Model # 2PK+
INSTRUCTION MANUAL

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Introduction

The **2PK+** is designed to enable the perfusion of whole cells, patch pipettes and sharp electrodes during electrical recordings and/or imaging studies. The key element in the success of the technique is the inclusion of a quartz microperfusion capillary. The quartz capillary is coated with polyamide and is extremely flexible and durable. Its durability allows it to be positioned near the tip of the recording pipette. Quartz has excellent low noise characteristics which helps to limit the amount of noise introduced during perfusion. This insures that high quality recordings can be obtained even as perfusion is taking place.

Perfusion in the 2PK (Pipette Perfusion Kit), the predecessor to the **2PK+**, was achieved by simply increasing the amount of negative pressure applied to the patch pipette, This caused the perfusion solution to be drawn into the patch pipette after the negative pressure reached a sufficient level to overcome the restriction to flow caused by the ID of the quartz microcapillary. A precision vacuum control unit was employed so that the flow could be achieved without destruction of the cell or the patch.

In order to be able to perfuse whole cells, increasing suction would cause the contents of the cell to be pulled into the pipette possibly clogging the tip of the pipette. To overcome this obstacle, the 2PK+ uses positive pressure and a pressure vessel that permits fluid to be injected into the recording pipette under pressure. The cell can be protected by leaving the pipette open to atmosphere. Conversely, negative pressure can be applied to neutralize the positive pressure so that the cell experiences no net change in pressure.

The process of cell perfusion is achieved when the lumen of the recording pipette is open to the interior of the cell. Fluids and other objects are free to pass between the cell and the pipette and visa versa. There is a natural tendency for fluid in the pipette to mix into the cell. However, the 2PK+ assures the mixing by setting up a stream of solution that flows from the perfusion capillary into in recording pipette and then out into the cell. The increased volume of fluid can be displaced up the pipette. Since such a small volume of fluid is transferred for a particular perfusion event, many perfusions are possible before the pipette is backfilled to the rim. Also it can take as long as an hour for a previous solution to back diffuse down the pipette (see **Reference Articles**).

With the 2PK+, it is possible to perfuse whole cells, patch pipettes and sharp electrodes by changing the recording media in the pipette and the internal milieu of the cell. In some instances it may be as though there is an IV directly into the cell whereby the researcher has continuous access to the interior of the cell for the introduction of agonists, antagonists and fluorescent dyes.

Parts List

☐	Pressure/Vacuum Generator	
☐	9 Volt DC wall power supply	☐ 120 ☐ 230
☐	Perfusion Pipette Holder	
☐	Accessories kit	☐ Pressure Vessel (1)
		☐ 3 reservoirs 0.125ml
		☐ Tube and double luer valve assembly (1)
		☐ PE tube (2 pieces of PE-10, 1' long)
		☐ Silver wire (3 pieces, 2" long)
		☐ Butane torch (1 with instructions)
		☐ Sand paper (1)
		☐ 30 ga needle (4)
		☐ Vacuum grease (1)
		☐ Quartz tubing (20 pieces, 100 micron ID)
		☐ Beem capsules (10 large, 10 small)
		☐ Alligator clips (4)
		☐ Polystyrene block (1)
		☐ Input hose (2 pieces 1/8" id TYGON tubing)
☐	Instruction Manual	
	Options	
☐	MRC-6 with 3 reservoirs	

Components

1) Pressure / Vacuum Generator

Used to give precise control of pressure and vacuum for perfusion. It consists of two precise 20 turn pressure regulators and an internal input regulator set to 15 PSI. An LCD display indicates the pressure being monitored (in mmHg) as selected via the mode switch. Pressure and vacuum cannot be monitored simultaneously. Pressure and vacuum readings can only be taken when the system is hooked in with the other components. The vacuum side should not be left open to air when on. This may introduce contaminants such as dust that will decrease performance. A five micron filter is incorporated in the vacuum line to prevent damage to the system in event the pipette is damaged and fluid is sucked back.

Vacuum is created by a venturi pump (positive air flow creates vacuum). The vacuum pressure regulator adjusts the air flow into the venturi which regulates the vacuum pressure. Both vacuum and pressure are measured.

When the unit is in operation it will sound like there is an air leak as it always makes a hissing noise. The higher the vacuum/pressure setting the louder the hiss. This is part of normal operation.

The vacuum pressure generator is designed to produce at least 55 mm/Hg of pressure and vacuum respectively (The pressure side can go higher but it is not recommended for safe operation of the generator). This can give an effective pressure difference to the perfusion solution of 100 mm/Hg. Enough to make it fly into the pipette!

2) Power Supply

This is a 9V DC wall adapter used to power the pressure/vacuum generator.

3) Perfusion Pipette Holder

The perfusion pipette holder is specifically designed for this purpose. It is made of polycarbonate and is available in different configurations to match any amplifier or pipette size (customer specified). Just like a standard patch pipette holder it has a port for a suction tube. It also has an additional port where a PE tube can enter and easily access the pipette lumen. The PE tube can be locked in to keep the holder sealed.

4) Positive Pressure Vessel

The positive pressure vessel is used for whole cell perfusion. It allows the user to push rather than suck solutions into the pipette. For whole cell perfusion, adding suction to the pipette might cause the pipette tip to clog. The pressure vessel has a port for pressure to enter and another one for the PE tube that carries the perfusion solution to the pipette. The perfusion solution is sealed into the micro vial at its bottom. Solutions can be changed simply by unscrewing the vial and screwing in another. The volume is deliberately kept small since so little is required to perfuse a cell and since some substances are considered precious. As an option, the ***MRC-6*** can be used with the positive pressure vessel to provide for multiple solution changes. See the MRC literature & manual for more information.

5) Output Hose and Luer Valves

This is the required assembly to connect the perfusion pipette holder and the positive pressure vessel together with the rest of the system. The luer valves are used to divert pressures toward and away from components and also to open the system to atmosphere or connect to mouth or syringe suction.

6) Silver Wire

It is used to provide an electrical connection from the pipette to the head stage of the amplifier. It should be chlorided before use.

7) Polyethylene Tubing - (PE)

Thin polyethylene tubing is used for the linkage of the quartz capillary to the positive pressure vessel. PE tubing provides an inert and low volume transfer pathway for perfusion solutions. Its narrow ID means that dead volumes are kept to a minimum when solutions are changed.

