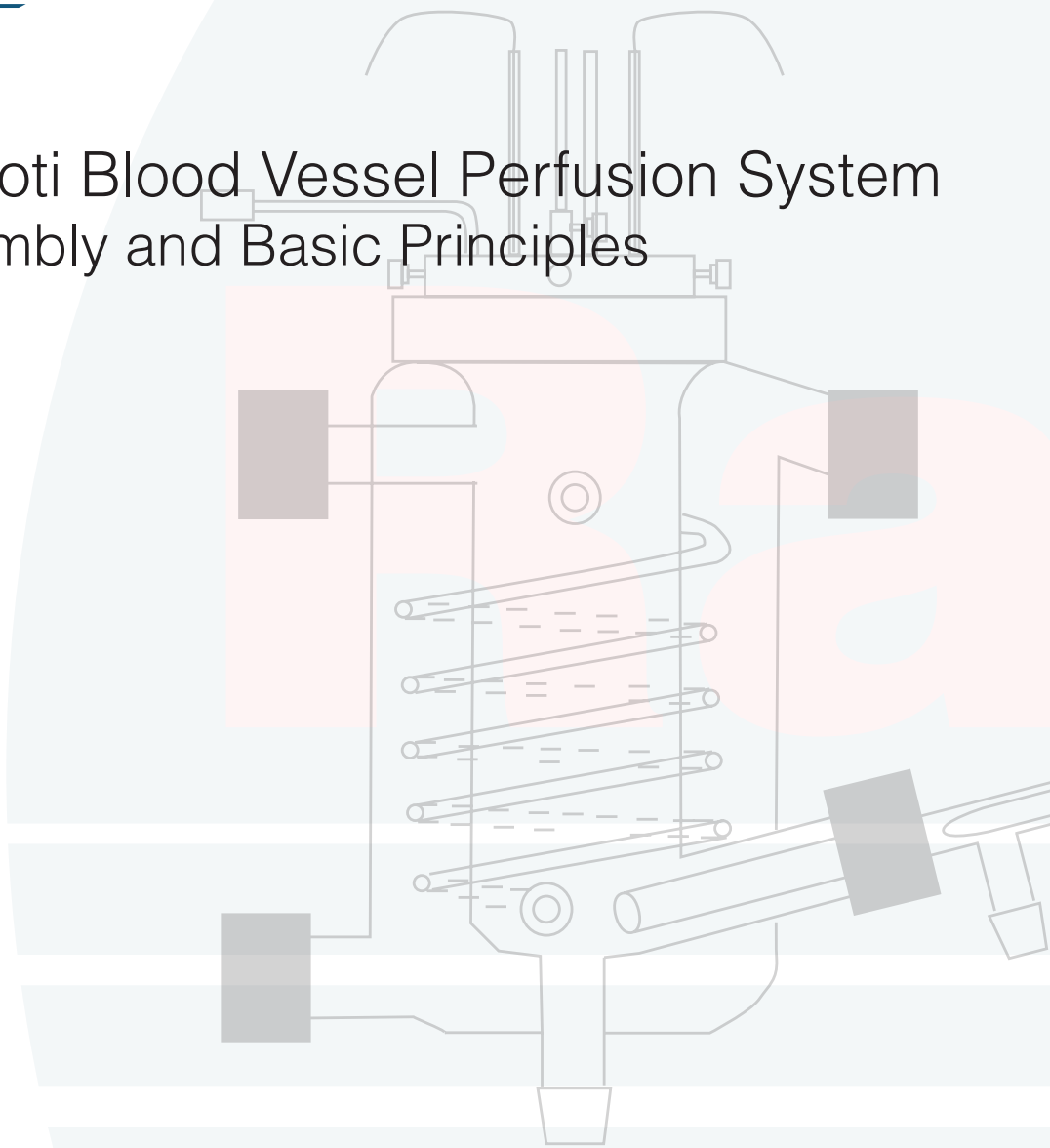
158700-1 Radnoti Blood Vessel Perfusion System

Assembly and Basic Principles



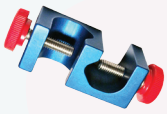
www.radnoti.com info@radnoti.com

v. 14

Radnoti

www.radnoti.com info@radnoti.com

List of Components

Description	Qty	Part #	
Base only, for 2-bar stand	1	159950-B2	
Stabilizer Bar only, for 2-Bar stand	1	159950-C2	
Rod 24” Long Stainless Steel	3	159950-24	
Rod 12” Long Stainless Steel	1	159950-12	
Ring Clamp for 500ml water jacketed reservoir	1	159953-500	
Ring Clamp 100ml Buffer reservoir	1	159953-100	
Ring Clamp bubble trap	1	120149-RC	
Ring Clamp Blood Vessel Perfusion Chamber	1	159953-25	
Universal Stand Clamps	5	159952	
3 Way Stop Cocks	3ea	120721	
Micro Pressure Transducer	1	159905	

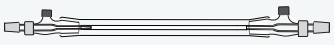
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

List of Components

Description	Qty	Part #	
Water-Jacketed Reservoir 500ml	1	120142-0	
Oxygenating Bubbler with Dual Inlet port (For 500ml Reservoir)	1	140144-0	
Blood Vessel Perfusion Chamber	1	158730	
Bubble Trap	1	130149	
Buffer Reservoir 100ml	1	1581-100	
Radnoti Flex Tube	2	130155-48 130155-60	

