

# MYOGRAPH SYSTEM – 114PN

USER GUIDE VOL.3.7

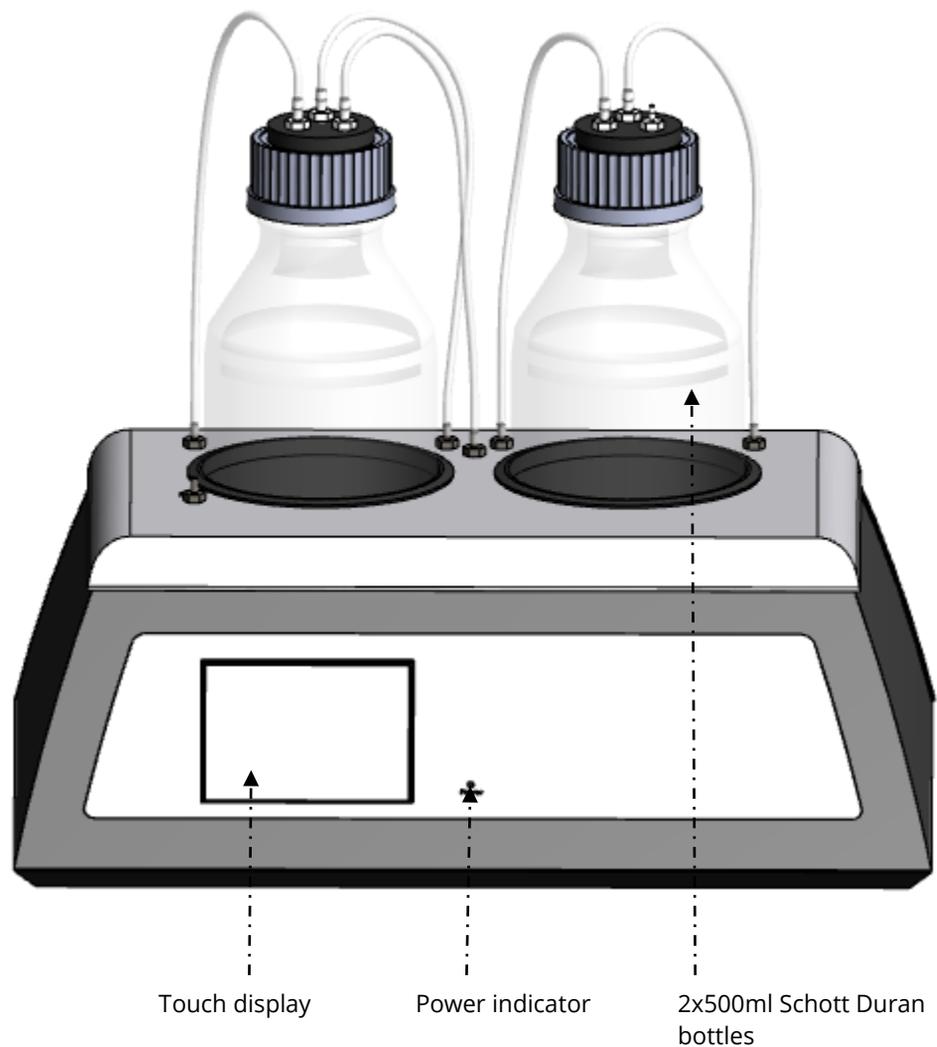


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