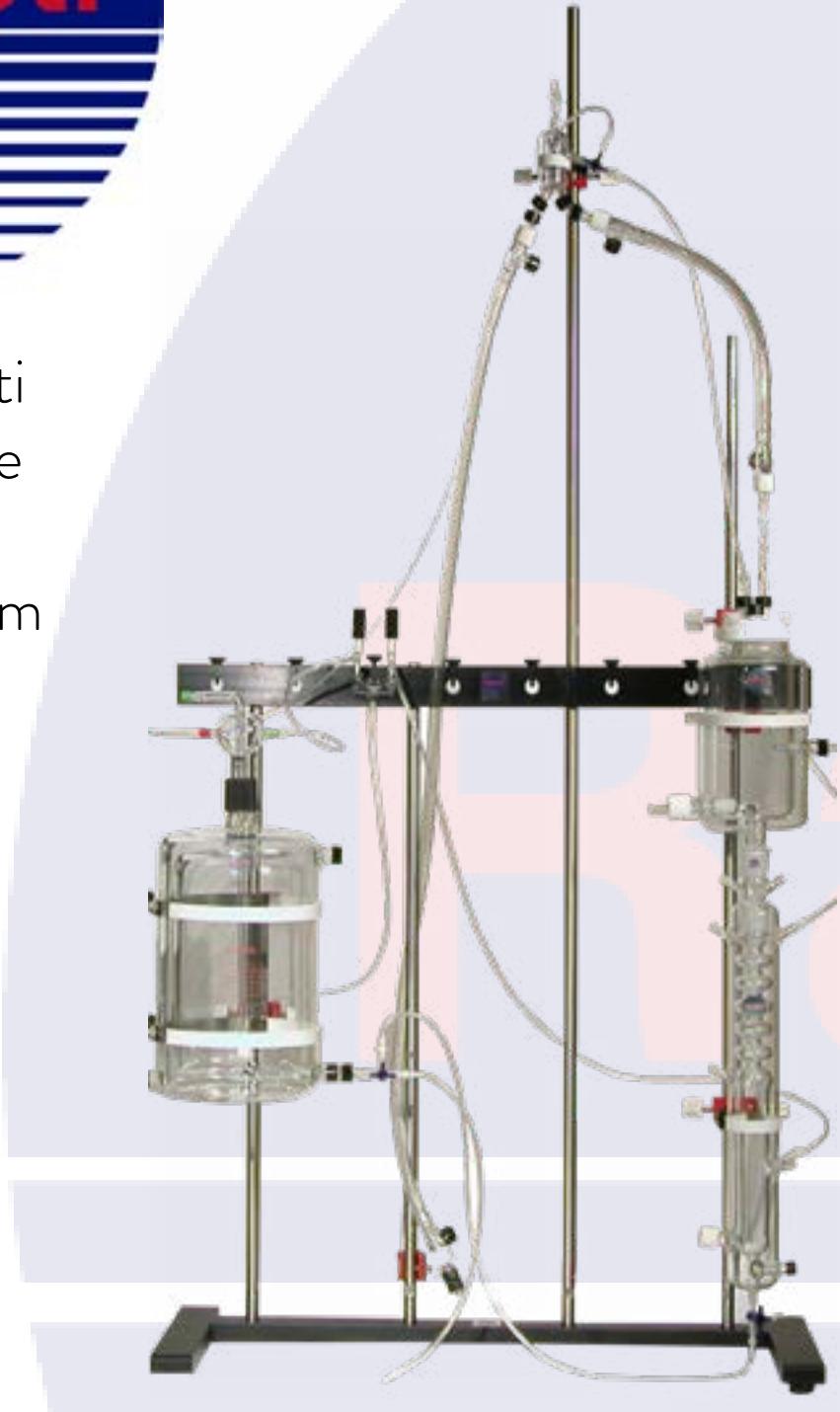




120102EZ Radnoti  
Constant Pressure  
Recirculating  
Langendorff System





I want to personally thank you for purchasing this quality Radnoti product. For four decades we have been designing and manufacturing the world's most advanced tissue/organ isolation workstations and accessories.

We believe that our commitment to continuous improvement and quality assurance sets us apart from other companies in this field. We hope you'll agree. So if you experience any problems with anything you purchase from us, or if you want to suggest an improvement in any of our products, then please feel free to contact me directly at desmond@radnoti.com

Thank you again for the confidence you have shown in us.

Yours faithfully,



Desmond Radnoti, CEO  
desmond@radnoti.com

# Radnoti



**Radnoti LLC**  
541 Edna Place  
Covina, CA 91723  
(800) 428-1416 (626) 357-8827  
Fax. (626) 303-2998

**Radnoti Ltd.**  
8 Terenure Place  
Terenure Dublin 6 West  
D6W Y006 Ireland  
+353 1 524 2111 Fax. +353 1 443 0784

# 120102EZ Radnoti Langendorff System

Purchase Order#

---

Sales Order#

---

Ship Date

Month      Day      Year

Inspected by:

Packaged by:

---

---

Electronics/Electromechanical

Item #	Serial #	Warranty Period

Glassware and Hardware Warranty: Inspect upon receipt and contact info@radnoti.com if there are any issues with shipping damage.

Electronics Electromechanical: Please reference above listed items and serial numbers.

# 120102EZ Radnoti Langendorff System

## Table of Contents

Plan Your Experiment	37
Keys to Success	38
Experiment Options	39
Assembling the Lab Stand	40
Ring Clamps	41
Connecting Luer Fittings	43
Connecting the Perfusion Tubing (FlexTube)	45
Radnoti Quick Disconnect Tubing (Q.D.)	46
Connecting the Water Jacket Tubing	47
Connecting the Radnoti Thermal Circulator	48
Connecting the Perfusion Tubing (Tygon)	49
Connecting the Perfusion Tubing (Silicone)	51
Connecting the Gas Tubing	52
Connecting Pump Heads to Peristaltic Pump	57
Peristaltic Pump Motor Quick Start	58
Radnoti Thermal Circulator Quick Start	60
Valve Flow and System Location	61
Priming the System	
Priming the System (recirculating mode)	
11 Preparation of the Donor	37
11 Langendorff Mode	38
12 Mounting of the Heart	39
13 Stabilizing the Heart	40
16 Left Ventricular Pressure (LVP)	41
18 The Frank Starling Law of the Heart	43
19 Aortic Flow In/Out	45
20 Apical Force	46
21 ECG	47
22 Pacing	48
23 Ion Selective Electrodes and Accessories	49
24 Operating Modes of the Radnoti System	51
25 Trouble Shooting	52
26 System Maintenance	57
28 Cleaning the System	58
29 Typical Buffer/Perfusion Solution	60
31 Disclaimer	61
32	
35	

This document's contents apply to the following Radnoti products:

120102EZ Radnoti Langendorff System

120102EZ-220 Radnoti Langendorff System (220/230V option)

120102EZ-NP Radnoti Langendorff (No peristaltic pump option)

# 120102EZ Radnoti Langendorff System

## List of Components

Description	Qty	Part #	
Base only, for 4-bar stand	1	<a href="#">159950-B4</a>	
Stabilizer Bar only, for 4-Bar stand	1	<a href="#">159950-C4</a>	
Rod 24" Long Stainless Steel	5	<a href="#">159950-24</a>	
Rod 12" Long Stainless Steel	2	<a href="#">159950-12</a>	
Double Ring Clamp for Reservoir	1	<a href="#">120141-2</a>	
Ring Clamp (Bubble traps)	1	<a href="#">120149-RC</a>	
Ring Clamp (Oxygenating Chamber)	1	<a href="#">159953-10</a>	
Ring Clamp (Hi-Tech Heart Chamber)	1	<a href="#">159953-MEM</a>	
Universal Stand Clamps	6	<a href="#">159952</a>	

# 120102EZ Radnoti Langendorff System

## List of Components

Description	Qty	Part #	
Water-Jacketed Reservoir 2 Liter	1	<a href="#">120142-2</a>	
Oxygenating Bubbler with Inlet port (For 2 Liter Reservoir)	1	<a href="#">140143-2</a>	
High-Tech Heart Chamber (Including Cannulas #140153-OST and 140154-OST)	1	<a href="#">140150</a>	
Bubble Trap	1	<a href="#">130149</a>	

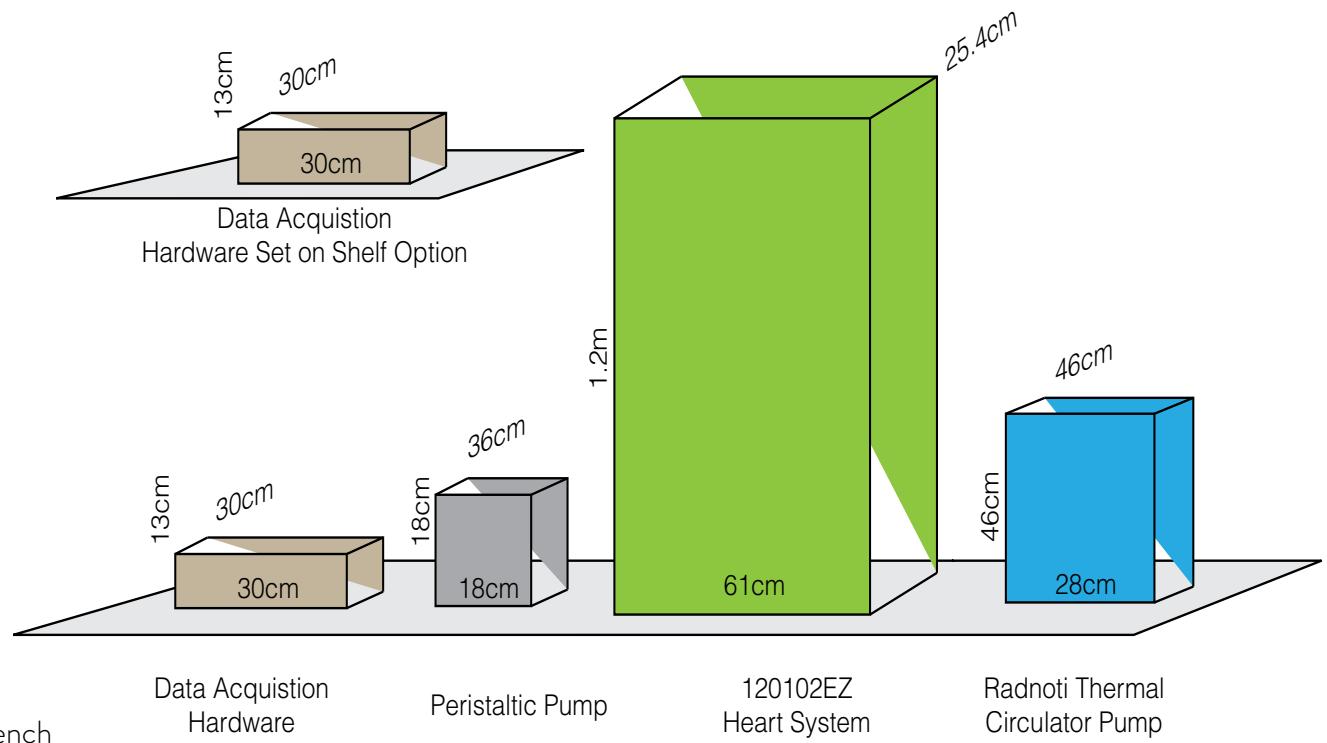
# 120102EZ Radnoti Langendorff System

## List of Components

Description	Qty	Part #	
Oxygenating Chamber	1	<a href="#">120144</a>	
Adapter, Heart, 24mm Female-Male	1	<a href="#">120147</a>	
In-Line Inject Port	2	120151EA	
Q.D. "Y" Adapters	2	<a href="#">120162</a>	
TNV Manifold, 2 Port	1	<a href="#">120168</a>	
Reservoir Filling Funnel	1	<a href="#">120140A</a>	

# 120102EZ Radnoti Langendorff System

## System layout space considerations and requirements



The lab bench should be sturdy and well supported across its length and depth. The recommended area dedicated to your system is 1.8m x 60cm. When choosing your system location, if equipped with shelves or cabinets above the bench, be sure they do not obstruct the vertical height of the apparatus. Remember that it must be free of obstruction for up to 1.2m in height for the first 60cm of depth measured from the front of the bench top.

### Power Requirements

You will need power supplies to support the following electronic components:  
Computer/Monitor/Data Acquisition System 110/115V 220/230V

Peristaltic Pump 110 /115V 220 / 230V

Radnoti Thermal Circulator 110 / 115V 220 / 230V

**DO NOT USE POWER STRIP (use a separate circuit to computer)**

# 120102EZ Radnoti Langendorff System

## System layout space considerations and requirements

---

The Radnoti Thermal Circulator #170051G in most cases can be placed on the same bench as the rest of the system. If vibration is an issue to the intended experimental protocols, then the unit can be located on a cart separate from the lab bench. It is important to maintain relative elevation of the circulator with regards to the system so as to maximize the pump's efficiency.

The Radnoti Peristaltic Pump should be placed in close proximity to the system, keeping dead volume low and allowing the user to easily adjust the flow rate.

The data acquisition system is connected to the computer via USB 2.0. If shelves exist on the lab bench, consideration should be given to placing the unit up on the shelf as an added safety measure as this apparatus is considered a wet lab set up. Alternatively, a cart may be used. Placing the system on a shelf or cart also serves to reduce the potential for artifact noise in the signal when recording. If separation is not possible in this fashion, use of the digital filters within the software will resolve this issue.\*

When choosing a location for the data acquisition system or analog-to-digital converter, be mindful of instrumentation connection cables and cable length limitations.

Ambient temperature should be in the range of 50° to 104° F (10° to 40° C) with a maximum relative humidity not to exceed 80%.

\*Digital filters or digital filter adjustment capability are software/hardware-dependant and will depend on data acquisition system being used.

### **Recommended Consumables**

A good quantity of paper towels

5 – 10 liters of distilled water - **Double DI Water is not recommended**

Manometer (not mandatory - value may be calculated using 13.64 mm of water to 1 mmHg)

Scissors and/or razor knife

Amount of time recommended to allot to assembly:

Depending on level of instrumentation allow 2-4 days for initial set up.

# 120102EZ Radnoti Langendorff System

## Keys to Success

---

Other than mishandling the heart during excision, making an error in the formulation of a perfusion solution, adding a toxic agent to a perfusion solution, or the occurrence of arrhythmias, the most common cause of contractile failure in both working (ejecting) and Langendorff hearts is contamination.

Contamination can take two forms: particulate or bacterial. The source of bacterial contamination may be the perfusion apparatus itself, where inadequate washing at the end of an experiment allows bacteria to thrive. Alternatively, the perfusion solution may be the cause with particulate impurities in reagents or bacterial contamination that occur during preparation or storage. In both instances the problem can be alleviated by:

- Filtration (sterile filter 0.45 µm) in the course of preparation and inclusion of 1 µm filters in recirculating perfusion circuits to remove precipitants and denatured proteins.
- Making up fresh (not storing) glucose- or substrate-containing buffers, which are good bacterial growth media. If buffers must be stored, glucose and calcium should be added just before use to reduce bacterial growth (from glucose) or precipitants (from calcium phosphate crystals).
- Thoroughly washing the perfusion apparatus with dilute, low phosphate detergent, followed by distilled water after every day of use. Pay special attention to cleaning the aerators, stopcocks, injection ports and devices inserted into the heart, such as cannulae and balloon catheters.

If contamination of the perfusion apparatus does occur, it may be possible to remove it by washing with acid (0.1 M HCl) or detergent, but usually it is better to dismantle, wash, and sterilize all components. However, a well designed and properly washed apparatus with well-prepared perfusion solutions can be used for years without contamination occurring.

## Plan Your Experiment

---

Prior to the use of the system it is important to plan your experiment.

- Select a buffer solution.
- Determine signals to be obtained.
- Instrument your system for signal-generating equipment.
- Calibrate signal generating equipment.
- Determine signal strength based on normal physiological values.

# 120102EZ Radnoti Langendorff System

## Experiment Options

There are a great number of physiological parameters that can be measured in the Isolated Perfused Heart preparation.

A pressure measurement while in constant flow mode will show the resistance of the heart, indicating vasodilation or vasoconstriction.

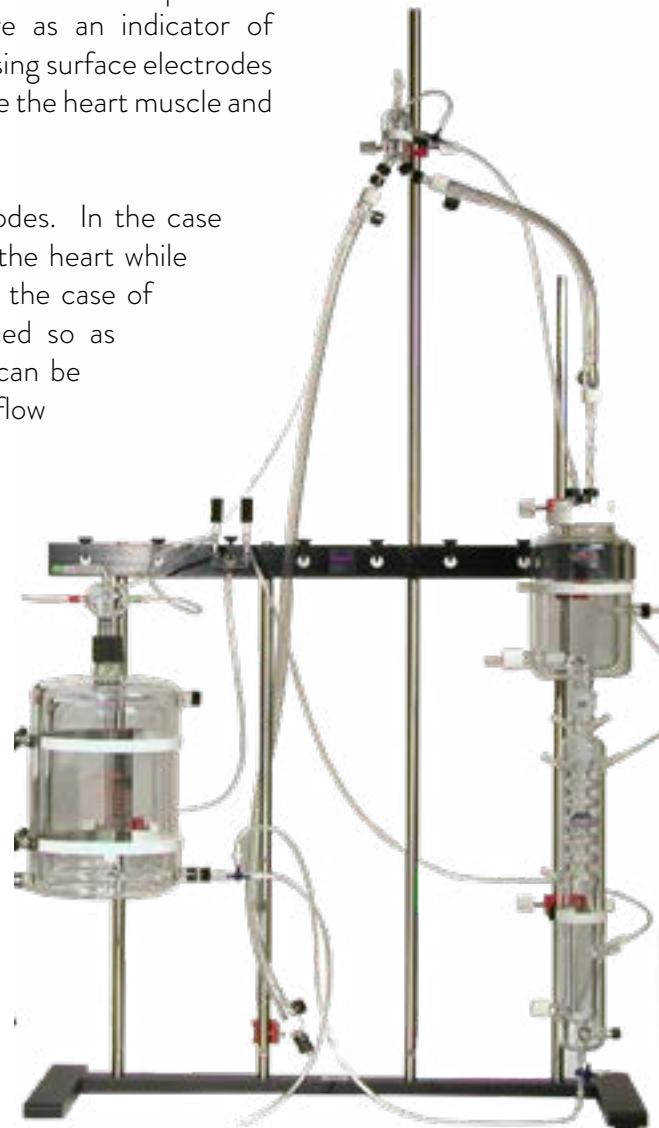
A left ventricular pressure (LVP) measurement made using a combination of pressure transducer, flexible balloon catheter and latex balloon can serve as an indicator of contractile force. Electrocardiograms (ECGs) are readily obtained using surface electrodes of monopolar or bipolar construction, or needle electrodes that pierce the heart muscle and are of interest in studies involving arrhythmias.