8) Sand Paper

Used for one particular technique for removing carbon debris from the quartz capillary after melting. It can also be used to bevel the tip of the quartz perfusion capillaries.

9) 30 Gauge Needle

Thirty gauge needles are provided to enable the priming of the perfusion tube (PE tube) and the quartz capillary. This assembly must be filled with the pipette filling solution before a seal is made. This prevents air from entering the pipette and also prevents premature introduction of the perfusion solution.

10) Vacuum Grease

It is used to enhance various seals on the pipette holder and positive pressure vessel if necessary.

11) Quartz Tubing

Quartz tubing is used for the perfusion canula to carry the perfusion substance down through the pipette to the tip. The quartz is provided in raw form and can be pulled and cut before use. It is coated with polyamide and thus has a brown appearance. As long as the polyamide coating is present, the quartz is extremely flexible.

12) Beem Capsules

Used for holding solutions for patch pipette perfusion.

13) Polystyrene Block

A Polystyrene block is used to hold the positive Pressure vessel or the Beem capsules during experiments. It provides a convenient way to hold these small components near the perfusion pipette and also provides valuable electrical insulation to further reduce noise.

14) Additional Reservoirs

Three additional reservoirs are provided for the positive pressure vessel. They allow for quick change of solution.

15) Alligator Clips

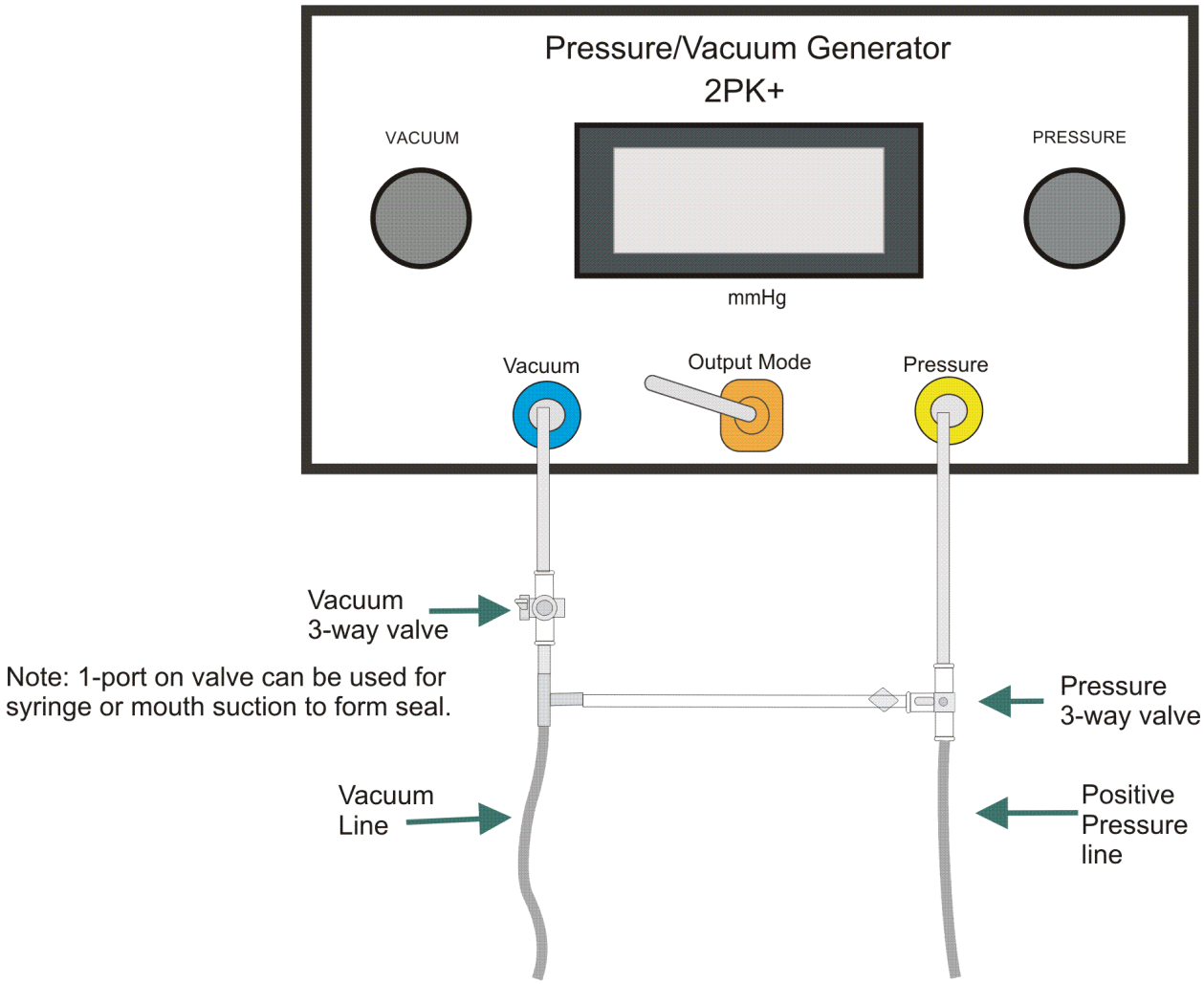
These are used for pulling the quartz capillaries. One may be used to pin the quartz up while one, two, or three are used to weigh down the other end.

16) Butane Torch

The butane torch provides a small size flame of adequate temperature to melt the quartz tubing at around 700^0C. The torch will run for about 15 minutes on a full charge. It can be recharged using regular butane that is used in cigarette lighters. Torched is shipped empty for safety reasons.

