Campden Instruments Ltd.

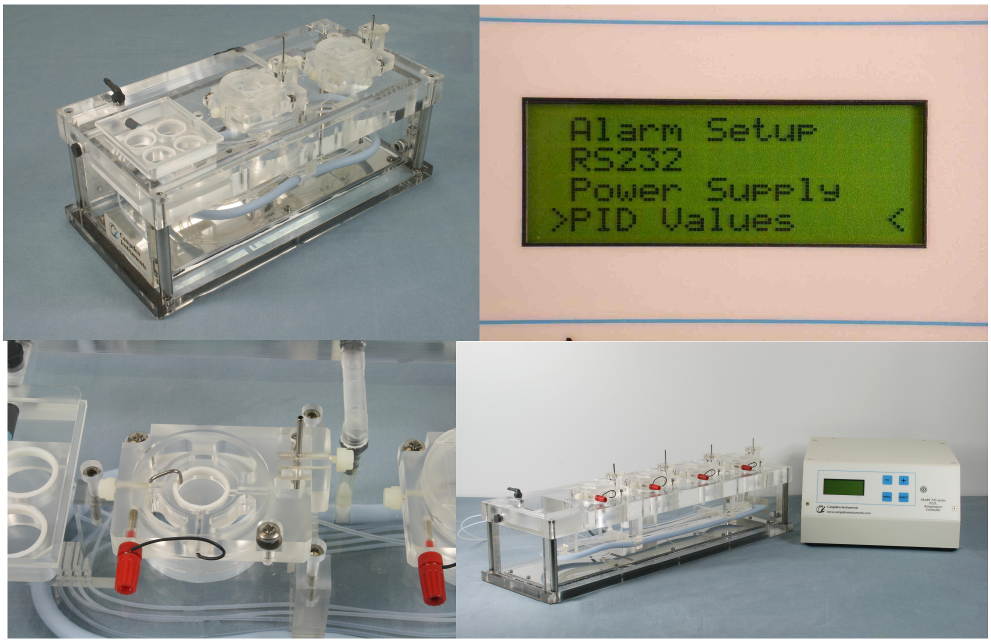
A Lafayette Instrument Company

INSTRUCTION MANUAL

7450 Model series
IN-VITRO SLICE CHAMBERS

and

HIGH RESOLUTION TEMPERATURE CONTROLLER
(Electrophysiology and Biochemistry models)
(Dual, Quad and Hex chambers)



May 2012

DCN-1695

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Contents

The purpose of this manual is to allow the user to achieve expertise in the use of the Instrument and to give the maintenance technician an insight into maintaining the instrument in peak operating condition.
Please read and understand the information contained in this manual before using the instrument. Only competent and capable personnel should use the instrument.

This document should be retained for future reference as it contains the name and address of the manufacturer within the EC

PACKAGING
Please retain the original packaging for future use.
Instruments will not be accepted for service or repair unless the unit has been adequately and properly packaged. Additionally instruments will not be accepted without prior authorisation and have been certified as being uncontaminated with any material that may be hazardous to the health of service personnel. Returns Authorisation and Decontamination Certificate forms can be obtained by contacting Campden Instruments.

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EC DECLARATION OF CONFORMITY

Name and address of Manufacturer:

Campden Instruments Limited
PO Box 8148
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UK

Description of Instrument:

SLICE CHAMBER & TEMPERATURE CONTROLLER

Model Type/Number:

7450 é é é é é é ..

Serial Number:

7450 é é é é é é .

The instrument specified above complies with the relevant health and safety requirements of the following:

1. EC Directive(s):
   Electromagnetic Compatibility Directive 89/336/EEC
   The Low Voltage Directive 73/23/EEC
2. UK Regulations:
   Electricity at Work Regulations 1989
3. European Standards
   EN 50081-1: 1992 Electromagnetic compatibility generic emissions standard part 1
   EN 50082-1: 1992 Electromagnetic compatibility generic immunity standard part 1

Additionally, the health and safety requirements of the following British and harmonised European Standards have been incorporated in the design of the above instrument:

BS 2771:part 1:1986 (EN 60 204:part1:1985)
BS 5304:1988

1. Introduction

Campden Instrumentsô 7450 series slice chambers have been designed to be capable of being operated as either an interface (Haas type) chamber or as a submerged (Oslo type) chamber.
Additionally the chambers may be supplied suitable for research into either cell electrophysiology or biochemistry.

The slice chamber is available in two or four chamber configurations. Additionally a six-chamber version is available for biochemistry use only. Electrophysiology chambers are spaced for suitable access by micromanipulators. Materials used in the chambers and tubing have been carefully chosen to suppress bacterial growth and the electronics are carefully designed to eliminate electromagnetic noise and should not be altered in any way.

Each chamber is removable from the unit and can be replaced if required. Chambers are available manufactured in clear acrylic (Perspex®/Plexiglas®) or p.t.f.e (Teflon®) materials. P.t.f.e. has the advantage of having a lower incidence of drug adhesion. For electrophysiology research, reference electrodes (Ag/AgCl) can be fitted to each chamber and terminated on the front edge of the chamber.

Perfusate tubing to the chambers is threaded through support pillars immersed in a temperature controlled heated water bath allowing the perfusate to be brought to the desired temperature equally for each slice.
Each assembly also has a holding chamber integrated into the top plate so that slices can be stored ready to hand whilst they are recovering from the slicing operation. The holding chamber should be filled with perfusate and can be aerated with carbogene gas. The holding chamber contains a removable slice holder. A cover plate over the holding chamber reduces heat losses via this area.