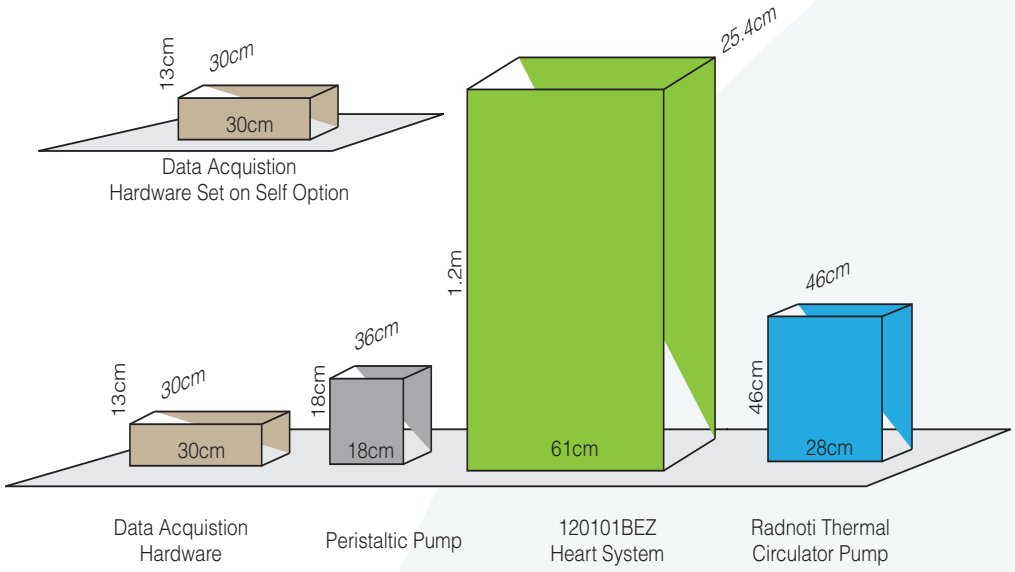
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

www.radnoti.com info@radnoti.com

Radnoti

System layout space considerations and requirements



Lab Bench

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. Height based on constant pressure mode with hydrostatic pressure head of 1 meter. Height requirement will lessen for lower hydrostatic pressure head or constant flow modes. 2nd 24" rod can be removed to reduce height to 24" 61cm in constant flow mode.

Dimensions may vary depending on manufacture and model of referenced equipment, Pre existing equipment already in place in the lab or equipment procured from source other than Radnoti.

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

Power requirements may vary depending on manufacturer and model of referenced equipment, Pre existing equipment already in place in the lab or equipment procured from source other than Radnoti.

DO NOT USE POWER STRIP (separate circuit to computer)

Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

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 pumps 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 then 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 locating the Data Acquisition interface or analog to digital converter Be mindful of instrumentation connection cables and cable length limitations.

Ambient temperature should be in the range of 50^{F} – 104^{F} degrees
(10^{C} to 40^{C} degrees) with a max 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 13.64mm of water to 1mmHg)
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.

Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Keys to Success

Other than mishandling the sample during excision, making an error in the formulation of a perfusion solution, adding a toxic agent to a perfusion solution, Contamination is the leading cause of experimental failure.

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 (5µm or sterile filter with 0.45µm filter) in the course of preparation and inclusion of 1µm 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 sample, such as cannulas 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 generation.
- Calibrate signal generating equipment.
- Determine signal strength based on normal physiological values.

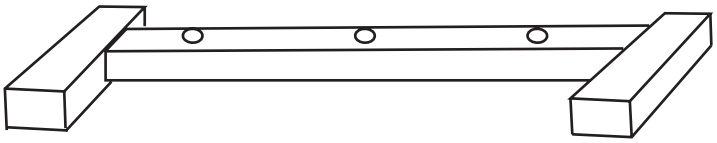
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

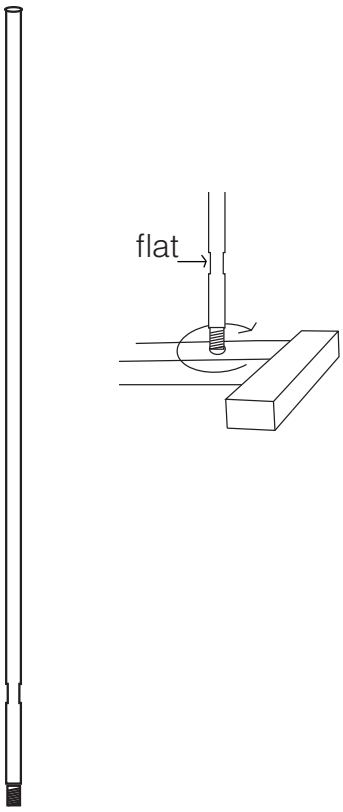
Radnoti

www.radnoti.com info@radnoti.com

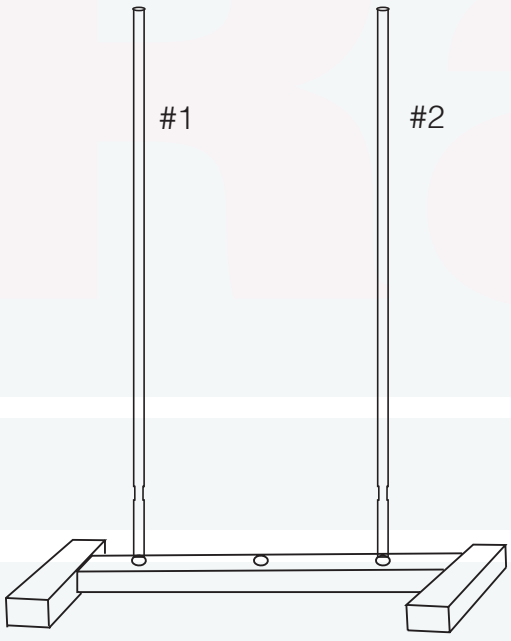
Assembling Lab Stand



Place 2 bar lab stand on lab bench and make sure all 4 of the rubber feet are making contact with your bench surface.