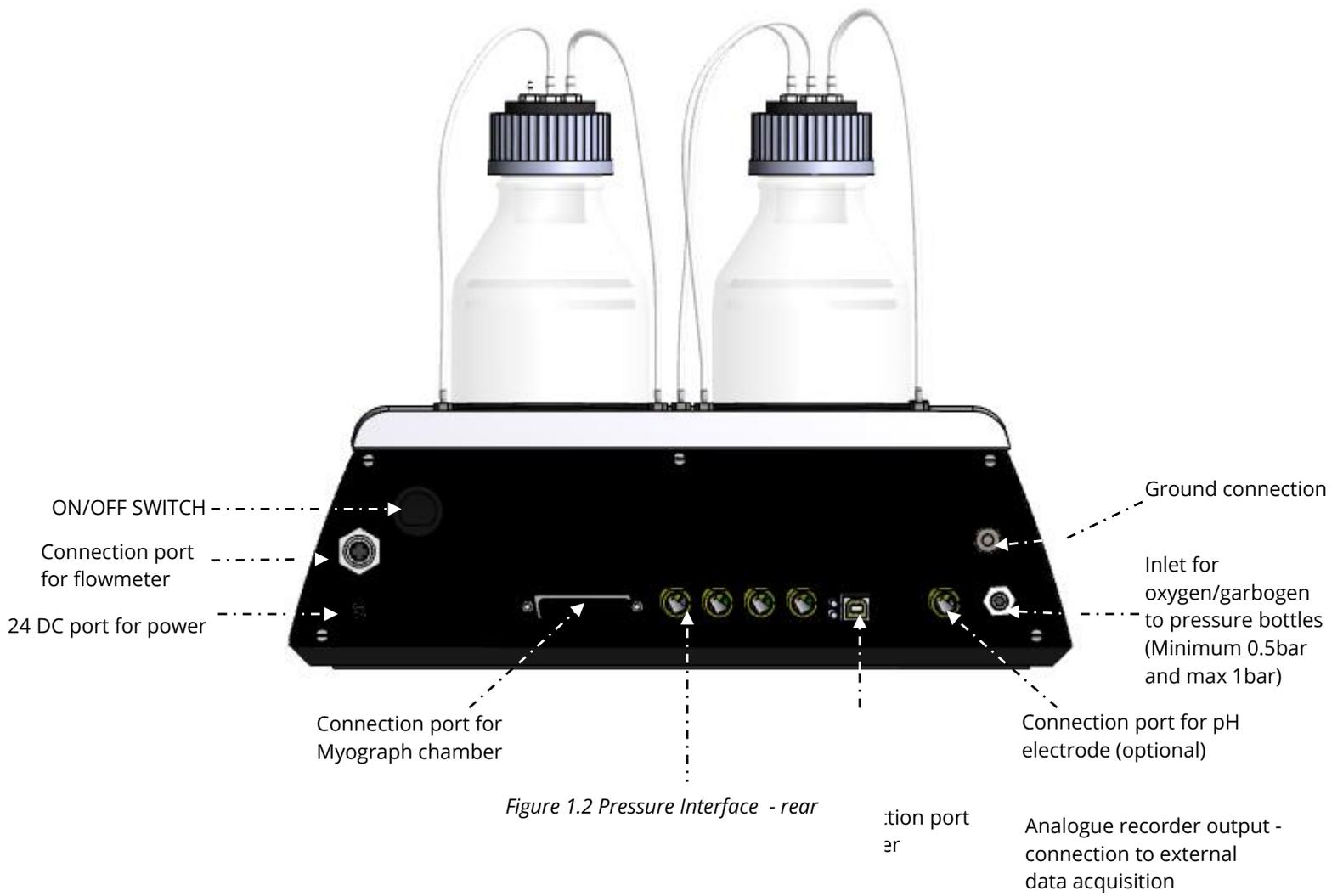
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# CHAPTER 1 - SYSTEM OVERVIEW