Oxygen consumption can be determined with dual oxygen electrodes. In the case of Working Heart, one is placed in the perfusate stream entering the heart while the second is placed in the perfusate stream exiting the heart. In the case of Langendorff, or retrograde perfusion, the second would be placed so as to monitor the effluent leaving the coronary sinus. This effluent can be removed via peristaltic pump and the sensor placed in line of the outflow stream.

Similarly, ion selective electrodes can be placed in the effluent or perfusate stream or oxygenation chamber of the Radnoti Isolated Perfused Heart apparatus, thus permitting measurement of pH and other cations and anions.

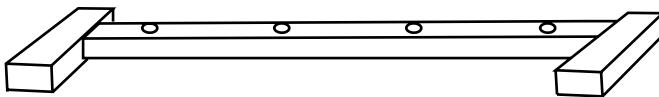
Radiolabeled compounds can be used for metabolic studies, as well as the release or uptake of various ions or substrates.

Optical studies measuring intracellular constituents have been performed on the fluorescence of endogenous or exogenous fluorescent compounds.

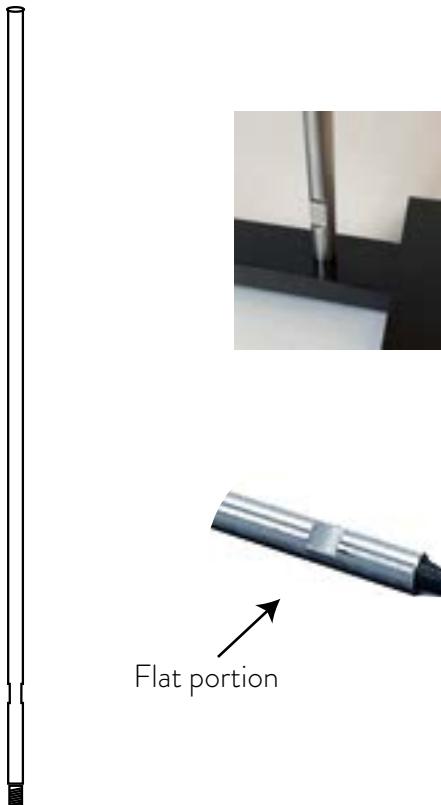


# 120102EZ Radnoti Langendorff System

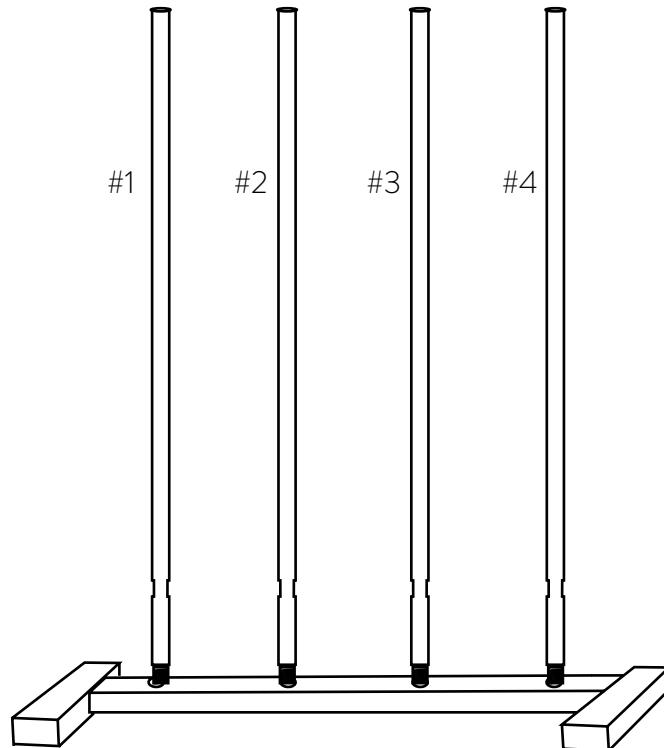
## Assembling the Lab Stand



Place 4-bar lab stand on lab bench and make sure all four of the rubber feet are making contact with your bench surface.

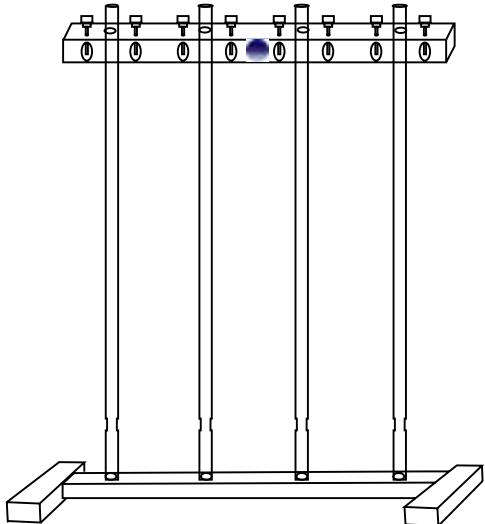


Secure four 24" stainless steel rods to the lab stand. You will notice that the threaded end of the rod has a flat portion which enables you to use a 3/8" wrench to make sure they are securely fastened. For further instruction we will refer to the 24" stainless steel rods from left to right as rods 1 through 4.



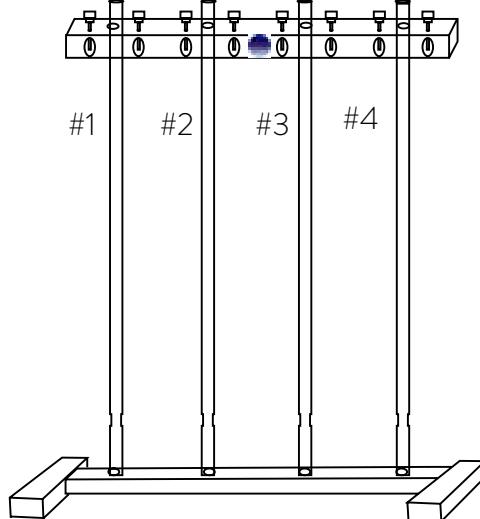
# 120102EZ Radnoti Langendorff System

## Assembling Lab Stand



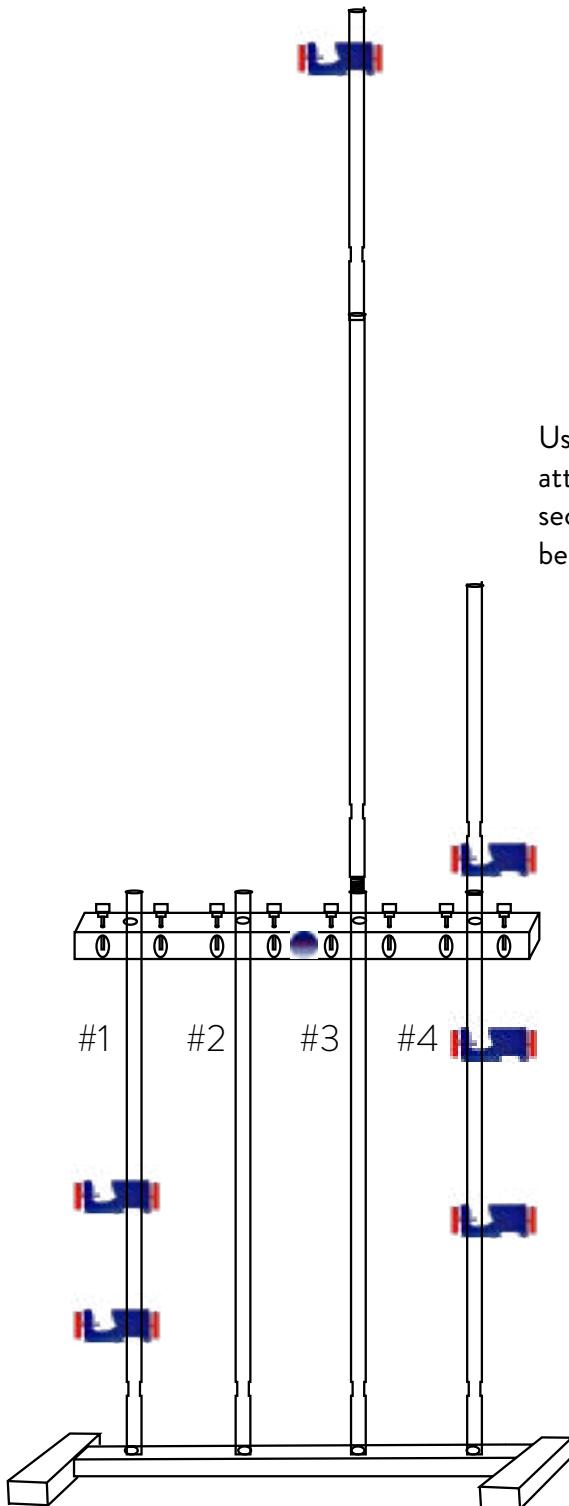
With 4 of the stainless steel rods securely attached to the 4 bar lab stand base, you may now slide on the stabilizer bar. With the Radnoti logo facing you will notice 4 holes at the top of the stabilizer bar that contain thumb screws. Make sure that all 4 thumb screws are unscrewed to the point where the screw does not intrude on the 1/2" hole into which the rod will slide. Gently slide the stabilizer bar over the 4 rods and secure with the 4 thumb screws approximately 1" from the top of the rod height. Be sure to check all 4 thumb screws are finger tight.

The system includes 2 12" stainless steel rods and 1 more 24" stainless steel rod. Screw the remaining 24" stainless steel rod on top of rod #3. Now attach a 12" stainless steel rod on top of the 24"stainless steel rod you just put on top of rod #3. The last 12" stainless steel rod will be screwed on top of rod#4.



# 120102EZ Radnoti Langendorff System

## Assembling Lab Stand



Using the 6 universal stand clamps that are provided with this system, attach as shown. Using the thumb screws, hand tighten so they are securely attached. The location is an estimate and does not need to be exact as you may find adjustment necessary.



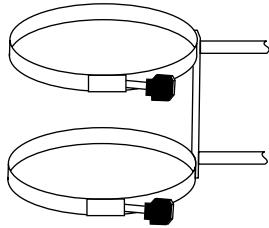
# 120102EZ Radnoti Langendorff System

## Ring Clamps

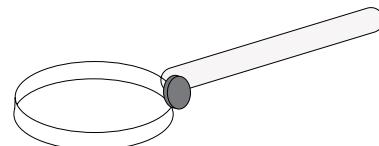
There are two types of ring clamps used with Radnoti systems. The single ring clamp is used with most of the glass components. The double ring clamp is used for components larger than 1 liter in volume. This system contains one double ring clamp which is used with the two liter reservoir.

The ring clamps use a worm gear mechanism that has a thumb screw attached. To open the ring clamp, simply unscrew the knurled knob. Insert the glass component into the clamp at roughly the center of the body. Insert the end of the clamp into the worm gear and finger tighten only.

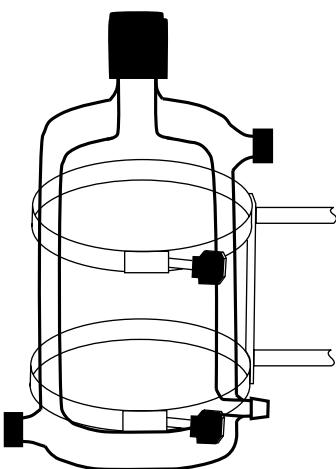
Do not over tighten as this could lead to breakage. Once the ring clamp has been attached to the glass component, you can then slide the rod of the clamp into the universal stand clamp and securely fasten to the lab stand rod.



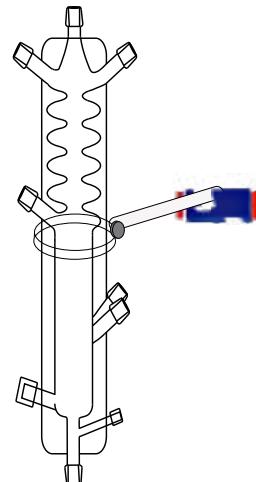
Double Ring Clamp



Single Ring Clamp



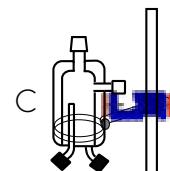
Double Ring Clamp  
attached to glass component



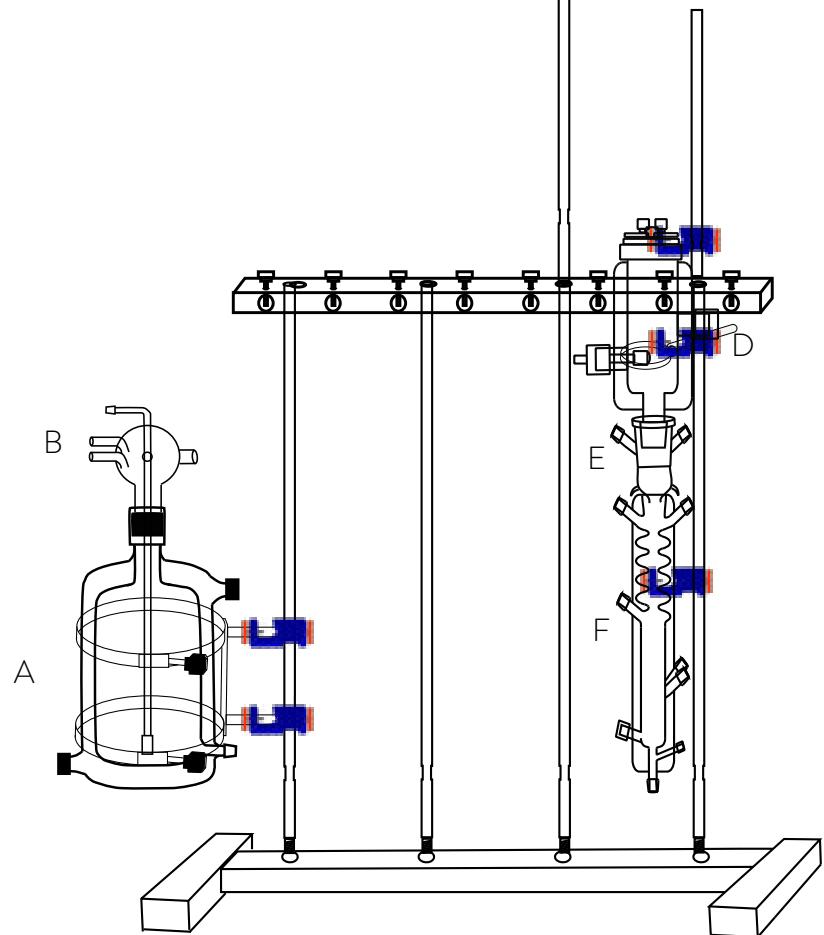
Single Ring Clamp  
attached to glass component

# 120102EZ Radnoti Langendorff System

## Placement of Glassware



	Glass Component	Corresponding Ring Clamp	Qty
A	Water Jacketed 2 liter Reservoir	#120141-2 Double Ring Clamp	1
B	Oxygenating Bubbler	N/A	1
C	Compliance Bubble Trap	#120149RC	1
D	Hi-Tech Heart Chamber	#159953-MEM	1
E	24mm Heart Adapter	N/A	1
F	Sheet Flow Oxygenator	#159953-10	1



# 120102EZ Radnoti Langendorff System

## Connecting Luer Fittings

Radnoti Working Heart systems come with the Radnoti tubing adapter kit. This provides the user with a “make any” connection as it relates to your Radnoti perfusion system. The kit comes in a sturdy red plastic container so you can keep things organized.

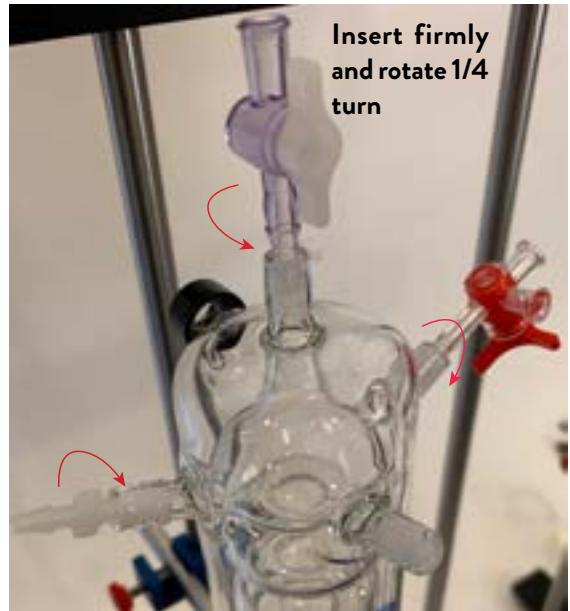
Your kit will come with a full set of preassembled tubing with fittings applicable to most applications. This kit contains the parts necessary to modify the system for your applications, should you need to.



### Types of Luer connections:



When assembling your system, use locking connections for tubing and valves with LuerLok fittings.



Use slip Luer connections for connections to glassware and any other Luer taper fittings.