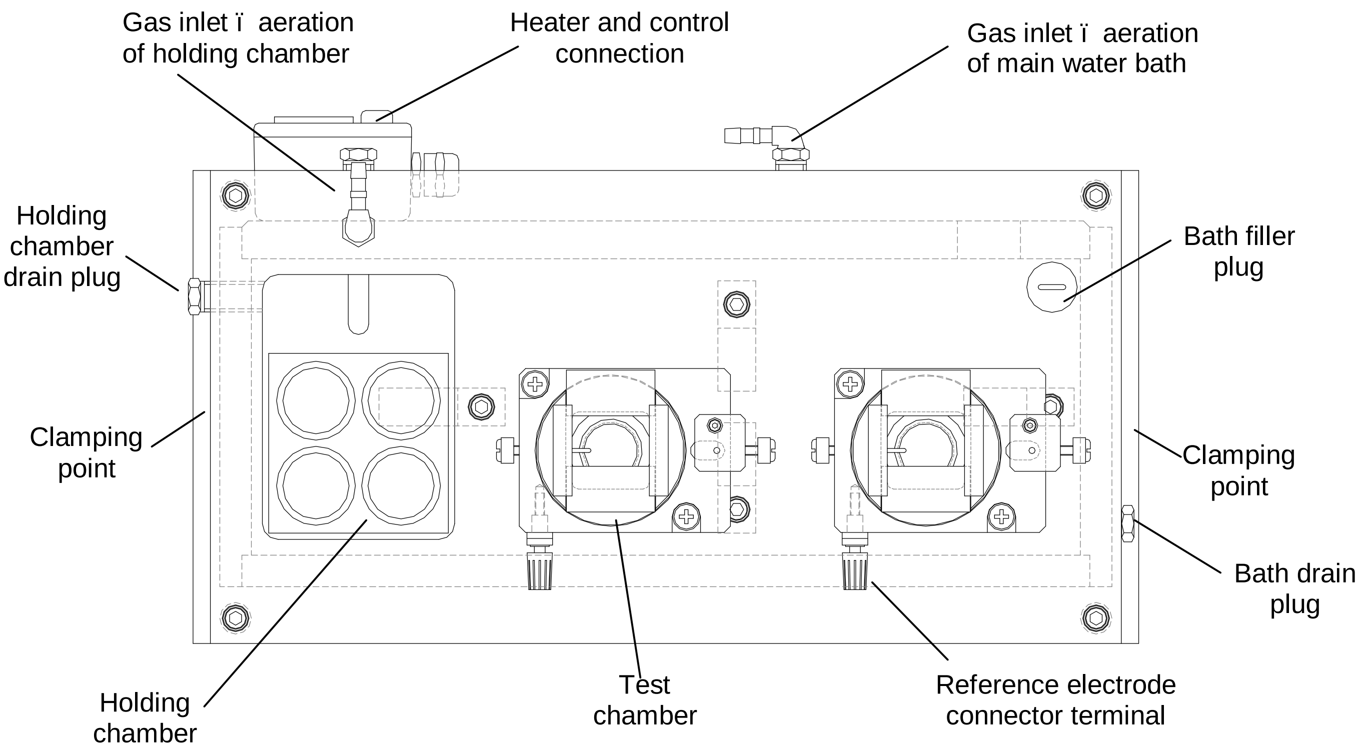
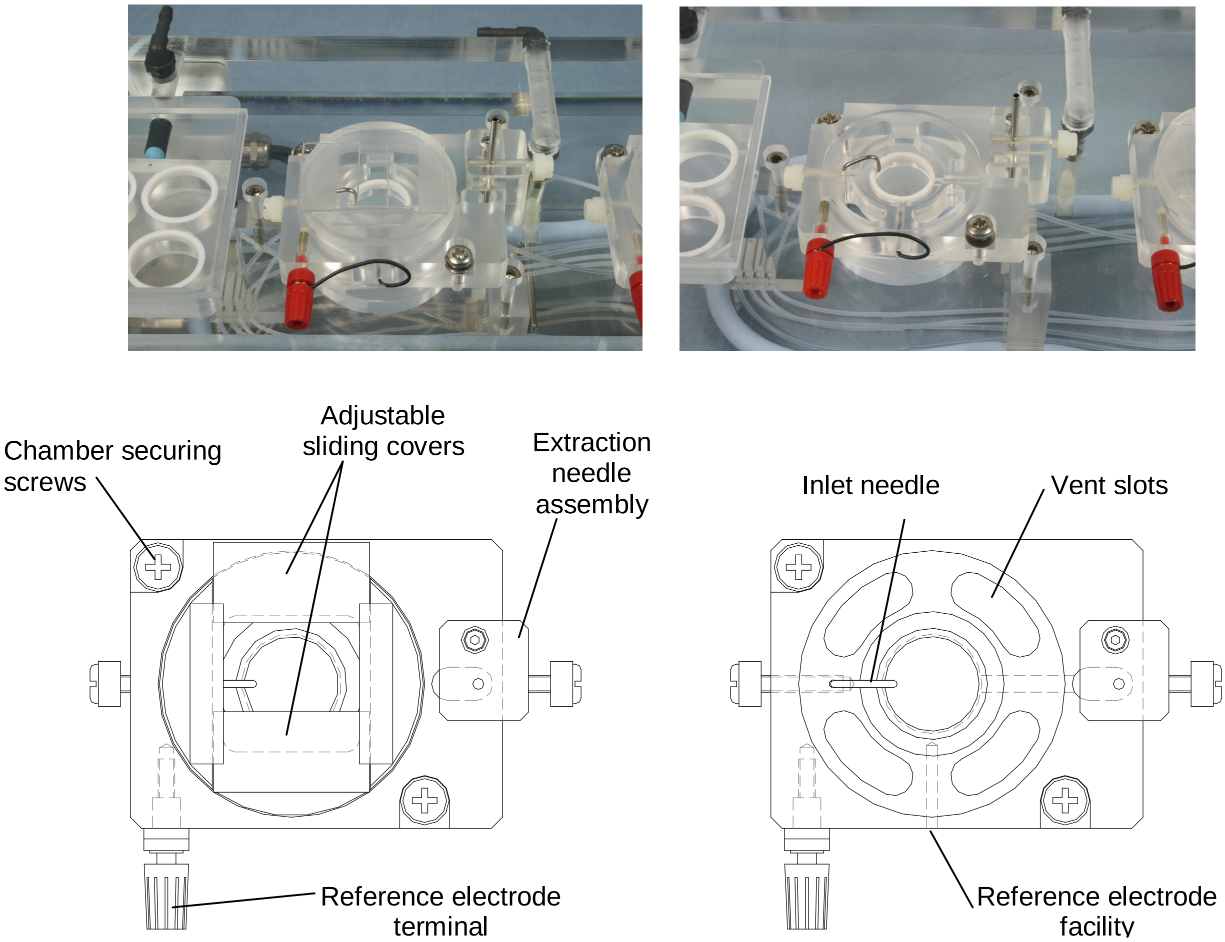


Figure 1. 2 channel chamber

When the water bath top plate is removed, all tubing and the tubing support posts are also withdrawn. This arrangement gives clear and easy access for threading the perfusate tubes and cleaning the water bath.
The water in the bath can be aerated (to reduce perfusate degassing) by connecting a suitable air pump or carbogene gas supply to the inlet port on the rear of the top plate.

Vent slots in the chamber allow warm moist air to be ducted up from above the water bath so that when the chamber is being used in interface mode, an adjustable cover retains a warm, moist atmosphere over the slices in the chamber and prevents the slice from drying out.



Chamber with covers

Chamber with covers removed

Figure 2. Chamber detail

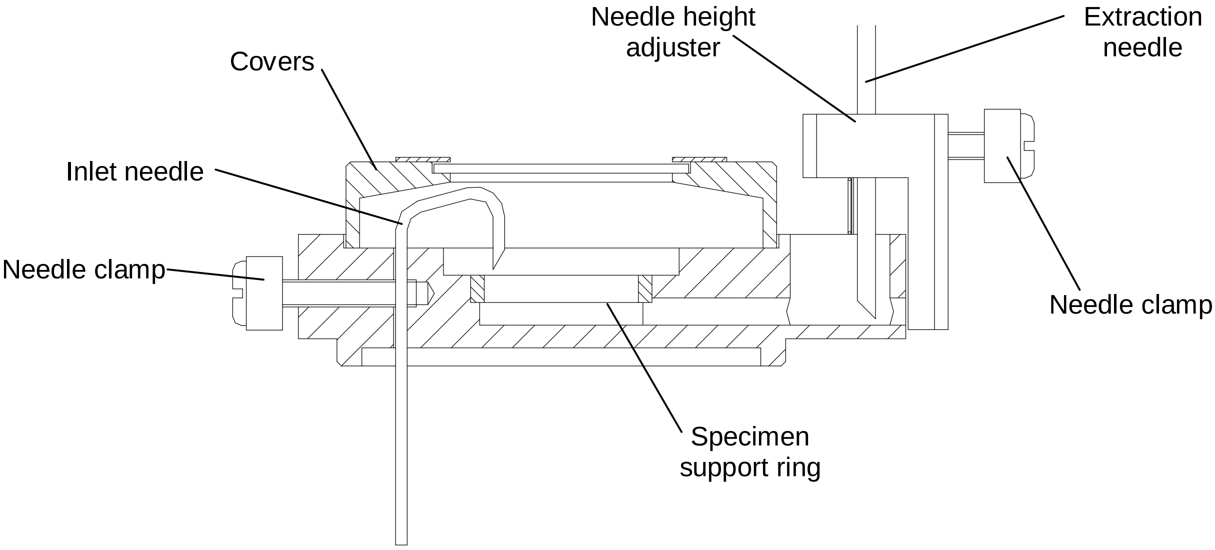


Figure 3. Cross section through chamber

The specimen is supported on a nylon net and the perfusate is fed on to the specimen. By feeding the perfusate onto the specimen from above, any degassing that does occur will be harmlessly vented to atmosphere.

A heater element attached to the stainless steel bath bottom plate provides heating for the water. Temperature feedback is via a thermistor probe located within the bath, below the aeration tubing where it will not be affected by the rising air bubbles.
If there is insufficient water in the bath and overheating occurs, a thermal cut-out will disconnect power from the heater element. Power will remain disconnected until the main supply to the heater controller is switched off and switched back on.

Electrical connections for the heater and water bath probe are via a single connector on the rear wall. The connector also has provision for electrical grounding. A separate ground terminal is provided.
The heater control unit provides the power for the heating element and the water bath probe provides the necessary feedback for PID type control. A separate temperature probe (745CTP) can also be connected to the heater control. The 745CTP probe is designed to give a high resolution and accurate temperature measurement. It can be inserted into an individual slice chamber to provide an accurate indication of the temperature, facilitating the determination of the temperature offset between the heated bath and the chambers. The 745CTP probe can also be used to provide accurate temperature readings in the holding chamber.
A separate output on the heater control unit gives an adjustable DC voltage for use with a heater pad. In this mode, the 745CTP may be set to feed back the temperature and form part of the PID control loop.