## 1.1 INTERFACE



*Figure 1.1 Pressure Interface - front*



## 1.2 114PN PRESSURE MYOGRAPH

### 1.2.1 PRESSURE MYOGRAPH - 114PN Chamber

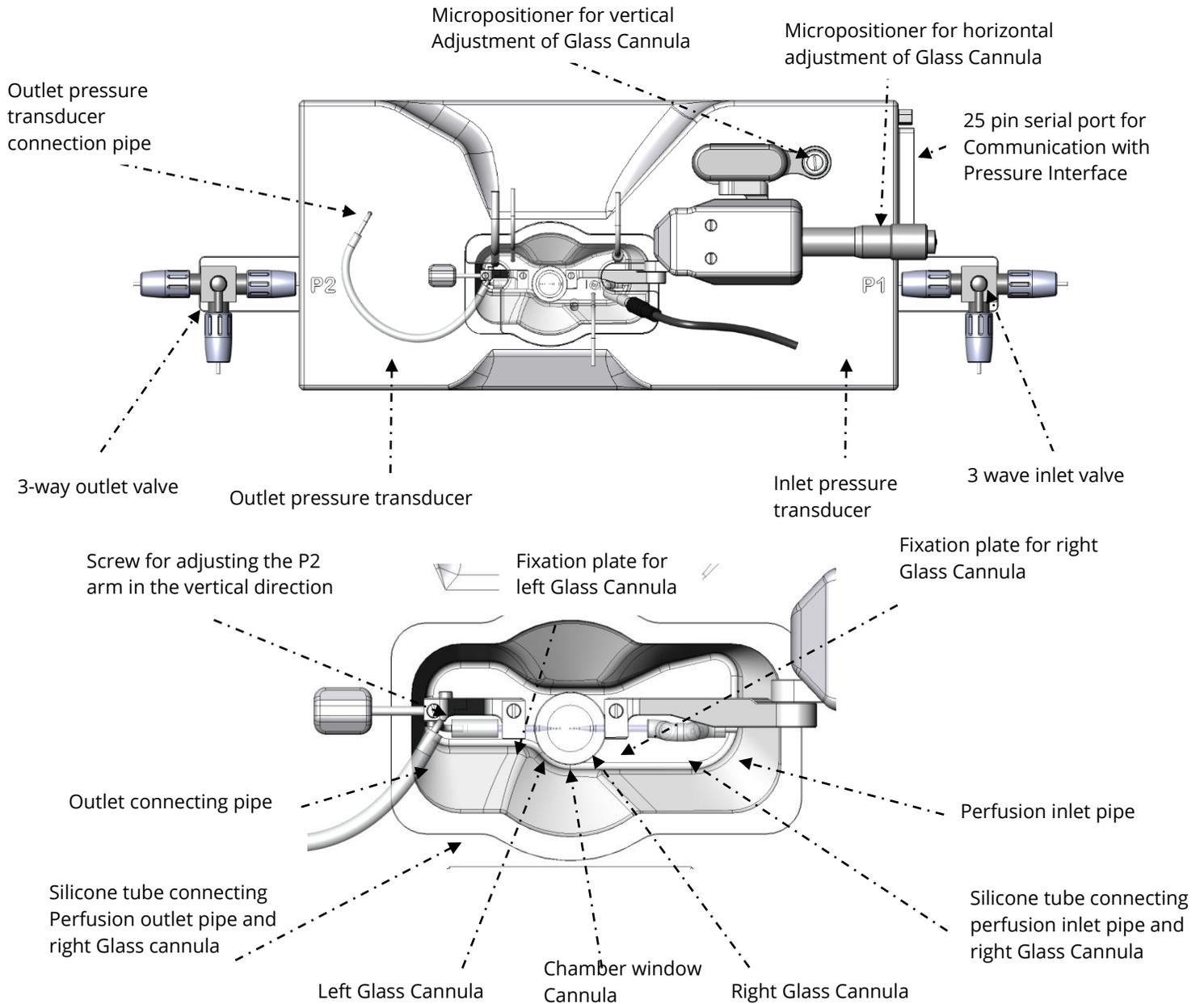


Figure 1.3 Pressure Myograph - 114PN with close-up detail of the chamber

# CHAPTER 2 - SET-UP THE PRESSURE MYOGRAPH

## 2.1 ADJUSTMENT OF THE GLASS CANNULAS

### **General adjustments (see figure 2.1)**

General adjustments are performed using the two micropositioners on top of the 114PN Pressure Myograph (Figure 2.1). The horizontal micropositioner “A”, is used to define the length between the two Glass Cannulas and thereby the horizontal stretch of the mounted blood vessel. The vertical micropositioner “B”, is used to adjust the vertical position of the right Glass Cannula and thereby the vertical position of the mounted blood vessel.

### **Pre-experimental alignment**

To make the general adjustments as easy as possible during an experiment, it is important to make an alignment of the two Glass Cannulas prior to the mounting of the blood vessel making sure they are completely aligned.

### **Right Glass Cannula (see figure 2.1)**

Horizontal positioning of the right Glass Cannula is adjusted by using the micropositioner “A”.

Vertical positioning of the right Glass Cannula is adjusted with the micropositioner “B”. Longitudinal positioning of the right Glass Cannula in the glass- cannula-holder is adjusted by loosening of screw “C”.

### **Left Glass Cannula (see figure 2.1)**

Horizontal positioning of the left Glass Cannula in the Glass-Cannula-holder is adjusted by carefully loosening screw D”. Vertical positioning of the left Glass Cannula is adjusted by carefully loosening screws “F” (Figure 2.1C) to move closer or more far away from the bottom of the chamber. Longitudinal positioning of the left Glass Cannula in the glass-cannula-holder is adjusted by gently loosening of screw “E”.