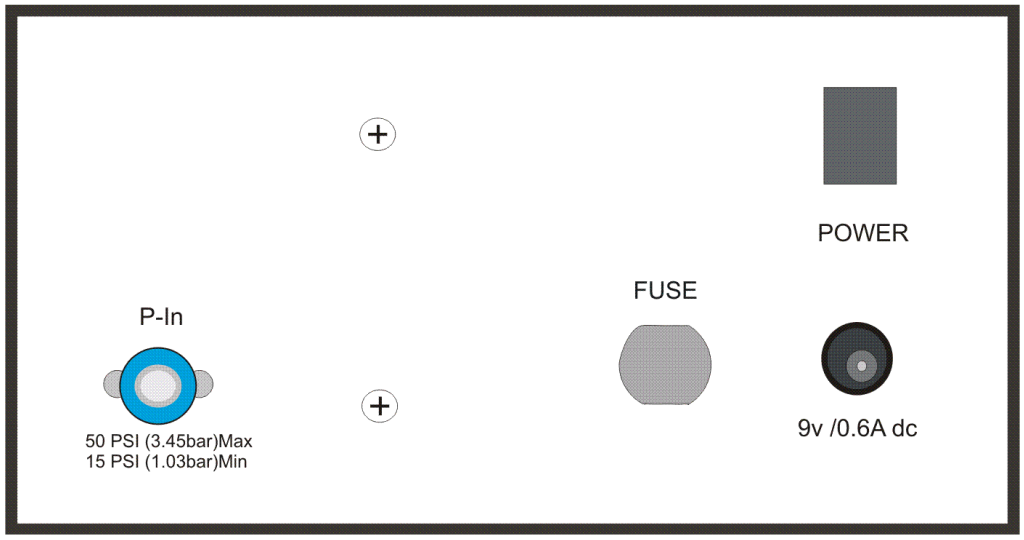
Set-up

Upon opening the kit, first check to be sure that all parts on the check list are included. If any part is missing or damaged contact the supplier immediately.



The tubing and luer valve assembly must be connected to the front of the pressure/vacuum generator (PVG). Connect the assembly as shown in the above diagram.

The *Vacuum Line* will get connected to the pipette holder side port. The *Positive Pressure line* will connect to the ***Pressure Vessel*** when performing whole cell perfusion.



The rear panel of the 2PK+ Pressure/Vacuum Generator has 2 connections.

1. ***Power In*** - Connect the 9V dc wall adaptor power supply to this port.

2. ***Input Pressure*** – Connect positive pressure either from a tank or house air. The pressure used must be between the stated pressure ranges. The recommended pressure value is 25PSI.

Before air or gas is supplied, test the unit by turning on the power. With no pressure supplied the pressure on the LCD should be zero or below +/- 1.0 mmHg. The power switch is a push-in and lock type. Power should be turned off when the unit is not in use. It is also a good practice to turn off the air supply to the unit when it is not required.

The vacuum line should not be left open while vacuum is on. This can cause dust and debris to enter the vacuum mechanism and reduce performance.

Quartz Capillary Preparation

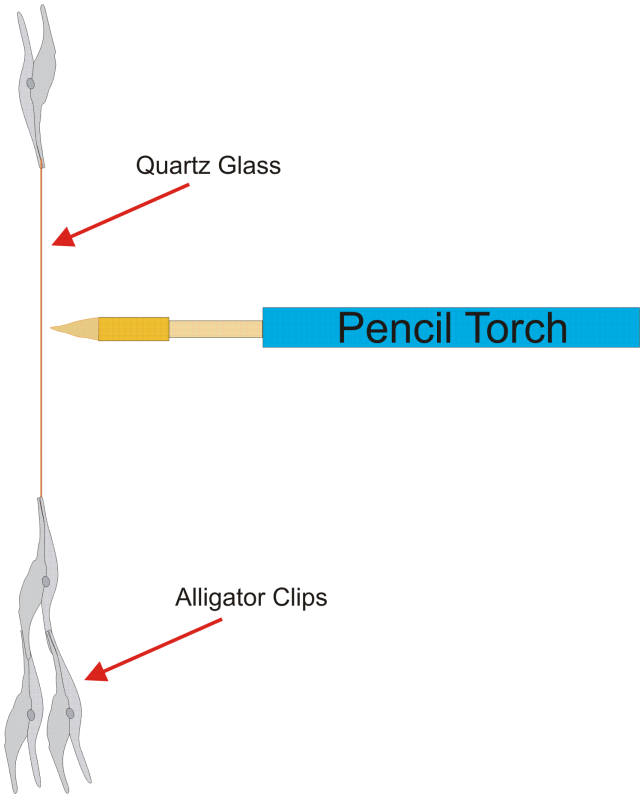
The quartz capillary (supplied) necessary for perfusion can be used as is or can be made to fit further down the pipette electrode. The quartz capillary supplied is 100um ID and 230um OD. If used as is, the quartz capillary will not be able to get close to the cell as possible. To get close to the cell and speed up the response rate the quartz capillary needs to be modified.

Below are instructions to prepare the quartz capillary to reach further down the pipette.

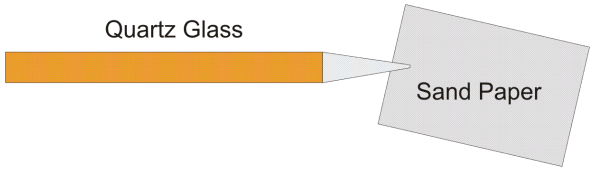
(Note: Many users find that the quartz capillary can be used without pulling the tip smaller. It is up to the user to determine if this method will work. The smaller pulled tip can fit further down the pipette, but once the polyimide is burned off the tip can break. The un-pulled tip is more robust, but will not fit as far down the pipette, it will give faster flow, however, than the pulled one.)

Remove a length of quartz tubing from the vial and suspend it vertically about eight inches (20cm) above a clean work bench using one of the alligator clips. Use one, two, or three other alligator clips to weight down the other end. Be sure that the capillary hangs straight and steady.

Using the butane torch at a high flame, heat the capillary at the midpoint until it melts and the lower end falls. Move the flame away quickly when this happens so as not to destroy the upper strand. The polyimide will burn away and reveal the quartz glass underneath. The exposed quartz is very hard and brittle.

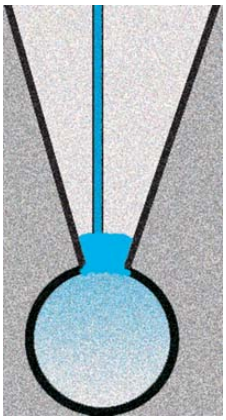


The carbon on the pulled part of the quartz must be removed. This can be done with a small piece of sandpaper or a micro knife. A dissecting microscope is very helpful. In addition, the capillary can be cleaned with a sonicator and methanol. Finally, the tip of the quartz is cut back using a pair of scissors, wire cutters or other implements. The final tip size should be no less than 35µm. Larger sizes can be experimented with. With the advent of the positive pressure vessel, it may be possible to perfuse without pulling the quartz capillary. However the small size of the tip will help place it further down into the pipette tip. It should not be necessary to make a fresh quartz capillary for each experiment. One capillary should last through several experiments before requiring a change.