**Insert firmly and rotate 1/4 turn to ensure a tight connection.**

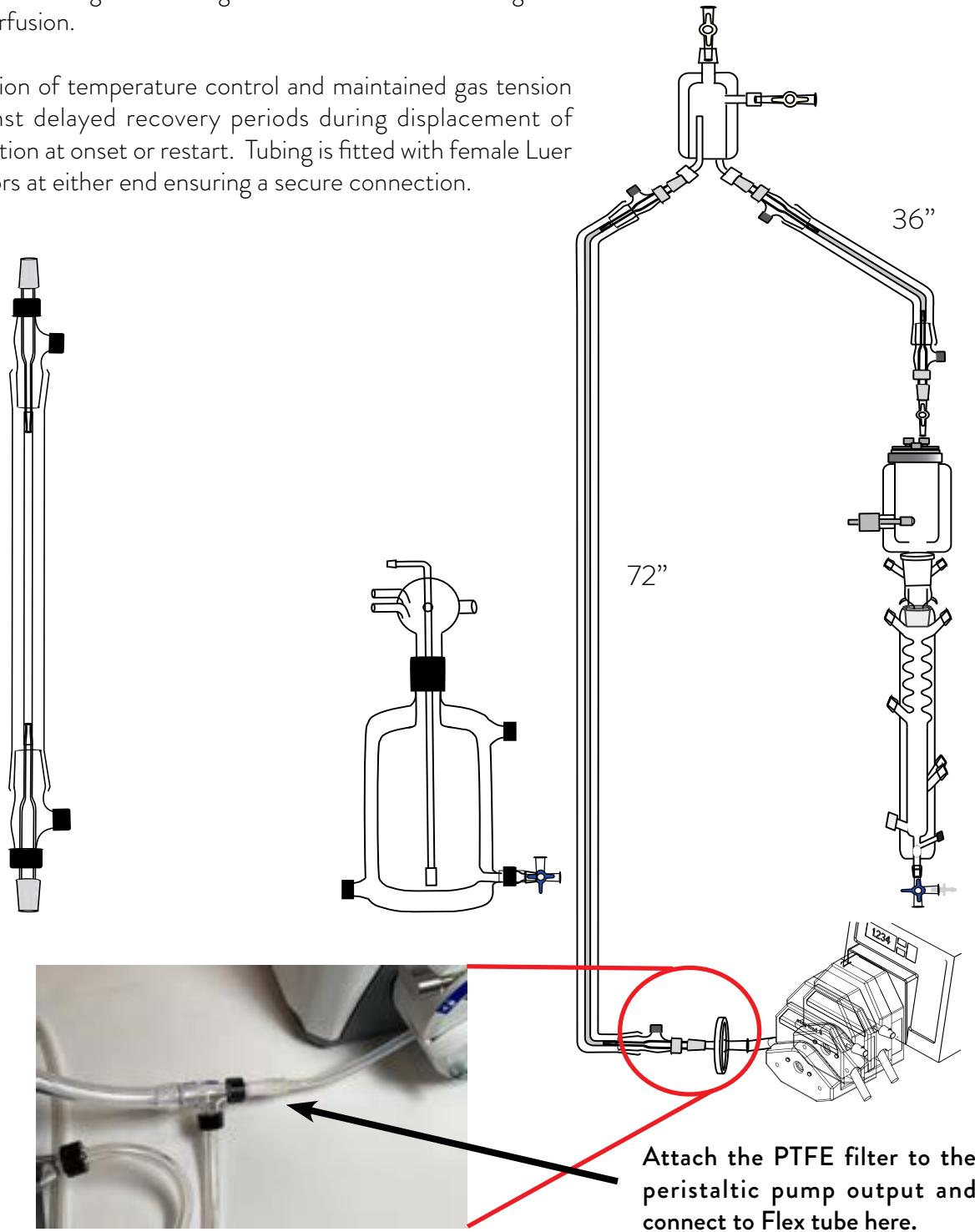
# 120102EZ Radnoti Langendorff System

## Connecting the Perfusate Tubing (FlexTube)

Radnoti Flex Tube Water Jacketed tubing is used for all critical flow lines of the system. The water jacket maintains temperature.

The inner Teflon tubing maintains gas tensions in low-flow or global ischemia reperfusion.

The combination of temperature control and maintained gas tension ensures against delayed recovery periods during displacement of perfusion solution at onset or restart. Tubing is fitted with female Luer lock connectors at either end ensuring a secure connection.



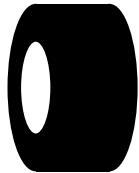
# 120102EZ Radnoti Langendorff System

## Radnoti Quick Disconnect Tubing (Q.D.)

The Radnoti Q.D. tubing system consists of three parts:

- The #120159 Tygon (Water Jacket tubing)
- The #160196 Q.D. Threaded Cap
- The #120160 Q.D. Insert Sleeve

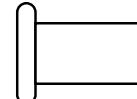
Q.D Threaded Cap



Tygon (Water Jacketed Tubing)



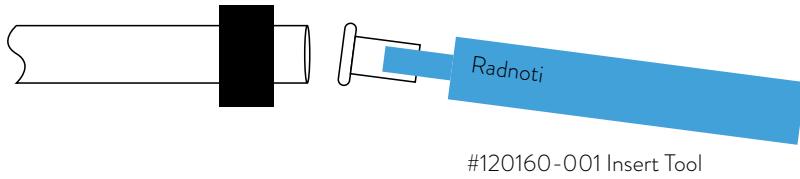
Q.D. Insert Sleeve



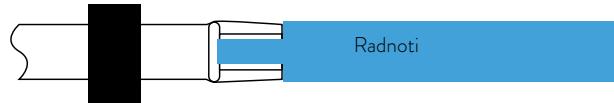
All of the water jacket tubing comes pre-assembled with the system. Should you need to make modifications or replace tubing we have included 10 ft (3 m) of spare #120159 Tygon tubing.

To make the Q.D. fitting you will need the Radnoti Insert Tool #120160-001, Q.D. Threaded Cap and Q.D. Insert Sleeve. These can be found in your supplied #120140-B Radnoti Tubing Adapter Kit.

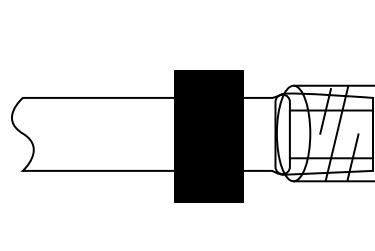
First slide the Q.D. Threaded Cap, hole side first, onto the tubing. Then, as shown on the diagram, place the Q.D. Insert Sleeve onto the tool. Approach the tubing at an angle with the tool and firmly push into the tubing so as to be flush with the tubing as set by the tool.



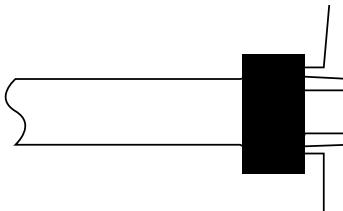
#120160-001 Insert Tool



Remove the tool and moisten the tip of your newly created water jacket tubing with some water and insert into the threaded port of your glass component.



Slide the Q.D. Threaded Cap onto the glass threads and tighten. Do not over tighten as this can lead to breaking the thread.



# 120102EZ Radnoti Langendorff System

## Connecting the Water Jacket Tubing

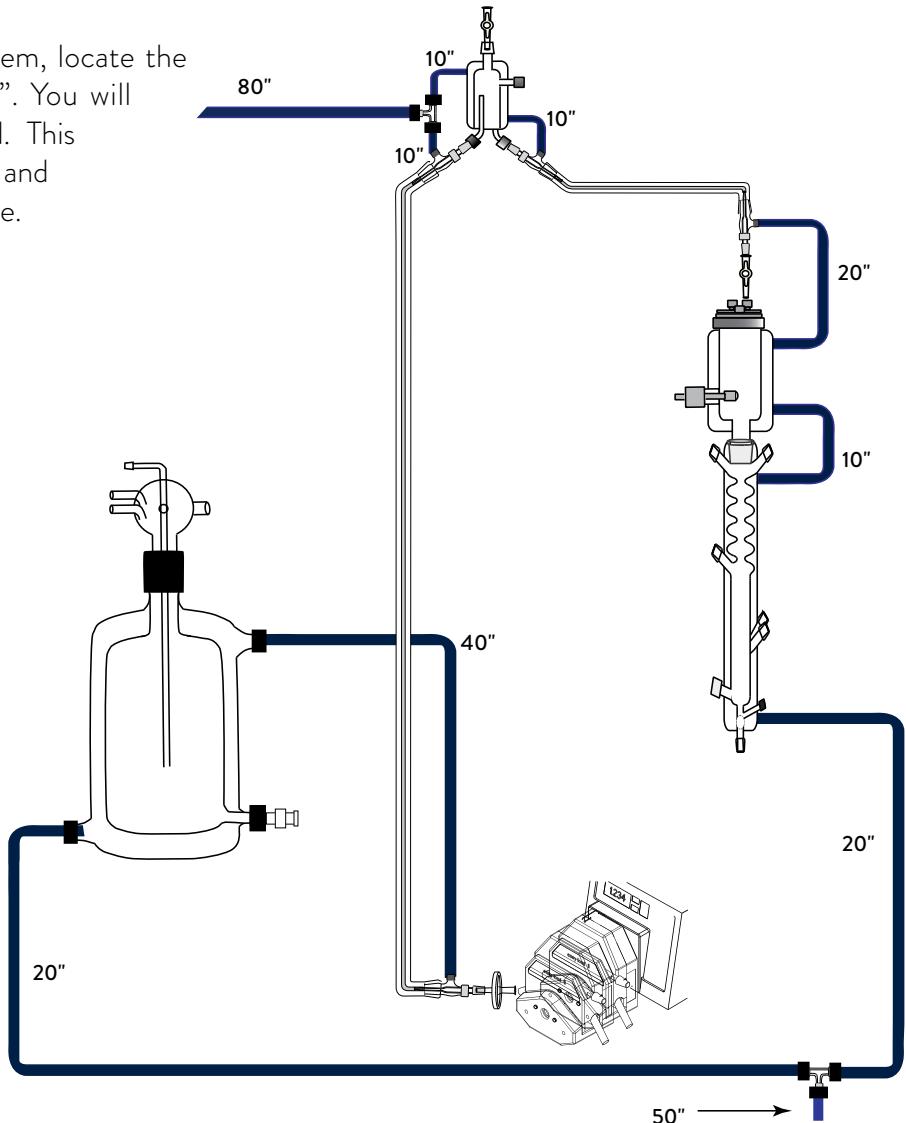
The water jacket tubing is used to circulate water from the Radnoti Thermal Circulator throughout the system to establish physiological temperature. As you will see in the diagram, the inflow of water flows from the bottom of the glass component to the top. The reason for this is to displace the air out of the glass component, reducing thermal loss.

In the tubing kit supplied with your system, locate the package labeled "Water Jacket Tubing". You will find that each of the tubes are numbered. This number indicates the length of the tube and corresponds with the diagram on this page. All water jacket connections use the Radnoti Q.D. type fitting.

For best results when using the Radnoti Q.D. fittings, moisten the end of the tubing with water and push into the glass threaded port on your component. Then screw down the black cap.

Do not overtighten as this will result in damage to the Q.D. cap or glass thread.

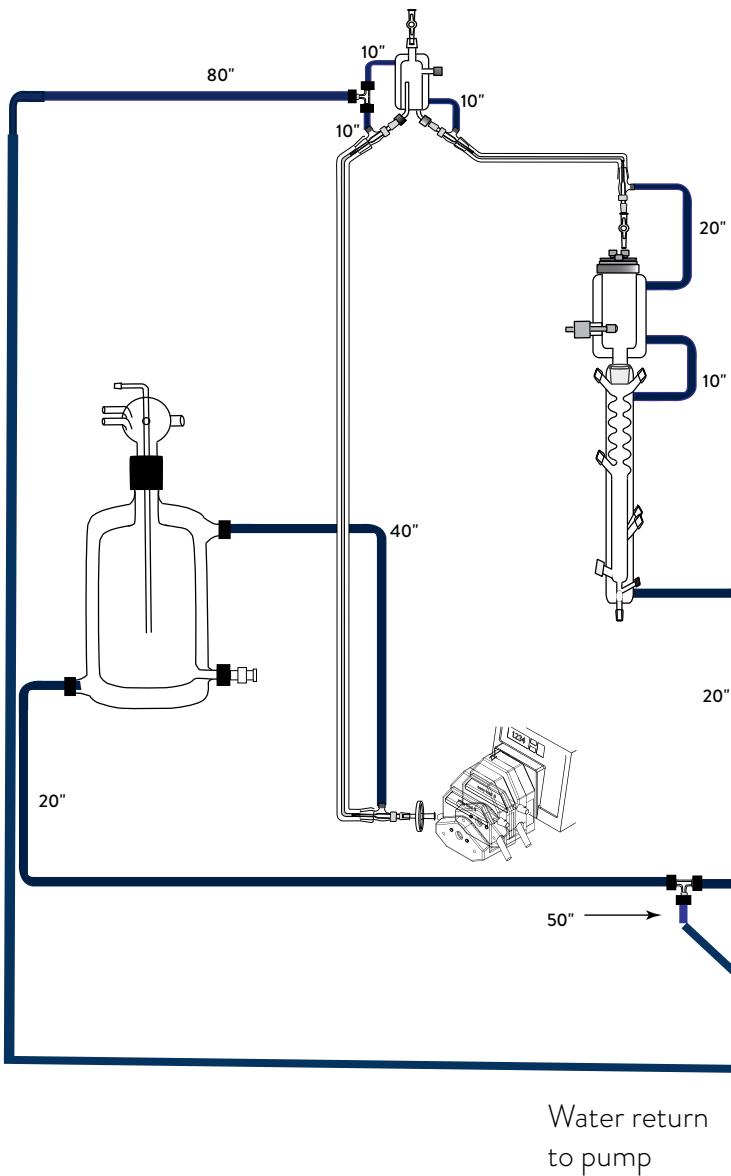
Once you have connected the tubing to match the figure on the right, we would strongly recommend you connect the water circulating pump and test the water jacket connections before adding additional components.



# 120102EZ Radnoti Langendorff System

## Connecting the Radnoti Thermal Circulator

The pump output and return water jacket lines connect to the Radnoti Thermal Circulating water bath via the attached Radnoti Q. D. tubing adapters.



Remove the black screw caps from the adapters on the pump head and set aside. The tubing lines are assembled.

Connect the lines as indicated.

The Radnoti Thermal Circulator pumps 16 L/min.

**Be sure to test all waterjacket connections with the pump on before beginning your experiment.**

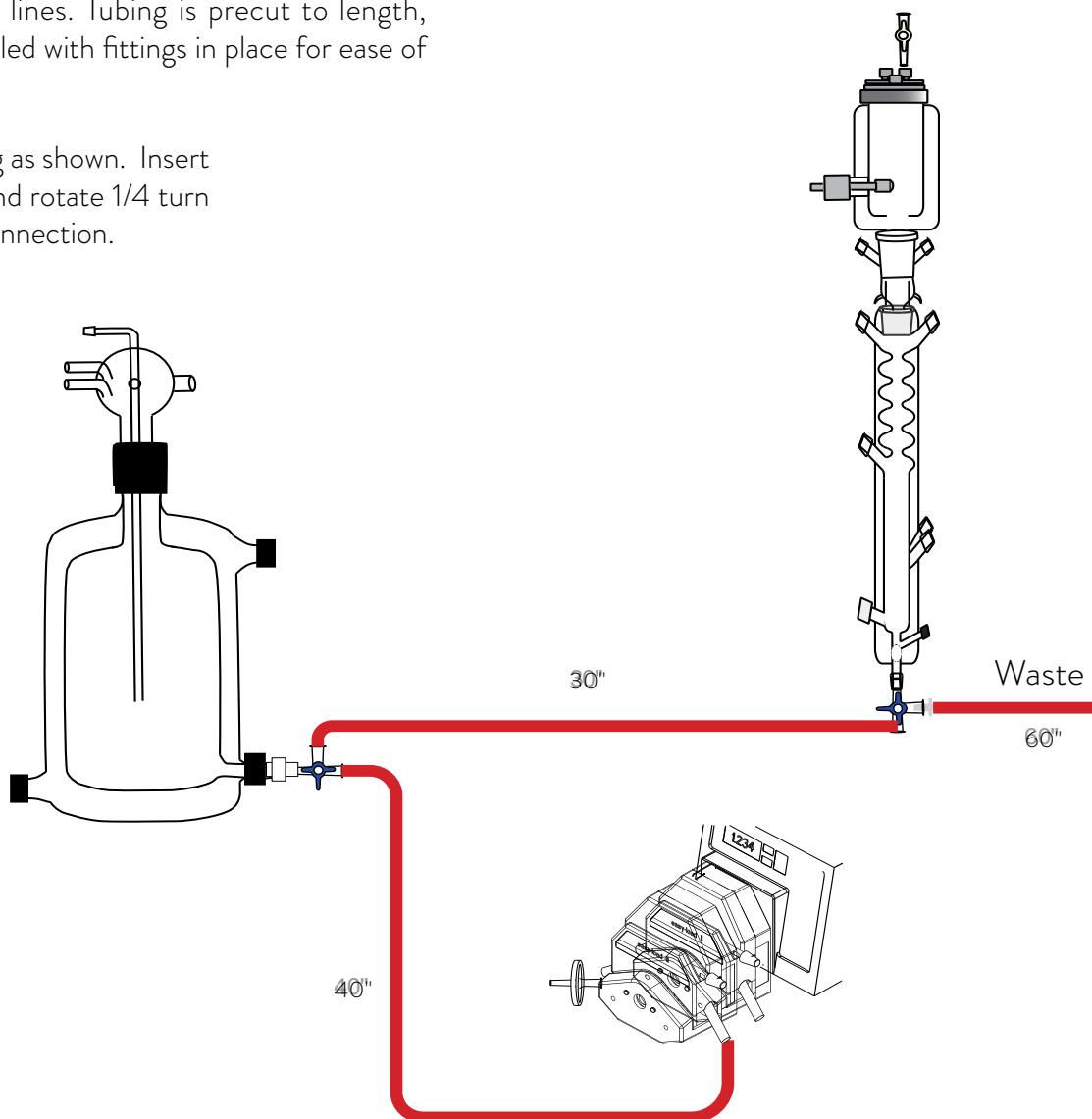
# 120102EZ Radnoti Langendorff System

## Connecting the Perfusate Tubing (Tygon)

Radnoti uses Tygon® ND tubing formulation. This tubing is specifically designed to meet the performance and regulatory requirements of critical medical applications. The tubing meets USP Class VI criteria and withstands Ethylene Oxide (EtO), gas, and gamma sterilization.

The non-wetting surface permits more complete drainage of tubing lines. Tubing is precut to length, labeled and assembled with fittings in place for ease of assembly.

Connect the tubing as shown. Insert Luer taper firmly and rotate 1/4 turn to ensure a tight connection.