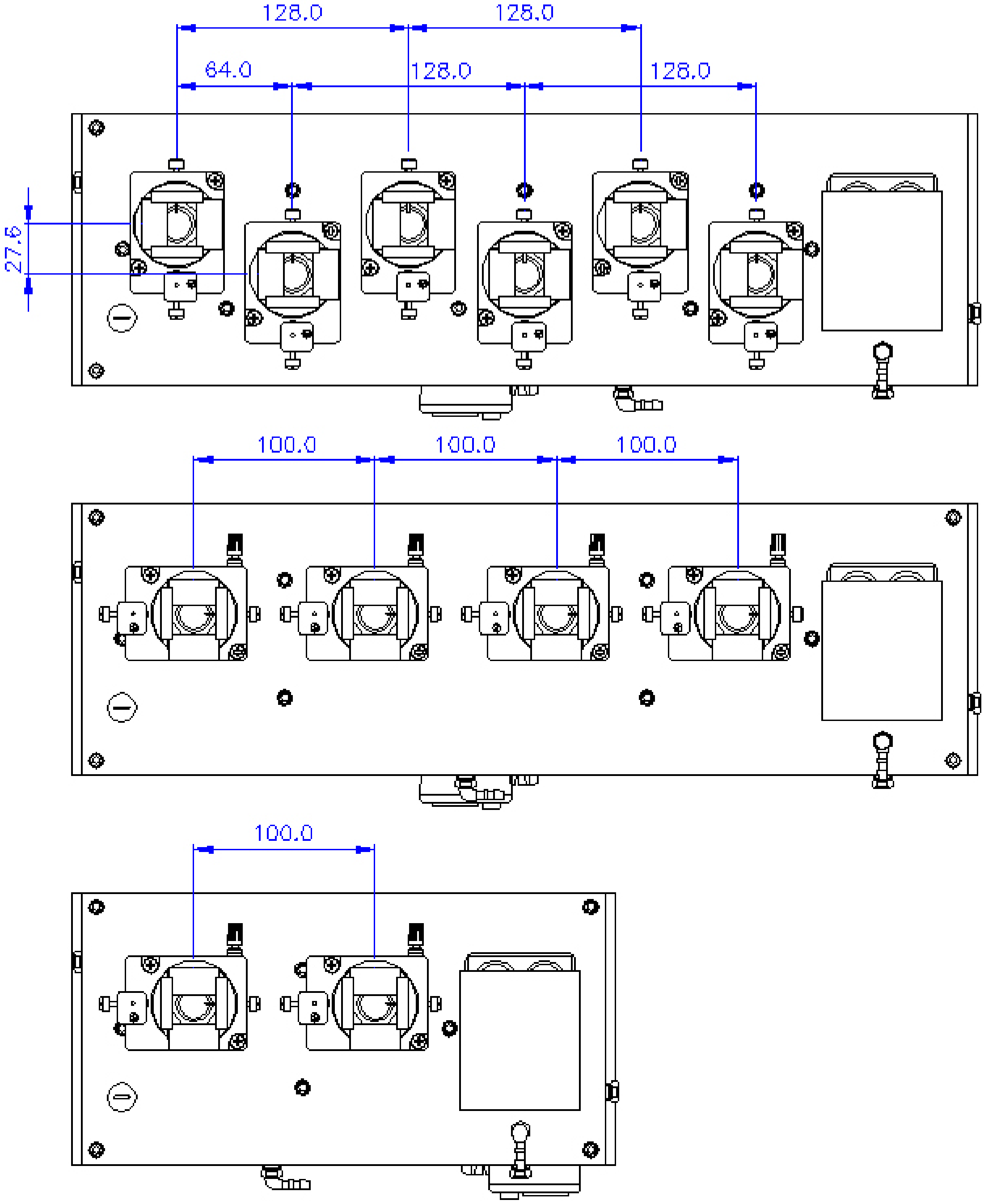


Figure 4. Diagram showing the layout of the chambers

2. Set up and Operation

A note about tubing

The use of the correct tubing to match the application is vital to the success of your experiments. Certain drugs tend to adhere to the walls of the delivery tubing. This can make cleaning difficult, sometimes to the point where it is impossible to removes traces of drug and the only option is to replace the tubing in order not to contaminate subsequent experiments. Different types of tubing are used in different areas of the chamber according to use. In this manual we refer to tubing by simple generic names, e.g. Tygon or p.t.f.e; in reality there are various grades of these tubes available, each manufactured using different polymer ingredients. The choice of polymer, again, can have a significant effect on the integrity of an experiment. The grades of tubing offered by Campden Instruments are the result of feedback from many researchers in different fields and can be used in almost any application without undue concern. A great deal of time and research has been spent developing our chambers so that they may give reliable results however all this can come to nought if the incorrect grade of tubing is used.

The appendix at the end of this manual gives part numbers for replacement tubing offered by Campden Instruments.

If you require further advice on the choice of tubing please contact us.

Practical considerations regarding tubing and electrical cables.

Researchers have noted that loose tubing and electrical cables from both the chamber control and from independent equipment such as patch clamp probes etc., can significantly affect the readings if they are inadvertently knocked, moved or strained during the experiment. It is worthwhile paying very close attention to the routing and securing of such tubes and cables; they should be routed away from busy areas and suspended or restrained without undue strain in such a way they will not be liable to disturbance during your experiments.

2a Fitting and replacing Ag/AgCl reference electrodes. (Electrophysiology only)

If the unit has been ordered for electrophysiological use, it will be supplied with Ag/AgCl reference (ground) electrodes already fitted. Where the unit is intended for biochemistry use reference electrodes are unnecessary and are not supplied. To replace the Ag/AgCl electrode, disconnect the electrode wire from the terminal on the front edge of the chamber (see figure 3). The old electrode can be removed and a replacement fitted. Feed the electrode wire into the hole and carefully pull it through. Just before the electrode enters the hole, smear silicone sealant over the rear of the electrode and then push it into the hole until its front face is flush with the wall of the chamber. Check that the insulating sleeve over the pellet wire is in place otherwise electrical shorting may occur due to condensation.

There must be no silicone over the front of the electrode. If this happens, noise problems may result.

A chamber supplied for biochemistry use can be converted to electrophysiological use by using the following procedure to fit a reference electrode. Remove the chamber by removing the two chamber securing screws (see figure 2). The hole for the Ag/AgCl pellet is only partially drilled through. The hole to accept the pellet should now be drilled through using a 2.2mm diameter drill (see figure 2) until it breaks through into the chamber. Fit a pellet as described above. Terminate the electrode wire on the reference electrode terminal.

Re-fit the insert into the top plate and secure with the two securing screws. Repeat for the other inserts.

2b. Fitting perfusate supply tubing

Remove four socket cap screws (one in each corner) that secure the top plate to the water bath and lift off the top plate. The top plate locates the tubing support posts.

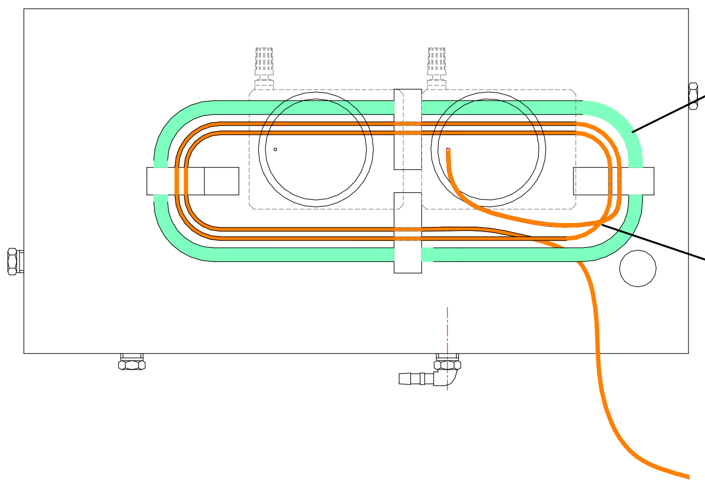


Figure 5. Aeration and Perfusate tube routing (typical)

P.t.f.e. (Teflon®) tubing should be threaded through the holes in the support posts. With the single or two channel models, the tubing should be looped round approximately twice to ensure adequate heat transfer, whilst with the four channel models, one full loop should be sufficient.
A typical routing for the tubing in a two-channel model is shown in figure 5; note that for clarity only one length of tubing is shown. For uniformity of heat transfer, take care that the tubes to each chamber are the same length and follows similar routes through the posts. The inner end of the tubing should be attached to the inlet needle of each chamber with a short length of silicone tubing (see figure 6). When the top plate is replaced, the loose ends of the tubing should be routed out of the unit via two recesses in the top of the bath rear wall.