Whole Cell Perfusion Set Up

For an experiment in which whole cell perfusion is to be performed, the 2PK+ must be assembled as follows:



1. Connect the positive pressure line to the pressure vessel port.
2. Connect the vacuum line to the pipette holder barb port.
3. Connect the pipette holder to the headstage.
4. Place the PE-10 tubing down the 30^{O} port on the holder until it is even with the silver wire.
5. Place a quartz perfusion capillary made in accordance with the directions above, into the PE tube adjacent to the silver wire.
6. Adjust the perfusion capillary by pulling and pushing the PE-10 tube at the 30^{O} port until its tip is just ahead of the silver wire.
7. Using a thirty gauge needle and syringe, fill the PE tube/quartz capillary with the pipette filling solution.
8. Place the recording electrode over the quartz capillary and silver/silver chloride wire and seal it into place with the thumb screw.
9. Use the PE tubing at the 30^{O} port to push the quartz capillary down to the tip of the pipette. The tip of the capillary should sit at a point where its tip is roughly half the diameter of the patch pipette. Tighten the 30^{O} port to hold the perfusion capillary in position and to maintain a good seal (add vacuum grease if necessary). The rubber gasket of the 30^{O} port can be sealed on the quartz tube, the PE tube or the interface of the two. Be sure it is very tight when sealing on the quartz tube. Here vacuum grease should be employed for a good seal. In general, never let the PE tube make contact with the solution in the recording pipette.
10. Prepare solutions for perfusion in the reservoirs for the positive pressure vessel. You should begin with the pipette filling solutions. As experience is gained it may be possible to start with a test solution as long as the PE tube and perfusion capillary are first filled with the pipette solution just in case there is any pre-flow.
11. Seal the reservoir into the positive pressure vessel while the PE tube is still loose. This will prevent the pressure produced when sealing from starting premature perfusion.
12. Insert the PE tubing down the top of the Pressure Vessel until it is at the bottom of the reservoir. The PE tube connecting the pipette holder to the pressure vessel should be kept as short as possible. This speeds up perfusion and helps reduce stray capacitance which is a source of noise.
13. Set up the polystyrene block so that the positive pressure vessel can be placed in it and held there during an experiment.
14. Set the pressure 3-way valve with the OFF position toward the vacuum side.
15. Set the vacuum 3-way valve with the OFF position toward the unused port. The unused port can be used to form a seal using a syringe attached to it or by mouth suction.
16. The system is now ready to do a whole cell perfusion.
17. Form a seal to the cell as usual. Be sure that the positive pressure vessel is ported to vacuum as in the diagrams to prevent premature perfusion when going on-cell.
18. Rupture the cell membrane using current (tweaking the electrode) or increased suction. A syringe may be necessary for this, **DO NOT PORT HIGH PRESSURE FROM A SYRINCE TO THE 2PK+ PRES./VAC. GENERATOR AS IT MAY DAMAGE THE PRESSURE TRANSDUCER.**

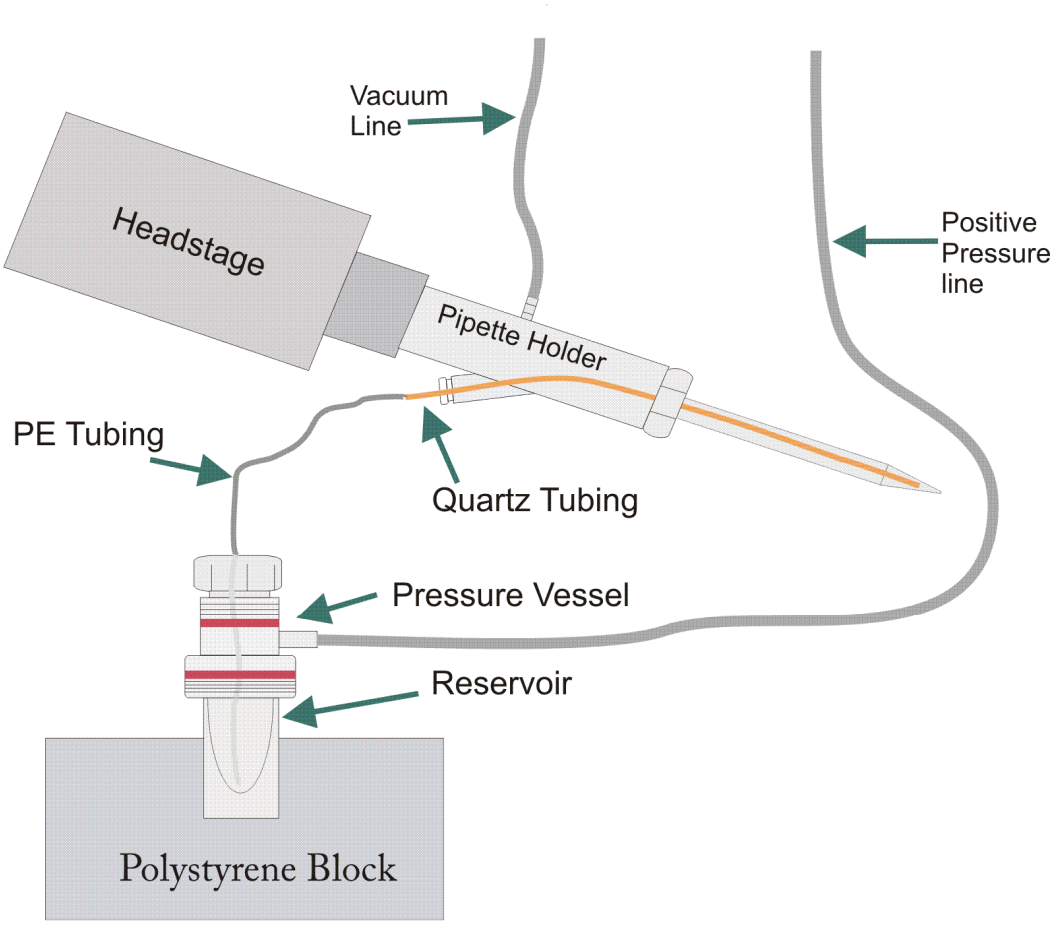
19. To perfuse whole cells, open the vacuum side of the pipette holder to atmosphere and increase positive pressure on the pressure vessel (Be sure to use proper valve configuration --see **Diagrams**). Positive pressure can also be offset using vacuum. Switch modes on the Press./Vac. Generator back and forth to supply exact opposite pressures at first. Be advised that the pressure that the perfusion solution experiences are equal to the absolute value of positive and negative pressures applied to the system. Example: +10 mm/Hg applied to the positive pressure vessel and 10mm/Hg applied to the suction port on the pipette holder gives the perfusion solution an effective pressure force to enter the pipette of 20mm/Hg. Using the 2PK+ at