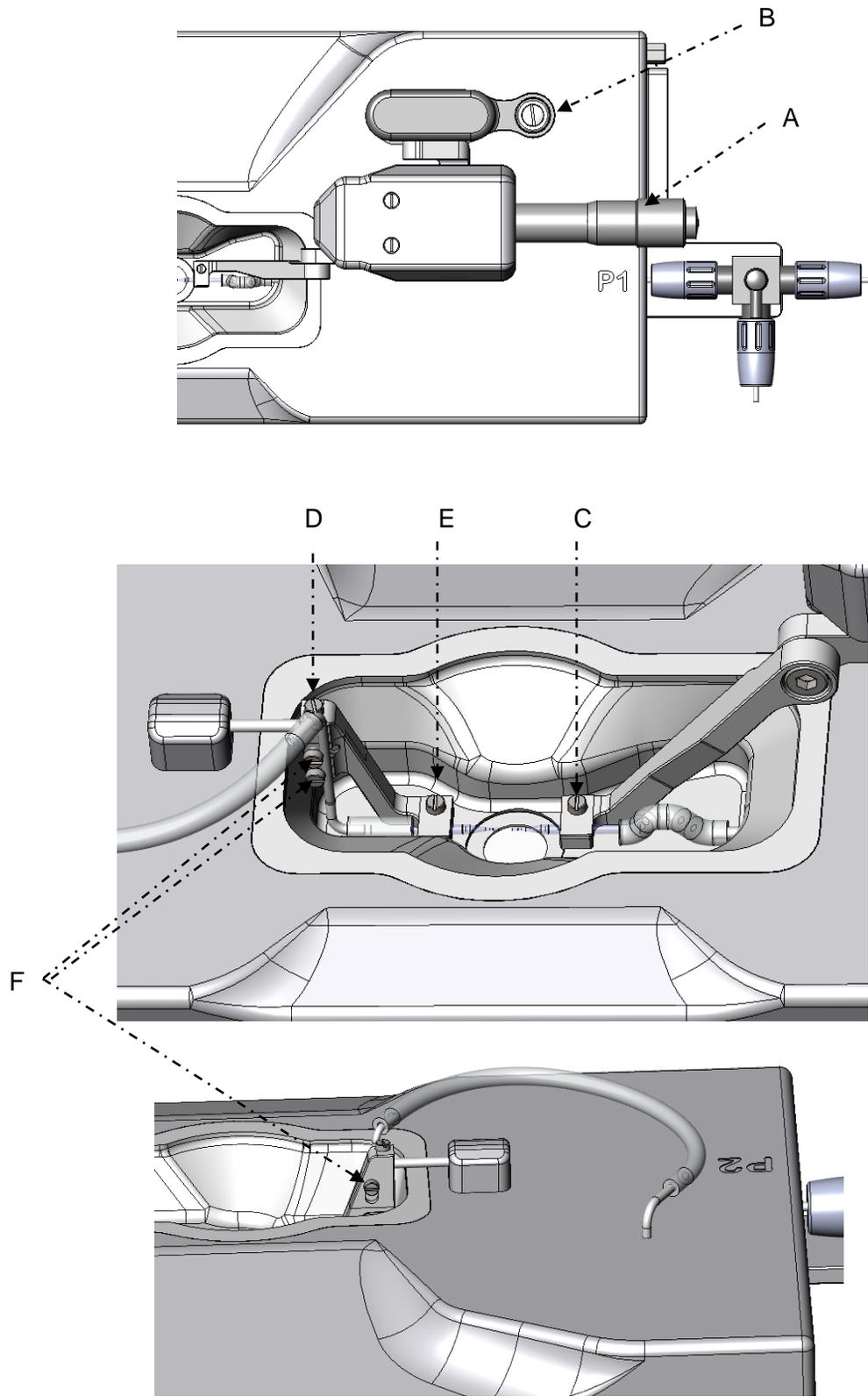
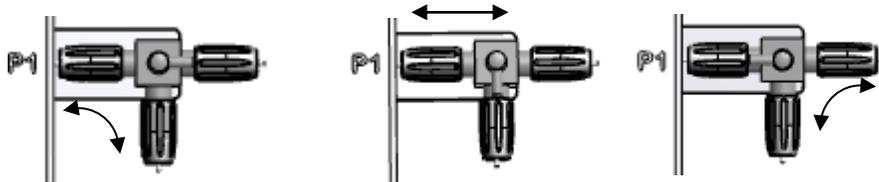


Figure 2.1 Pressure Myograph - 114PN with close-up detail of the chamber

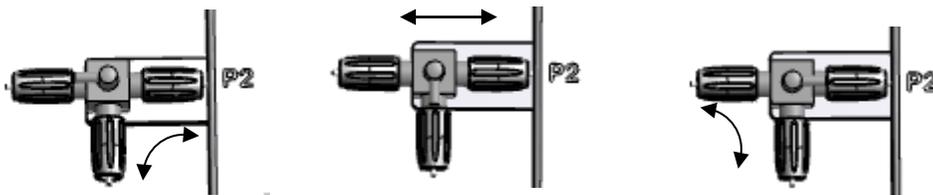
*NOTE: BE CAREFUL NOT TO DAMAGE THE TIP OF THE GLASS CANNULAS DURING THE GENERAL ADJUSTMENT.*

## 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 2.2 and figure 2.3 below.



*Figure 2.2 Three-way valve flow settings at P1. The arrows indicate the flow direction*



*Figure 2.3 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 small vessels in the 114PN Pressure Myograph.