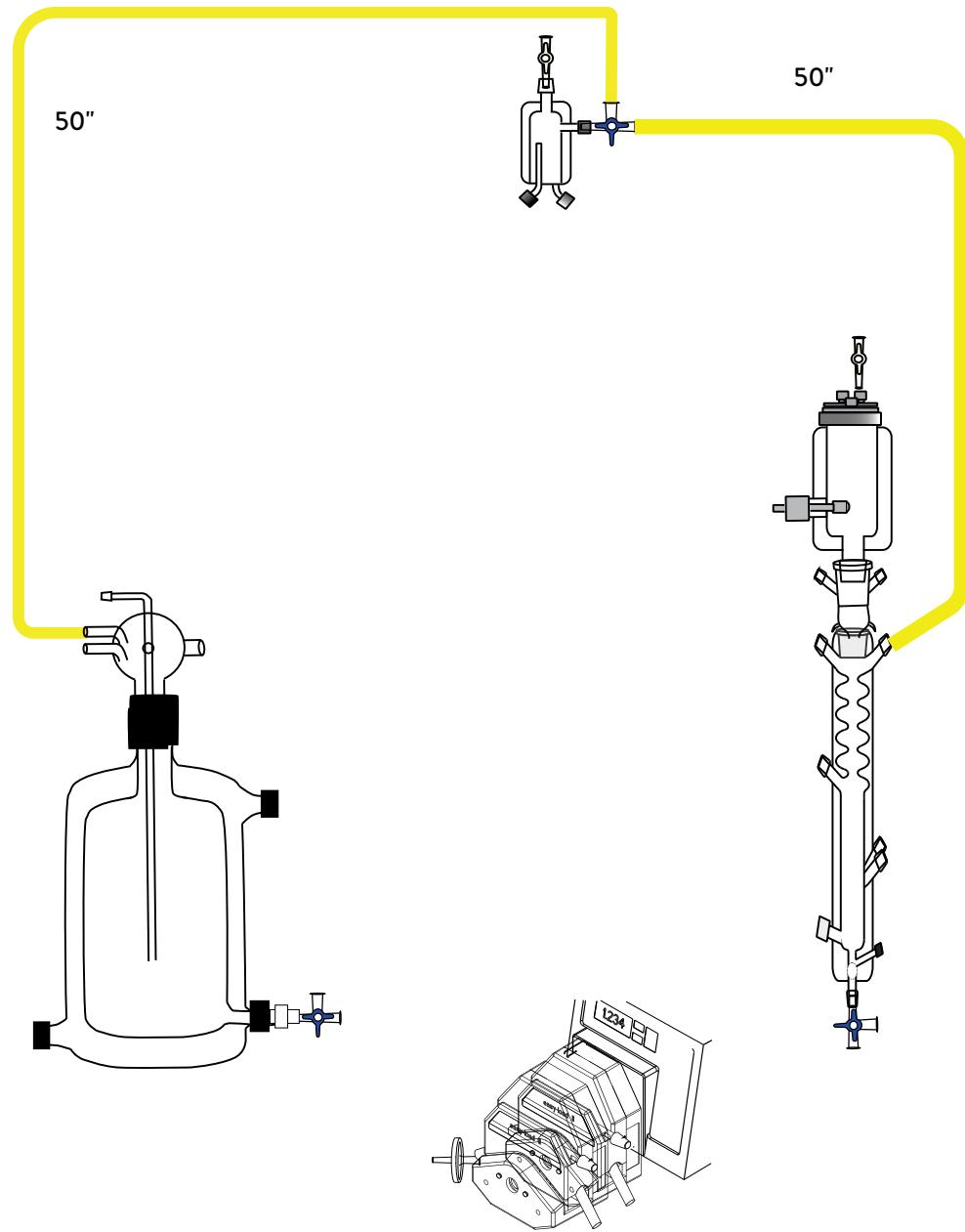
# 120102EZ Radnoti Langendorff System

## Connecting the Perfusate Tubing (Silicone)

The silicone tubing in the 120101BEZ system is primarily used for overflow from a glass component or when the fluid needs to be returned. The walls of the silicone tubing have much less surface friction and permit even flow.



Connect the tubing as shown below. Slide the silicone tubing over the female Luer Lok thread to attach.



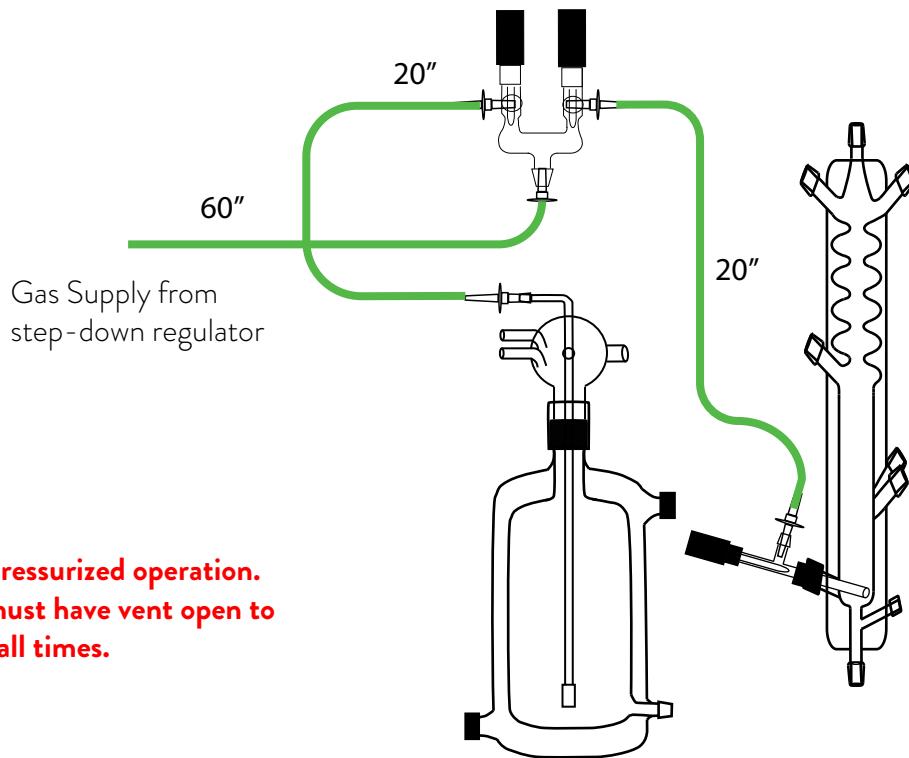
# 120102EZ Radnoti Langendorff System

## Connecting the Gas (95% O<sub>2</sub> - 5% CO<sub>2</sub>) Tubing

The gas mixture is delivered via a step-down regulator and gas cylinder (user supplied). You will want a step-down regulator that can deliver 5 to 10 psi maximum with flow control. Gas flows to the system are nominal.

From the step-down regulator, the 120168 2-way Teflon needle valve Y manifold allows adjustment and balancing of the gas flow between the 140143-2 Oxy Bubbler and the 120144 Sheet Flow Oxygenator. Ground glass female Luer fittings for inflow and outflow allow easy connection of tubing lines. The glass rod fused to the manifold body allows for easy mounting on the lab stand wherever it is most convenient.

Connect the tubing as shown. Adjust the gas flow to each component based upon the needs of your experiment.



**Glassware is not rated for pressurized operation.  
All chambers being gassed must have vent open to atmosphere at all times.**

# 120102EZ Radnoti Langendorff System

## Connecting Pump Heads to Peristaltic Pump



This L/S EASY-LOAD II Pump Head, when combined with a MASTERFLEX® L/S® drive or compatible system, is designed to provide a simple, easy-to-use peristaltic pump system. The pump head accepts several different tubing sizes for a wide range of flow rates. The unique over-center cam design and automatic tubing retention allow quick tubing changes and greatly reduced maintenance time.

Each pump is supplied complete with a 15" (38 cm) length of tubing and one mounting hardware package.



The L/S EASY-LOAD II Pump Head is compatible with most MASTERFLEX® L/S® drives with a standard tang interface.



Align the tang and mount the pump head using the screws provided.



Pump Heads can be mounted in tandem (up to four), depending on the torque capabilities of the drive. Special mounting hardware is required.

# 120102EZ Radnoti Langendorff System

## Connecting Pump Heads to Peristaltic Pump



### TUBE LOADING

1. Be sure the pump drive is turned off.
2. Rotate the lever to the left to open the pump.
3. Load the correct size tubing. Center the tubing between the retainers.
4. Rotate the lever to the right to close.

### **WARNING:**

Stop the drive when changing the tubing or its position in the rotor mechanism (the rotor is partially exposed when the **LOADING LEVER** is in the open position).

NOTE: For optimum tubing life, keep tubing straight where it enters and exits the pump.

Flow rates for Radnoti pump head model 170112.

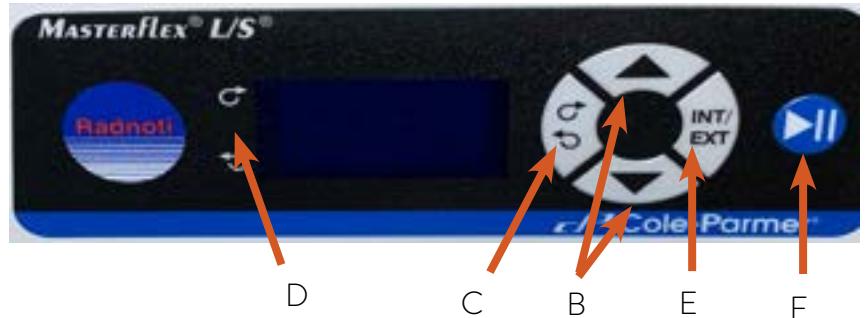
L/S Tubing	mL per rev	mL/min @ 600RPM
L/S 13	0.06	36
L/S 14	0.21	130
L/S 16	0.8	480
L/S 25	1.7	1000
L/S 17	2.8	1700
L/S 18	3.8	2300

Radnoti systems are supplied with L/S 16 unless other is specified by the customer.

# 120102EZ Radnoti Langendorff System

## Peristaltic Pump Motor Quick Start

- A. Power (ON/OFF) switch: Turns the unit ON or OFF.
- B. SPEED KEYS: Sets the speed of the pump (RPM). The higher the number the faster the speed of the pump.
- C. FLOW DIRECTION KEY: Sets the direction of the rotation of the pump drive Clockwise/Counterclockwise.
- D. An LED annunciator shows the active direction. The motor is brought to a controlled stop before reversing direction.
- E. INTERNAL/EXTERNAL KEY: This key changes the mode of operation for the drive. Internal (Local) operation through the front panel keypad is designated by "INT" while external (Remote) operation is designated by "EXT". Pressing and releasing this key will toggle between the two operating states.
- F. START/PAUSE KEY: When depressed, this key toggles the motor on and off.



# 120102EZ Radnoti Langendorff System

## Radnoti Thermal Circulator Quick Start

For the long-term reliability of water baths it is important to use oxygenated water that is free from ions and minerals that can cause corrosion of stainless steel. We recommend the use of distilled water and deionized water from modern ion exchange systems that do not use salt backflushing to regenerate the ion-exchange cartridges.

Stainless steel is protected from corrosion by a layer of chromium oxide. If the layer is damaged, oxygen present in water can reform the oxide layer. If the water is still or de-oxygenated, and the oxide layer is damaged, ions can corrode the stainless steel tank. If a water bath has been unused for some time, or water boiled, we recommend changing to fresh distilled water or correct deionized water.

Water normally contains calcium or magnesium ions. Deionized water has most ions removed as indicated by its conductivity level; the more pure the water the lower the conductivity.

It is important to use only deionized water from an ion exchange system with replaceable cartridges.



# 120102EZ Radnoti Langendorff System

## Radnoti Thermal Circulator Quick Start

The temperature of the bath liquid can be set using the **S** button:

1. With the display showing the bath temperature, press the **S** (section) button. The display will flash indicating that it can be set to the desired temperature.
2. Use the main dial to set the desired temperature.
3. If no key is pressed for 10 seconds then the display will revert back to showing the bath temperature and the set temperature will remain at its original value.
4. Press the **S** button to store the requested value and the display will revert to showing the bath temperature. If the temperature selected is higher than the current liquid temperature, the heater light will come on.

For the full list of features, refer to the manual that is supplied with the pump.

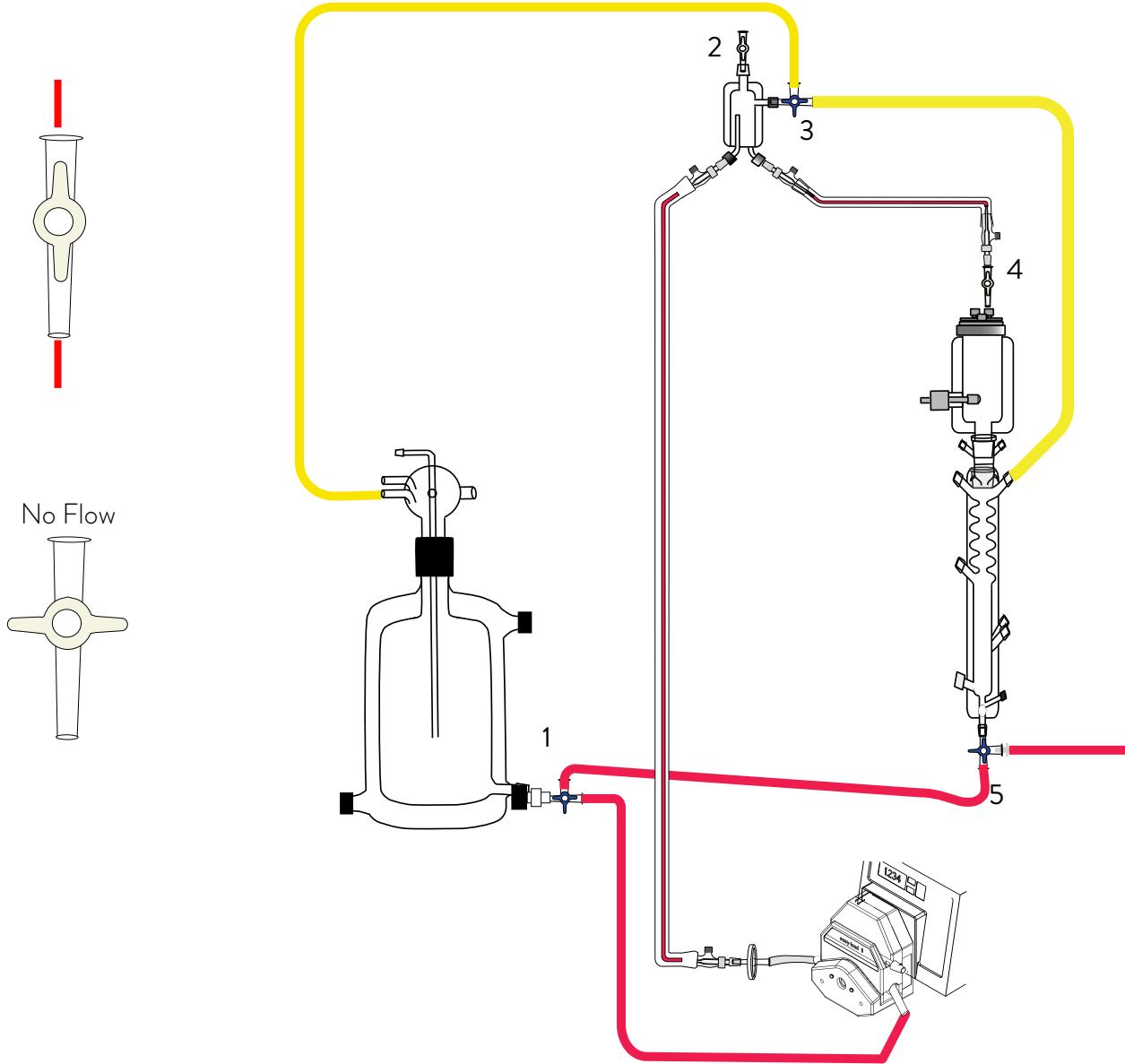
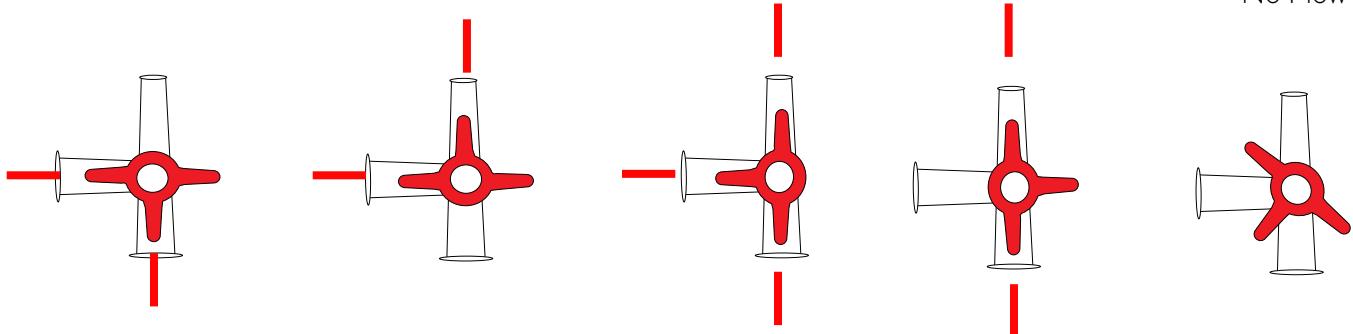


# 120102EZ Radnoti Langendorff System

## Valve Flow and System Location

Valve Flow Overview (details for Priming the System on following pages)

No Flow

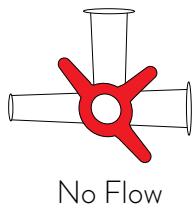


# 120102EZ Radnoti Langendorff System

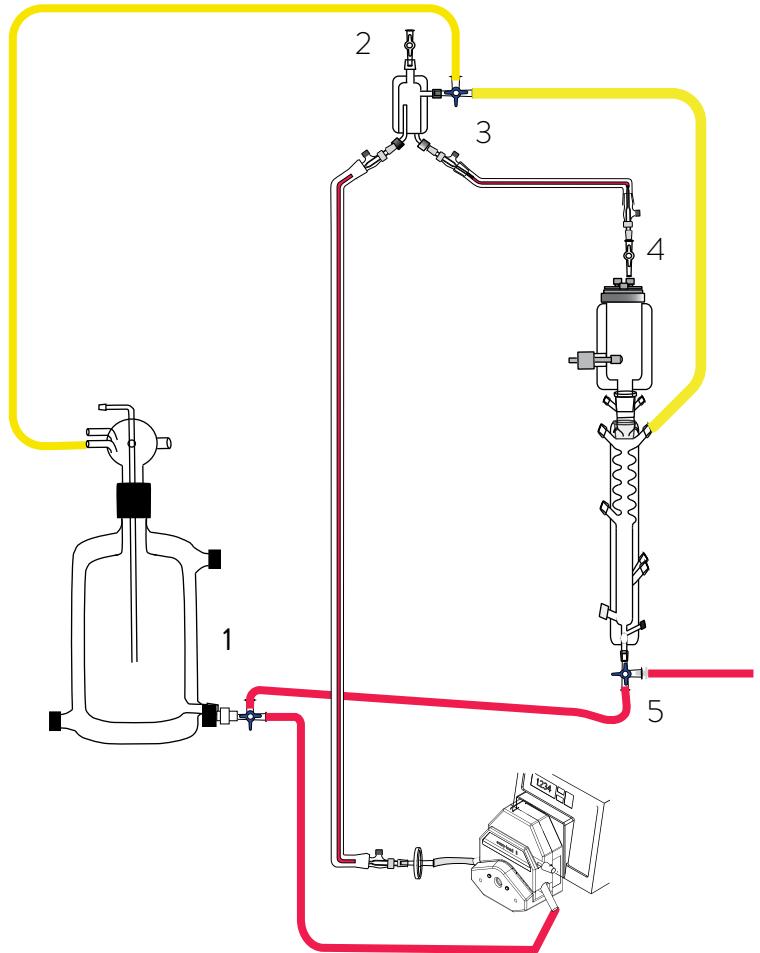
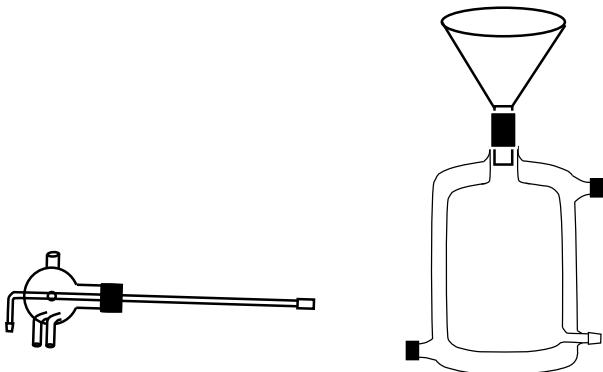
## Priming the System

Priming the system requires the water jacketed reservoir to be filled first.