its maximum, an effective pressure gradient of over 100mm/Hg can be achieved! In some cases it may be necessary to balance pressures, in other cases simply opening the pipette to atmosphere is enough. 10-20mm/Hg should be enough to start flow.
Please note: (1) With time, the user typically develops a feel for his/her particular set up. (2) Rarely will positive and negative pressures be matched for perfusion to take place. This it is a good starting point for first time users. The user may find that perfusion takes place with a few mmHg of positive pressure and two or three times the amount of negative pressure or vice versa. It must be discovered empirically since each experimental set up is different.

20. Be sure to neutralize all pressures after perfusion has taken place so as not to over fill the recording pipette or introduce air bubbles. To neutralize, open the pipette holder suction and the positive pressure vessel to atmosphere using the luer valves.
21. When changing reservoirs on the positive pressure vessel be sure that there is no suction being applied. Also be sure the PE tube is loose and the positive pressure line is open to atmosphere at the luer valve so that premature perfusion does not take place.

    Those who need to maintain some suction on the pipette to hold the seal should take special care to adjust the system to allow this without causing perfusion.

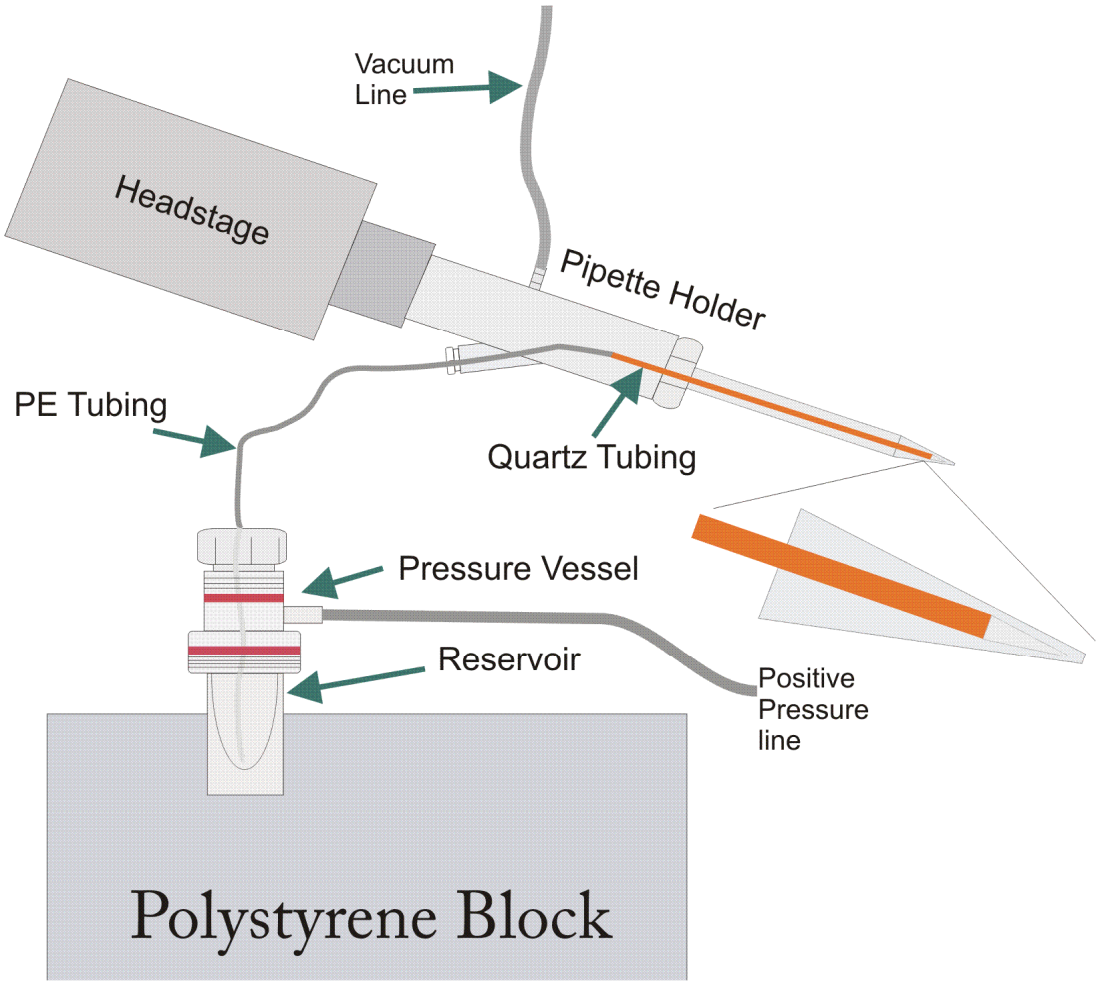
Whole Cell perfusion Setup with unprepared Quartz Capillary



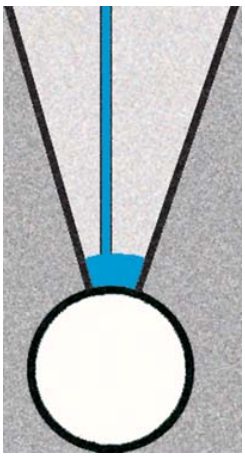
The above diagram shows the system used with the quartz tube in its original size (not prepared).The quartz tube has a 100um id and will allow for higher flow rates when used in this manner. However, this also impedes the quartz tube from getting close to the cell as the pipette narrows at the end. Being closer to the cell increases the response rate.

The diagram below shows the system used with a prepared quartz tube. As you can see the quartz is able to go further down the pipette when the quartz tube is pulled and a fine tip is formed.

Whole Cell perfusion Setup with prepared Quartz Capillary



Perfusion Patch Pipettes



The set up for pipette perfusion is similar to the set up for whole cell perfusion except it is a little simpler. The positive pressure vessel and the positive pressure side of the 2PK+ does not need to be used, however it can be incorporated once practice is gained in the technique.