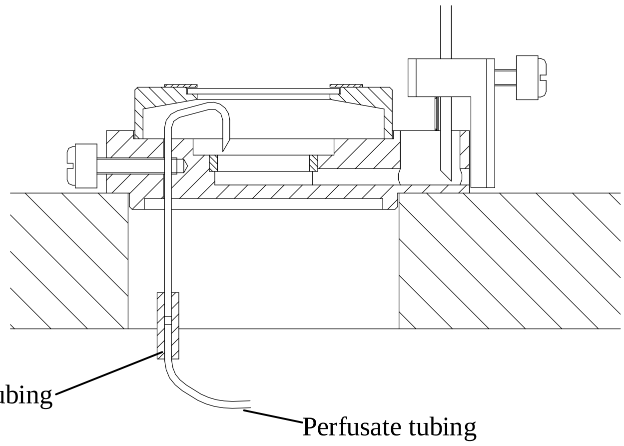


Figure 6. Perfusate connections

2c. Bath water

The bath should be filled with clean water. The bath can be filled by temporarily removing the top plate or by removing the filler plug and using a funnel. The water level should be between the two lines scribed on the sidewall of the bath. Whilst the experiment is in progress it may be necessary to top-up the water level, in this instance the filler plug should be used so that the experiment is not interrupted. As fresh water is introduced, the temperature of the water in the bath will be affected therefore it is advisable to top-up with small amounts of water at frequent intervals rather than large amounts infrequently. For optimum performance, the water level should be maintained between the two scribed lines.
Water can be drained from the bath by unscrewing the drain plug in the sidewall. The filler and drain plugs must not be over-tightened to avoid damaging the bath. The use of plumbersôp.t.f.e tape on the plug threads is advised.

2d. Perfusate removal

The level of the perfusate in the chamber controls whether the chamber operates in interface or submerged mode. The extraction needle should be adjusted by loosening the clamp screw as shown in figure 7, roughly positioning the needle and retightening the screw. Fine adjustment of the perfusate level is accomplished by turning the adjuster screw as required.
Tests have shown that the extraction needle will perform better if it has been ówettedô by immersing it in water for 24 ï 48 hours before using it. This is because steel as used in the needleõs construction is naturally hydrophobic. Wetting it for a period reduces this hydrophobicity and allows the needle to be more sensitive to the height of the fluid in the chamber and so control the liquid height closely.

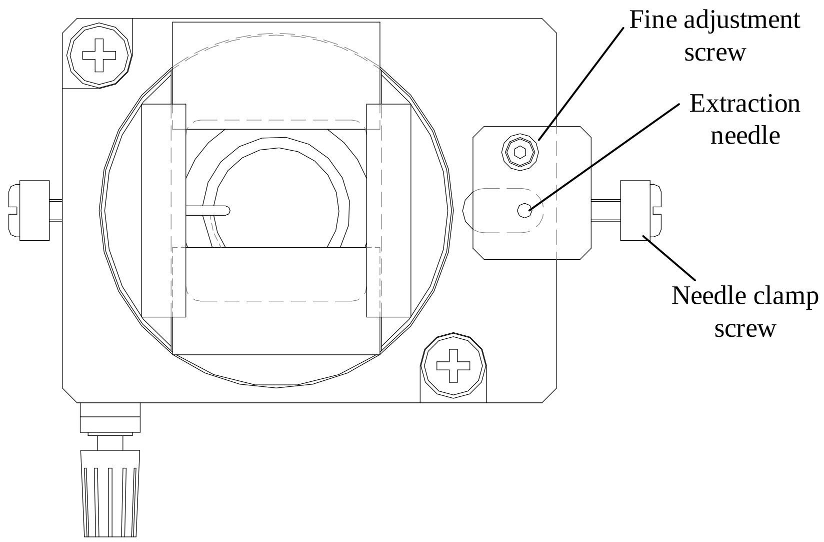


Figure 7. Perfusate extraction

The upper end of the extraction needle should be connected to a peristaltic pump. The pump should be set to extract at a greater flow rate than the incoming flow rate. The needle tip has been reground to a specific angle that allows the needle to continuously ósipô the perfusate from the chamber once it has been wetted without breaking the meniscus contact with the fluid in the chamber.

2e. Aeration of the water bath

It is advisable that the water in the bath is aerated with either air or carbogene. Aeration of the water will reduce the amount of degassing of the perfusate as it flows through the tubes within the bath. Connect a suitable gas supply via flexible tubing to the gas port on the rear of the bath; alternatively, an air pump may be used. Control the amount of bubbling in the bath: It should occur continuously throughout the porous tube to ensure good aeration of the water. If the rate is too low, the perfusate running through the p.t.f.e tubes may degas; if the rate is too high then efficient heating may be impaired. Please note that because the aeration tube is porous it will become waterlogged whilst there is no air supply, therefore when the air supply is connected there will be a short delay whilst the incoming air ejects the water. A short burst of higher pressure air (than is to be used during the experiment) will clear the water from the tube effectively.

2f. Aeration of the Holding chamber

The holding chamber should only be aerated using carbogene gas. The supply should be connected to the inlet on the top of the top plate. Adjust the gas flow to give the required aeration. Aeration should not be too great or the flow of heat from the main chamber below will not be sufficient to maintain the temperature at a suitable level. The cover for the holding chamber should always be used in order to prevent undue heat loss.

2g. Temperature setting

Fill the bath with clean water to between the level markers on the sidewall and replace the top plate. Connect the heater controller to the bath. Set the heater controller to heat the bath water to the desired level. There will be a temperature offset between the water bath and the slice chambers and this must be taken into account. The offset will depend on the volume of water, the amount of gas bubbling through the bath, perfusate flow rate, etc. The exact chamber temperature (and thus the temperature offset) can be ascertained by running a dummy experiment and measuring the chamber temperature with the temperature probe 745CTP. Detailed instructions for the operation of the heater controller are given below.
It should be noted that both submerged and interface chambers maintain the slices under ideal conditions as long as there are no outside influences. Outside influences can have a large effect on temperature stability. Doors opening and closing, open windows, technicians breathing over the apparatus can have dramatic effects. Care should be taken to avoid such extraneous influences. If close, direct observation is essential then a breath deflector may be beneficial.

2h. Chamber Preparation

Nylon nets: Glue a piece of nylon net to each ring. Best results are obtained using French stockings cleaned 2-3 times in a washing machine to remove any coatings or fabric treatment from the material. Stretch the nylon net over the opening of a large glass bottle. Put some fast acting glue (e.g. Loctite 406 + Loctite 770 Activator) on the edge of each ring and glue them to the nylon net. After drying, cut out the rings together with the glued nylon net.

Position the rings (with the nylon nets at the bottom) in the chambers. Adjust the heights of the inlet and extraction needles as required and commence superfusion. When the superfusion is stable and the temperature is at the set value place the specimen on the nylon net. Ensure that the specimen is completely submerged (for submerged operation) or in contact with the surface of the perfusate (for interface operation); adjust the extraction needle more or less into the inner well to get the correct perfusate level.

Chamber covers: The chamber cover incorporates two sliding doors. Once the specimen has been placed on its net the cover can be put in place. The doors allow the atmosphere above the specimen to be maintained and controlled ï warm, moist, oxygenated air is allowed to rise through the vent slots in the chamber from the heated water bath below. The doors can be opened to allow probes to be positioned in the specimen or observations to be made of the specimen as the experiment progresses. The cover can be rotated and the doors can be adjusted as required.

After setting up the superfusion, covers and temperature, the experiment can commence.