You will notice that the threaded end of the rod has a flat which enables you to use a wrench to make sure they are securely fastened. For further instruction we will refer to the 24” stainless steel rod’s from left to right as rods 1 through 2.

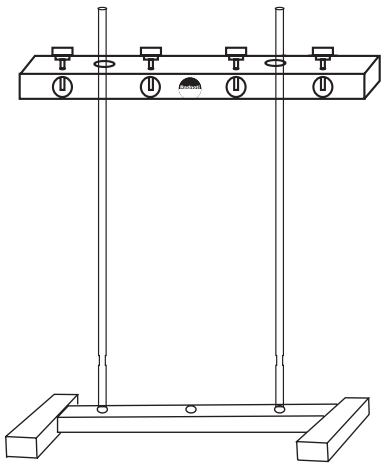


Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

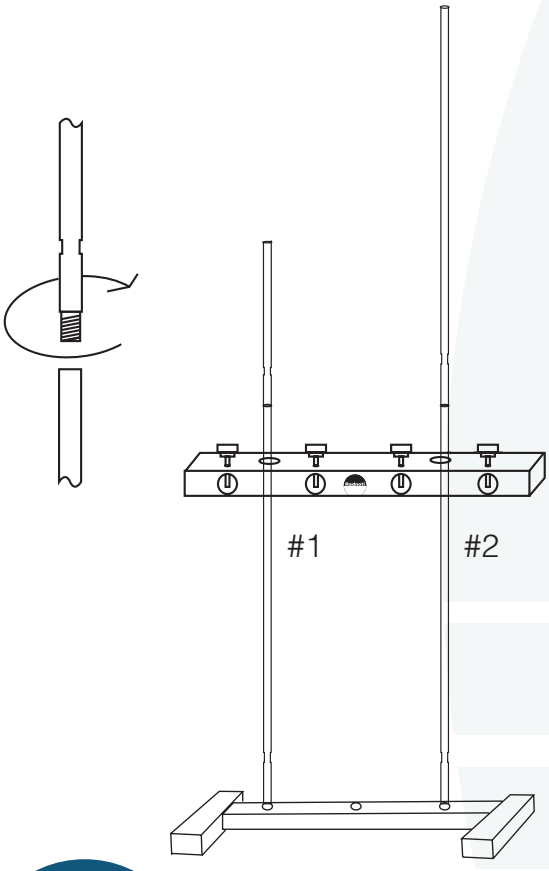
Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

Assembling Lab Stand



With 2 of the stainless steel rods securely attached to the 2 bar lab stand base, you may now slide on the stabilizer bar. With the Radnoti logo facing you will notice 2 holes at the top of the stabilizer bar that contain thumb screws. Make sure that the 2 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 2 rods and secure with the 2 thumb screws approximately 1" from the top of the rod height. Be sure to check all thumb screws are finger tight.



The system includes 1 12" stainless steel rods that we will now screw into rod #1. There is also a third 24" rod that we will now screw into rod #2. As before, make sure that the rods are securely fastened using the flat at the bottom of the rod.

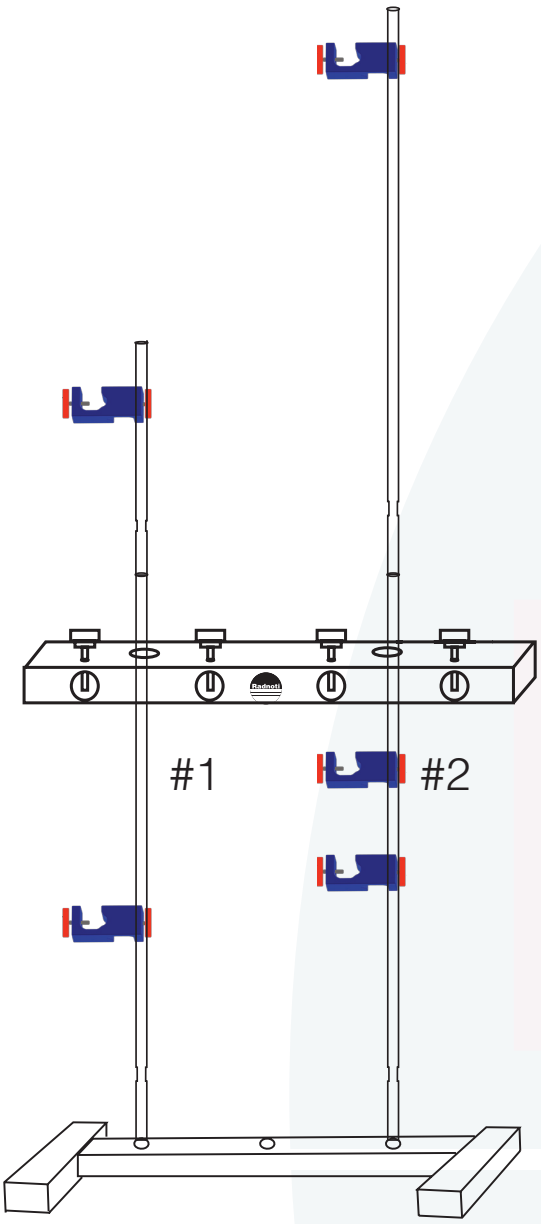
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

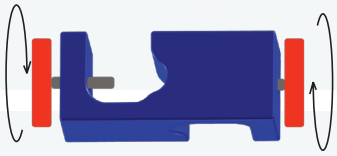
Radnoti

www.radnoti.com info@radnoti.com

Assembling lab Stand



Using the 5 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.