### 3.1.1 PREPARATION

1. Prepare your PSS. 60mM KPSS can be used to test contractions, and 16mM KPSS can be used to cause relaxations (usually 16mM KPSS only works in small arteries for relaxation).
2. Make at least 4 loops with nylon suture to use for securing the vessel to the mounting Glass Cannulas. Double loops are best and will prevent the loops from slipping loose once tightened (Do NOT use

single or triple loops for the suture).

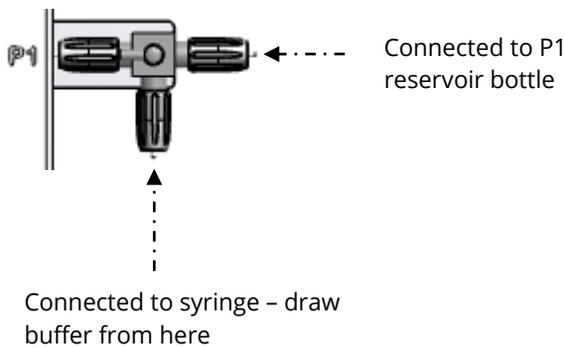
3. Pre-heat PSS to 37°C, making sure the PSS is bubbled with carbogen (5% CO<sub>2</sub>, 95% O<sub>2</sub>) or PraxAir (5% CO<sub>2</sub>, 21% O<sub>2</sub>, balance N<sub>2</sub>) for at least 20 to 30 minutes.
4. If desired, rinse the mounting chamber and all tubing that sees buffer with double-distilled H<sub>2</sub>O.
5. Fill the P1 reservoir bottle in the Pressure Interface with pre-warmed PSS. DO NOT FILL MORE THAN 300ML. Keep 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.
6. Purge the 114PN system with pre-heated (37°C) and gassed buffer (Appropriate running buffer) as described in the 114PN Pressure Myograph System User Manual page (IMPORTANT STEP)

### 3.1.2 MOUNTING

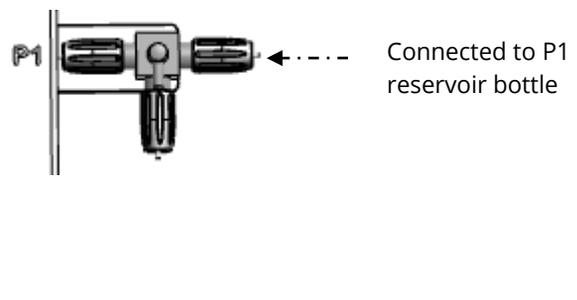
1. Isolate small vessel of interest.
2. Clean the vessel as best as possible of perivascular fat and tissue.
3. Place the small artery into a small container of cold or room temperature PSS.
4. Place 3-4.0 ml. of cold or room temperature PSS in the mounting chamber.
5. Have a syringe with buffer attached to the P1 3-way valve, but the P1 and P2 3-way valves should be closed towards P1 and P2 glass cannulas.
6. Position the sutures appropriately on both Glass Cannulas so that they are ready for use when the vessel is mounted. Place 2 loops on each Glass Cannula in case one breaks or fails.
7. Carefully transfer the vessel from the small container to the mounting chamber. Using a large bore pipette tip and pipette or an eye-dropper, carefully suck up the isolated vessel and transfer it to the chamber. This will minimize handling and the potential for damaging the vessel.
8. Continue to mount the vessel on the Glass Cannulas. Start with the P1 side. Once the P1 side of the vessel is mounted on the Glass Cannula, secure the vessel to the Glass Cannula by tightening the two loops around the vessel onto the Glass Cannula.
9. Make sure the vessel is mounted in the correct direction. Flow through the vessel is directional, so proximal and distal ends for flow will need to be noted somehow. Mount the proximal end to flow on the P1 Glass Cannula.
10. 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 valve.
11. 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 damaging the transducers. This will also help opening the distal end if it is pinched shut. Close the P1 3-way valve towards the vessel to keep the vessel from

collapsing.

12. Position the P1 Glass Cannula with attached vessel to facilitate mounting of the vessel on the P2 Glass Cannula. Gently mount the vessel on the P2 glass cannula, without pulling it off the P1 glass cannula. Secure the vessel as described in step 8.
14. Once the vessel is secured on the P2 Glass Cannula, open the P1 3-way valve and 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 myograph cable). The vessel should slightly fill and bulge from the small pressure added. Close P1 3-way valve towards the vessel to maintain a slightly pressurized vessel. Transfer the mounting chamber to the microscope and attach all the tubing.
15. 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.
16. 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.

