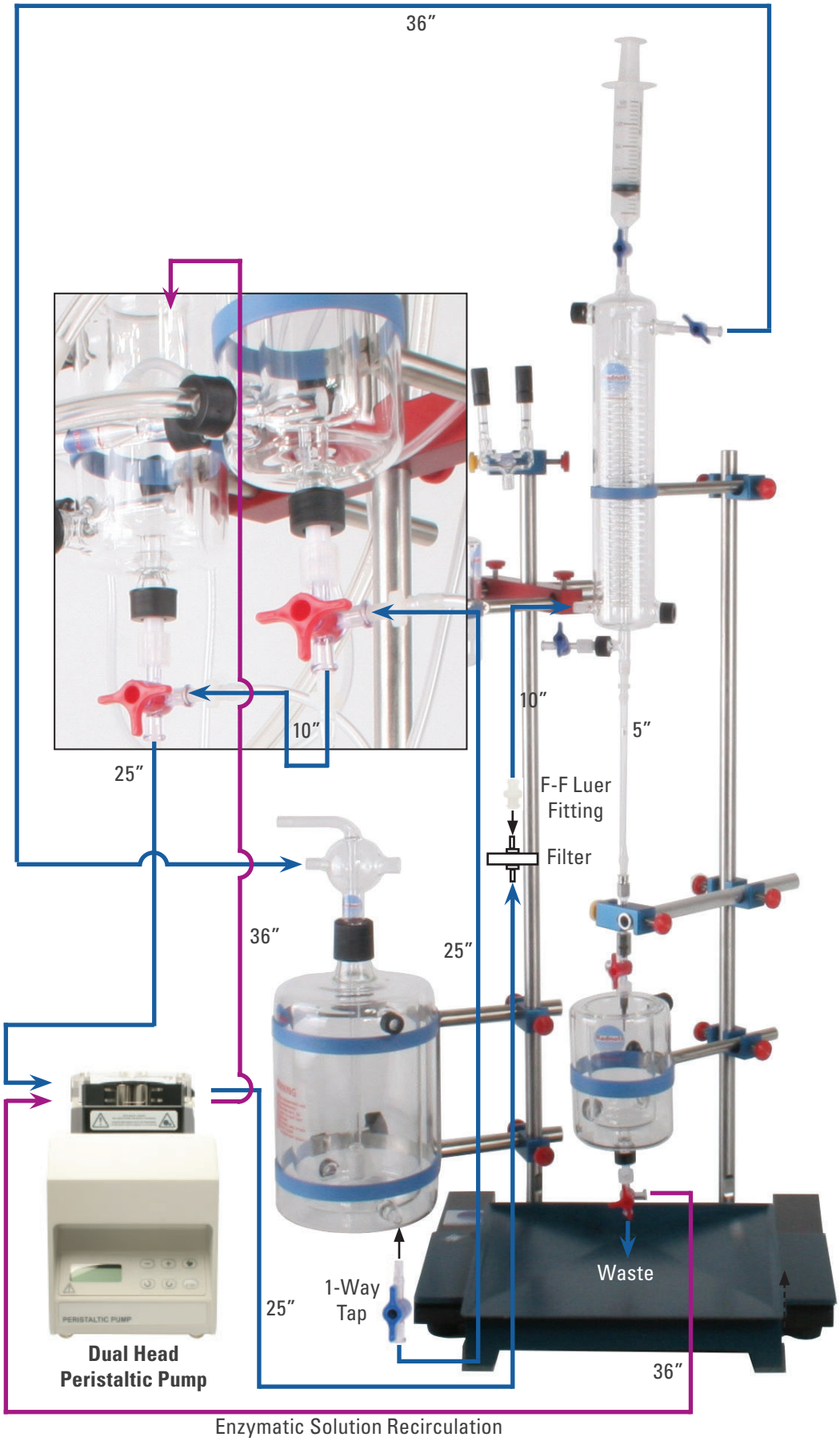
NOTE:

A dual head peristaltic pump is recommended to utilize the full features of this apparatus. A Minipuls 3 Peristaltic Pump will be used to illustrate the perfusion connections.