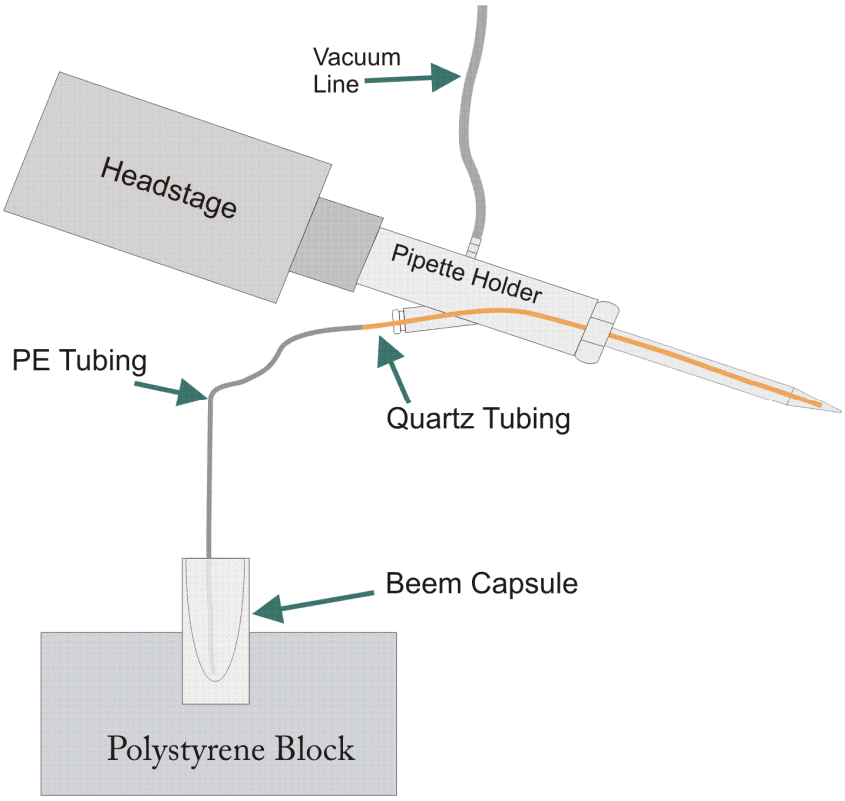
For pipette perfusion, set up the perfusion pipette holder as below and lead the PE tube to the polystyrene block where it will sit in a Beem capsule of solution, since only suction is necessary for perfusion.

1. Prepare materials for perfusion in a small Beem capsule and place it in the polystyrene block holder.
2. Connect the pipette holder to the headstage.
3. Place the PE-10 tubing down the 30^{O} port on the holder until it is even with the silver wire.
4. Place a quartz perfusion capillary made in accordance with the directions above, into the PE tube adjacent to the silver wire.
5. Adjust the perfusion capillary by pulling and pushing the PE-10 tube at the 30^{O} port until its tip is just ahead of the silver wire.
6. Using a thirty gauge needle and syringe, fill the PE tube/quartz capillary with the pipette filling solution.
7. Place the recording electrode over the quartz capillary and silver/silver chloride wire and seal it into place with the thumb screw.
8. Use the PE tubing at the 30^{O} port to push the quartz capillary down to the tip of the pipette. The tip of the capillary should sit at a point where its tip is roughly half the diameter of the patch pipette. Tighten the 30^{O} port to hold the perfusion capillary in position and to maintain a good seal (add vacuum grease if necessary). The rubber gasket of the 30^{O} port can be sealed on the quartz tube, the PE tube of the interface of the two. Be sure it is very tight when sealing on the quartz tube. Here vacuum grease should be employed for a good seal. In general, never let the PE tube make contact with the solution in the recording pipette.
9. Lower the pipette into the bath and place the end of the PE tube into the Beem capsule containing the first solution to be perfused.
10. Patch the cell as normal. If you are using the positive pressure vessel, be sure to port suction to it as well so that there will be no pre-flow of solution.
11. When ready for perfusion simply turn over suction control to the vacuum generator on the 2PK+. Increase vacuum on the pipette holder. 20-30 mmHg should be enough to cause perfusion. Use more if necessary. If using the positive pressure vessel, increase pressure to the positive pressure vessel, but be sure to balance it off with at least 10 mmHg greater negative pressure to maintain the patch. **IT SHOULD BE NOTED THAT POSITIVE AND NEGATIVE PRESSURES USED IN CONJUCTION WILL ALMOST NEVER BE EVEN**. One may find that 10 mmHg suction might be balanced by 40mmHg pressure or vice versa for example. These levels must be determined empirically in each set up.
12. When perfusion is to be stopped, return the system to normal suction pressure for maintaining the patch. If using the positive pressure vessel, port suction (negative pressure) back to it to prevent additional filling of the pipette.

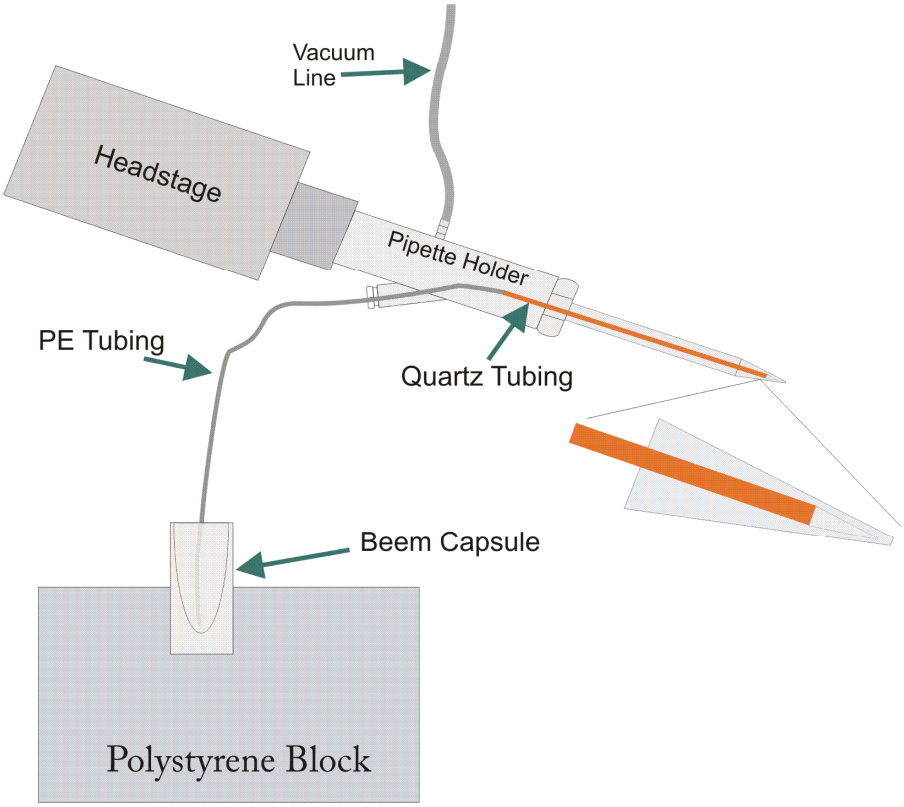
Perfusion solutions can be changed anytime perfusion is stopped. Depending on the rate of perfusion, they might be able to be changed without introducing an air bubble. Perfusion capillaries should be changed as often as needed. They should last through at least a week's worth of experiments.