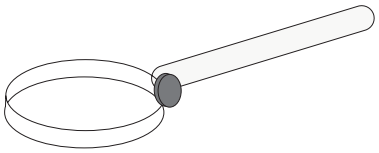


Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

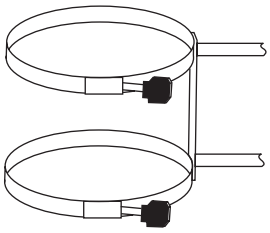
Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

Ring Clamps

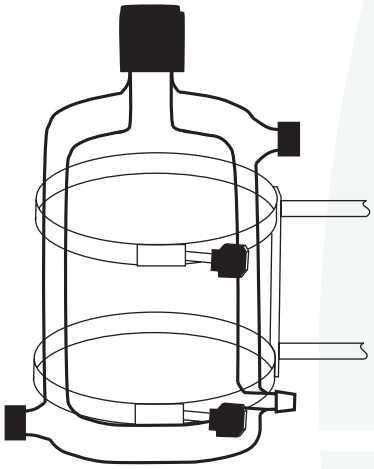


Single Ring Clamp

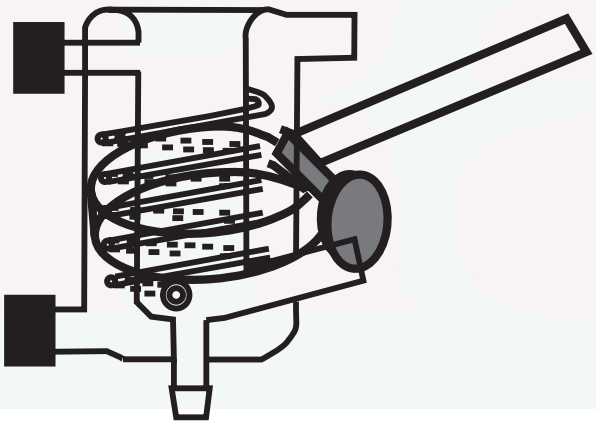


Double Ring Clamp

There are two types of ring clamps used with Radnoti systems, the single ring clamp and the double ring clamp. The single ring clamp is used with most of the glass components while the double ring clamp is used for components larger than 1 liter in volume. This system contains only 1 double ring clamp which is used with the 2 liter reservoir. The ring clamps use a worm gear ring clamp 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
attached to glass component



Single Ring Clamp
attached to glass component

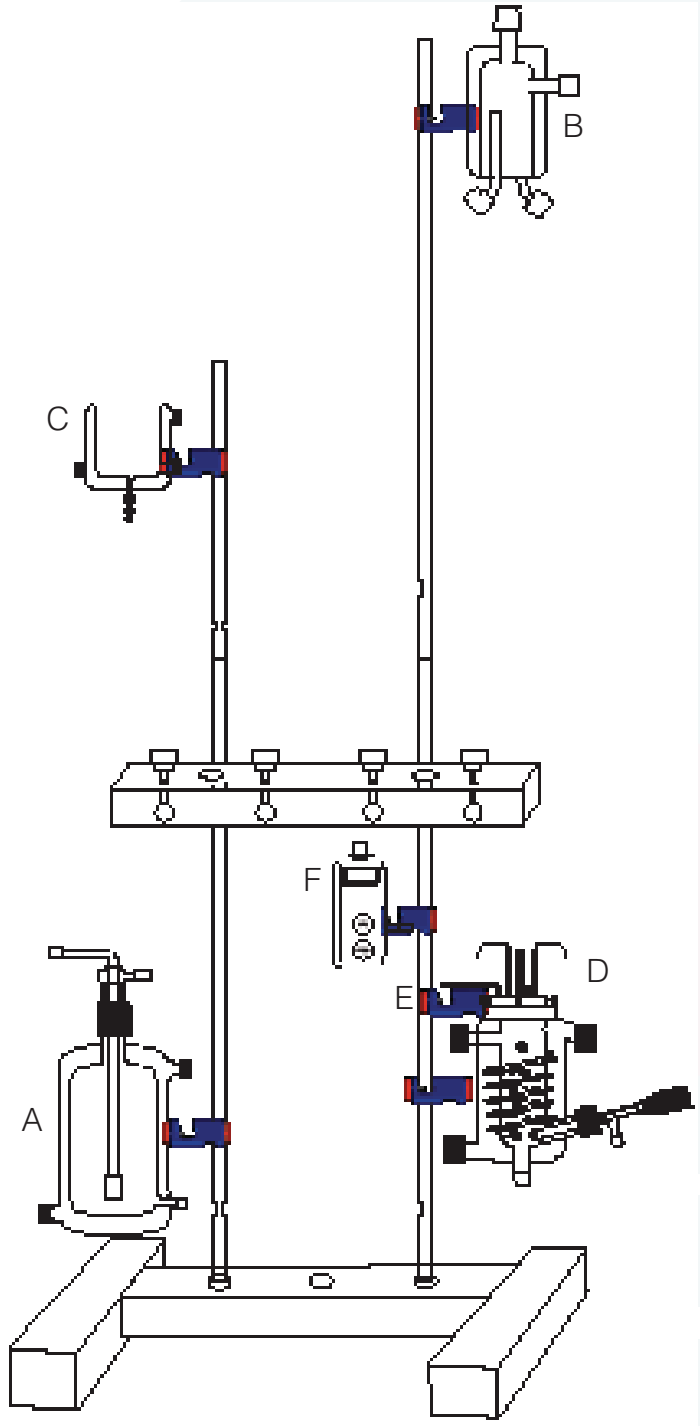
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

