

MYOGRAPH SYSTEM - 110PXL

USER GUIDE VOL. 3.6



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CHAPTER 1

1.1 INTERFACE

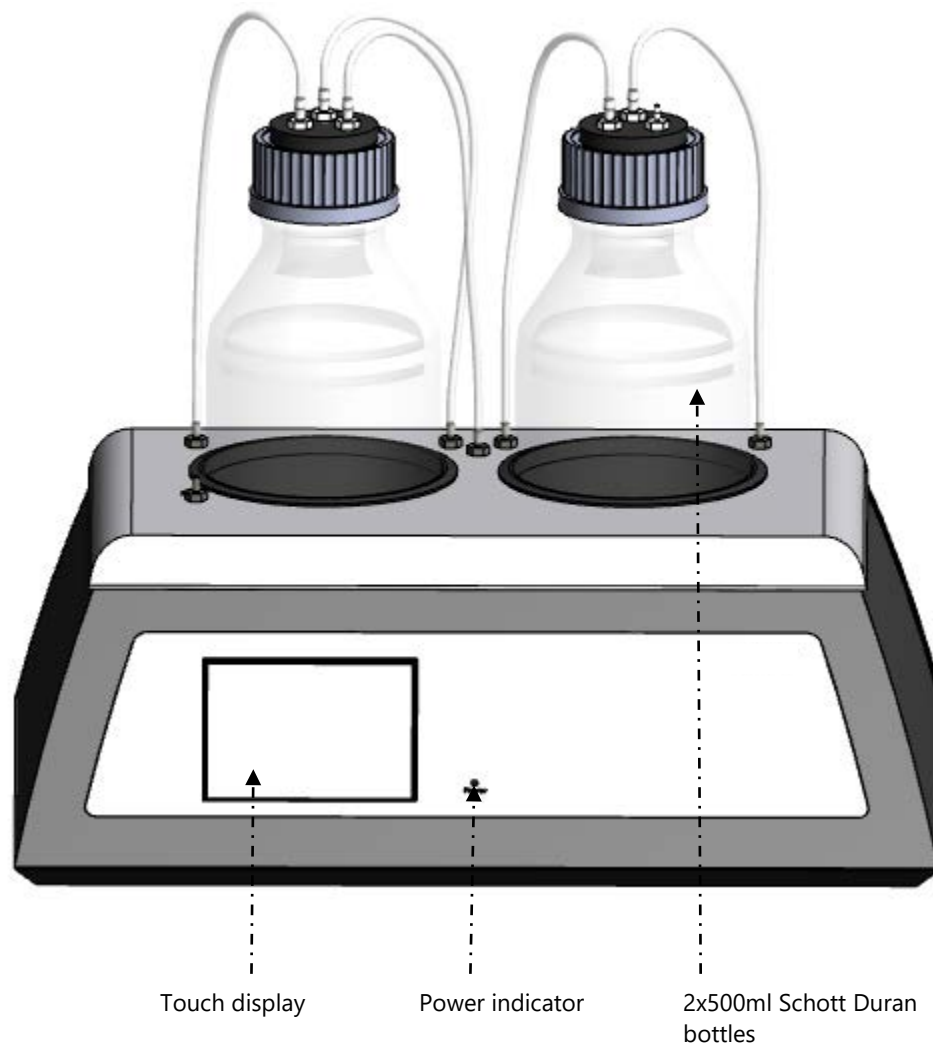


Figure 1.1 Pressure Interface - front

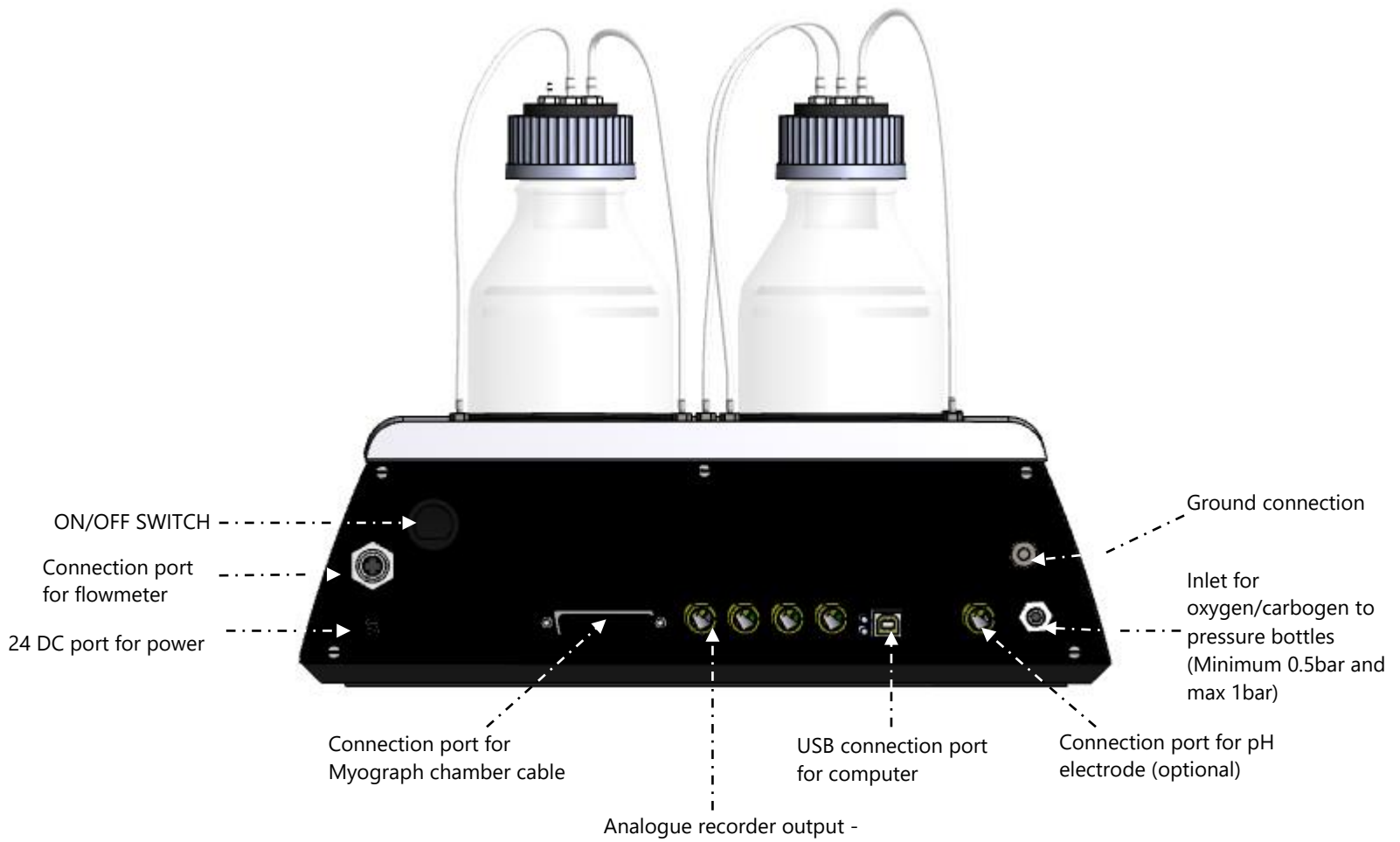


Figure 1.2 Pressure Interface - rear

1.2 110P-XL PRESSURE MYOGRAPH CHAMBER