Caution should be used when changing solutions so as not to introduce air bubbles. It is a good idea to open both sides of the system to atmosphere giving zero flow. It may be possible to change solution under low suction (if necessary to maintain a seal) without introducing air bubbles if one works quickly. Again, individual factors related to the whole experiment will determine this. It is a good idea to practice changes under suction to see if the outcome is satisfactory.

Patch perfusion Setup with unprepared Quartz Capillary



Patch perfusion Setup with prepared Quartz Capillary



Helpful Hints

- Try to use filtered solutions for perfusion. De-gas all solutions slightly before use by gentle warming.

- Either pressure or vacuum can be monitored at a time. It is not possible to monitor both simultaneously. The mode switch will not introduce bias to the pressure measurement.

- The 2PK+ is designed to measure down to +/- 0.1 mmHg. It can be affected by changes in atmospheric pressure, i.e. weather or wind conditions. As a rule we claim that the pressure measurement is accurate to 0.5mm/Hg. As the measuring capability is 0.1mm/Hg in fact greater accuracy is possible, but we feel that measurement of less than 0.5mm/Hg should be used as a reference.

- The advent of the positive pressure vessel may obviate the need to pull the quartz capillary to a fine tip -- this should be determined empirically. Bear in mind that the smaller the tip of the perfusion capillary, the closer to the tip of the recording pipette it can be placed.

- Shutting off the instrument does not stop gas flow. Turn off gas at the source when the unit is not in use.

- Perfusion in both whole cell and pipette mode can take seconds to minutes. Times can be changed by altering perfusion capillary tip size, pressure/vacuum settings and PE tubing length. Other factors like cell size, type, and temperature have a great effect on perfusion speeds.

- Dozens of perfusions are possible before backfilling the pipette to the suction line. Longer/larger pipettes should be used for long and complex experiments. ALA Scientific can provide perfusion pipette holders with dual 30^{O} ports but we do not recommend them since an additional tube in the recording pipette will add more noise.

- Be sure to keep the pipette holder clean. Once a week it should be washed in distilled water and methanol and dried in a desiccator. The same is true for the positive pressure vessel. Both items are made of polycarbonate, so that frequent cleaning is necessary for low noise recordings.

- To perfuse sharp electrodes we recommend using the whole cell approach. The sharp electrode may be less efficient for actual whole cell perfusion due to its small tip diameter. However, it should be possible to perfuse the electrode.

Limited Warranty

ALA Scientific Instruments agrees to warranty this product for one year from the date of shipment. Said warranty covers all parts and labor necessary to remedy defects in workmanship and/or materials. Coverage is limited to repair or replacement of parts. Items not covered under this limited warranty include external tubing, pipette holders, pressure vessel, butane torch, connectors, internal filters, luer valves and all disposable parts. All units returned to ALA Scientific Instruments for repair, whether under warranty or not, must be returned freight prepaid. Freight collect will be refused and will result in longer repair times.

ALA Scientific Instruments Inc. assumes no liability for damage to or resulting from the use of this product including damage to other equipment, personal property and persons using this instrument. The user is responsible for using this instrument in accordance with this manual for the intended purpose of internal pipette and whole cell perfusion. Usage that is inconsistent with this intent may result in forfeiture of warranty. Please consult ALA Scientific Instruments if considering alternative applications.

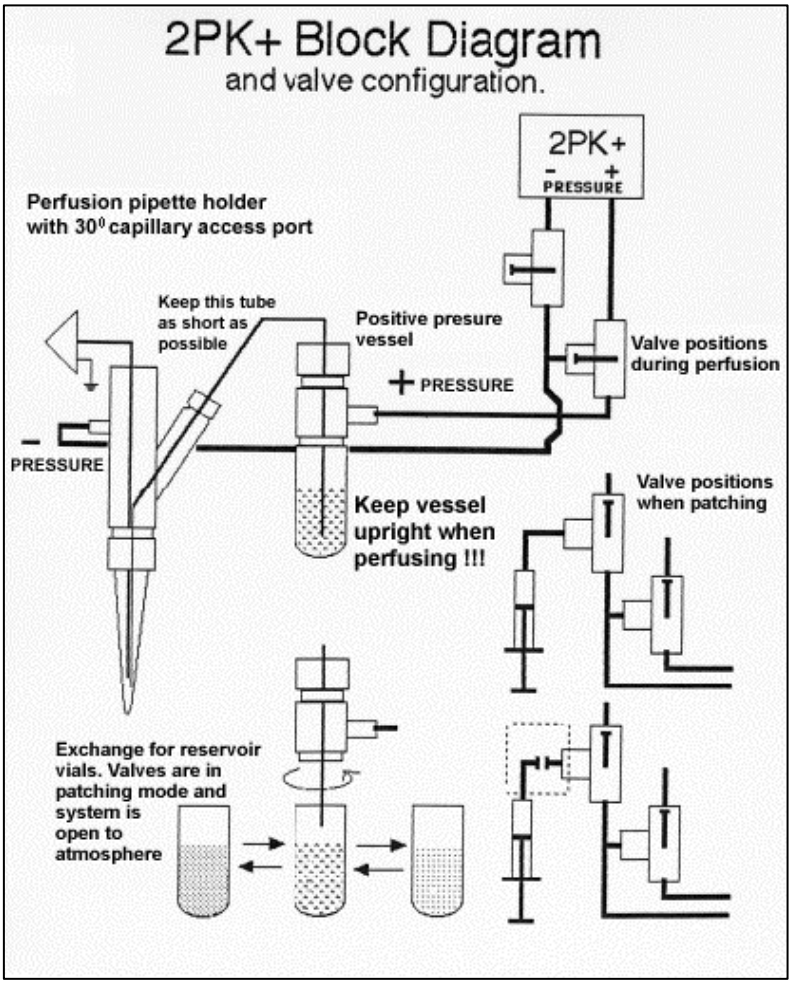
Please note: It is the buyer's responsibility to inspect this instrument upon receipt for possible damage that has resulted from shipping and to report any claim to the carrier within three business days.