Set Valve 1 to all closed.



- Remove the Oxy-bubbler and set gently on lab bench.
- Your system came with a fill funnel specifically designed for use with the Water Jacketed Reservoirs.
- Attach the fill funnel to the reservoir and fill; remove funnel and replace Oxybubbler.
- Begin gassing the reservoir with 95/5% O<sub>2</sub>/CO<sub>2</sub> to maintain gas tension and buffer pH level.



**NOTE: Glassware is not rated for pressurized operation. All chambers being gassed must have vent open to atmosphere at all times.**

# 120102EZ Radnoti Langendorff System

## Priming the System

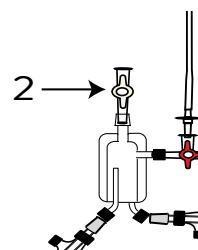
We recommend adding 10cc syringe barrels to the compliance bubble trap to aid in the priming process.



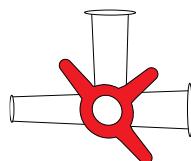
Set valve 2 to the open position.



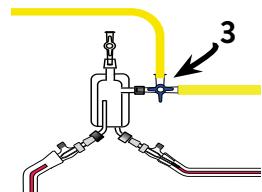
Open



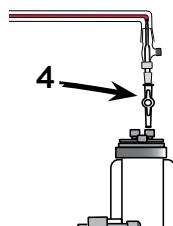
Set valve 3 to closed.



No Flow



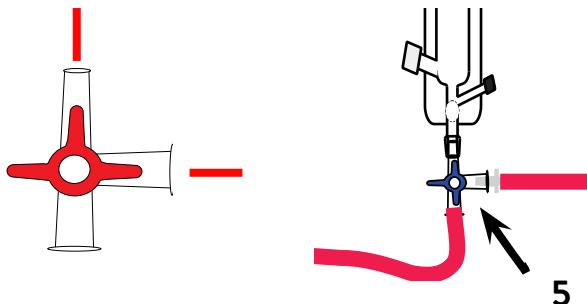
Set valve 4 to the open position.



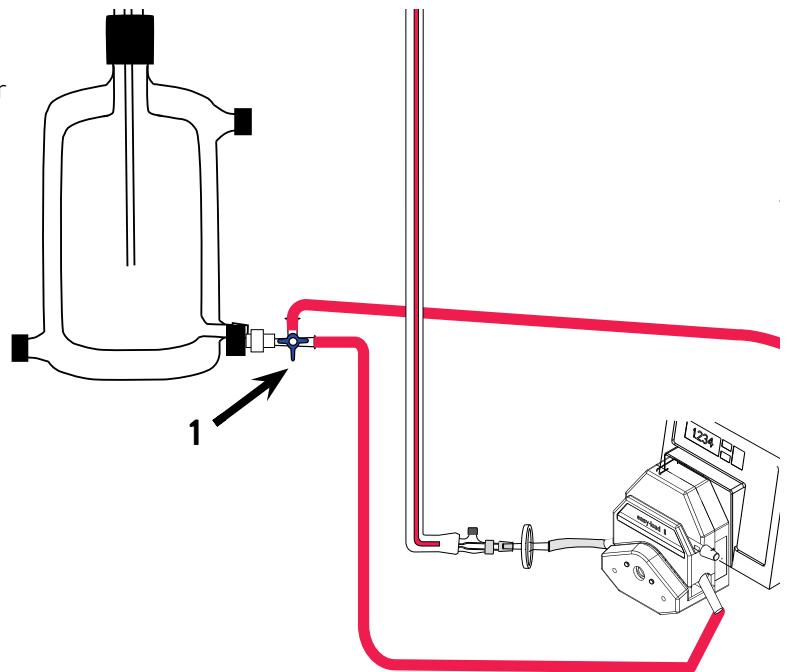
# 120102EZ Radnoti Langendorff System

## Priming the System

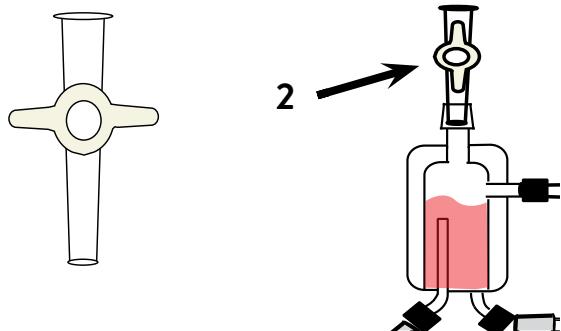
- Valve 5 should be set to direct flow to waste..



- Open Valve 1 to direct flow from the reservoir to the peristaltic pump.
- Turn on the peristaltic pump



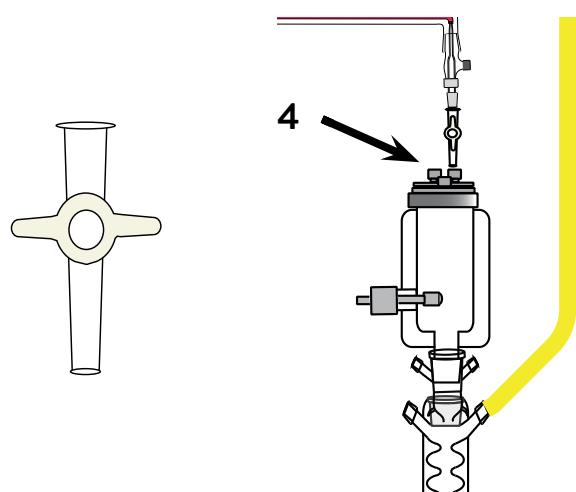
- When Bubble Trap is 2/3 full close valve 2.



# 120102EZ Radnoti Langendorff System

## Priming the System

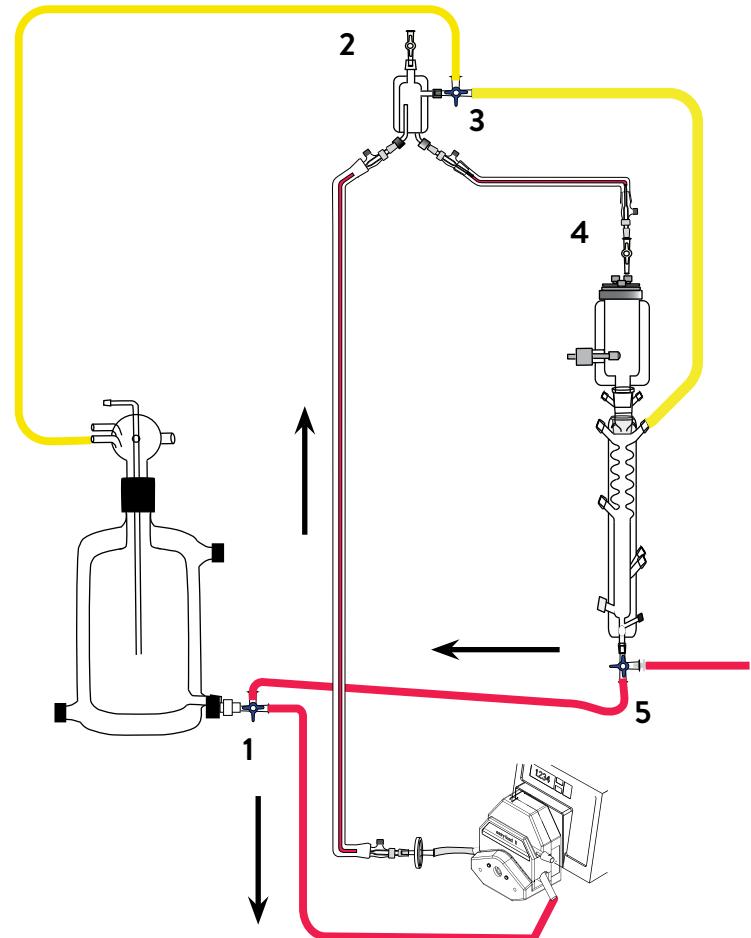
- Close Valve 4 and turn off peristaltic pump.
- **The system is now primed and ready for the heart..**



## Priming the System (recirculating mode)

After the heart is in place and you wish to switch over to recirculating mode:

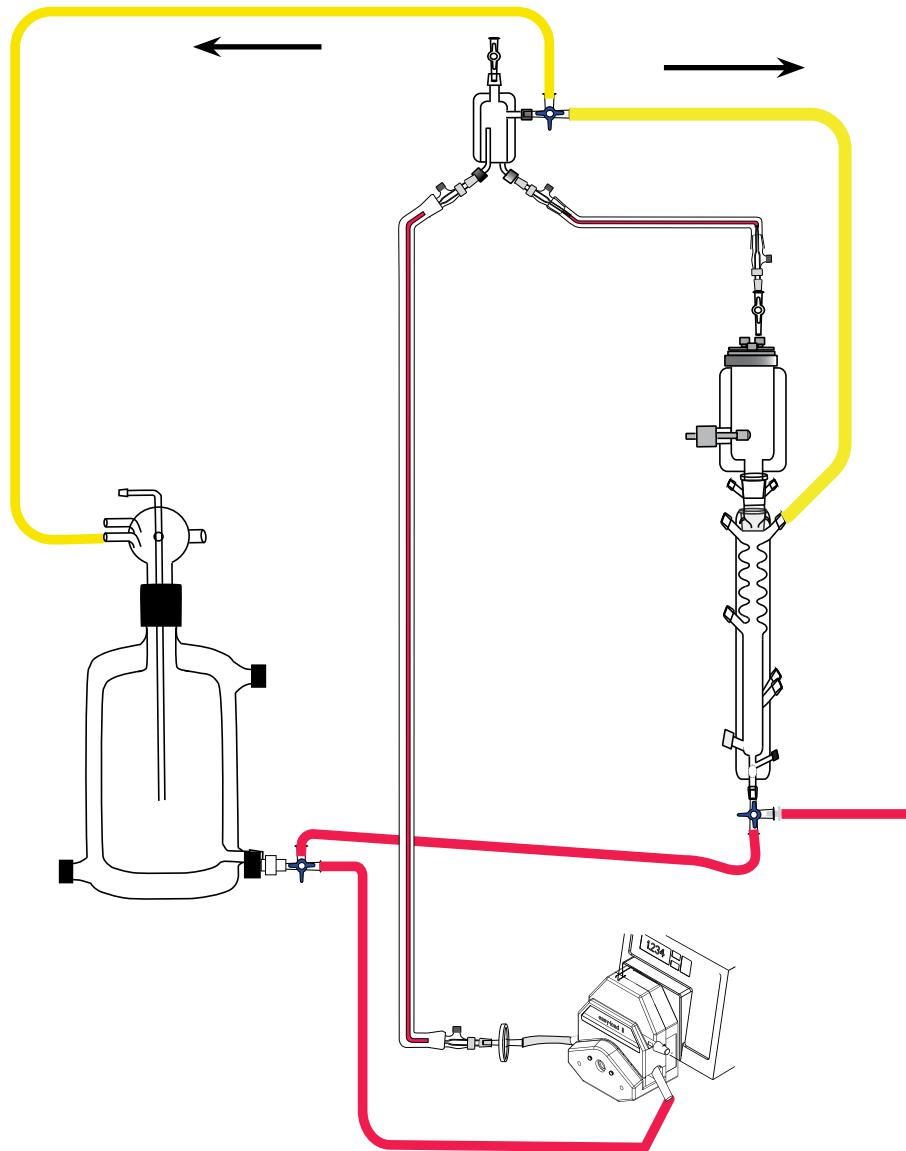
- Change valve 1 to block flow from the reservoir and accept flow from the oxygenating chamber.
- Change valve 5 to direct flow towards the three way valve at the perfusate reservoir outlet.



# 120102EZ Radnoti Langendorff System

## Priming the System (constant pressure mode)

- When running in constant pressure mode, the bubble trap compliance chamber maintains the pressure head.
- The pump speed is normally set to 2x the anticipated flow rate.
- Excess buffer is directed via the compliance port to either return to the buffer reservoir during initial priming, or return below the heart to the oxygenating chamber in recirculating mode



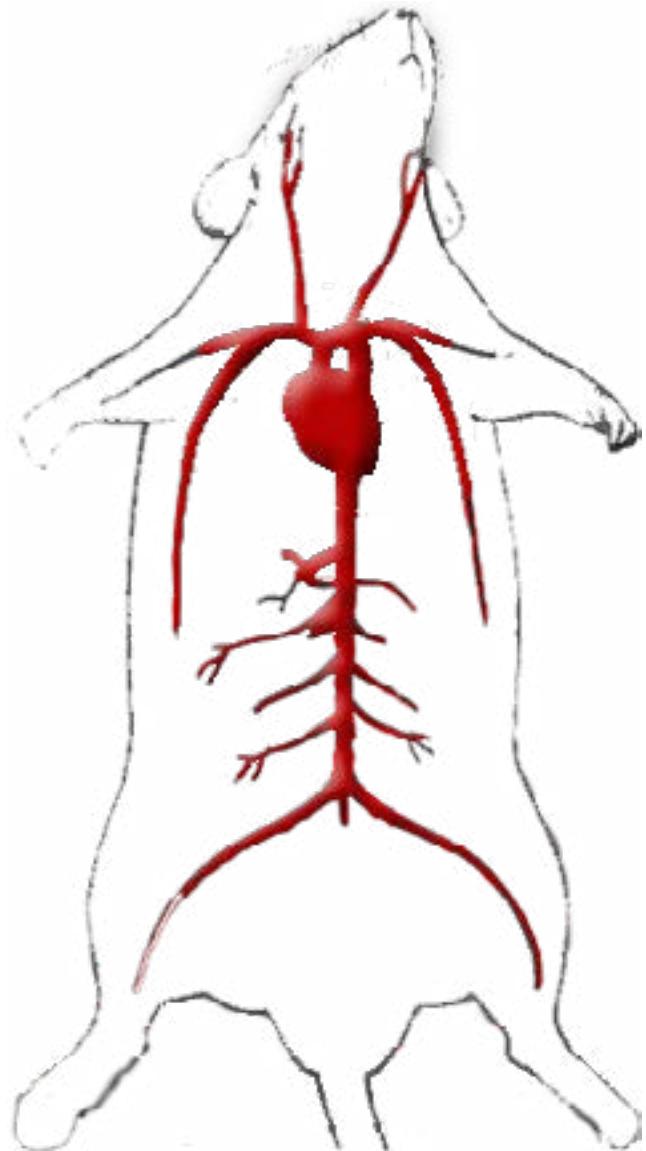
# 120102EZ Radnoti Langendorff System

## Preparation of the Donor

The anesthetic(s) used will depend on: the donor, potential problems with side effects in the experimental protocol, the extent of the surgical procedures and the regulations of your Animal Care and Use Committee. The most common are barbiturates, such as nembutal or thiopental, ketamine/xylazine, ethyl ether and common volatile surgical anesthetics. The latter two can present potential personnel hazards due to fire or intoxication.

Carbon dioxide and euthanasia solutions should not be used due to the danger of an anesthetic overdose causing severe or prolonged cardiac impairment or hypoxia. Unless there is an overriding experimental concern, the donor should be heparinized prior to surgery to reduce the formation of emboli in the vasculature.

Cardiac removal may be performed by a simple medical incision (median sternotomy) with the blunt end of a pair of blunt-sharp pointed scissors to open the thoracic cavity. This is followed by exposure of the heart, opening of the pericardium, support of the organ, and removal of the heart by cutting across the arch of the aorta and the vena cava. Care should be taken not to cut the aorta so short as to impair mounting on the cannula. The heart may be placed in a beaker of chilled, heparinized perfusate to arrest the beating of the heart.