2i. Control of superfusion inflow

There are two principle ways to control the inflow of the superfusion medium (perfusate). The flow can be controlled by gravity through a suitable restrictor or by using a peristaltic pump. See figures 8 and 9

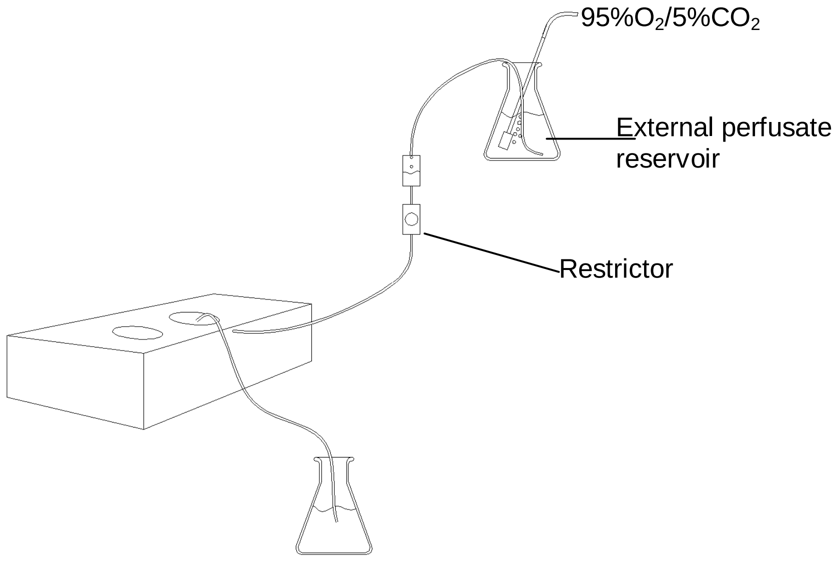


Figure 8. Perfusate feed via gravity

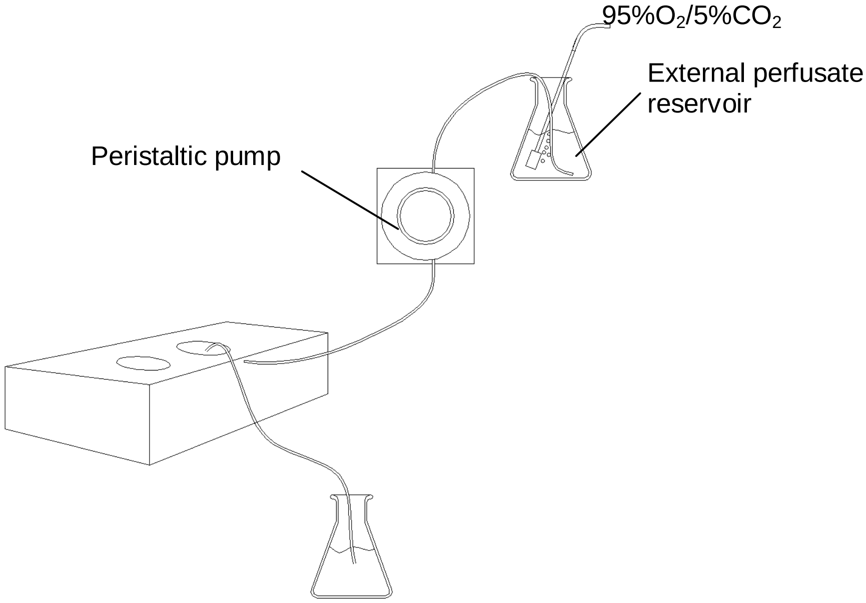


Figure 9. Perfusate feed via peristaltic pump

The perfusate (e.g. artificial cerebrospinal fluid - a.c.s.f) should be oxygenated in a fluid reservoir as shown in figures 8 and 9 with Carbogene (95%O_2/5%CO_2). The fluid reservoir should be heated to about 28 ï 30°C otherwise too much of the Carbogene will be degassed when the fluid is heated as it flows through the tubes in the chamber bath and may adversely affect the tissue slice and your experiments. If you use a preheating stage for the reservoir, ensure that the preheating temperature is not higher than 32°C otherwise not enough oxygen will be dissolved into the perfusate.
The perfusate tubing in the bath is made from p.t.f.e. P.t.f.e is chemically inert and helps ensure that substances with a high adhesion to plastic materials will not bind. If drug adhesion is likely to be a problem you should also use p.t.f.e tubing outside the chamber. Additionally you should wash all tubing for some hours with distilled water after the experiment.

2j. Holding chamber preparation

Each chamber is supplied with a holding chamber insert to hold the slices suspended in aerated a.c.s.f. The insert has four chambers, each having an inner p.t.f.e. ring to support nylon netting. Nylon netting should be attached to each ring as described above in the section óChamber Preparationôto support the specimens whilst they recover.

3. Temperature Controller

THE UNIT MUST NOT BE OPERATED UNLESS THE WATER BATH CONTAINS WATER OR SERIOUS DAMAGE WILL OCCUR

Before connecting the unit to a mains supply, the unit must be set for your particular voltage supply. The voltage is set by prising out the fuse holder drawer and re-inserting it such that the voltage legend for your supply is aligned with the mark on the inlet moulding. See Figure 10.

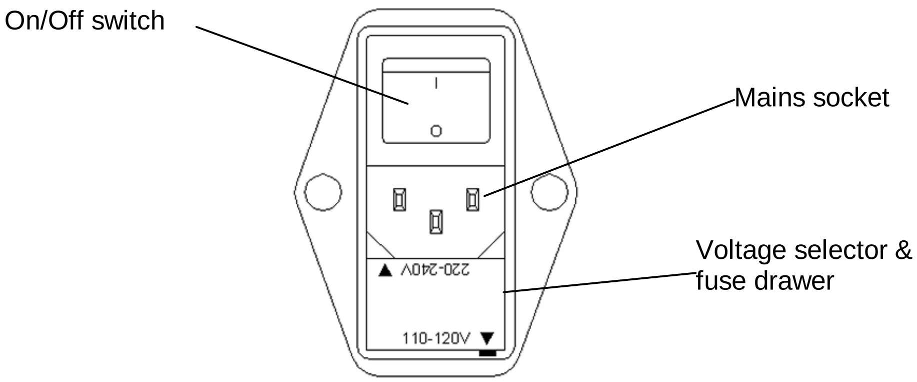


Figure 10. Mains inlet/voltage selector (example shown is set to 110-120V)

The inlet moulding accepts a standard IEC socket. Where possible a standard mains lead - IEC socket/mains plug ï suitable for your mains outlet will have been supplied with the instrument. The instrument must not be operated unless it is connected to a suitably earthed (grounded) mains supply

3a. Connections

PLEASE NOTE: - Do not connect the external power outlet and the slice chamber to the controller at the same time. This may result in damage to the temperature controller.

Connect the heater controller as shown in figure 11.

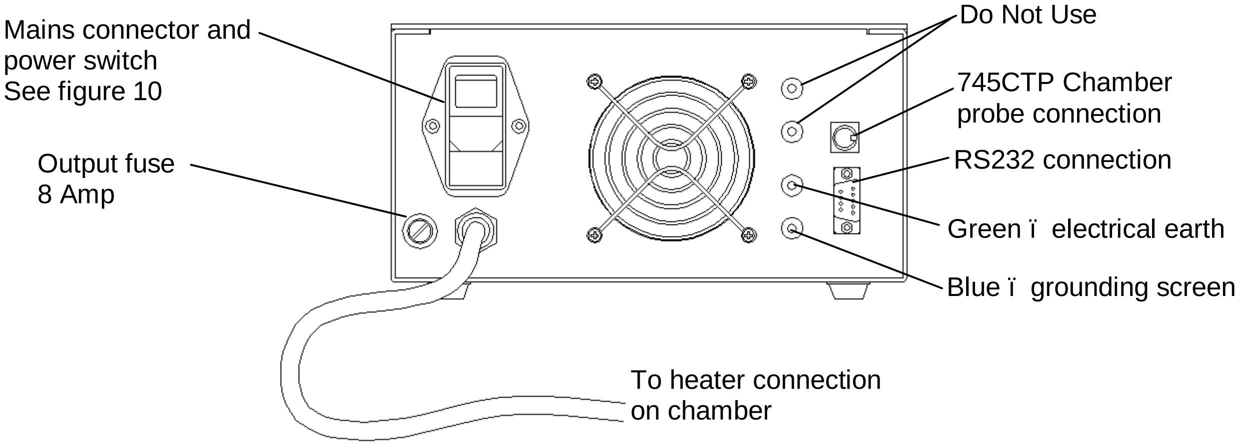
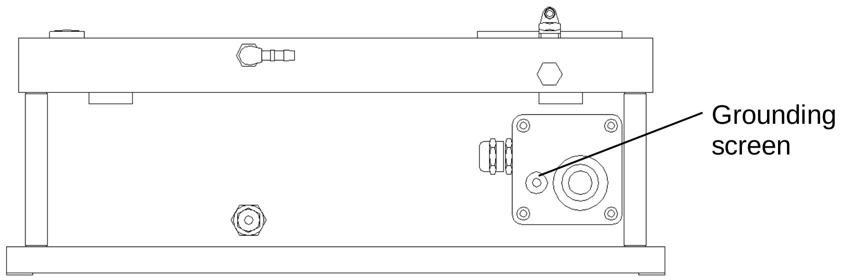


Figure 11. Rear view of heater controller

The trailing lead should be connected to the mating connection on the rear of the slice chamber.
The separate 745CTP-chamber probe should be connected to the chamber probe connection
The RS232 port allows connection to a computer so that data can be collected automatically. This facility is explained in the appropriate section RS232.
The red and black sockets provide power for an alternative heater pad if required with or without PID temperature control using the chamber probe. Connection of an alternative heater pad and a chamber at the same time may result in damage to the temperature controller.
The green socket is available to provide an electrical earthing point for the alternative heater pad if required. It is connected internally to the earth terminal of the mains power lead.
The blue outlet is connected internally to the screens of the chamber heater and probe cables. It may be connected to a Faraday cage to minimise electrical noise. A similar connecting point is available on the chamber adjacent to the heater connection ï see the illustration below.