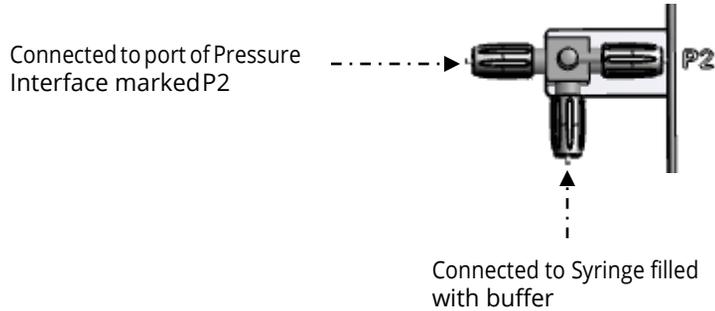


*Figure 3.1a Filling tube from P1 to P1 3-way valve*



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

17. 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.

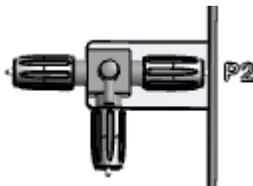


18. Make sure the Flow is turned ON before starting this step. Use the buffer that was drawn from the bottle to charge the remainder of the system. The buffer should be pushed GENTLY from the 3-way valve all the way to the waste bottle. When the buffer starts dripping in the waste bottle, stop pushing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

*IMPORTANT:*

*BE CAREFUL WHEN CHARGING THIS PART OF THE SYSTEM. EXCESSIVE PRESSURE CAN DAMAGE THE INTERNAL 3RD HIDDEN TRANSDUCER IN THE PRESSURE INTERFACE. AVOID EXCESSIVE PRESSURE (>250MMHG) WILL DAMAGE THE INTERNAL PRESSURE TRANSDUCERS IN THE PRESSURE SYSTEM.*

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



### 3.1.3 EQUILIBRATION

1. Turn on the heat, which should be preset to 37°C.
2. Start aerating PSS in the chamber with oxygen
3. Set pressure and flow ON and set P1 to 20mmHg and P2 to 5mmHg. This will cause a flow gradient and perfuse the vessel with some of the newly charged buffer. Make sure that the charged lines DO NOT have air bubbles, otherwise endothelium will be stripped from the lumen. Perfuse for 2 to 3 minutes.
4. Set MyoVIEW or interface in the No Flow state and set P1 and P2 pressure to 10mmHg.
5. Increase pressure by 10mmHg every 5 minutes until target pressure is reached. For cerebral arteries from rats, this may be 60 to 70 mmHg. For mesenteric resistance arteries, this would be 60mmHg.
6. 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.
7. Continue to pressurize the vessel.
8. After 40 minutes from the start of heat and pressurization, do another wash, as in step 6.
9. Once 60 minutes have passed, the wake-up or standard-start protocol can be initiated.

### 3.1.4 EXAMPLE OF WAKE-UP OR STANDARD-START PROTOCOL

*NOTE: SEE APPENDIX 1 FOR BUFFER RECIPES.*

After dissection of a given tissue and all the handling and mounting procedures the tissue needs to be 'activated' and 'woken' 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 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

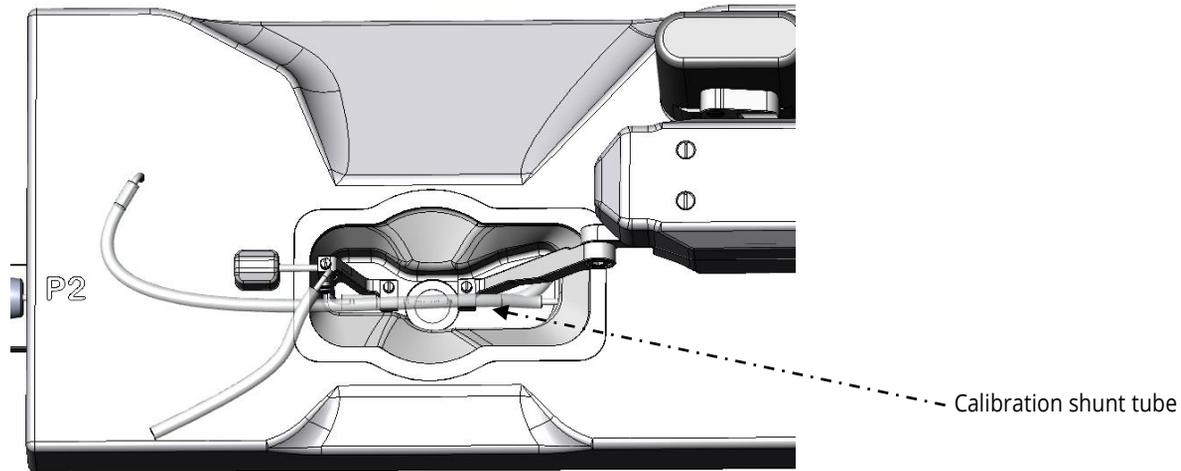
The Pressure Myograph is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and that the instructions are followed at all times.