# 120102EZ Radnoti Langendorff System

## Langendorff Mode

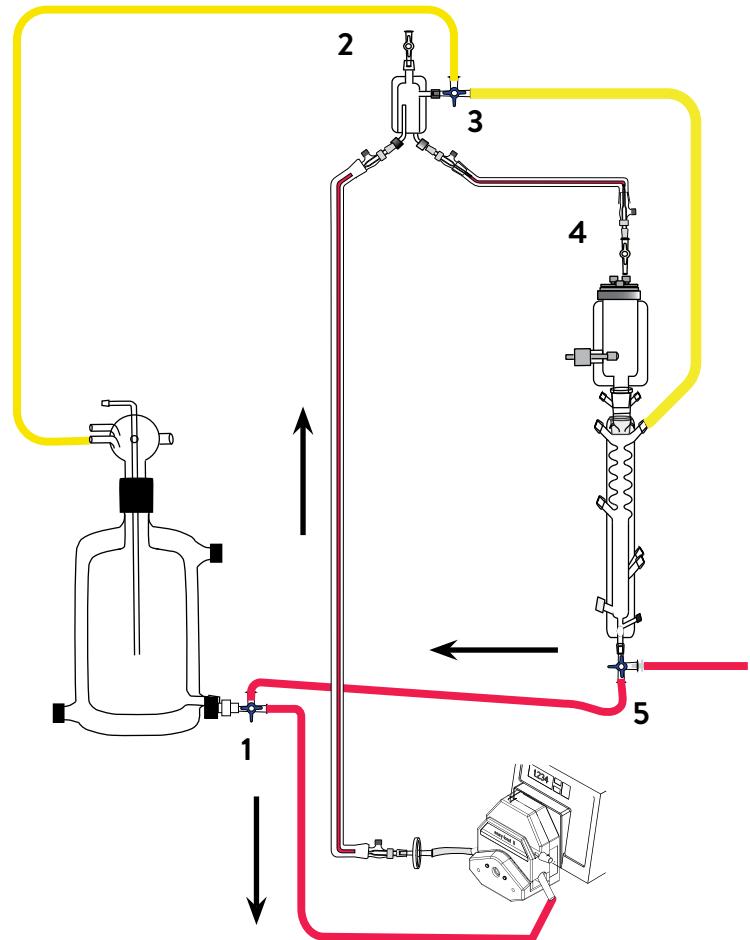
Before any working heart experiment can be initiated the heart must be stabilized in Langendorff mode.

The Langendorff heart preparation involves the cannulation of the aorta. The buffer solution is then delivered retrograde via the aorta either at a constant flow rate (delivered by the peristaltic pump) or a constant hydrostatic pressure (usually in the range of 60-100 mmHg).

In both instances the aortic valves are forced shut and the perfusion fluid is directed into the coronary ostia thus perfusing the entire ventricular mass of the heart and draining into the right atrium via the coronary sinus.

In order to prepare the system for the heart, you need to configure it to a constant pressure, non-recirculating mode.

Set the peristaltic pump rate to 1.5 - 2X anticipated flow rate. This will insure that buffer supply to the aortic bubble trap will not run empty, thus allowing air-free buffer delivery.



# 120102EZ Radnoti Langendorff System

## Mounting of the Heart

Using a pair of blunt tipped tweezers - some researchers prefer to use two pairs - slide the aorta up onto the aortic cannula. The aorta needs to be high enough to be secured but not so far as to occlude or block the ostia. Care must also be taken to avoid puncturing the aorta. The heart can be held on the cannula with a blood vessel clamp such as Dieffenbach serafine.

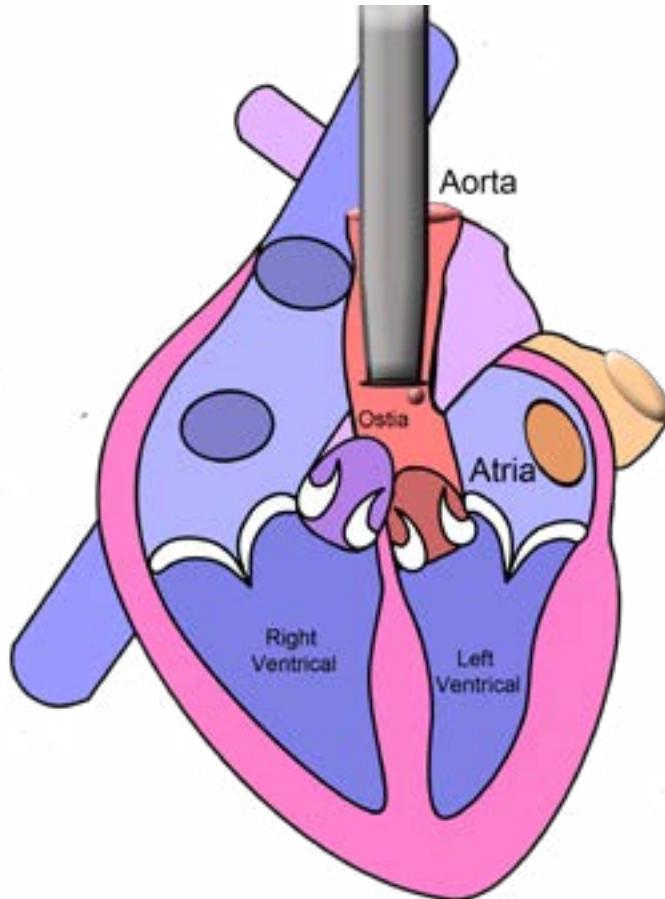
At this point, valve 7 should be opened to the aortic cannula.

The heart should be secured with silk sutures. In the case of the flanged cannula, the aorta is slid down the cannula so that the tie is against the flange.

The most critical part of the preparation is to minimize the time from the removal of usual perfusion in the donor to the reperfusion of the heart.

Since this normally highly metabolically active organ has only the oxygen and substrate contained in the vessels at the time of removal to sustain itself, this time should be kept to a minimum.

Perfuse in this mode until such time as the heart has been flushed and begins equilibration process.



# 120102EZ Radnoti Langendorff System

## Stabilizing the Heart

Once mounted on the cannula and reperfusion has begun, the heart should begin to beat strongly within seconds.

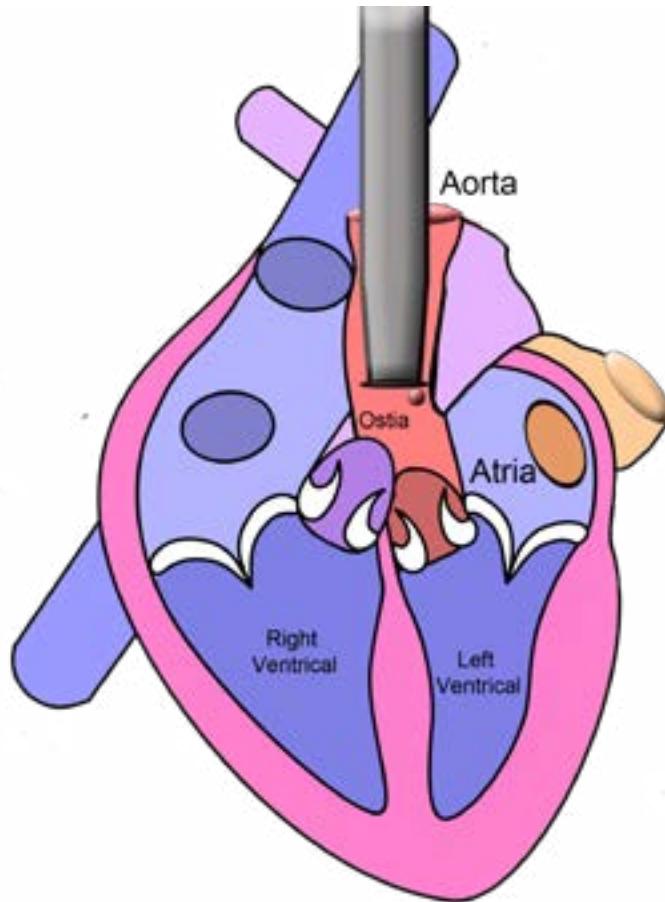
In constant pressure mode the heart is autoregulating flow, reducing the risk of under perfusing or over perfusing. If a constant flow mode is used, perfusion pressure should be carefully monitored to avoid under perfusion or over perfusion.

Perfusion rates are about 3-15 mL/g heart weight for constant flow systems for saline based buffer solutions. For both constant pressure and constant flow systems, the initial pressure should be about 50-60 mmHg for most mammalian hearts, dependent on the donor, heart rate (pacing), oxygen delivery and work output.

Physiologically normal perfusion pressures of 80-100 mmHg, as in blood-perfused hearts, are not used in saline-perfused hearts due to enhanced edema and potential valve damage.

The heart will stabilize rapidly and most experiments can begin within 10-15 minutes after the preparation has been mounted and the various monitoring systems attached.

The heart should be functional for several hours, although it is prudent to reduce the experimental time as much as possible. Preparations will suffer edema if uncompensated by a plasma expander concomitant with protein loss from the heart.



# 120102EZ Radnoti Langendorff System

## Left Ventricular Pressure (LVP)

Left ventricular pressure (LVP) is a valuable data set obtained from the isolated heart in Langendorff modes via a saline-filled balloon, balloon catheter and pressure transducer.

Selection of the appropriate balloon and balloon size is imperative to the successful setup for LVP measurements. Balloon material should be such that the balloon is compliant so as to avoid artefact due to balloon material stiffness. Any resistance is to be due to ventricle vs. balloon.

For rat and larger hearts Radnoti offers an array of balloon sizes molded in thin wall latex (see LVP Balloons). For mouse heart there are no commercially available balloons. Users typically fabricate a balloon from cellophane or a condom tip.

The selection of balloon size is also a major contributor to successful LVP measurements. Balloons should be slightly larger than the maximum expanded volume of the ventricle to avoid the effects of measuring the resistance of the balloon to stretch.



Species	Body weight in kg	Balloon Size	Volume (mL)	Diameter x Length
Rat	0.1 - 0.2	3	0.03	3 x 7
Guinea Pig	0.3 - 0.4	4	0.05	4 x 8
Pig	0.5	5	0.1	5 x 9
Rabbit (small)	0.7	6	0.2	6 x 10



The proper filling of the balloon and insertion into the left ventricle is required.

The balloon is usually secured to the flexible cannula with a small suture. The balloon must be filled and all air bubbles removed. This can be accomplished by assembling the parts while submerged in a container of the appropriate saline. We recommend the use of a Luer threaded syringe for this application.

Fill the syringe with the appropriate saline and attach to the catheter. When withdrawing the plunger on the syringe the balloon will collapse on itself. If necessary purge any drawn air from the syringe. A 3-way stopcock between the syringe and the catheter will aid in this procedure.

Gently push the fluid into the balloon catheter assembly so as to distend the balloon slightly. Inspect visually for trapped air bubbles. If present, draw back on the syringe to dislodge trapped air bubbles or pockets and purge the air from the syringe and fill balloon again.

Sometimes it is necessary to tap or flick the catheter to break free small air bubbles.

# 120102EZ Radnoti Langendorff System

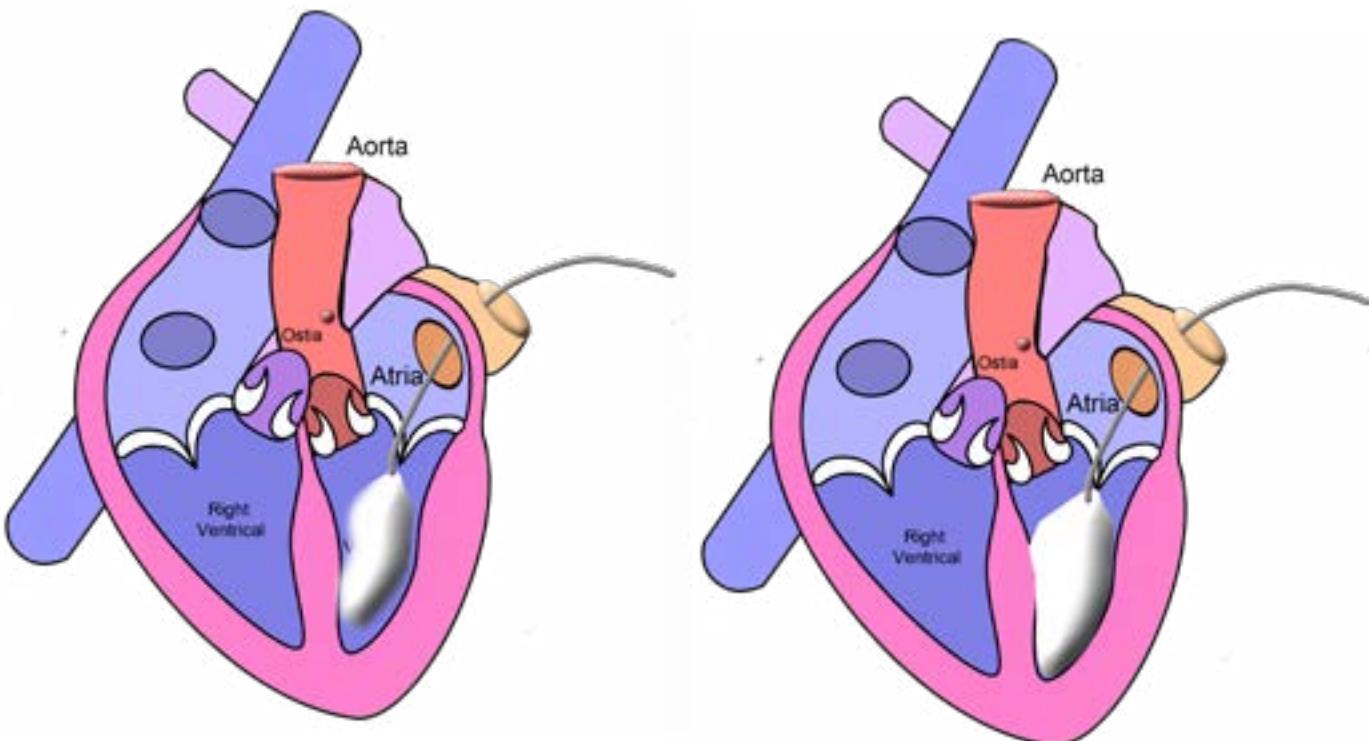
## Left Ventricular Pressure (LVP)

At this point you can connect the pressure transducer. It is recommended that a 3-way stopcock is used at the top of the flexible balloon catheter. With the balloon occluded and using a syringe with a small hypodermic needle, place a droplet of fluid in the open stopcock port.

This will displace any small amount of air in the port, while surface tension will hold the droplet in place. Once the droplet is in place, insert the pressure transducer. Now open the stopcock to the pressure transducer, rather than the balloon.



The balloon catheter may be inserted into the chamber through an accessory port in the chamber or an unused port in the lid. Placement of the balloon into the left ventricle is performed by either insertion by passage through the left atrium, or by passing the catheter through the wall of the left ventricle. In order to make it easier to insert, it is advised that you draw the fluid back from the balloon in order to collapse it.

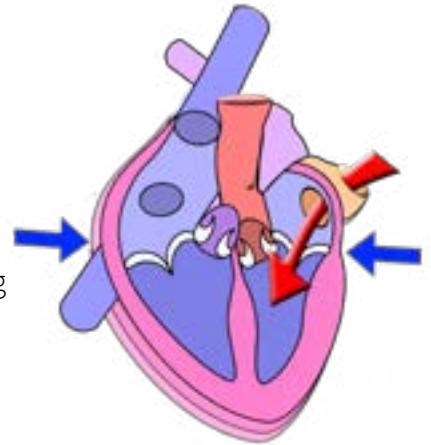


# 120102EZ Radnoti Langendorff System

## The Frank Starling Law of the Heart

The Frank Starling Law of the Heart (also known as Starling's law or the Frank-Starling mechanism) states that the more the ventricle is filled with blood during diastole (end-diastolic volume), the greater the volume of ejected blood will be during the resulting systolic contraction (stroke volume).

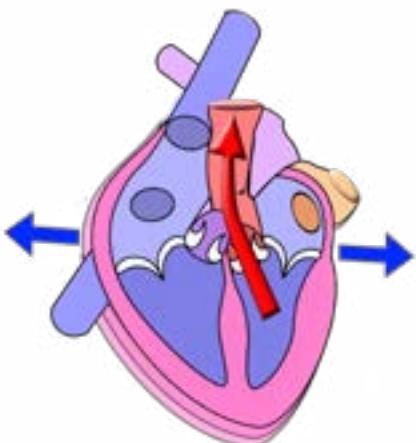
This means that the force of contractions will increase as the heart is filled with more blood and is a direct consequence of the effect of an increasing load on a single muscle fiber. In particular, such increased load stretches further the myocardium and enhances the affinity of troponin C for calcium, hence increasing the contractile force.



The force that any single muscle fiber generates is proportional to the initial sarcomere length (known as preload), and the stretch on the individual fibers is related to the end-diastolic volume of the ventricle. In the human heart, maximal force is generated with an initial sarcomere length of 2.2 micrometers, (a length which is rarely exceeded in the normal heart).

Initial lengths larger or smaller than this optimal value will drop the force of the muscle owing to less overlap of the thin and thick filaments for larger values, and more overlap of the thin filaments for smaller values.

This can be seen most dramatically in the case of a premature ventricular contraction. The premature ventricular contraction causes early emptying of the left ventricle (LV) into the aorta. Since the next ventricular contraction will come at its regular time, the filling time for the LV increases, causing an increased LV end-diastolic volume. Because of the Frank Starling Law, the next ventricular contraction will be more forceful, causing the ejection of the larger-than-normal volume of blood, and bringing the LV end-systolic volume back to baseline.



For example, during vasoconstriction the end-diastolic volume increases, increasing preload. This will increase stroke volume. The heart will pump what it receives.

The above is true of healthy myocardium. In the failing heart, the more the myocardium is dilated, the weaker it can pump, as it then reverts to Laplace's Law.