Note that it is advisable to use only one of these points (not both) in order to avoid earth-loop problems.

The various connections and their uses are explained more fully in the appropriate section of this manual.

3b. Operation

Power-up
Connect the heater controller to the slice chamber as outlined above and switch the controller on at the main switch on the rear of the unit.
Refer to figures 12, 13 and 14.

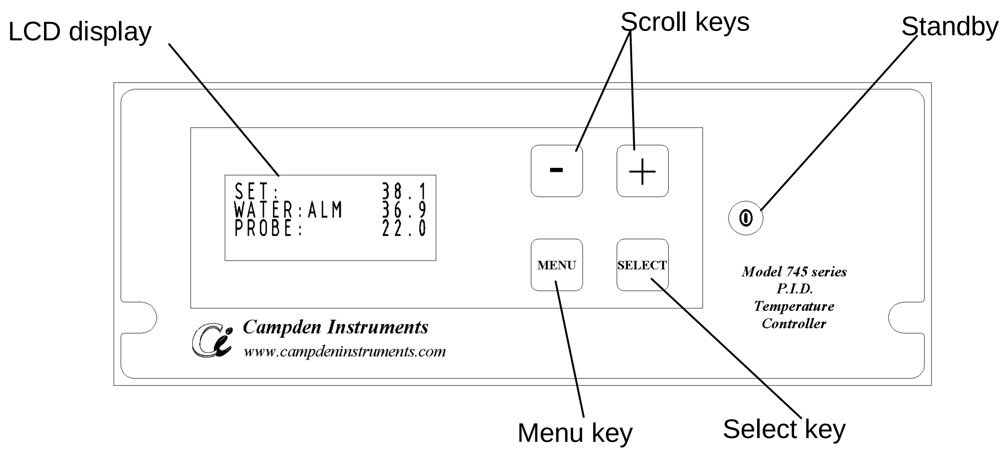


Figure 12. Controller front panel (as used with a slice chamber)

Press the standby key and the LCD display will be illuminated. The actual parameters displayed will depend upon how the unit was last used. Figure 12 shows a typical display with the unit configured to be used with its associated slice chamber whilst figures 13 and 14 show typical displays as if the unit had been configured to power an independent heater.

As shown in figure 12, the unit has been set up for use with a slice chamber where the desired temperature is 38.1 degrees, the current water temperature in the water bath is 36.9 degrees with an alarm set and the chamber probe is reading 22.0 degrees.
If the unit has not been connected to either the slice chamber or 745CTP chamber probe, the display will show ŒERRORô against the offending connection line.

The SET figure indicates the desired temperature in the outer water bath. To change the value press either the **+** or **–** keys. Each momentary press will increment (or decrement) the number to the right of the decimal point by one count. A sustained press (press and hold) will cause the number to the right of the decimal point to count through 10 points and then the numbers to the left of the decimal point to count continuously until either the key is released or the limits of the available settings have been reached.
To set a particular value, press and hold the appropriate +/- key until the approximate value has been reached and then fine tune the value by repeated momentary presses of the appropriate key. The controller will then provide power to the chamber heater to heat the water until that value has been reached. Note that the controller does not have a facility to cool the water in the outer chamber.
The limits of the temperature settings are between 25 and 50^{o} Celsius. The unit may also be set to operate in degrees Fahrenheit, this will be explained later.

The second line ŒWATERô indicates the current temperature of the water in the outer chamber. An alarm may be set to warn if the water temperature, once it has reached the set value, subsequently drifts away (+ or -) from that set value by a preset amount. The alarm condition is indicated by a buzzer and by flashing the temperature value on and off. The display will also show ŒPress Selectô Pressing the select key will reset the alarm. Note that the temperature feedback loop is provided by the thermistor probe in the outer water bath, not by the 745CTP chamber probe.

The third line shows the current temperature experienced by the 745CTP chamber probe. An alarm may be set to warn if the probe temperature, once it has reached the desired value, subsequently drifts away from that desired value. The desired value is set by pre-setting a lower and upper temperature condition. The alarm condition is indicated by a buzzer and by flashing the temperature value on and off. The display will also show ŒPress Selectô Pressing the select key will reset the alarm.

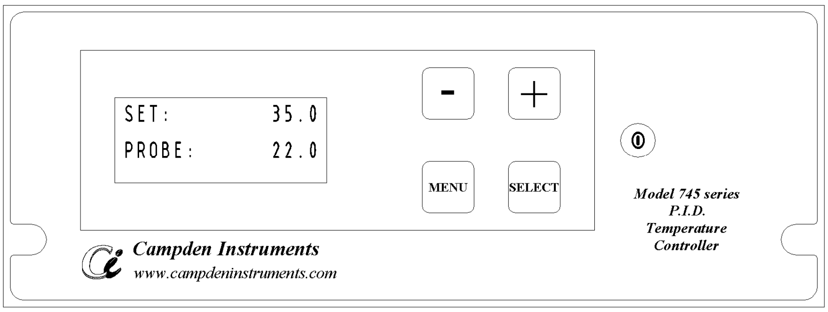


Figure 13. Controller front panel (as used with heater pad and feedback)

If the controller has been set up as a power supply for use with a heater pad and with the 745CTP chamber probe providing temperature feed back the display will be as shown in figure 13.
The first line ï SET ï shows the desired temperature. The set temperature may be adjusted as described above using the + or ï keys.
The line ï PROBE ï shows the current temperature experienced by the 745CTP chamber probe.

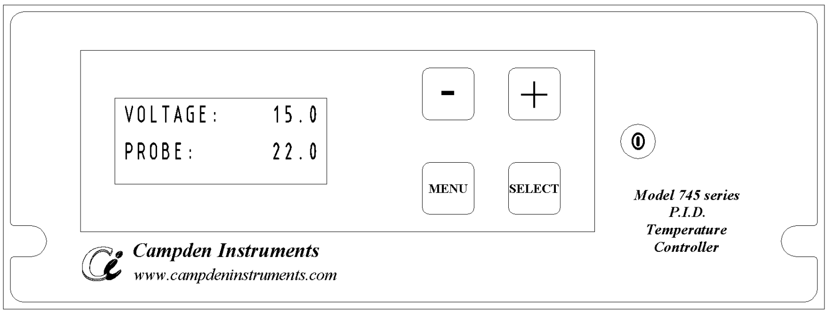


Figure 14. Controller front panel (as used with a heater pad)

If the controller has been set up as a power supply for use with a heater pad but with the 745CTP chamber probe not providing control feed back the display will be as shown in figure 14.
The first line ï VOLTAGE ï shows the voltage available at the red/black sockets on the rear of the unit. The voltage may be adjusted in 0.5V increments by pressing either the + or ï keys.
The line ï PROBE ï shows the current temperature experienced by the 745CTP chamber probe.

3c. Configuring the controller

As mentioned briefly in earlier sections, the controller may be configured to operate in different modes and use either degrees Celsius or Fahrenheit, may have alarms set to indicate temperature drift, etc. The controller can be configured by changing the various options available via menus and sub-menus.

To access the Menu screen, apply power to the controller and press the stand by key.
Press the MENU key and the screen will change to that shown in figure 15.

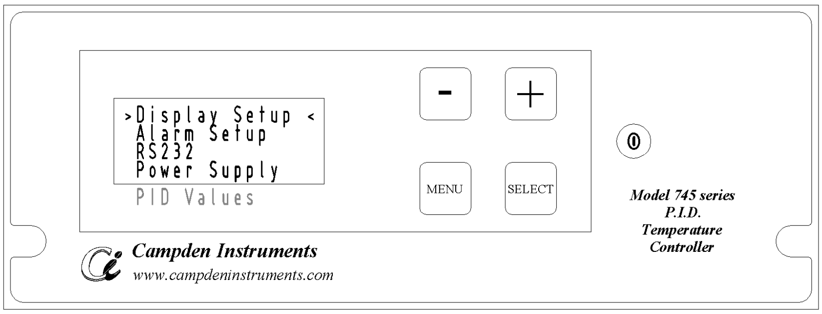


Figure 15. Controller front panel ï Menu screen

The sub-menus can then be accessed using the + and ï keys to scroll the > < cursors up and down the display and pressing the SELECT key. 5 sub-menus are available although the screen can display only four ï the fifth display, PID Values (shown in grey in figure 15), is accessed by scrolling the > < cursors down.
The following sections outline the options within each sub-menu.