1. Confirm the placement of the assembled perfusion pump in relation to the rest of the cardiomyocyte isolation apparatus. Connect the pump's power supply and turn the pump on. Note the direction of rotation.
2. Connect a 1-Way Tap to the outlet of the Radnoti 1.0 L Water Jacketed Reservoir for control of the buffer flowing out from the reservoir. Next, connect one end of a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 25") to this 1-Way Tap (see Figure 3-7). Connect the other end of this tubing to the 3-Way Tap of the nearest Enzyme Buffer Reservoir.
3. Join both 3-Way Taps of the Enzyme Buffer Reservoirs with a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 10").
4. Insert a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 25") into the free 3-Way Tap luer of the second Enzyme Buffer Reservoir. Connect the free end of this perfusate tubing with the the peristaltic pump's tubing. The corresponding interfacing accessory (dependent on peristaltic pump type) will be required.
5. Place this pump tubing in one of the pump's head. Ensure that the placement of the pump tubing will correspond to the correct direction of rotation, which will draw in the buffer.
6. Similarly, join the other end of the pump tubing to a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 25"). Connect the other end of this perfusate tubing to a suitable disposable filter. Connect the other side of the filter to a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 10"), which a Female-Female Luer Fitting may be required.
7. Connect the other end of the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 10") to the inlet of the Radnoti Heating Coil w/ Bubble Trap (10 mL). Ensure that the outlet (at the bottom) of the Heating Coil is connected to the Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 5"), which its other end is attached to the Cannula Shaft. Join the Heating's Coil's overflow port (at its side top) to the flow return inlet (female luer that flows into the reservoir) of the Oxygenating Bubbler with a Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 36").
8. Connect another Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 36") to the 3-Way Tap at the bottom of the heart chamber for the option to recirculate one of the enzmyatic solution when required. Connect the free end of this tubing to another pump tubing. Similarly, connect the other end of the pump tubing to another Radnoti Tygon Perfusate/Gas Tubing (0.093" x 0.156", 36"). Insert the free end of this perfusate tubing directly into (from the top) the Enzyme Buffer Reservoir, which enzymatic solution will be recirculated.
9. Properly place the pump tubing in the other available pump head to recirculate when required.

Figure 3–7

Connecting perfusate tubings for perfusion



NOTE:

Connections to the in-line filter may vary depending on the the type of filter used. The supplied Radnoti Tubing Adapter Kit includes a range of fittings.

Buffer Filling

1. Ensure that the apparatus is fully assembled, clean and ready. Next, ensure that the taps connected to the outlet of each Water Jacketed Reservoir are closed.
2. Release and lift the Oxygenating Bubbler with Inlet from the Water Jacketed Reservoir. Insert the Reservoir Filling Funnel and fill the reservoir with the desired volume of buffer.
3. Remove the Reservoir Filling Funnel and insert the Oxygenating Bubbler with Inlet back into the reservoir.
4. Turn on the gas and begin gassing the reservoir with 95/5% O_2/CO_2 to maintain gas tension and buffer pH level.

NOTE:

The screw of the Universal Stand Clamp that holds the ring clamp of the Heating Coil, can be loosened to allow twisting of the Heating Coil to further aid the release of any trapped air bubbles.

Priming

1. Ensure that both 3-Way Taps of both Enzyme Buffer Reservoirs are turned off to stop any solution flowing out from them.
2. Fill both Enzyme Buffer Reservoirs with the required enzymatic solutions.
3. Temporarily disconnect the tubing attached to the bottom luer of the 3-Way Tap that is attached to one of the Enzyme Buffer Reservoir. Briefly and gently turn the 3-Way Tap to only allow flow downwards, hence using some of the enzymatic solution flowing out to remove any trapped air bubbles. Reconnect the tubing and similarly repeat for the other Enzyme Buffer Reservoir.

Initially turning off 3-Way Taps of Enzyme Buffer Reservoirs

4. Ensure that both 3-Way Taps of both Enzyme Buffer Reservoirs are still turned off and in the position that only allows the buffer to flow through them from the reservoir.
5. Ensure that the Peristaltic Pump is properly set up as outlined in Chapter 4. Note the Peristaltic Pump's rotation setting and ensure that Pump Tubing is securely placed in the right alignment that will pump buffer out from the reservoir as the pump drive turns.
6. Ensure that the 3-Way Tap at the Aortic Cannula is closed.

NOTE:

The 3-Way Tap of the Aortic Cannula is opened, while the Heating Coil's overflow port is closed when cannulating an isolated heart.