Placement of Components

	Components	Corresponding Ri
A	Water Jacketed 500ml liter Reservoir	#159953-500
B	Bubble Trap	#120149-RC
C	Buffer Reservoir 100ml	#159953-100
D	Blood Vessel Perfusion Chamber	#159953-25
E	Blood Vessel Perfusion Lid	
F	Micro Pressuer Transducer	Univ Stand Clamp wit



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

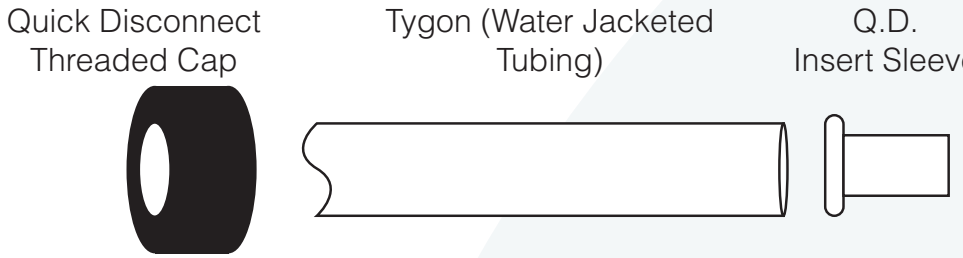
Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

Radnoti Quick Disconnect Tubing (Q.D.)

The Radnoti Q.D. tubing system consists of 3 parts - the #120159 Tygon (Water Jacket tubing) - the #160196 Quick Disconnect Threaded Cap and the #120160 Q.D. Insert Sleeve.

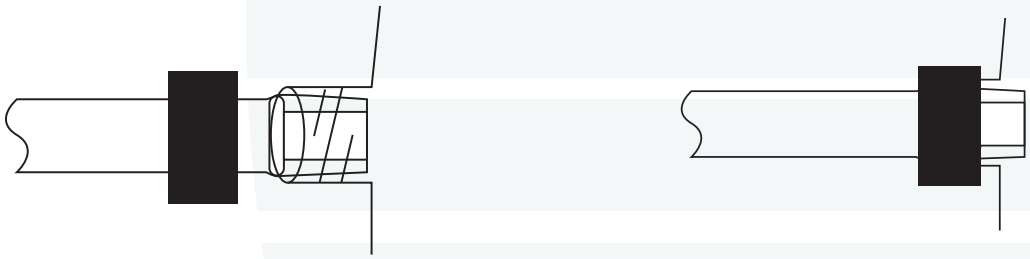


All of the water jacket tubing comes pre-assembled with the system. Should you need to make modifications or replace tubing we have included 10ft (3m) of spare #120159 Tygon tubing for you.

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.

Remove the tool and moisten the tip of your newly created water jacket tubing and insert into the threaded port of your glass component. Slide the Quick Disconnect Threaded Cap onto the glass threads and tighten. ***Do not over tighten as this can lead to breaking the thread.***



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

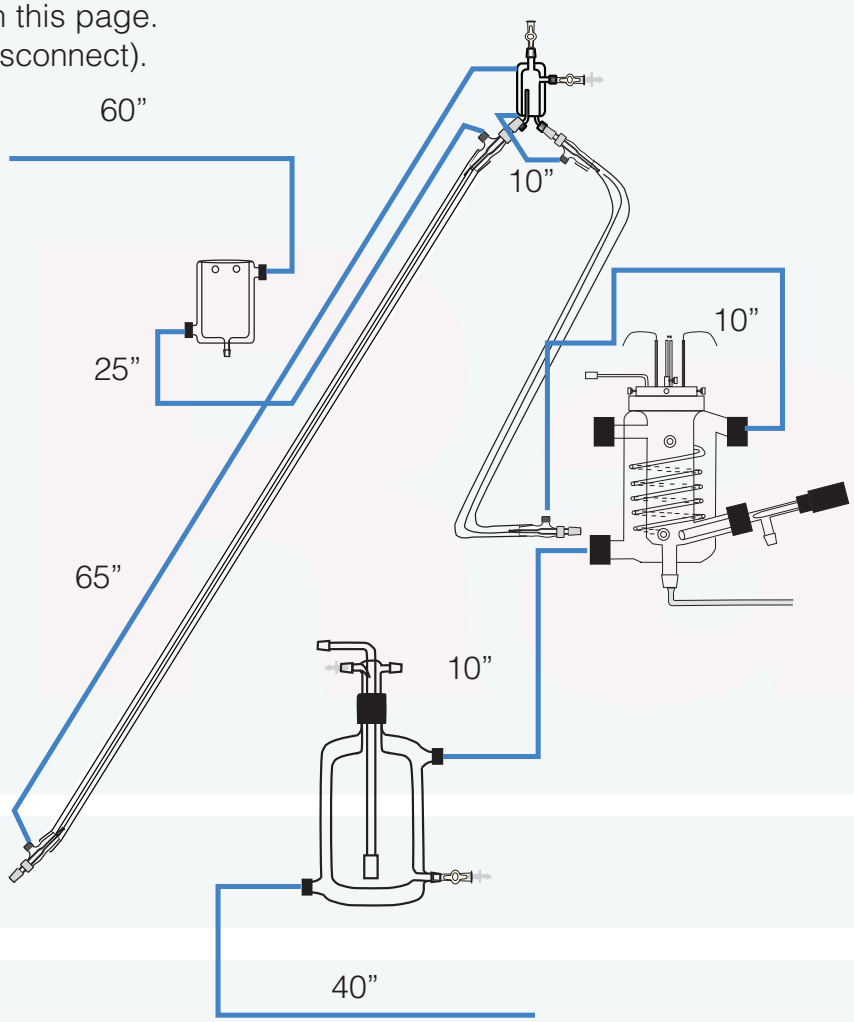
Connecting The Water Jacket Tubing

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. The connections use the Radnoti Q.D. (Quick Disconnect).