1. Display Setup
The unit can display the output of the 745 CTP chamber probe with 1 or 2 decimal point resolution and the controller can be set to operate in either Celsius or Fahrenheit.

745 CTP Chamber probe resolution.
The chamber temperature probe resolution may be set to either 1 or 2 decimal places. Changing the resolution will not affect its accuracy.
Press MENU and using the ï and + keys move the cursors (>é . <) to the line ôDisplay Setupô
Press SELECT and the lines RES and Units are shown.
Move the cursors to the RES line and press SELECT. The cursors will change from > < to = =. The resolution of the chamber probe may now be toggled between 1 decimal place and 2 decimal places using either the + or ï keys. When the resolution has been set as desired, press either the MENU or SELECT key.

Pressing the MENU key will change the display back to the main menu screen. Press MENU again to get back to the normal operating display and mode
Pressing the SELECT key will change the display back to the Display Setup screen.

Temperature scale
Move the cursors to the Units line and press SELECT. The cursors will change from > < to = =. The temperature scale may now be toggled between Celsius (shown by ôDeg Cö) and Fahrenheit (shown by ôDeg Fö).
When the appropriate selection has been made, press either the MENU or SELECT keys.
Pressing the SELECT key will change the display back to the Display Setup screen.
Pressing the MENU key will change the display back to the main menu. Press MENU again to get back to the normal operating display and mode.

2. Alarm Setup
The unit has two alarms available to provide indication of abnormal operation.
As the water bath is heated the controller monitors the situation by a PID algorithm. When the temperature in the water bath reaches the set temperature the controller attempts to maintain the water at that temperature. An alarm within the controller may be set so that if an event causes the water in the bath to drift away from that temperature by a predetermined amount an alarm will operate. The chamber probe may also have an alarm condition. This is defined by entering the upper and lower temperature limits of the probe alarm envelope.
If the alarm has been set is subsequently triggered, a buzzer will sound and the appropriate display line (water or probe) will flash on and off. The display will also show ôPress Selectö Pressing the select key will reset the alarm.

To access the alarm menu press the MENU key to get to the menu screen. Using the + and ï keys scroll the > < cursors to the line Alarm Setup and press the SELECT key.

The display will change to show the Alarm screen.
The first line shows which alarms are enabled. The selection may be toggled between NONE, PROBE, WATER and BOTH. Press SELECT and use the + and ï keys to select the desired output. Press SELECT again to get back to the Alarm set up screen.
The second line shows the tolerance value about the set point of the water bath alarm. To change the alarm value scroll the cursors to the appropriate line and press SELECT. The + /- tolerance can now be adjusted between +/- 0.1 and +/- 2.0^{o} Celsius (or +/-0.2 and +/-3.6^{o} Fahrenheit).
The third and fourth line show the upper and lower envelope conditions of the probe alarm. To change the alarm value scroll the cursors to the appropriate line and press SELECT. The alarm value can now be changed. Note the lower limit will not be allowed within 0.2^{o} C or above of the upper limit and visa versa.
When the appropriate selections have been made, press either the MENU or SELECT key.
Pressing the SELECT key will change the display back to the Alarm Setup screen.
Pressing the MENU key will change the display back to the main menu. Press MENU again to get back to the normal operating display and mode.

3. RS232
The heater controller has the facility to output the water temperature and chamber probe readings to a PC.
From the normal operating display press MENU
Using the + and ï keys scroll the > < cursors to the line RS232 and press SELECT
The first line RS232 shows what data is to be outputted. This may be toggled between NONE, PROBE, WATER and BOTH. Press SELECT and use the + and ï keys to select the desired output. Press SELECT again to get back to the RS232 set up screen.
The second line shows how often the data is to be collected and sent. Using the SELECT and +/- keys this may be toggled between 1-second (1 SEC), 1-minute (1 MIN) and 10-minute intervals (10 MIN) or POLL. When set to POLL, the calling computer determines when the data is collected and sent.
The third line allows the output resolution of the chamber probe to be set. It can be toggled between 1 decimal place and 2 decimal places.
When the appropriate selections have been made, press either the MENU or SELECT key.
Pressing the SELECT key will change the display back to the RS232 screen.

Pressing the MENU key will change the display back to the main menu. Press MENU again to get back to the normal operating display and mode.

3a. RS232 PC set-up
Data from the temperature controller can be received on any RS232 interface program for example Microsoft® HyperTerminal® (which can be found off the Windows® start menu ï programs ï communications). The link requires a 9pin male-female null modem cable. The communication should be configured as; baud rate - 9600, Data bits - 8, Parity ï None, Stop bits ï 1, Flow control ï None.
With the communications link in place and the interface software running; switch on the temperature controller. The text óCampden Instruments Ltd. 745 Temperature Control.ô should appear on the screen.
Data will be collected as defined by the settings on the control unit. Data from the Temperature probe, the water bath or both can be collected. When selected for a 1 second output, the data will sent from the controller followed a carriage return (ASCII ó\r\nô) every second. When 1 minute is selected, data will be sent every minute etc. If Poll is selected the controller will wait until the host PC polls for the data to be sent. Sending the ASCII character ópô to the controller requests the probe temperature and sending the ASCII character ówô to the controller requests the water temperature.

4. Power Supply

PLEASE NOTE: - Do not connect the external power outlet and the slice chamber to the controller at the same time. This may result in damage to the temperature controller.

The heater controller can be used independently of the slice chamber. For example it may be used to power a heater pad. Two modes are possible:

a. It can be used to simply supply a DC voltage where the only control is by manually varying the voltage output.
b. It can be used supply a DC voltage but the chamber probe is used to provide a feedback loop to the PID algorithm which, in turn, controls the voltage output in order to maintain a set temperature.

Press MENU to enter the menu screen and use the + and ï keys to move the > < cursors to the line Power Supply and press SELECT.
Three options are now available:

óExt PSUô this may be SELECTed and then toggled between ON and OFF.
If it is set to OFF then the controller is configured for use with a slice chamber and the Red and Black output sockets on the rear of the controller are ineffective.
If it is set to ON then the chamber output is ineffective. The red and black output sockets on the rear of the controller are available for use. Red is + VDC and black is 0v.

óFeedbackô determines whether the chamber probe is to be included in the feedback loop.
If óFeedbackô is toggled to ON the chamber probe forms the feedback loop in the PID control. The PID control will vary the voltage output according to the feedback. The normal operating display will be as shown in figure 13 and the set temperature can be adjusted using the + and ï keys.
If óFeedbackô is toggled to OFF the controller will simply supply a DC voltage at the red and black sockets. The normal operating display will be as shown in figure 14 and output voltage can be adjusted by using the + and ï keys. The voltage available can be adjusted between 5VDC and 27VDC

óMax Vô determines the maximum voltage available when the unit is operated with both óExt PSUô and óFeedbackô loop set to ON. It may be adjusted between 5 VDC and 27VDC using the + and - keys.

When the appropriate selections have been made, press either the MENU or SELECT key.
Pressing the SELECT key will change the display back to the Power Supply screen.
Pressing the MENU key will change the display back to the main menu. Press MENU again to get back to the normal operating display and mode.

5. PID Values.
The controller works using the PID principle. When the controller is used with a standard slice chamber there should be no need to modify any of the values as these have been determined experimentally during extended trials during the product development stage. If the controller is to be

used in Power Supply mode with a heater mat and with temperature feedback from the chamber probe it may well be advantageous to modify the PID settings to obtain optimum performance from the system. Small changes in PID values can have a dramatic effect on performance. Campden Instruments has tried various heater mats during the productôs development stage and will be pleased to give advice on likely values to be used as a starting point for a particular heater mat.

Press MENU to enter the menu screen and use the + and ï keys to move the > < cursors to the line ôPID Valuesô and press SELECT.

The first line is the P (proportional) value. This may be SELECTed and adjusted using the + and ï keys between 1 and 100.
The second line is the D (derivative) value. This may be SELECTed and adjusted using the + and ï keys between 0 and 100.
The third line is the I (integral) value. This may be SELECTed and adjusted using the + and ï keys between 0 and 100.
The fourth line allows the unit to be reset to the default values. If Default is SELECTed and toggled to YES, the PID values will be reset to the defaults.