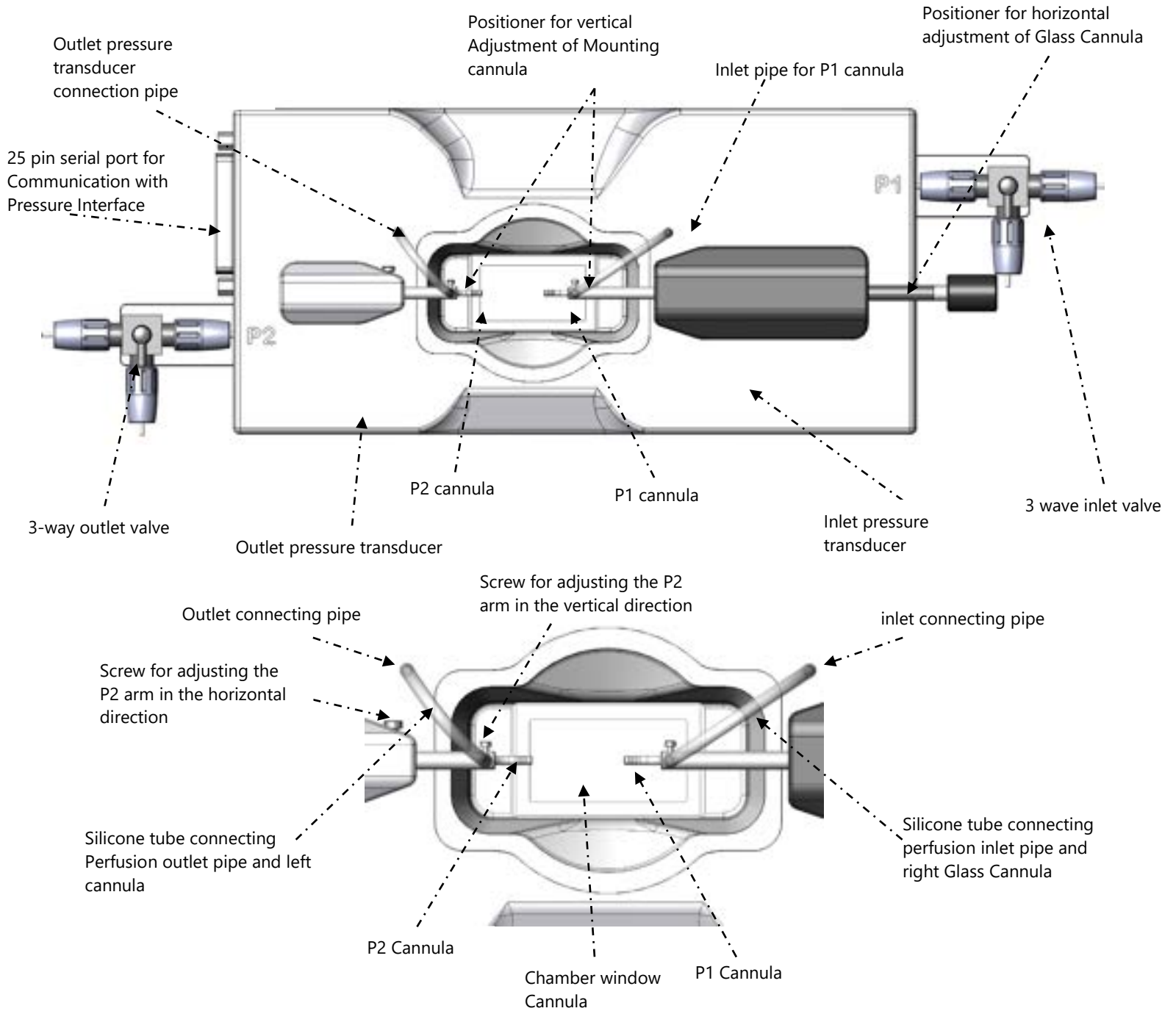


Figure 1.2 Pressure Myograph – 110P-XL with close-up detail of the chamber

CHAPTER 2 - SET-UP THE PRESSURE MYOGRAPH

2.1 ADJUSTMENT OF THE CANNULAS

It is important to make an alignment of the Mounting Cannulas prior to the mounting of the blood vessel.