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

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



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

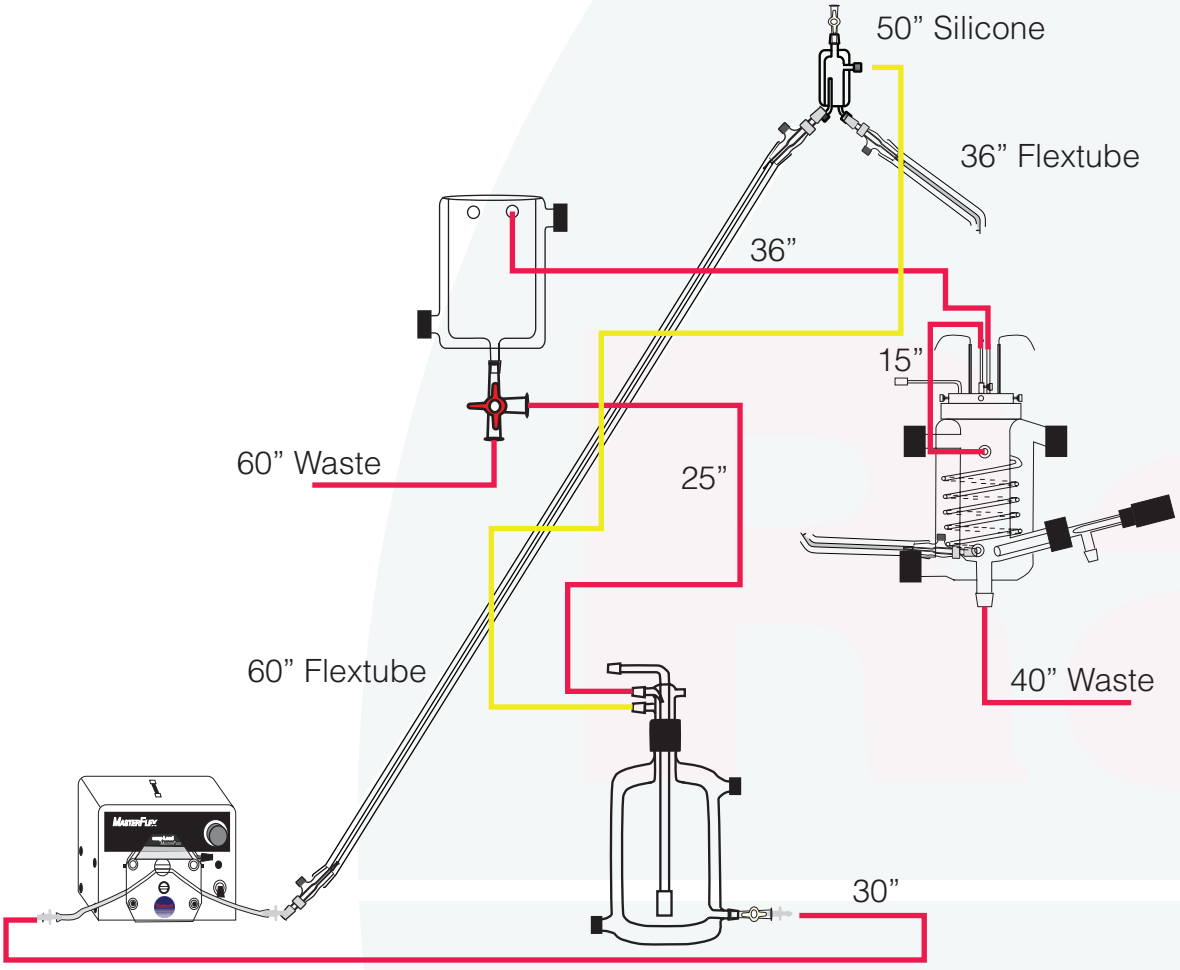
Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

Connecting The Perfusate Tubing (Tygon)

The perfusate tubing is what transports your buffer through the various components of your system. Critical runs are accommodated via water jacketed flex tube assemblies. Overflow or return lines, gravity fed are typically Silicone tubing due to favorable the flow characteristics. A typical flow loop would consist of outflow of the buffer reservoir connected to peristaltic pump, delivered to bubble trap (most cases a flex tube assembly is used for this run) out flow of the bubble trap to the sample input or cannula (in most cases a flex tube assembly is used for this run) outflow of the sample to waste, or recirculated back to supply reservoir or alternate reservoir.