### 4.1 CLEANING THE 114P PRESSURE MYOGRAPH

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

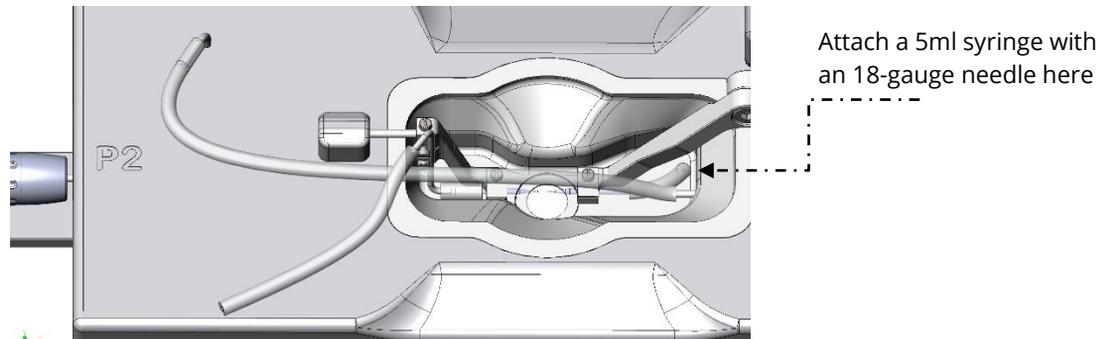
1. After completing an experiment, remove the vessel mounted on the Glass Cannulas.
2. Remove buffer from the tissue chamber.
3. Turn off heat.
4. Dump any buffers in the bottles (both P1 and P2 bottles). P1 bottle is the bottle that starts with buffer. P2 bottle is the waste bottle. If looking at the 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. If using Glass Cannulas for small vessels, then insert the Calibration Shunt tube that came with your

system to bypass the Glass Cannulas in the same fashion you would use the shunt to calibrate your pressure transducers (see figure in following section).

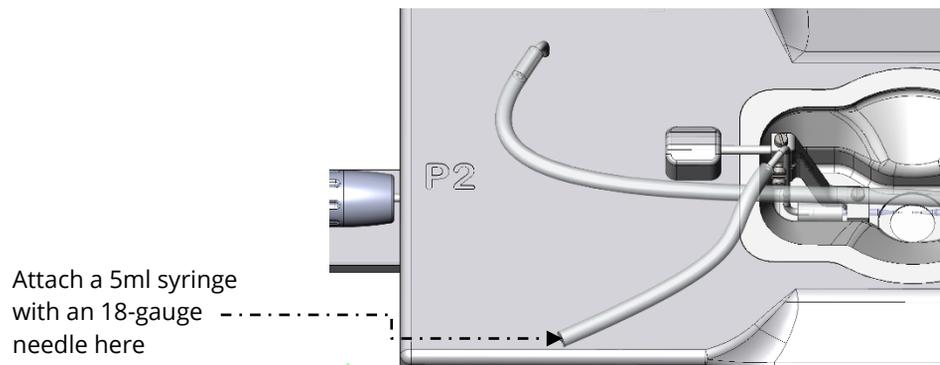


8. In the Pressure Interface menu set the Pressure P1 and P2 as follows either directly on the Pressure Interface or using MyoVIEW:
  - P1 = 150mmHg
  - P2 = 70mmHg
  - Set the Pressure ON
  - Set Flow ON
9. Let the pressure regulator run until all the buffer in the tubing's is replaced with air in the perfusion circuit.
10. Turn off the Pressure and the Flow. Rinse the P1 Schott bottle carefully with distilled water to remove salt. Add 100ml distilled water in 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 settings, run for at least 20 minutes to clean the perfusion circuit with distilled water.
13. While the above cleaning program is running:
  - a. Rinse the 114P chamber with distilled water 3 times.
  - b. Add 7ml distilled water to the chamber.

- c. Attach a 5ml syringe with an 18-gauge needle 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 2-5ml distilled water into the P1 glass cannula and silicone tube. Detach the needle from the silicone tube.