Right Cannula P1 (see figure 2.1)

The horizontal micropositioner “A”, is used to define the distance between the two Cannulas and thereby the horizontal stretch of the mounted blood vessel. The vertical position of the P1 cannula is adjusted by loosening screw “B” (Figure 2.1). and thereby the vertical position of the mounted blood vessel.

Left Cannula P2 (see figure 2.2)

Horizontal positioning of the left Cannula P2 is adjusted by carefully loosening screw “B” (Figure 2.2). Vertical positioning of the left Cannula is adjusted by carefully loosening screw “A” (Figure 2.2)

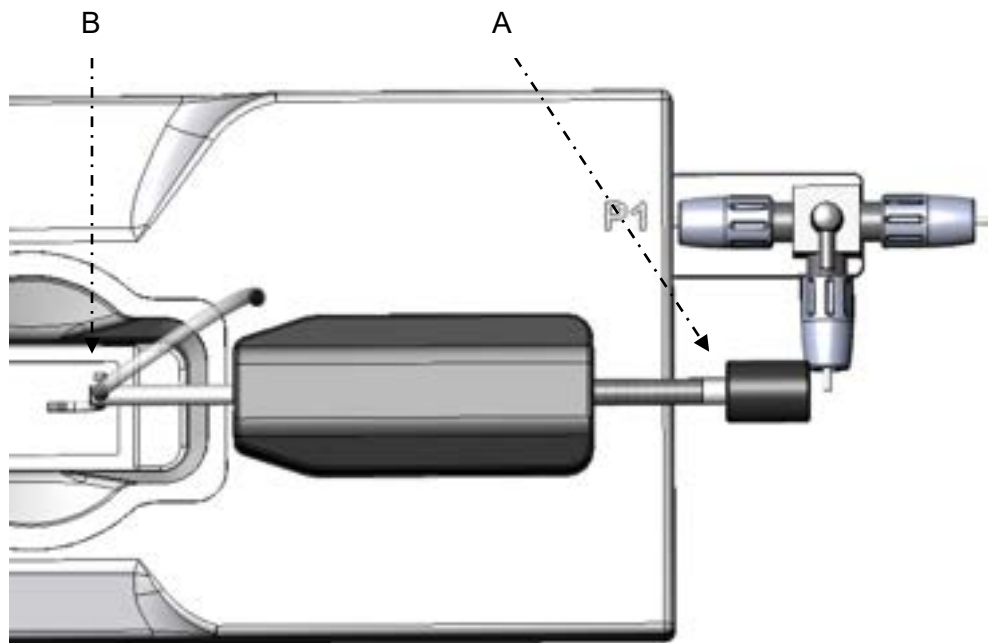


Figure 2.1 Adjustment of P1 cannula

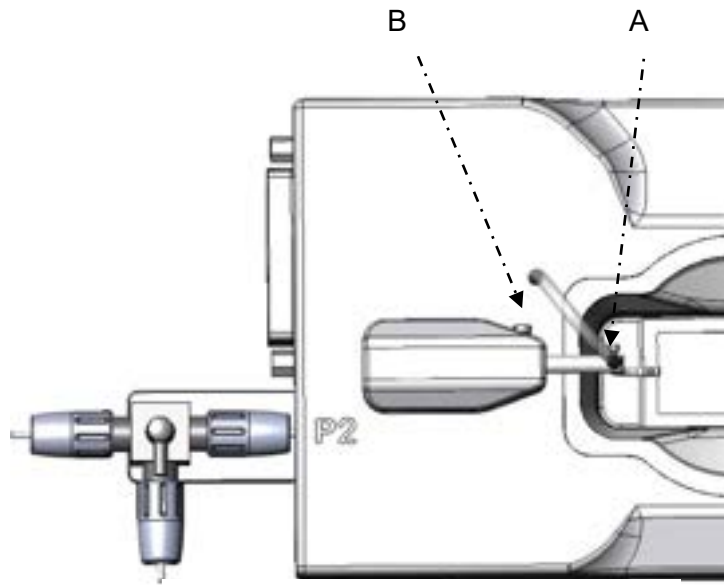


Figure 2.2 Adjustment of P2 cannula

2.2 3-WAY VALVE ADJUSTMENTS

The 3-way valves on each side of the Pressure Myograph chamber (at P1 & P2) have three different settings to control the in- or outlet flow to the pressure transducers. The flow settings are illustrated in figure 2.3 and figure 2.4 below.

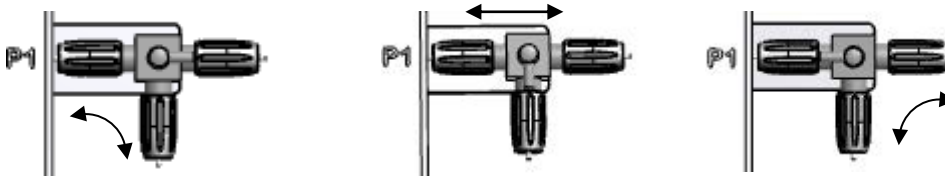


Figure 2.3 Three-way valve flow settings at P1. The arrows indicate the flow direction.