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

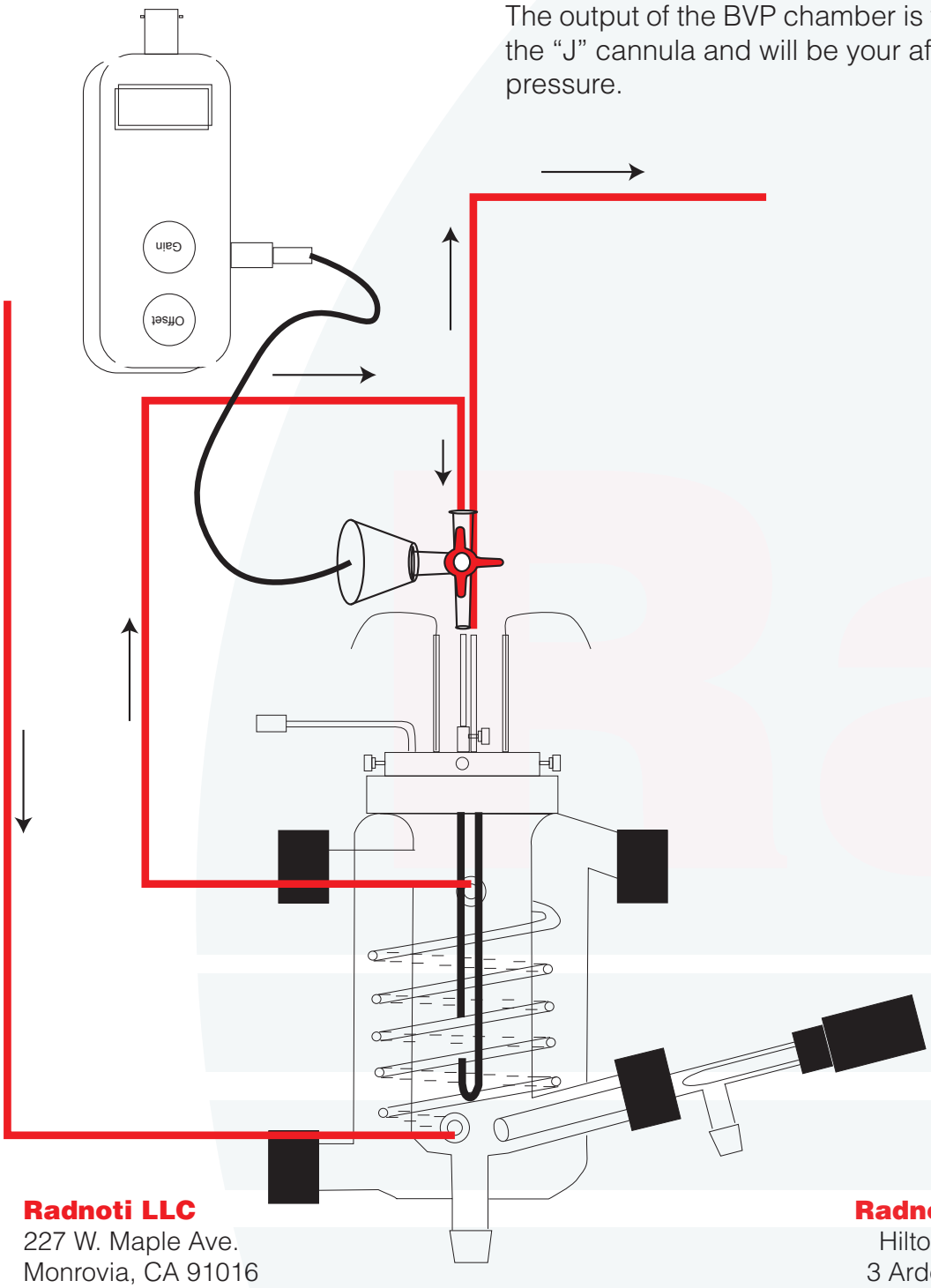
www.radnoti.com info@radnoti.com

Blood Vessel Chamber Inset

The Radnoti Micro Pressure Sensor is connected to the 3 way stop cock to monitor inflow pressure.

The output of the BVP chamber is through the “J” cannula and will be your after load pressure.

Your 36” flextube coming from the bubble trap will connect to the internal coils of the BVP Chamber. The out of the internal coil will feed your straight cannula.



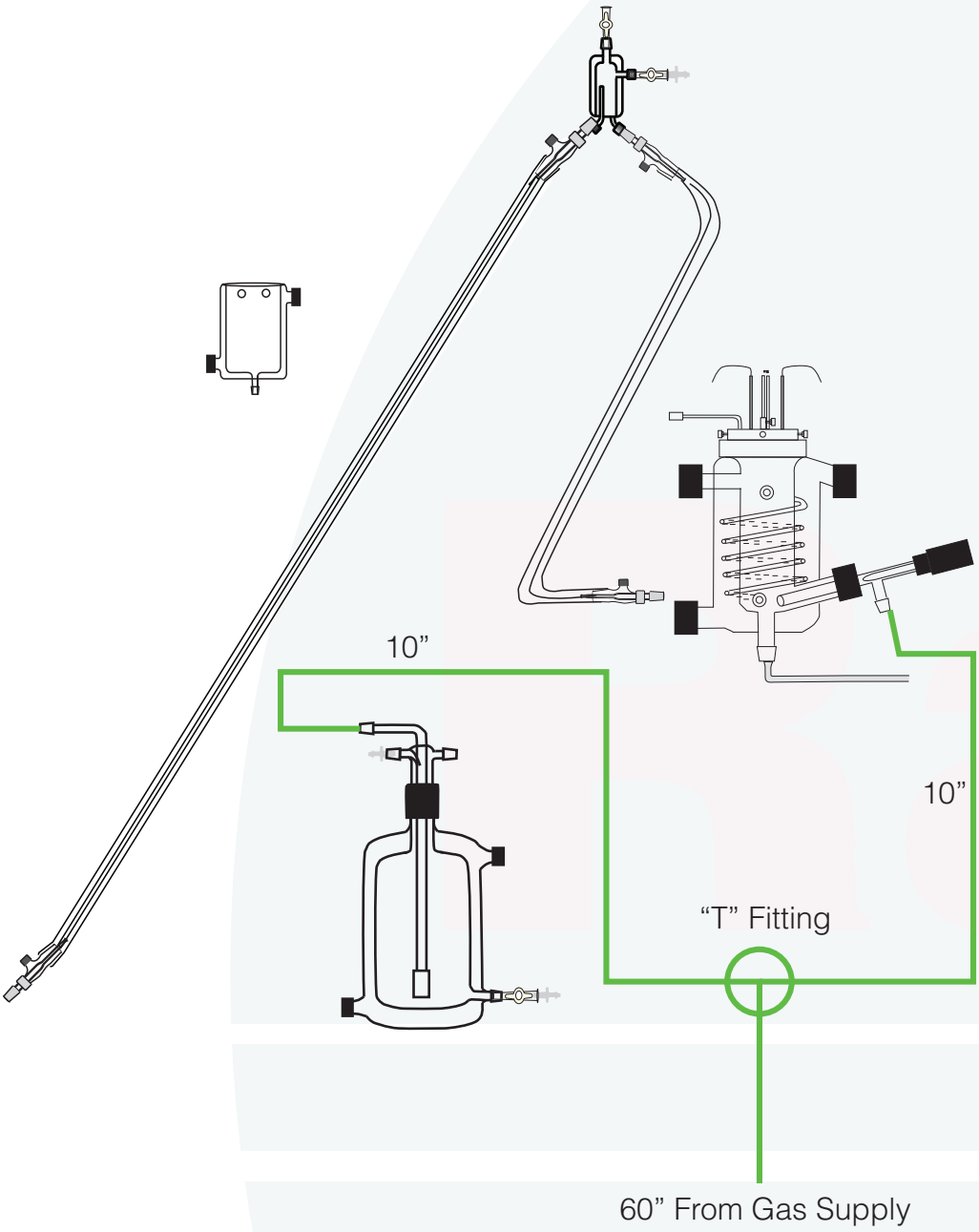
Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