7. Ensure the side injection/measurement port of the Heating Coil is closed (i.e. via a 1-Way Tap), but its overflow port is opened.
8. Temporarily disconnect the 20 mL Damping Syringe with its 1-Way Tap.
9. Ensure there is sufficient buffer in the reservoir.
10. Start the Peristaltic Pump to pump the buffer up to the Heating Coil. Ensure pumping speed is sufficient to fill the Heating Coil.
11. Fill the Heating Coil and release any trapped air bubbles through its top. Gently tap the Heating Coil to dislodge any adhering air bubbles.
12. Open and close the 3-Way Tap at the aortic cannula to release any hidden air bubbles.
13. Continue filling the Heating Coil until reaching sufficient buffer. Reattach the 20 mL Damping Syringe as previously mentioned.
14. Adjust the pump speed to ensure a smooth recirculation via the Heating Coil's overflow port to place it on standby.
15. Ensure all solutions are sufficiently gassed.

Chapter 4

Electronics Setup

This chapter describes how to connect the required electronic components to the cardiomyocyte isolation apparatus, which include the Thermal Water Circulator and Minipuls 3 Peristaltic Pump.

It is recommended that all electronic components be set up on a ‘dry’ table, separate to that used for the ‘wet’ myocyte apparatus.

Water Heating

This cardiomyocyte isolation apparatus is designed to be compatible with a range of heated water bath recirculators. Please read the corresponding manual to identify and confirm the circulating inlet and outlet, and be familar with its operation. Accordingly, set the heating temperature as desired, such as 37 °C.

Perfusion

This section describes how to assemble the electronic components that are related to perfusion. The apparatus is designed to be perfused at a constant perfusion flow. Additional glassware and accessories will need to be additionally purchased if constant perfusion pressure is required.

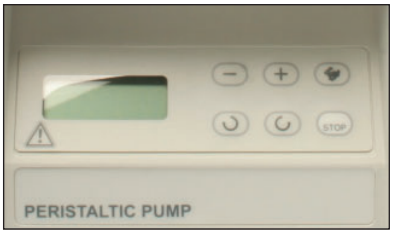
Peristaltic Pump

Several dual head peristaltic pumps may be used with this apparatus. The Minipuls 3 Peristaltic Pump is described as an example.

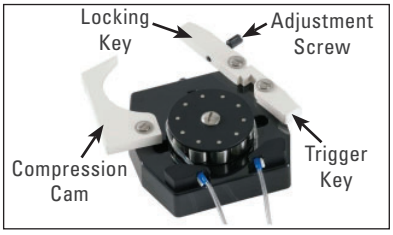
NOTE:

The power switch is at the rear of the Minipuls 3 Peristaltic Pump.

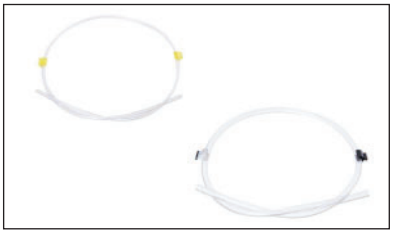
1. Familiarize yourself with the pump controls on its keypad that are located on its front.
   - The **+** and **-** keys are used to set the pump speed.
   - Pressing the **RABBIT** key on the pump sets the pump speed to maximum (48.0 rpm). Pressing the RABBIT key again returns the pump speed to the preset value.
   - Pressing the **STOP** key will halt the pump.
   - Pressing the **Forwards** and **Backwards** pump direction keys changes the direction of the pump's rotation.
2. Unlock each channel of the head by pressing the Trigger Key towards the roller barrel. Position the the correct tubing around the rollers.
   - 'Yellow & Yellow (1.42 mm ID)' for mouse hearts.
   - 'Black / White (3.16 mm ID)' for rat hearts.
3. Swing the Compression Cam back and snap the Locking Key. Using the Adjustment Screw, adjust the cam pressure on the tubing to the minimum necessary to ensure pumping of the fluid.
4. When the pump is not in use, release the Compression Cam by pressing on the bevelled corners of the Trigger Key and slacken the tubing. This increases the life of the tubing.



Keypad of Peristaltic Pump



Pump Head



Pump Tubings

Chapter 5

Cleaning

This chapter describes the cleaning and maintenance of the cardiomyocyte isolation apparatus.

Cleaning

After the experiment has been completed, care should be taken to scrupulously clean the equipment. It is important to remember that the solutions that can sustain the heart and muscle will also provide excellent media for bacteria.