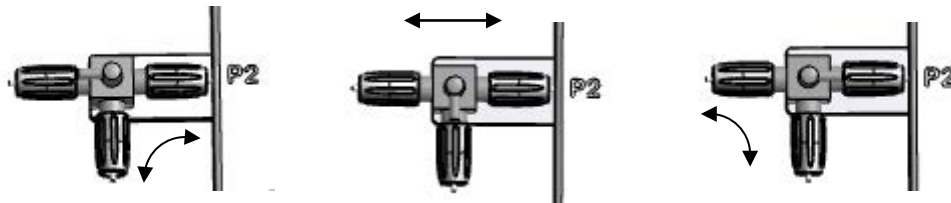


Figure 2.4 Three-way valve flow settings at P2. The arrows indicate the flow direction.

CHAPTER 3 - EXPERIMENTAL SET-UP

This section shortly describes the basic technique of mounting a vessel in the Pressure Myograph chamber.

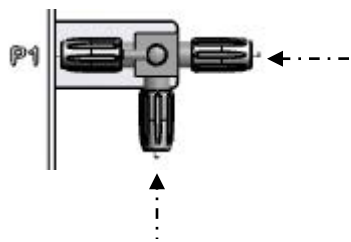
3.1 PREPARATION

1. Prepare your PSS. 60mM KPSS can be used to test contractions
2. Make at least 4 loops with silk suture (#400658) to use for securing the vessel to the mounting Cannulas. Single loops are enough to prevent the loops from slipping loose once tightened (Do NOT use double or triple loops for the suture).
3. Pre-heat PSS to 37°C, making sure the PSS is bubbled with carbogen (5% CO₂, 95% O₂) or PraxAir (5% CO₂, 21% O₂, balance N₂) for at least 20 to 30 minutes.
4. Fill the P1 reservoir bottle in the Pressure Interface with pre-warmed PSS. DO NOT FILL MORE THAN 300ML. Keep the other end of the tubing from the bottle, attached to port on top of the bottle marked P1, higher than the bottle, otherwise capillary action will draw buffer from the bottle and will leak all over the bench.
5. Purge the 110P-XL system with pre-heated (37°C) and gassed buffer (Appropriate running buffer) as described in the 110P-XL Pressure Myograph System User Manual (IMPORTANT STEP)

3.2 MOUNTING

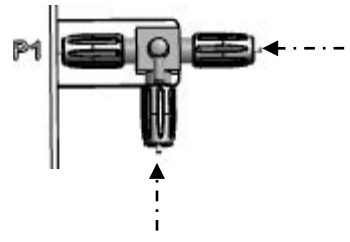
1. Isolate a vessel of interest.
2. Clean the vessel as best as possible of perivascular fat and connecting tissue.
3. Place the artery into a small container of cold or room temperature PSS. Fill 8ml of cold or room temperature PSS in the mounting chamber or enough to cover the cannulas.
4. Have a 20ml syringe with buffer attached to the P1 3-way valve, and the P1 and P2 3-way valves should be closed towards P1 and P2 cannulas.
5. Place 2 loops on each Cannula in case one breaks or fails. Position the sutures appropriately on both Cannulas so that they are ready for use when the vessel is mounted on the cannulas.
6. Carefully transfer the vessel from the small container to the 110P-XL chamber. Minimize handling and potential for damaging the vessel.

7. Continue to mount the vessel on the Cannulas. Start with the P1 side. Once the P1 side of the vessel is mounted on the Cannula, secure the vessel to the Cannula by tightening the two loops around the vessel onto the Cannula.
8. Make sure the vessel is mounted in the correct direction. Mount the proximal end on the P1 Cannula.
9. Once secured, gently rinse the vessel of any blood. Before washing the lumen, gently push some buffer from the syringe through the P1 3-way valve to the port that is not connected to the vessel to bleed out any air that might have been trapped in the 3-way valve.
10. Open the valve towards the vessel and gently push buffer through to wash out any blood in the lumen. If the chamber is connected to the interface, P1 and P2 pressure can be monitored not to exceed 150mmHg during the wash and prevent breaking the transducers. Close the P1 3-way valve towards the vessel to keep the vessel from collapsing.
11. Position the P1 Cannula with attached vessel to facilitate mounting of the vessel on the P2 Cannula.
12. Gently mount the vessel on the P2 cannula, without pulling it off the P1 cannula. Secure the vessel as described in step 8.
13. Once the vessel is secured on the P2 Cannula, open the P1 Cannula and very gently push more buffer into the mounted vessel. Look at the pressure Interface during this process to monitor P1 and P2 pressure not exceeding 150mmHG (remember to plug in the cable). The vessel should slightly fill and bulge from the small pressure-head. Close P1 3-way valve towards the vessel to maintain a slightly pressurized vessel.
14. Fill the tubing leading from the P1 reservoir bottle (marked P1 at the top of the bottle) with the buffer that has been pre-warmed and pre-bubbled. The easiest way to do this is to attach a syringe to the P1-3way valve as illustrated and draw buffer from the bottle. The 3-way valve should be positioned as shown in figure 3.1a.
15. Once the buffer is drawn through the tubing and no air bubble can be seen turn the 3-way valve as shown in figure 3.1b to close towards the syringe.