Connecting the Gas Tubing



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Radnoti

www.radnoti.com info@radnoti.com

Radnoti Micro Pressure Transducer

Remove protective Luer Cap before using Sensor

Connect 8 pin power supply connection to amplifier.
Connect output BNC connector to recording device.
Remove protective cover from sensor.
Plug sensor connector into amplifier.

Calibration technique 1:

Make sure to let the amplifier warm up for about 10 minutes before calibration.

The output of the amplifier is 0 to about 3.2 volts full scale. This allows for precise calibration of 10mv/mmHG.

1. Connect a pressure calibration bulb and gauge to the pressure transducer.
2. Turn the Gain control fully clockwise.
3. Zero the amplifier to about 000 on the readout using the Offset control.
4. Inflate the system to 150mmHG and hold.
5. Turn the gain control until the display shows 150.
6. Deflate to zero and readjust to 000
7. Inflate again to 150mmHg and readjust Gain.
8. When pressure is at zero the readout should show 000 and when at 150 the readout should show 150.

Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

Calibration technique 2:

If a monometer is not available for calibration or you wish to simply calibrate on the system you can perform the calibration with a known pressure head.

1. Open the transducer to atmosphere and set zero as in calibration technique 1.

2. Close the transducer to atmosphere and open to known fluidic hydrostatic pressure head.

The pressure head can be determined by the difference in elevation between the sensor and the open highest point of the hydrostatic pressure head. Pressure is calculated as 13.64mm of elevation = 1mmHg of pressure.

Example: if the distance from the sensor and the hydrostatic hipoint (overflow of bubble trap) is 682mm then the pressure would be 50mmHg (682/13.64=50)

It is important that there not be a fluid path bellow the sensor as it will draw a negative pressure equal to the elevation difference of its outlet and the sensor.

Please Note:

The tip of the fitting is filled with a pressure transferring gel. It is important not to disturb this gel as it may degrade the performance of the transducer.
It is very important not to drop the fitting on its open end. At the tip of the fitting there is a small glass housing that may be damaged.

Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784

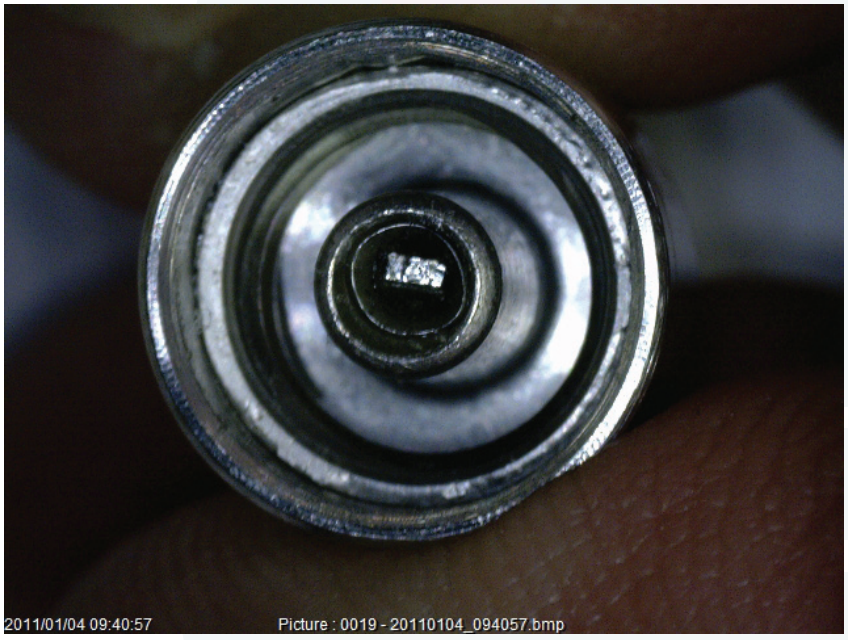
Radnoti

www.radnoti.com info@radnoti.com

Radnoti Micro Pressure Transducer

DO NOT TAMPER OR REMOVE PRESSURE TRANSFERRING GEL

IMAGE BELOW SHOWS GEL REMOVED



Radnoti LLC
227 W. Maple Ave.
Monrovia, CA 91016
(800) 428-1416 (626) 357-8827
Fax. (626) 303-2998

Radnoti Ltd.
Hilton House
3 Ardee Road
Rathmines Dublin 6 Ireland
+353 1 524 2111Fax. +353 1 443 0784