# 120102EZ Radnoti Langendorff System

## The Frank Starling Law of the Heart

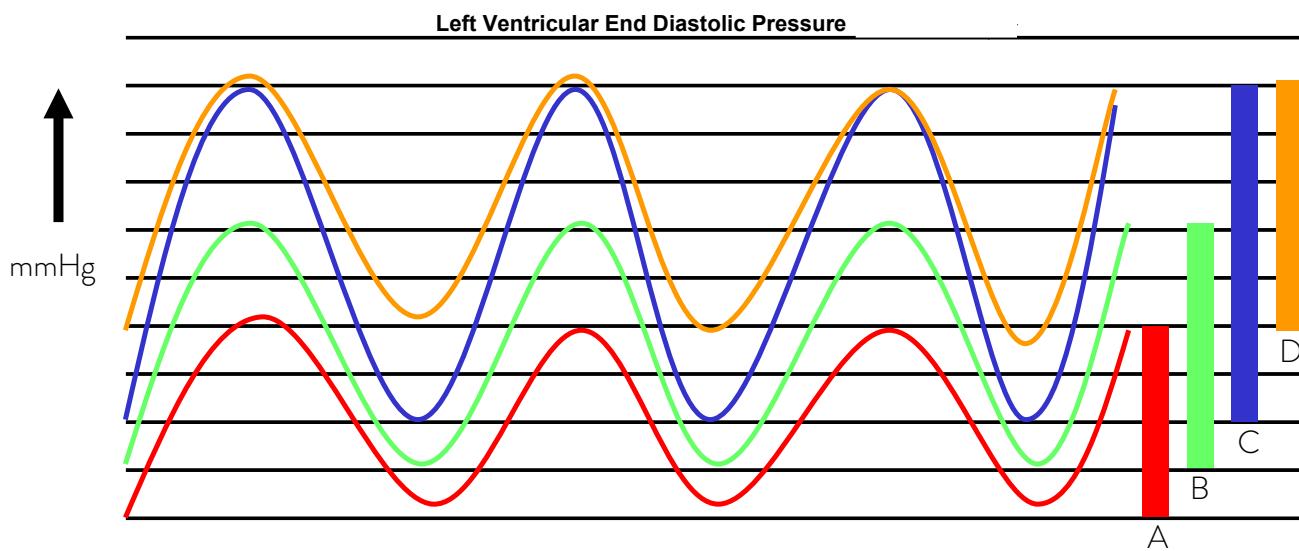
Optimize the preload to obtain accurate maximum developed pressure measurements. This is a combination of both the resting pressure (systole) and maximum developed pressure (diastole).

The preload on the balloon should be increased gradually while monitoring the developed pressure. You will see a distinct pressure wave as you begin to increase preload to the balloon as seen in the **RED** (A) trace.

Gradually increasing preload will increase end developed pressure, as shown in the **GREEN** (B) trace. We recommend you increase preload by increments of 2 mmHg and evaluate maximum developed pressure and systolic pressure. Continue with this process until such time as an optimum developed pressure is achieved while maintaining a physiological normal systolic pressure.

The **BLUE** (C) trace indicates the approximate preload and maximum developed pressure for a 250-300 g adult rat.

The **ORANGE** (D) trace indicates that preload has increased too far. This is depicted in the trace as an acceptable maximum developed pressure but an abnormal systolic or preload pressure. This would also be an indication of a balloon size being too small for the donor heart.



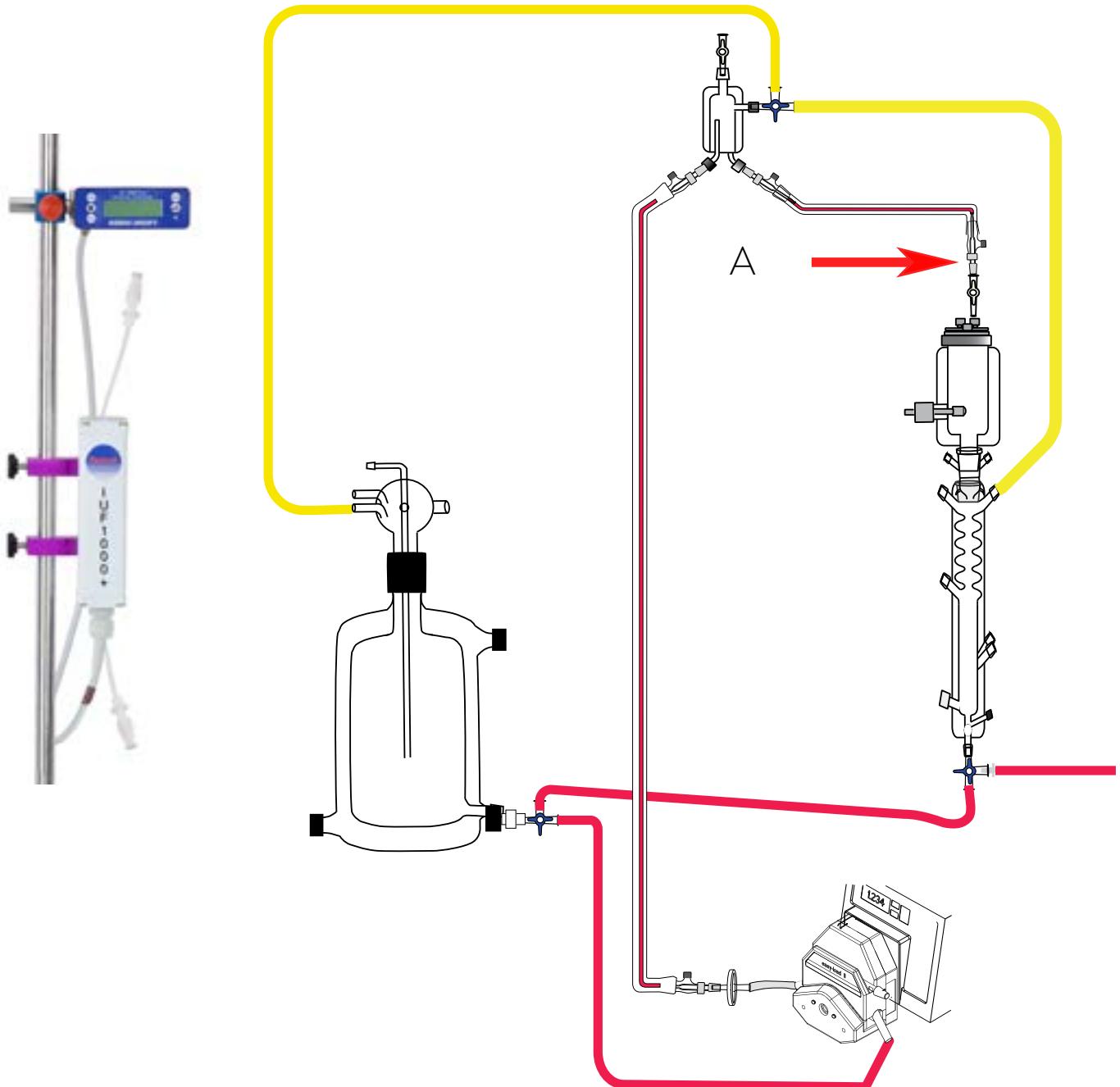
# 120102EZ Radnoti Langendorff System

## Aortic Flow In/Out

Flow measurements can be made with the addition of a Radnoti IUF-1000+ Flow Meter.

See diagram for recommended placement of the transducer for each measurement.

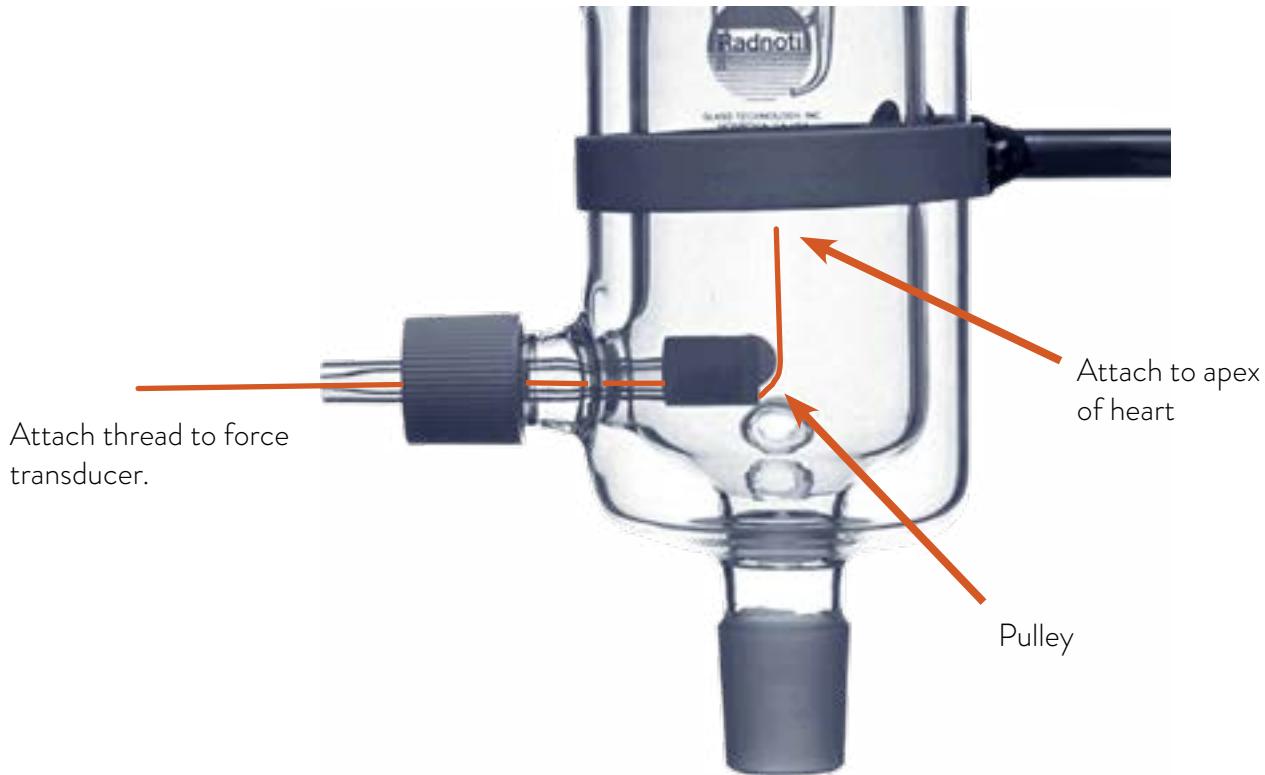
A: Aortic in flow (Retrograde)



# 120102EZ Radnoti Langendorff System

## Apical Force

The simplest measurement of contractile force is made using a force transducer tied to the apex of the heart with a pulley in-between the heart and the transducer. In this system a measurable amount of force is lost in a rotational motion as the heart contracts, which some investigators compensate for with a three-point mount.



Any number of force transducers may be mounted to the stand in a straight path to the port in the heart chamber.

Please see your force transducer's operating manual for connection to data acquisition device and calibration.

# 120102EZ Radnoti Langendorff System

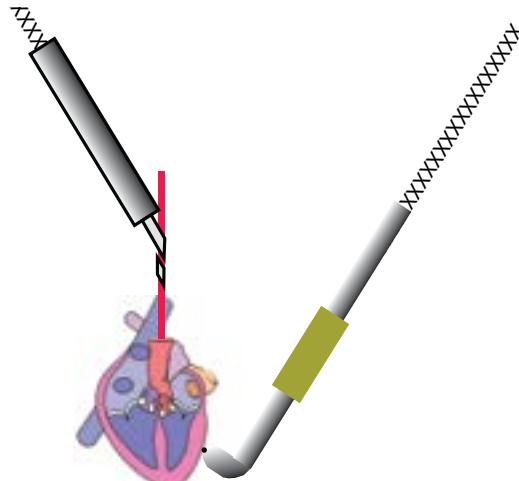
## ECG

The Radnoti 140155 ECG electrode set is designed for recording monophasic action potentials during isolated perfused heart experiments. The spring-loaded platinum-tipped lead will maintain constant contact with the beating heart. This type will not cause tissue damage that can occur with suction-type or impaled lead-type electrode sets. The second lead tip is of malleable platinum wire for ease of wrapping around the aorta or steel aortic cannula.

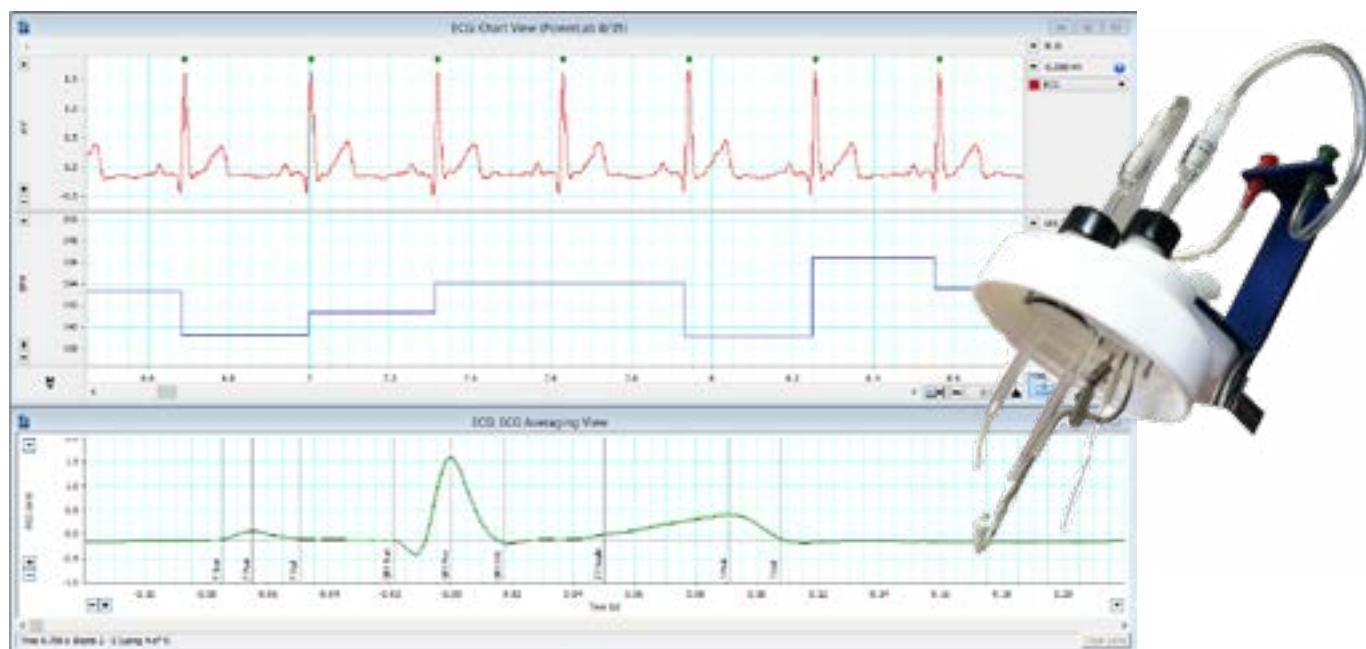
For placement under the Radnoti Hi-Tech heart chamber lid via ball-and-swivel clips or in open-top chambers. Each electrode lead has coaxial wire with ground for connection to bioamplifier. Shielded coaxial wire reduces noise artifacts during recordings.



Radnoti ECG electrode set



Suggested lead placement.



# 120102EZ Radnoti Langendorff System

## Pacing

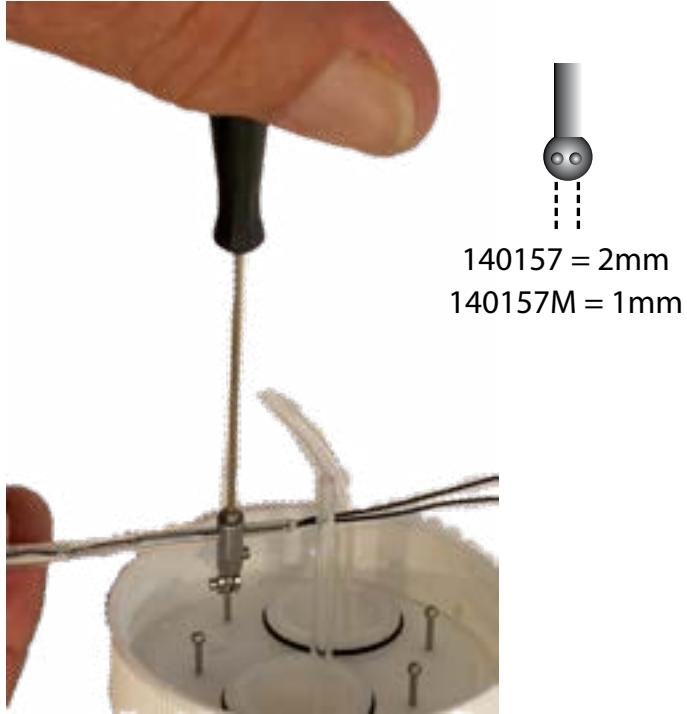
The experimenter must decide whether the heart will be paced or allowed to beat spontaneously. Pacing is used to maintain a standard contractile response and metabolic demand. Spontaneous beating may permit the experimenter to measure changes in heart rate and rhythm that will occur with various drugs or manipulations.

To pace a heart, the stimulus rate must exceed the natural cardiac pacemaker rate. Often the sinoatrial node is crushed or the right atrium excised to eliminate the contribution of the primary intrinsic pacemaker. Pacing voltage is determined as a set percentage (normally 110-150%) above the voltage required to capture (pace) the heart and should not have to exceed 3-5 V, with a duration of 0.1 to 1.0 msec.

Pacing may also be used to induce arrhythmias in attempts to measure changes in fibrillation threshold.

The Radnoti 140157 and 140157M Pacing electrode is designed specifically for use during isolated perfused heart experiments. The 140157M has the tips placed closer together (1mm) for mouse hearts.

Designed for ease of use, the spring-loaded platinum-tipped leads to maintain constant contact with the beating heart. This prevents damage to tissue or cells as may occur with suction-type or impaled-lead type electrode sets. The lead tips are of 99.99% pure platinum. These leads are designed for placement under the Radnoti Hi-Tech heart chamber lid via ball-and-swivel clips or in open-top chambers.



# 120102EZ Radnoti Langendorff System

## Ion Selective Electrodes and Accessories

The Radnoti [Water Jacketed Electrode Holders](#) provide a convenient in-line electrode placement solution that offers a temperature controlled measurement point when tied into your system's water jacket circuit.

Threaded Luer lock ports are provided for secure placement in your perfusate circuit. Each port is provided with three caps:

- One blank cap for closing off the port when not in use.
- One cap with a septa which allows for injection directly into the perfusate when upstream of your sample.
- One cap with a through-hole cap in combination with an o-ring for water tight seal for Micro Electrode.



Single port shown with optional microelectrode.