Connected to syringe – draw buffer from here

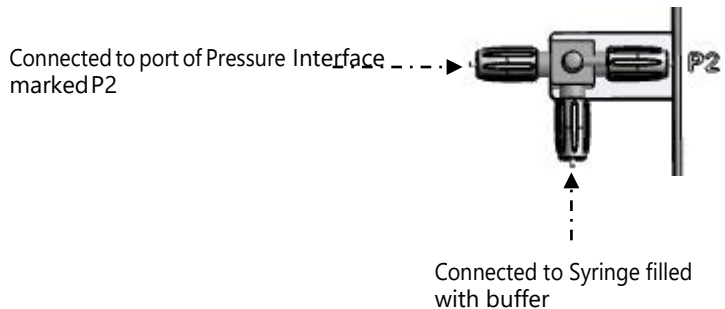
Figure 3.1a Filling tube from P1 to P1 3-way valve



Connected to syringe – draw buffer from here

Figure 3.1b After the tube has been filled and there is NO air inside, then close the 3-way valve toward the syringe.

16. Disconnect the syringe and move it to the P2 side of the chamber, but DO NOT EMPTY THE SYRINGE. Attach to the 3- way valve as shown.

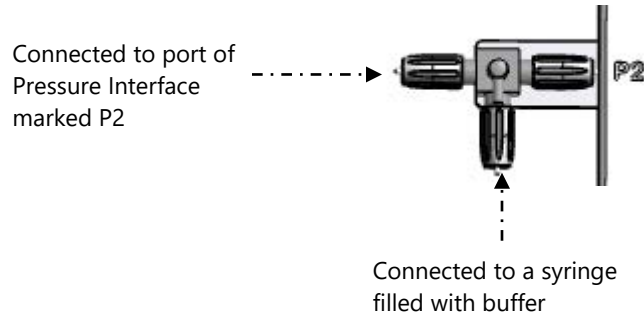


17. Make sure the Flow is turned ON before starting this step. Use the buffer that was drawn from the bottle to charge the remainder of the system. The buffer should be pushed GENTLY from the 3-way valve all the way to the waste bottle. When the buffer starts dripping in the waste bottle, stop pushing buffer.

IMPORTANT:

BE CAREFUL WHEN CHARGING THIS PART OF THE SYSTEM. EXCESSIVE PRESSURE CAN DAMAGE THE INTERNAL 3RD HIDDEN TRANSDUCER IN THE PRESSURE INTERFACE.

18. Close the 3-way valve toward the syringe as shown below.
19. The system is charged and ready to use.



3.3 EQUILIBRATION

1. Turn on the heat, which should be preset to 37°C.
2. Start aerating PSS in the chamber with oxygen
3. Set Flow OFF, Set pressure ON and set P1 to 20mmHg. Wait 5 minutes.
4. Increase the P1 pressure by 10mmHg every 5 minutes until target pressure is reached. These steps can be programmed into the MyoVIEW Sequencer to be performed automatically.
5. After the first 20 minutes after starting the heat and pressurization, remove the old PSS and replace it with new PSS that should be pre-warmed and oxygenated.
6. Continue to pressurize the vessel.
7. After 40 minutes from the start of heat and pressurization, do another wash, as in step 5.
8. Once 60 minutes have passed, the wake-up or standard-start protocol can be initiated.

3.4 EXAMPLE OF WAKE-UP OR STANDARD-START PROTOCOL

NOTE: SEE APPENDIX 1 FOR BUFFER RECIPES.

After the dissection of a given tissue and all the handling and mounting procedures, the tissue needs to be 'activated' and 'waked' up to get proper contraction and responses during coming experiments. To do this, a couple of contractions are important e.g. performed with potassium and noradrenaline. Below is an example of how a wake-up protocol could be performed, but other protocols could be as relevant as this one as long as the mounted tissue is stimulated for contraction and dilation a couple of times. Furthermore, for vessels it is important that you test the endothelium function before initiating your experiments e.g. as described in the following section.

1. Once the vessel is equilibrated, remove the old PSS buffer and add KPSS buffer to cause a contraction.
2. Allow the vessel to contract until a plateau is reached.
3. Wash the KPSS buffer out using PSS buffer until the baseline diameter is reached.
4. Repeat the KPSS contraction and washes.
5. An agonist such as noradrenaline can be added (10^{-6} M final in the bath) to cause a contraction. However, the vessel should also have developed some myogenic tone. The amount of tone will depend on the vessel used.
6. Once the vessel is contracted, Acetyl-Choline (10^{-5} M final in the bath) can be added to assess endothelial function.
7. Wash the vessel 5 to 6 times over 20 to 30 minutes.
8. Vessel is now ready for experiments.