If the unit is damaged in shipment it is the buyer's responsibility to file a claim with the carrier. ALA Scientific Instruments will assist as much as possible.

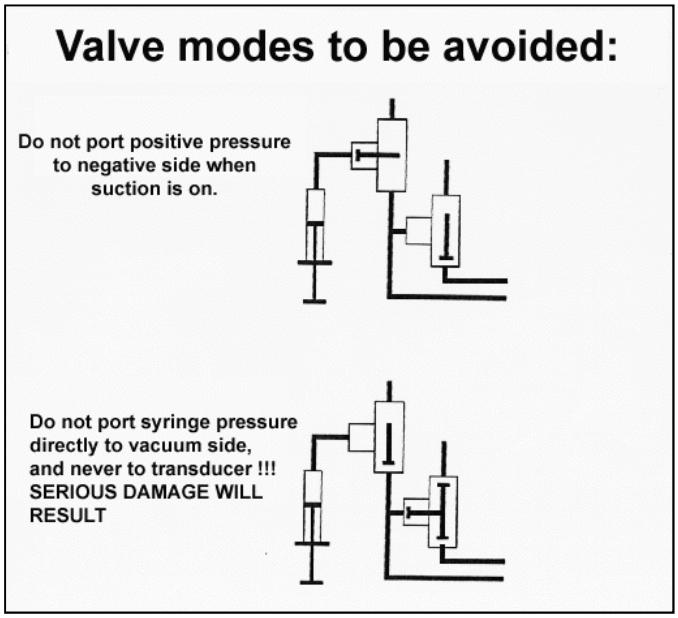
This instrument is designed for non-clinical use only!

Diagrams

Block Diagram



Valve Modes to be Avoided



Specifications

Wall Power Supply Output	9VDC/100mA (center = +)
Power Consumption	0.08W
MAX. Input Pressure	50 PSI (345 kPa)
MIN. Input Pressure	15 PSI (100kPa)
MIN. Output Pressure	0
MAX. Output Pressure	50 mmHg (6.66kPa)
MAX. Output Vacuum	-40 mmHg (-5.33kPa)
Input Pressure Connector	1/8in ID tube barb
Output Pressure Connectors	Luer lock
Dimensions	16 cm x 15.8 cm x 8.5 cm (LWH)
Weight	1 kg
Fuse	100mA (Located inside generator)

This instrument complies with all applicable standards for light industrial machinery at the European Union designated by the CE mark

NOTE on Pressure Supply:

A pressure pump producing 2-4ATM of pressure may be substituted for the air/nitrogen cylinder. The output of the pump must be filtered to be free of condensation so that moisture will not get into the 2PK+ and harm the regulators and pressure transducer.

Pressure Conversion Table

Conversion table for millimeters of mercury to kilopascals

mHg	kPa	mmHg	kPa	mmHg	kPa
1	.1333	28	3.733	55	7.333
1.5	.1999	28.5	3.8	55.5	7.4
2	.2666	29	3.866	56	7.466
2.5	.3333	29.5	3.933	56.5	7.533
3	.3999	30	4	57	7.6
3.5	.4666	30.5	4.066	57.5	7.666
4	.5333	31	4.133	58	7.733
4.5	.5999	31.5	4.2	58.5	7.8
5	.6666	32	4.266	59	7.866
5.5	.7333	32.5	4.333	59.5	7.933
6	.7999	33	4.4	60	8
6.5	.8666	33.5	4.466	60.5	8.066
7	.9333	34	4.533	61	8.133
7.5	.9999	34.5	4.6	61.5	8.199
8	1.067	35	4.666	62	8.266
8.5	1.133	35.5	4.733	62.5	8.333
9	1.2	36	4.8	63	8.399
9.5	1.267	36.5	4.866	63.5	8.466
10	1.333	37	4.933	64	8.533
10.5	1.4	37.5	5	64.5	8.6
11	1.467	38	5.066	65	8.666
11.5	1.533	38.5	5.133	65.5	8.733
12	1.6	39	5.2	66	8.8
12.5	1.667	39.5	5.266	66.5	8.866
13	1.733	40	5.333	67	8.933
13.5	1.8	40.5	5.4	67.5	9
14	1.867	41	5.466	68	9.066
14.5	1.933	41.5	5.533	68.5	9.133
15	2	42	5.6	69	9.2
15.5	2.066	42.5	5.666	69.5	9.266
16	2.133	43	5.733	70	9.333
16.5	2.2	43.5	5.8	70.5	9.4
17	2.267	44	5.866	71	9.466
17.5	2.333	44.5	5.933	71.5	9.533
18	2.4	45	6	72	9.6
18.5	2.466	45.5	6.066	72.5	9.666
19	2.533	46	6.133	73	9.733
19.5	2.6	46.5	6.2	73.5	9.8
20	2.666	47	6.266	74	9.866
20.5	2.733	47.5	6.333	74.5	9.932
21	2.8	48	6.4	75	10
21.5	2.866	48.5	6.466	75.5	10.066
22	2.933	49	6.533	76	10.132
22.5	3	49.5	6.6	76.5	10.2
23	3.066	50	6.666	77	10.266
23.5	3.133	50.5	6.733	77.5	10.332
24	3.2	51	6.8	78	10.4
24.5	3.266	51.5	6.866	78.5	10.466
25	3.333	52	6.933	79	10.532
25.5	3.4	52.5	7	79.5	10.6
26	3.466	53	7.066	80	10.666
26.5	3.533	53.5	7.133	80.5	10.732
27	3.6	54	7.2	81	10.8
27.5	2.796	54.5	7.266	81.5	10.866

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