The cleaning procedures will be dependent upon:

- types of chemicals and biological materials that are being used.
- types of measurements that are being made and what substances can interfere with those measurements.
- frequency of use of the equipment and number of users involved.

General Cleaning Tips

NOTE:

Bactericidal soaps may contain iodine or other materials which can affect isolated tissues and cells.

- Non-phosphate soaps are preferred since insoluble phosphates can form from calcium and magnesium in physiological salt solutions.
- Cleaning supplies and equipment (such as brushes) should be used only for cleaning this glassware and not used for other lab cleaning procedures.
- Often overlooked as a source of contamination is the water supply. This should be kept clean, the bath rinsed, and solution changed to reduce precipitate build up.
- Covering equipment to reduce airborne contamination from microbes and spores is useful.
- A convenient rule of thumb for testing for contamination in preparations that you have found reliable is that two consecutive experimental failures that are not explained by an obviously damaged sample, poor surgical or dissection techniques, or solution problems may be caused by perfusate contamination.

Shared equipment is the most difficult to maintain properly. In order to maintain equipment properly it is generally best to:

- assign the maintenance or the oversight of the equipment to one individual who will monitor equipment and maintain cleaning supplies.
- have written protocols posted with the equipment.
- have a logbook where cleaning dates, as well as notification of problems, suggestions, etc., can be recorded.

Cleaning Glass Items

- The glassware is borosilicate glass, which can be cleaned with a wide range of soaps, ethyl alcohol, dilute HCl or HNO_3 (0.1 M) or other solvents.
- Extensive flushing with distilled, deionized water to remove all traces of the cleaning agents and salts is recommended. Large glassware, such as reservoirs or assemblies can be flushed in place, but care must be taken to thoroughly clean aerators, stopcocks and associated parts.
- Aerators should be blown dry using gas or air at the final water rinse. If acid is used, the runoff water should not be more acidic than the normal water pH.
- As with the use of any chemicals, proper protective gear and training are essential to reduce personnel hazards and experimental and environmental contamination.
- Heated acid or chromic acid is generally not recommended due to personnel hazards and possible heavy metal contamination of the system.
- If very lipophilic substances (prostaglandins, ionophores, certain dyes, etc.) are used, rinses with ethyl alcohol or the most appropriate organic solvent can be used first, but this will necessitate thorough cleaning afterward to remove any traces of the organic solvent.
- Use of toxins, biohazard materials, and radiochemicals can present considerable complications to a generalized cleaning procedure. Having an apparatus and a contained area dedicated to these procedures reduces problems.
- Diluted bleach can be used on glassware but must be rinsed extensively.
- Glassware can be sterilized but all fixtures (such as aerators, stopcocks caps, etc.) should be removed prior to sterilization.
- The glass aerators can be cleaned with water or dilute acid if clogged.
- The use of water or gas under high pressure can result in damage to the glassware and personnel and therefore is not recommended.
- After a general soap and water rinse to remove soluble materials, cleaning with 0.1 M HCl or 0.1 M HNO3 for several hours or overnight, followed by an extensive water rinse, will usually remove most contaminants. If this does not work, 1 M acid can be tried. Again, if acid is used, the runoff water should not be more acidic than the normal water pH.
- Because the glass frit filaments are thin, high concentrations of acids, or especially alkalis, can destroy them and are not recommended.

Cleaning Non-Glass Items

- Initial cleaning of non-glass items should be with aqueous soap solutions. Depending upon the chemical resistance of the materials, the use of other solvents, cleaning procedures or sterilization may be possible.
- Areas and items to be especially well cleaned are the aerator, tubing, syringe ports, cannulae, pressure transducer fittings, septa, balloon, catheters, and electrodes (oxygen, pacing, ion selective, etc.).

Tubing Maintenance

NOTE:

Silicone tubing is very permeant to gases, so it should not be generally used to transport gassed solutions.

Tubing should be inspected at the pump head for wear. The interior of tubing can gradually be roughened during use and the abraded areas will form sites for bacterial growth. Tubing should be a high grade with low plasticizer leaching.

The use of disposable tubing and stopcocks will assist in cleanup, as will regular scheduling of these procedures, rather than intermittent experiments, if non-dedicated equipment must be used. When baths are used intermittently the lack of frequent cleaning and the lack of solutions rinsing out bacteria that are deposited in the tubing may result in a contamination problem when the system is finally used.