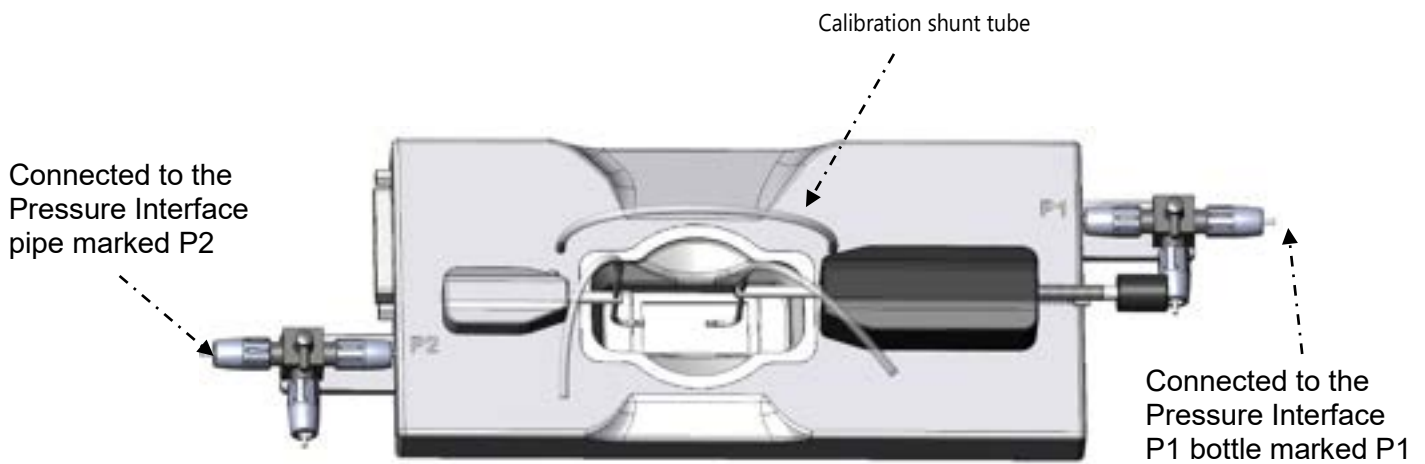
CHAPTER 4 - CLEANING AND MAINTENANCE

The Pressure Myograph is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and that the instructions are followed at all times.

4.1 CLEANING THE 110P-XL PRESSURE MYOGRAPH

NOTE: DMT STRONGLY RECOMMENDS THAT THE PRESSURE MYOGRAPH AND SURROUNDINGS BE CLEANED AFTER EACH EXPERIMENT.

1. After completing an experiment, remove the vessel mounted on the Cannulas.
2. Remove buffer from the tissue chamber.
3. Turn off heat.
4. Dump any buffers in the bottles (both P1 and P2 bottles). P1 bottle is the bottle that starts with buffer. P2 bottle is the waste bottle. If looking at the Pressure Interface from the front, P1 bottle is on the right and P2 bottle is on the left.
5. Put the bottles back in place and tighten the caps.
6. Make sure all the tubing is connected to the chamber and bottles.
7. Insert the Calibration Shunt tube that came with your system to bypass the Cannulas in the same fashion you would use the shunt to calibrate your pressure transducers (see figure in following section).



8. In the Pressure Interface menu set the Pressure P1 and P2 as follows either directly on the Pressure Interface or using MyoVIEW:

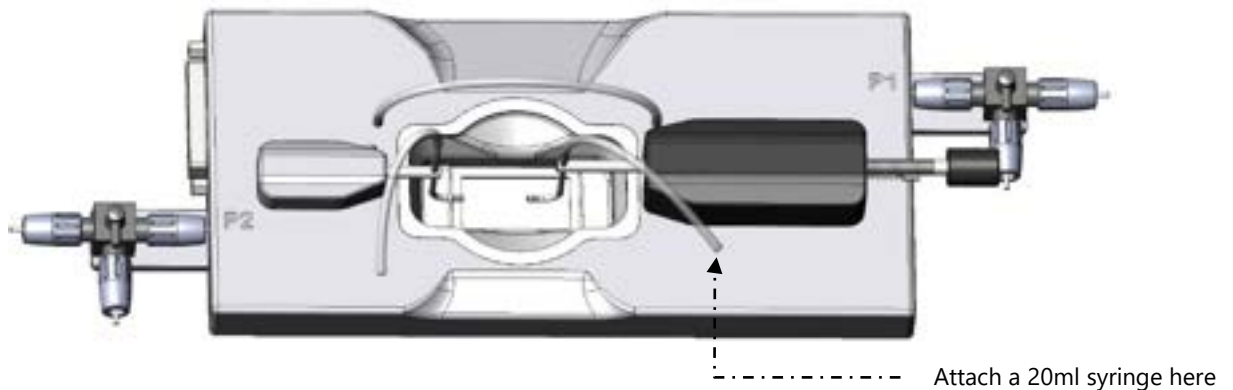
P1 = 150mmHg
 P2 = 70mmHg
 Set the
 Pressure ON
 Set Flow ON

9. Let the pressure regulator run until all the buffer in the tubing's is replaced with air in the perfusion circuit.
10. Turn off the Pressure and the Flow. Rinse the P1 Schott bottle carefully with distilled water to remove salt. Add 300ml distilled water to the P1 Schott bottle and tighten the cap again.
11. In the Pressure Interface menu set the Pressure P1 and P2 as follows either directly on the Pressure Interface or using MyoVIEW:

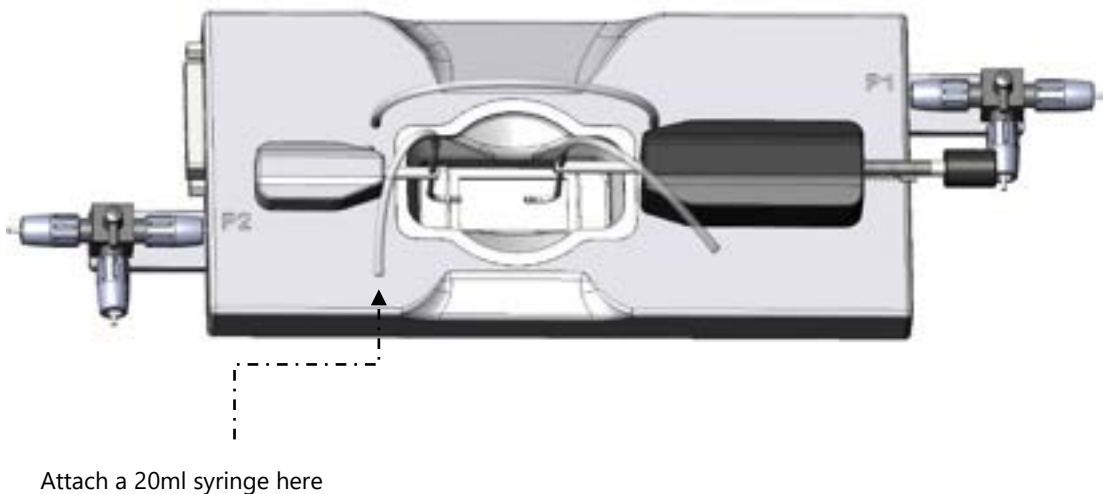
P1 = 150mmHg
 P2 = 70mmHg
 Set the
 Pressure ON
 Set Flow ON