- d. Attach a 5ml syringe with an 18-gauge needle to the silicone tube at the P2 side connected to the steel pipe going into the chamber. Use the syringe to drag 2-5ml distilled water into the P2 glass cannula and silicone tube. Detach the Syringe/ needle 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 5ml 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 air 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 CLEANING THE 114PN PRESSURE MYOGRAPH WITH 8% ACETIC ACID (OPTIONAL)

*NOTE: AN 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 114PN SYSTEM, HOWEVER FOR THE 112PP IN THE CLEANING MENU ALL 4 PRESSURE TRANSDUCERS CAN BE MONITORED. DMT THEREFORE RECOMMENDS THAT THE PRESSURE MYOGRAPH ALWAYS BE FLUSHED FIRST. PRESSURES ABOVE 250MMHG WILL DAMAGE THE PRESSURE TRANSDUCERS PERMANENTLY.*

*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 Glass 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 Glass 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 Glass 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.*

*BE VERY CAREFUL NOT TO DAMAGE THE GLASS CANNULAS DURING THE CLEANING PROCEDURE.*

*AFTER CLEANING, ALWAYS CHECK THAT THE GREASING AROUND THE TRANSDUCER PIN IS SUFFICIENT TO KEEP OUT THE BUFFER SOLUTION FROM THE TRANSDUCER COMPARTMENT.*

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.

### **4.3 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 <sub>2</sub> PO <sub>4</sub>	(136.09)	1.18	0.08	0.16	0.32	0.64
MgSO <sub>4</sub> ·7H <sub>2</sub> O	(246.498)	1.17	0.145	0.29	0.58	1.16
NaHCO <sub>3</sub>	(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 <sub>2</sub>	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of CaCl<sub>2</sub> (110.99) in double-distilled H<sub>2</sub>O. 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 CaCl<sub>2</sub> in approximately 80% of the desired final volume of double distilled H<sub>2</sub>O 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 H<sub>2</sub>O.
3. Add the appropriate volume of 1.0M CaCl<sub>2</sub> for the total volume of PSS being made (for example, 1.6mL of 1.0M CaCl<sub>2</sub> for 1 liter of buffer). Continue to stir the PSS while the CaCl<sub>2</sub> is being added.
4. Bring the solution up to the final volume with double-distilled H<sub>2</sub>O. 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% O<sub>2</sub> + 5% CO<sub>2</sub>) 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 <sub>2</sub> PO <sub>4</sub>	(136.09)	29.5	2.0	4.0	8.0	16.0
MgSO <sub>4</sub> ·7H <sub>2</sub> 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 <sub>2</sub>	(110.99)	40	20mL	40mL	80mL	160mL

1. Make a 1.0M solution of  $\text{CaCl}_2$  (110.99) in double-distilled  $\text{H}_2\text{O}$ . Filter-sterilize the calcium solution through a  $0.22\mu\text{m}$  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  $\text{H}_2\text{O}$  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  $\text{H}_2\text{O}$ .
3. Bring the solution up to the final volume with double-distilled  $\text{H}_2\text{O}$ . 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  $\text{H}_2\text{O}$ .
6. Add:
  - 1.09 g/L Glucose
  - 2.10 g/L  $\text{NaHCO}_3$
7. Add the appropriate volume of 1.0M  $\text{CaCl}_2$  for the total volume of PSS being made (for example, 1.6mL of 1.0M  $\text{CaCl}_2$  for 1 liter of buffer). Continue to stir the PSS while the  $\text{CaCl}_2$  is being added.
8. Bring the solution up to the final volume with double-distilled  $\text{H}_2\text{O}$ . Aerate the solution with carbogen (95% $\text{O}_2$  + 5% $\text{CO}_2$ ) 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
$\text{KH}_2\text{PO}_4$	(136.09)	1.18	0.08	0.16	0.32	0.64
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	(246.498)	1.17	0.145	0.29	0.58	1.16
$\text{NaHCO}_3$	(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
$\text{CaCl}_2$	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of  $\text{CaCl}_2$  (110.99) in double-distilled  $\text{H}_2\text{O}$ . Filter-sterilize the calcium solution through a  $0.22\mu\text{m}$  filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals except the  $\text{CaCl}_2$  in approximately 80% of the desired final volume of double distilled  $\text{H}_2\text{O}$  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  $\text{H}_2\text{O}$ .

3. Add the appropriate volume of 1.0M CaCl<sub>2</sub> for the total volume of PSS being made (for example, 1.6mL of 1.0M CaCl<sub>2</sub> for 1 litre of buffer). Continue to stir the PSS while the CaCl<sub>2</sub> is being added.
4. Bring the solution up to the final volume with double-distilled H<sub>2</sub>O. 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% O<sub>2</sub> + 5% CO<sub>2</sub>) for about 20 minutes.

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