#220510      #220520      #220530  
Single Port    Double Port    Triple Port

Be sure to order the appropriate [Ring Clamp](#) and [Universal ring stand clamps](#) for your configuration if necessary.

# 120102EZ Radnoti Langendorff System

## Ion Selective Electrodes and Accessories

Recommended placement for the Radnoti water jacketed electrode holders are listed below:

A. Aortic inflow

B. Effluent measurement

Available Micro Electrodes:

RGT-MI-410 Micro-combination pH microelectrode

RGT-MI-442 K<sup>+</sup> ion microelectrode

RGT-MI-600 Ca<sup>++</sup> ion microelectrode

RGT-MI-730 Dip-type O<sub>2</sub> microelectrode

RGT-MI-420 Na<sup>+</sup> ion microelectrode

RGT-MI-425 Na<sup>+</sup> ion microelectrode

RGT-MI-740 Dip-type NH<sub>3</sub>, (NH<sub>4</sub>)<sup>+</sup> microelectrode

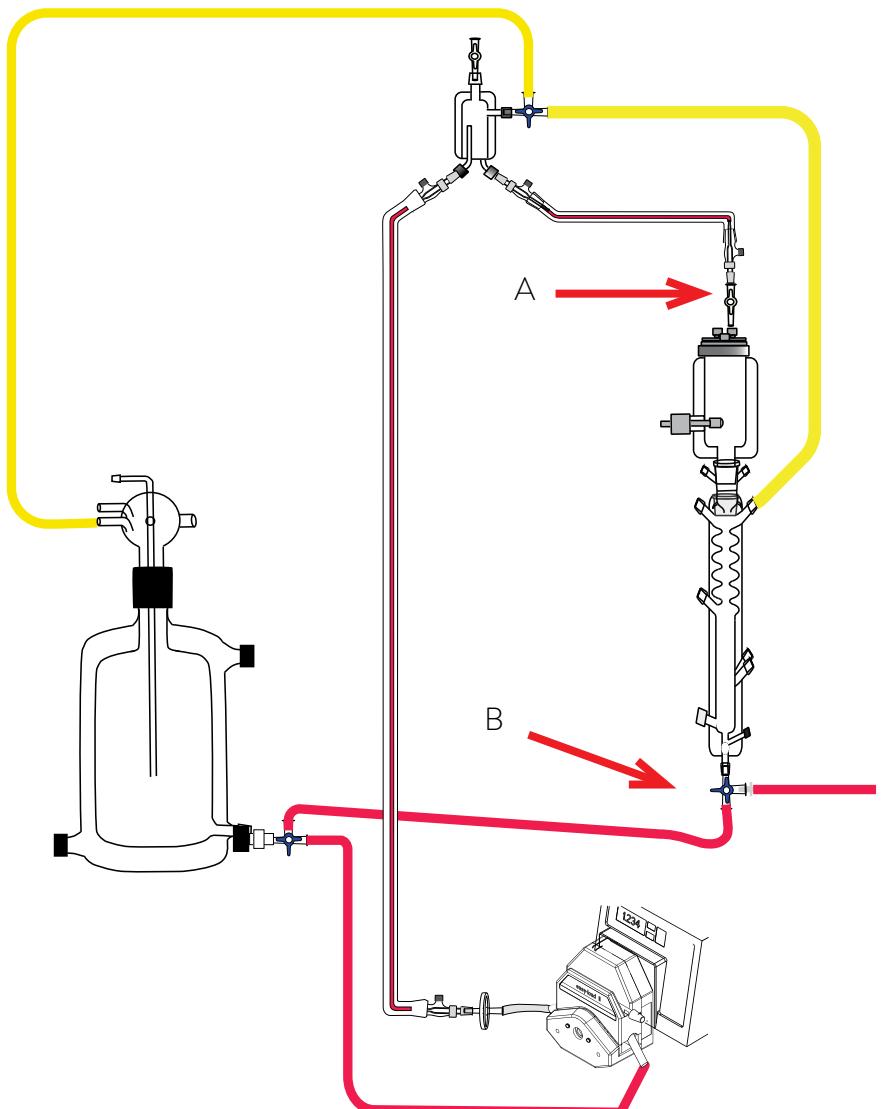
RGT-MI8-740 and #MI16-740 Flow-thru NH<sub>3</sub>,(NH<sub>4</sub>)<sup>+</sup> microelectrodes

RGT-MI-720 Dip-type CO<sub>2</sub> microelectrode

RGT-MI-915 and MI-905 Dip-type Conductivity microelectrodes

RGT-MV-ADPT Millivolt Adapter

RGT-O2-ADPT Oxygen Adapter



Typical microelectrode housing.

\*Images are not to scale

# 120102EZ Radnoti Langendorff System

## Operating Modes of the Radnoti 120102EZ Heart System

---

The Radnoti Isolated Heart Systems are offered in a number of configurations to allow you to purchase a system that fits your exact experimental needs. In moving forward with your research, our experience has shown that modularity and flexibility, alongside a large range of accessories are needed to meet ever-changing experiment requirements.

Primarily, there are two methods of isolated heart perfusion, retrograde perfusion, commonly referred to as Langendorff, and Working Heart perfusion.

Langendorff is the retrograde perfusion of the aorta. The tricuspid valve is forced closed allowing the flow of perfusate to supply the coronary arteries, via the ostium. This provides oxygen and nutrients which sustain the heart. When designing your experiment using a Langendorff preparation, there are two basic configurations that should be considered. Essentially, the researcher decides as to whether they require the heart to regulate flow or pressure.

In Constant Pressure Langendorff, the heart auto-regulates flow. It is set up so that a peristaltic pump provides perfusate to an elevated bubble trap, setting the hydrostatic pressure and then feeding down to the heart at a constant pressure. This in turn allows any changes in resistance to result in flow rate variations that are monitored using an accessory flow-meter.

Constant Flow Langendorff is where the heart auto-regulates pressure. It is set up so that the flow is held constant via the peristaltic pump speed. Changes in coronary resistance are detected as changes in pressure via an accessory pressure transducer which is placed in line at the aortic cannula.

Consideration should also be given as to whether a recirculating or non-recirculating configuration is chosen. Recirculating mode is achieved by collecting the effluent and pumping it to an oxygenator where gas is exchanged and perfusate routed back to the circulation loop via the peristaltic pump. Recirculating modes are often required when using expensive buffer solutions or monitoring metabolism.

In order to simulate as closely as possible the natural physiological norms, the Radnoti Working Heart System should be considered. In working heart mode in addition to the aorta, the left atrium is also cannulated. The heart is perfused through the atrium while the contracting left ventricle pumps the perfusate out through the aorta, against a user-set afterload pressure. This simulates the heart working the systemic circulation.

# 120102EZ Radnoti Langendorff System

## Trouble Shooting

### The Rapidly Failing Heart

The isolated heart preparation is normally very stable and reproducible once the experimenter has gained familiarity with it. A rapid deterioration unexpectedly occurring in two preparations consecutively is a strong indication of a problem.

Many times this failure is due to the growth of bacteria and the release of endotoxins into the perfusate.

Initial corrective measures should include:

- A thorough cleaning of the apparatus and replacement of tubing/fittings and replacement of solutions (which have a limited storage life in the refrigerator).
- A check of the water source.
- A check of aeration the appropriate gas mixture and pH of the aerated buffer at the normal operating temperature.
- Records should be kept of new purchases of substrate and salts. Certain toxic agents used by experimenters may be difficult to clean from the system and may require the use of organic solvents or the removal of tubing after each use, as well as the use of a separate reservoir.
- Tubing should be thoroughly pre-rinsed to remove plasticizers and the use of a high-quality silicone or Tygon tubing is recommended.
- Note that silicone tubing is extremely gas permeable; oxygen and other gas losses can be considerable.
- The use of high quality water is essential. Some experimenters use small amounts of EDTA (0.1 mM) to chelate trace heavy metals in suspect water supplies, although this is less of a problem with modern multiple cartridge ion-exchange systems.

# 120102EZ Radnoti Langendorff System

## Trouble Shooting

### Achieving Constant Pressure in Langendorff Mode

A constant pressure is set using gravity and the elevation of the Aortic Bubble Trap Compliance Chamber.

1 mmHg = 13.6 mm water.

Typically 1 meter of elevation is recommended. If you are having difficulty with setting and maintaining a constant pressure please check the following:

- Elevation of the Aortic Bubble Trap with relation to the heart.
- Vent port on Aortic Bubble Trap is open.
- Placing a 50 mL disposable syringe with the plunger removed at the vent port stopcock will greatly reduce the chance of spillage and help to dislodge bubbles that can vapor-lock the compliance port line returning to the reservoir. If this port is not in an open state, the fluid traveling down the compliance or overflow line returning to the supply reservoir will draw a negative pressure on the line feeding the aorta and distort pressure readings.
- Compliance port set to open returning fluid to supply reservoir. If this port is not in the open state then excess perfusate supplied from the reservoir via the peristaltic pump will be forced to the aortic cannula, over-pressurizing the heart and causing rapid failure.
- Peristaltic pump set to deliver perfusate at 1.5 – 2X anticipated flow rate. If the pump rate is set too low, the heart will consume buffer faster than the buffer is supplied and quickly drain the supply from the bubble trap. The flow rate should be set to at least 150 % of the anticipated flow rate with excess buffer returning to the supply reservoir via the compliance/overflow port at the aortic bubble trap.

# 120102EZ Radnoti Langendorff System

## Trouble Shooting

### Achieving Physiological Pressure in Constant Flow Mode

The constant flow model isolated heart is based on providing a fixed known flow rate to the heart via the peristaltic pump.

- The heart will autoregulate resistance and thus pressure. A pressure transducer is needed at the aortic cannula. Pressure readings should be within standard ranges for the species and heart weight.
- Verify fluid delivery rate from peristaltic pump. Delivery rate is a function of pump RPM and pump tubing size.
- Check elevation of the bubble trap. This should be no more than 100 mm from the heart unless required by the experimental model.
- High flow rates are needed to maintain heart. This typically indicates that the heart is compromised and in a state of vasoconstriction.
- It is recommended to review the procedure and protocol for harvesting of the heart.
- If there are no issues with harvesting, see Rapidly Failing Heart section.

# 120102EZ Radnoti Langendorff System

## Trouble Shooting

### Working Heart in Detail

It is important to stress that, *in vivo*, cardiac output is equal to the venous return from the lungs to the left atrium. In the isolated Working Heart the venous return is represented by the flow from the left atrial cannula.

An important point in using a working heart apparatus is that the left atrial perfusion line must be capable of delivering perfusion fluid at a rate sufficient to support the maximum cardiac output of a working heart at any particular preload. If the left atrial cannula is too small it will artificially limit the cardiac output of the preparation.

The problem is compounded by the pulsatile nature of atrial filling. This means that the atria only fill during about half of the cardiac cycle. To ensure that this problem does not arise and that left ventricular filling is not limited by inadequate left atrial inflow it is essential to check that the left atrial perfusion line can deliver a flow rate of at least twice the expected maximal cardiac output.

This is easily checked by running the apparatus without a heart attached and measuring the flow from the left atria line. A rate of at least 150 mL/min is recommended for a 1 g heart. Having flowed from the left atrial cannula into the left atrium, the perfusion fluid is ejected via the mitral valve into the left ventricle from where it is ejected through the aortic cannula against a hydrostatic pressure set via the compliance loop.

The afterload is determined by the height of the compliance reservoir above the aortic cannula. The compliance bubble trap contains a 2 mm diameter air bubble and mimics normal vascular elasticity. It is an essential component of the perfusion circuit, greatly increasing the chances of successful working heart function. In the course of left ventricular ejection, a portion of the perfusion fluid is forced into the coronary ostia and thereby perfuses the coronary vessels of the heart.

The coronary effluent exits from the right heart into the heart chamber where it may be sampled for assay or returned (via a roller pump) to the supply reservoir for re-oxygenation.

Depending on the species and experimental design, filling pressures are usually in the range of 7 cmH<sub>2</sub>O and after loads in the range 60-100 cmH<sub>2</sub>O or more. Under these conditions and using the heart from a 250 g rat, coronary flows of up to 25 mL/minute and aortic flows of 50-80 mL/minute can be expected.

These can be measured by timed collection into graduated cylinders or by optional flow meters. Summation of coronary and aortic flow gives the cardiac output.

### Working Heart in Detail, Continued

Hearts may be paced or allowed to beat spontaneously under which circumstances heart rate may be derived from a pressure recording which is usually via a side arm of the aortic cannula.

Because of the large volumes of perfusion fluid pumped by the heart, the working preparation usually operates in the recirculating mode and for this reason it is essential to have an in-line filter (1 µm porosity) in the circuit to remove any particulate contaminants which may originate from the heart, connecting tubing, glassware or perfusion solutions.

There is just one additional step following the establishment of perfusion (without the insertion of an LVP balloon), which is cannulation and secure tying off (without any leaks) of the left atrium via one of the orifices of the pulmonary veins.

The dimensions and relative positions of the aortic and atrial cannulae are critical to a successful preparation.

The Radnoti Heart Chamber will allow you to adjust the distance between cannula by rotating the cams on the heart chamber lid. Vertical position may be adjusted by the loosening of the locking cap on the cannula, setting the position, and then re-securing.

Once aortic and left atrial cannulation are accomplished, the aortic cannula stopcock is switched over and perfusion initiated by the left atrium whilst simultaneously opening up the aortic outflow line. In this way, oxygenated perfusion fluid from a constant pressure head left atrial perfusion reservoir (which is continuously filled by a roller pump from the Radnoti Buffer Reservoir) flows under gravity into the left atrial cannula.

The preload of the preparation is determined by the height of the overflow from the atrial perfusion bubble trap above the heart. This is usually set around 7 cm for isolated rat hearts but can be varied to suit other preparations or to allow construction of “Starling Curves” relating preload to cardiac function.

# 120102EZ Radnoti Langendorff System

## System Maintenance

---

After the experiment has been completed, the experimenter should take care 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:

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

Non-phosphate soaps are preferred since insoluble phosphates can form from calcium and magnesium in physiological salt solutions.

Note that bactericidal soaps may contain iodine or other materials which can affect isolated tissues and cells.

Cleaning supplies and equipment (such as brushes) should be used only for cleaning this glassware and not used for other lab cleaning procedures.

Questions and procedures noted here should be adjusted in accordance with your licensed procedures and the recommendations of your safety personnel.

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.

# 120102EZ Radnoti Langendorff System

## System Maintenance

---

Often overlooked as a source of contamination is the Water Circulator Supply. This should be kept clean, the bath rinsed, and solution changed to reduce precipitate build up.

Covering equipment to reduce air borne contamination from microbes and spores is useful.

Note that 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.

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 bath contamination.

## Cleaning the System

---

### Cleaning Glass Items

Much of the Radnoti apparatus is borosilicate glass which can be cleaned with a wide range of soaps, ethyl alcohol, dilute HCl or HNO<sub>3</sub> (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.

# 120102EZ Radnoti Langendorff System

## Cleaning the System

### Cleaning Glass Items, Continued

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 HNO<sub>3</sub> 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 the 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 should be inspected at the pump head for wear.

Note that 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.

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

# 120102EZ Radnoti Langendorff System

## Typical Buffer/Perfusion Solution

Typical buffer used for working heart applications:

	Suggested molarity (mM)	Add to distilled water to a final volume of 1 liter
NaCl	113	6.6037g
KCl	4.5	0.3355g
MgCl <sub>2</sub> •6H <sub>2</sub> O	1	0.2033g
NaHCO <sub>3</sub>	25	2.100g
NaH <sub>2</sub> PO <sub>4</sub>	1.6	0.2208g
CaCl <sub>2</sub> •2H <sub>2</sub> O	1.25	0.1838g
Glucose	5.5	0.9909g

For best results, use distilled or reverse osmosis purified water with resistance > 10 MΩ. Aerate this solution with 95% oxygen, 5% carbon dioxide and warm to 37°C, then adjust pH using NaOH to 7.35-7.40. Older literature references discussing these types of solutions often mention the use of small amounts of EGTA or EDTA to remove heavy metal contamination, which has become less of a problem when modern water purification systems are used.

Variations often are found in potassium (range 3.5-5.7 mM) and calcium concentrations (range 1-2.5 mM), besides modifications in salt concentrations used to adjust pH. Variations in perfusion calcium concentrations occur in the literature in part due to the difference in free calcium measurements (a serum/plasma calcium activity around 1-1.25 mM) versus total serum/plasma calcium (2-2.5 mM). To reduce tissue edema, oncotic pressure can be increased by adding albumin (or other plasma expanders) to the perfusion solution, but aeration of this modified solution causes foaming unless a membrane oxygenator is used.

## Typical Values

These values are obtained from a variety of sources and are displayed to demonstrate the approximate ranges of these values. Values are for adult animals. In vivo heart rate and blood pressure are taken at rest. Cation values are from serum. Left ventricular volume (LVV) is given for a balloon inserted into the left ventricle. CF (coronary flow) is given for a saline solution at 50-60 mmHg.

	Rate (bpm)	BP (mm/Hg)	Na (mM)	K (mM)	Ca (mM)	Mg (mM)	LVV (mL)	CF (mL/min/g heart)
Rat	330-380	129/91	140	5.7	2.6	1.1	0.1-0.2	8-10
Guinea Pig	280-300	120/70	145	7.4	2.6	1.2	0.1-0.2	5-8
Rabbit	205-220	110/73	155	4.6	3.5	1.6	0.4-0.7	2-5

## Disclaimer

These procedures and devices are intended for research and experimentation.

All statements, technical information and recommendations herein are based on tests and sources we believe to be reliable, but the accuracy or completeness thereof is not guaranteed.

Before using, user shall determine the suitability of the product for its intended use, and user assumes all risk and liability whatsoever in connection therewith.

Neither seller nor manufacturer shall be liable in tort or in contract for any loss or damage, direct, incidental, or consequential arising out of the use or the inability to use the product.

No statement or recommendation contained herein shall have any force or effect unless in an agreement signed by officers of seller and manufacturer.

# Radnoti



**Radnoti LLC**  
541 Edna Place  
Covina, CA 91723  
(800) 428-1416 (626) 357-8827  
Fax. (626) 303-2998

**Radnoti Ltd.**  
8 Terenure Place  
Terenure Dublin 6 West  
D6W Y006 Ireland  
+353 1 524 2111 Fax. +353 1 443 0784