12. Let the Pressure Interface with the above setting for at least 20 minutes or until the P1 bottle is empty to clean the perfusion circuit with distilled water.
13. While the above cleaning program is running
 - a. Rinse the 110P-XL chamber with distilled water 3 times.
 - b. Add 15ml distilled water to the chamber.

- c. Attach a 20ml syringe to the short silicone tubing on the P1 glass cannula which is easy to access because the Calibration Shunt tube has replaced its position in the chamber. Use the syringe to drag 10ml distilled water into the P1 cannula and silicone tube. Detach the syringe from the silicone tube.



- d. Refill the chamber with distilled water. Attach a 20ml syringe to the silicone tube at the P2 side connected to the steel pipe going into the chamber. Use the syringe to drag 10ml distilled water into the P2 cannula and silicone tube. Detach the Syringe from the silicone tube.



- e. Empty the chamber completely. When all the distilled water is removed from the chamber repeat step c and step d by dragging 20ml air into the P1 and P2 cannulas.
14. Stop the Flow and Pressure on the Pressure Interface or using MyoVIEW. Empty the P1 Schott bottle and tighten the cap on the P1 Bottle again.
 15. In the Pressure Interface menu set the Pressure P1 and P2 as follows either directly on the Pressure Interface or using MyoVIEW:

P1 = 150mmHg

P2 = 70mm Hg

Set the

Pressure ON

Set Flow ON

16. Let the pressure regulator run until the water in the tubing's is replaced with air in the perfusion circuit.
17. Turn off the Pressure and the Flow. Remove the P1 bottle from the Pressure Interface and let it dry. Remove the P2 bottle from the Pressure Interface. Rinse the P2 Schott bottle carefully with distilled water to remove salt and let it dry.
18. Remove the Calibration Shunt tube from the chamber and reconnect all the tubing's inside the chamber again.

4.2.1 CLEANING THE 110P-XL PRESSURE MYOGRAPH WITH 8% ACETIC ACID

NOTE: AN 8% ACETIC ACID WASH IS NOT NEEDED AFTER EACH USE. DMT ONLY RECOMMENDS THAT THE PRESSURE MYOGRAPH AND SURROUNDINGS BE CLEANED WITH ACETIC ACID IF SALT DEBRIS IS CLEARLY VISIBLE. USE THIS SPARINGLY. IF ONLY BUFFER HAS BEEN USED IN THE PERFUSION LINE, THEN THERE IS NO NEED TO EVER USE ACETIC ACID TO CLEAN THE TUBING, INTERNAL BUFFER LINES OF THE CHAMBER.

If an 8% acetic acid rinse is performed, then repeat these steps and make sure that the system is thoroughly washed with double- distilled water again to remove the acetic acid.

IMPORTANT:

BE VERY CAREFUL NOT TO DAMAGE PRESSURE TRANSDUCER P3 IF FLUSHING THE BUFFER CIRCUIT. THERE ARE NO READINGS FROM P3 VISIBLE ON THE PRESSURE INTERFACE FOR THE 110P-XL SYSTEM. PRESSURES ABOVE 250MMHG WILL DAMAGE THE PRESSURE TRANSDUCERS PERMANENTLY.

WARNING:

NEVER FLUSH THE AIR CIRCUITS (PORT NO. 2, 3 AND 4) ON THE PRESSURE INTERFACE BOTTLES WITH ANY KIND OF FLUID.

If the chamber still has salt, build-up then continue with the following procedure:

1. Fill the Pressure Myograph chamber to the edge with an 8% acetic acid solution and allow it to stand for a 1-2 minutes to dissolve calcium deposits and other salt build-up. Use a swab stick to mechanically clean all chamber surfaces.
2. Remove the acetic acid and wash the Pressure Myograph chamber and Cannulas several times with double distilled water.

3. If any kind of hydrophobic reagent have been used, which might be difficult to remove using step 1 and 2, then try incubating the chamber and Cannulas with 96% ethanol or a weak detergent solution (e.g. Treepol).
4. To remove more resistant or toxic chemicals, incubate the Pressure Myograph chamber and Cannulas with 1M HCl for up to 3 minutes.
5. Wash the Pressure Myograph chamber and Glass Cannulas several times with double distilled water.

IMPORTANT: BE VERY CAREFUL USING STEP 3 AND 4 REPEATEDLY AS STRONG REAGENTS CAN CAUSE EXTREME DAMAGE TO THE PRESSURE MYOGRAPH.

In cases of red or brown discolorations appearing on the chamber sides, the following cleaning procedure will work in most cases:

1. Incubate the Pressure Myograph chamber for 30 minutes with 20µl of a 2mM T-1210 Tetrakis-(2- pyridylmethyl)-ethylenedi- amine solution dissolved in double distilled water.
2. Use a swab-stick to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.
3. Wash the Pressure Myograph chamber several times with double distilled water.
4. Incubate the Pressure Myograph chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a swab-stick.
5. Remove the ethanol solution and wash a few times with double distilled water. Incubate the Pressure Myograph chamber with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.
6. Wash the Pressure Myograph chamber several times with double distilled water.