Warranty

Product Purchase and License Agreement

This Agreement is between ADInstruments NZ Ltd ['ADI'] and the purchaser ['the Purchaser'] of any ADI product or solution — software, hardware or both — and covers all obligations and liabilities on the part of ADI, the Purchaser, and other users of the product. The Purchaser (or any user) accepts the terms of this Agreement by using the product or solution. Any changes to this Agreement must be recorded in writing and have ADI's and the Purchaser's consent.

Responsibilities

The Purchaser and any others using any ADI product or solution agree to use it in a sensible manner for purposes for which it is suited, and agree to take responsibility for their actions and the results of their actions. If problems arise with an ADI product, ADI will make all reasonable efforts to rectify them. This service may incur a charge, depending on the nature of the problems, and is subject to the other conditions in this Agreement. ADI does not separately warrant the performance of products, equipment or software manufactured by third parties which may be provided to Purchaser as part of an overall solution. However, as further noted below, ADI will pass through to Purchaser all applicable third party warranties to the extent it has the right to do so.

ADI Product Hardware Warranty

ADI warrants that PowerLab Data Acquisition Units (PL prefix)[1] and Front-ends (FE prefix)[2] shall be free from defects in materials and workmanship for five (5) years from the date of purchase. Other PowerLab Data Acquisition Units[3], Front-ends[4] and Pods[5] shall be free of defects in material and workmanship for three (3) years from their date of purchase. ADI also warrants that ADI Specialized Data Recorders[6] and Instruments[7] shall be free of defects in material and workmanship for one (1) year from their date of purchase. If there is such a defect, as Purchaser's sole remedy hereunder, ADI will repair or replace the equipment as appropriate, and the duration of the warranty shall be extended by the length of time needed for repair or replacement.

To obtain service under this warranty, the Purchaser must notify the nearest ADI office, or Authorized Representative, of the defect before the warranty expires. The ADI or Representative office will advise the Purchaser of the nearest service center address to which the Purchaser must ship the defective product at his or her own expense. The product should be packed safely, preferably in its original packaging. ADI will pay return shipping costs.

Hardware Warranty Limitations

This warranty applies only to the ADI hardware specified in this document and used under normal operating conditions and within specification. Consumables, electrodes and accessories are not covered by this warranty. Third party equipment may be covered by the third party manufacturer's warranty. To the extent that ADI has the right to pass through any third party manufacturer warranties to Purchaser it will do so to the extent it is able to do so. Copies of applicable third party manufacturer warranties, to the extent they exist, are available upon request. The warranty provided here under does not cover hardware modified in any way, subjected to unusual physical, electrical or environmental stress, used with incorrectly wired or substandard connectors or cables, or with the original identification marks altered. Tampering with or breaking of the Warranty Seal will also void the warranty.

Product Types & Warranty Term

ADI manufactured products covered by a five (5) year warranty

[1] Data Acquisition Units: PowerLab C, C Series devices & 35 series with PL prefix
[2] Front-ends: ADI Front-end Signal Conditioners with FE prefix.

ADI manufactured products covered by three (3) year warranty

[3] Data Acquisition Units: PowerLab 26 series with ML prefix
[4] Front-ends: ADI Front-end Signal Conditioners with ML prefix.
[5] Pods: The entire range of ADI Pod Signal Conditioners.

ADI manufactured products covered by one (1) year warranty

[6] Specialized Data Recorders: Metabolic Systems (e.g., ML240 PowerLab/8M Metabolic System)
[7] Instruments: Blood FlowMeter, Gas Analyzers, NIBP System (excluding transducers), STH Pump Controller.

Third Party Products (Including Transducers)

Products not manufactured by ADI are covered by the manufacturer's warranty.

Accessories and Consumables

Accessories and Consumables are not covered by any type of warranty.

General Limitations

ADI products are produced to high standards, and should perform as described in the supplied documentation. There is a limited hardware warranty, and technical support is provided for all ADI products. Nevertheless, since ADI products could be affected by external factors (for instance, the computer system on which they run and other hardware and/or software provided by third parties), absolute performance and reliability of products and the overall solution cannot be guaranteed. No warranty, either expressed or implied or statutory, other than that expressly contained in this Agreement, is made in respect to ADI products or software, third party products or software, the overall solution or otherwise. The Purchaser therefore assumes all risks as to the performance and reliability of the products, the software, the solution and the results gained using them. ADI neither assumes or authorizes any person to assume on its

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Inquiries

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