4.2 MAINTENANCE OF PRESSURE MYOGRAPH CHAMBER PIPES

To prevent the pipes from being blocked by buffer salt deposits after an experiment, use the chamber cover to remove the cleaning solutions. Afterwards, remove the cover from the Pressure Myograph chamber and turn on the vacuum pump and vacuum valve for about 10 seconds. Wait to turn off the oxygen supply until turning off the vacuum pump. Wipe off any buffer remaining on the outside of the pipes using a piece of paper.

APPENDIX 1 - BUFFER RECIPES

Physiological Saline Solution (PSS)

1x PSS:

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	130	3.799	7.598	15.20	30.39
KCl	(74.557)	4.7	0.175	0.35	0.70	1.40
KH ₂ PO ₄	(136.09)	1.18	0.08	0.16	0.32	0.64
MgSO ₄ ·7H ₂ O	(246.498)	1.17	0.145	0.29	0.58	1.16
NaHCO ₃	(84.01)	24.9	1.05	2.10	4.18	8.37
Glucose	(180.16)	5.5	0.545	1.09	2.18	4.36
EDTA	(380)	0.026	0.005	0.01	0.02	0.04
CaCl ₂	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of CaCl₂ (110.99) in double-distilled H₂O. Filter-sterilize the calcium solution through a 0.22µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals except the CaCl₂ in approximately 80% of the desired final volume of double distilled H₂O while being constantly stirred. For example, if 1 liter of PSS is to be made, then dissolve all the chemicals in 800mL of double distilled H₂O.
3. Add the appropriate volume of 1.0M CaCl₂ for the total volume of PSS being made (for example, 1.6mL of 1.0M CaCl₂ for 1 liter of buffer). Continue to stir the PSS while the CaCl₂ is being added.
4. Bring the solution up to the final volume with double-distilled H₂O. Continue to stir the solution until the EDTA is fully dissolved. This takes about 15 minutes at room temperature.
5. Aerate the solution with carbogen (95% O₂ + 5% CO₂) for about 20 minutes.

25x Concentrated PSS (Stock solution):

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	3250	94.98	189.96	379.92	759.84
KCl	(74.557)	117.5	4.375	8.75	17.5	35.0
KH ₂ PO ₄	(136.09)	29.5	2.0	4.0	8.0	16.0
MgSO ₄ ·7H ₂ O	(246.498)	29.25	3.625	7.25	14.5	29.0
EDTA	(380)	0.65	0.125	0.25	0.50	1.0
CaCl ₂	(110.99)	40	20mL	40mL	80mL	160mL

1. Make a 1.0M solution of CaCl₂ (110.99) in double-distilled H₂O. Filter-sterilize the calcium solution through a 0.22µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals in approximately 80% of the desired final volume of double distilled H₂O while being constantly stirred. For example, if 1 liter of PSS is to be made, then dissolve all the chemicals in 800mL of double distilled H₂O.
3. Bring the solution up to the final volume with double-distilled H₂O. Continue to stir the solution until the EDTA is fully dissolved. This takes about 15 minutes at room temperature.
4. Store cold until use.

Before use:

5. Dilute the 25 x PSS stock solution 1:25 using double distilled H₂O.
6. Add:
1.09 g/L Glucose
2.10 g/L NaHCO₃
7. Add the appropriate volume of 1.0M CaCl₂ for the total volume of PSS being made (for example, 1.6mL of 1.0M CaCl₂ for 1 liter of buffer). Continue to stir the PSS while the CaCl₂ is being added.
8. Bring the solution up to the final volume with double-distilled H₂O. Aerate the solution with carbogen (95%O₂ + 5%CO₂) for at least 20 minutes. If necessary, wait further for the pH of the buffer to reach pH 7.4.

High potassium Physiological Saline Solution (KPSS)

1x 60mM KPSS:

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	74.7	2.18	4.36	8.72	17.44
KCl	(74.557)	60	2.24	4.48	8.96	17.92
KH ₂ PO ₄	(136.09)	1.18	0.08	0.16	0.32	0.64
MgSO ₄ ·7H ₂ O	(246.498)	1.17	0.145	0.29	0.58	1.16
NaHCO ₃	(84.01)	24.9	1.05	2.10	4.20	8.40
Glucose	(180.16)	5.5	0.5	1.00	2.00	4.00
EDTA	(380)	0.026	0.005	0.01	0.02	0.04
CaCl ₂	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of CaCl₂ (110.99) in double-distilled H₂O. Filter-sterilize the calcium solution through a 0.22µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals except the CaCl₂ in approximately 80% of the desired final volume of double distilled H₂O while being constantly stirred. For example, if 1 litre of PSS is to be made, then dissolve all the chemicals in

800mL of double distilled H₂O.

3. Add the appropriate volume of 1.0M CaCl₂ for the total volume of PSS being made (for example, 1.6mL of 1.0M CaCl₂ for 1 litre of buffer). Continue to stir the PSS while the CaCl₂ is being added.
4. Bring the solution up to the final volume with double-distilled H₂O. Continue to stir the solution until the EDTA is fully dis- solved. This takes about 15 minutes at room temperature.
5. Aerate the solution with carbogen (95% O₂ + 5% CO₂) for about 20 minutes.