Note regarding the principle of PID control and values of the P, I and D components.
The PID control algorithm attempts to control the response of the target by looking at three components in the system response:

1. The current error in the system, i.e. where the system is now and where it ultimately should be.
2. How the system has reacted in the past.
3. Attempts to predict what will happen in the future.

The current error in the system comes under the control of the P (proportional) component. The output of the controller is proportional to the error. A large P value is more aggressive, i.e. a large error will be met by a large response but may cause the system to overshoot. Conversely a small P value may not provide sufficient response and the system will be slow to react.

How the system has reacted in the past comes under the control of the I (integral) component. The controller output is proportional to the amount of time the error is present. The accumulative error, i.e. the sum of the past errors over a period of time, is used to calculate the integral and thence to correct the offset due to the P component. It also has a tendency to make the transient response worse, i.e. it tends to make the system oscillate. A large I value will reduce the response time but increases the likelihood of overshoot and increases the settling time.

The D (derivative) component of the control analyses the rate of change of the error and adds an element of control predicting how the system will react in the future. The controller output is proportional to the rate of change of the error. The net effect is to increase the stability of the system, reducing overshoot and improving the transient response. A large D value has decreases the likelihood of overshoot and decreases the settling time but may cause the system to react too slowly.

The three elements P, I and D are dependent upon each other; changing one element can change the effect of the other two. When tuning the system it is inadvisable to change more than one component at a time. Make small changes to each component in turn, checking the performance of the system to ascertain the effect of the change.

When setting PID values for a non-standard system, set the integral (I) and derivative (D) components to 0. Start with an arbitrary value for the proportional (P) component e.g. 50. Run the system. If the temperature overshoots the set point, reduce the value of the P term and retry from cool. If the temperature does not reach the set point, increase the value of the P term and retry from cool. Repeat this process until you find the point where the system is close to overshoot but still remains below the set point.
Increase the I component until any offset from the set temperature is removed. Retry from cool. If the system overshoots, it may be necessary to fine tune the P and I settings.
Increase the D component to improve the system response time as necessary.

3c. Practical considerations when operating the heater controller.

Use with a slice chamber

Once the unit is switched on the water in the bath will be heated to the set temperature. It should take approximately 35 minutes for a two-channel unit or 45 minutes for a four-channel chamber to reach the set temperature. Outside influences such as ambient temperature will affect this time.

Once the temperature in the water bath has stabilised, the 745CTP temperature probe should be used to ascertain the temperature offset between the water bath and the specimen chamber. Using this temperature difference the water óSETô temperature can be readjusted so that the specimen chamber reaches the desired temperature. The 745CTP probe can also be used to ascertain the temperature of the holding chamber.
The 745CTP probe is very sensitive and will react to extraneous influences such as draughts, opening doors, etc very quickly. If the probe is poorly positioned it may give unexpected and misleading indications.

Use with a heater pad

1. With feedback
If the unit is set up to be used with a heater pad using the 745CTP temperature probe as part of the PID feedback loop, the 745CTP probe must be placed in a suitable position where it will measure the target temperature. It should not be placed too early along the heat path nor too late. Care should be taken so that the probe will not be affected by outside influences that may upset the PID control algorithm. A poorly positioned probe exposed to large swings in temperature will feedback those variations to the controller and a stable temperature will not be achievable.
The PID values within the controller can be adjusted to take into consideration the system time constant (the length of time that it takes the system to react), heat loss and any other influences.

2. Without feedback
In this situation the controller will be operating in an open loop mode requiring intervention by the user to adjust the output voltage in order to control the heater pad temperature. Please bear in mind that any heat path has a time constant, i.e. the length of time it takes the system to react. If the time constant is large, any heat already in the system will continue to flow to the target and the target will continue to be heated until this latent heat is dissipated. Continuous monitoring of the situation will be required for satisfactory control and achieving a balanced system with near constant target temperature may be difficult and time consuming to achieve.

4. Fixing clamps

Should you wish to rigidly fix the unit to a test rig, fixing clamps are available in pairs.
Please note that the unit should only be clamped over its end flanges as shown in figure 16. As the unit is fitted with a thin magnetic rubber strip at each end, if the unit is clamped via the side flanges where it is unsupported, there is a strong possibility that serious damage will occur.

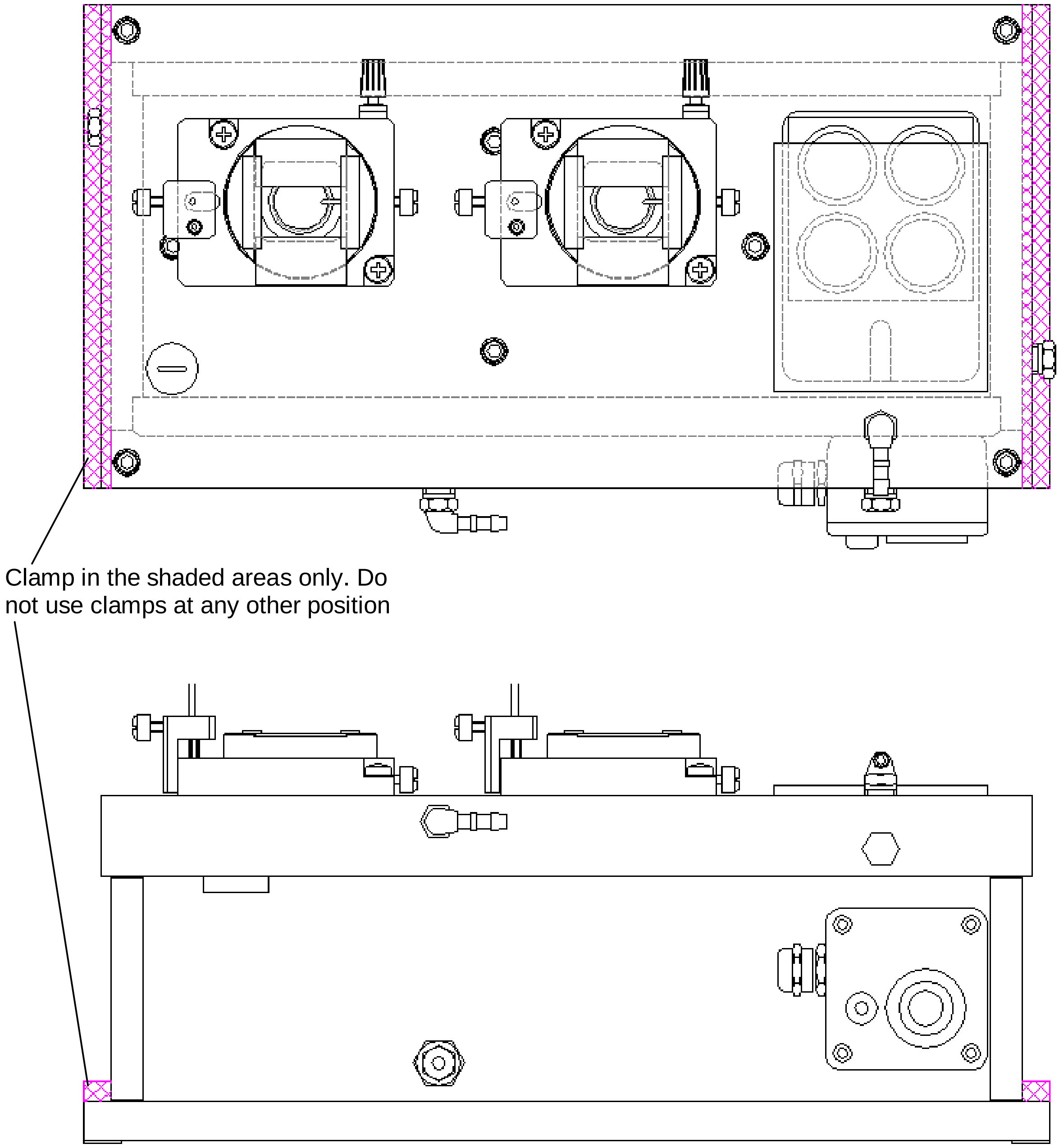


Figure 16. Clamping positions

5. Cleaning and maintenance

The water bath should be cleaned on a regular basis. Bacteria and algae will start to accumulate quickly and grow in the warm, moist conditions found within the bath.
Loosen the four screws in the bath top plate and remove the plate from the main water bath. All tubing, including the gas supply, is mounted on pillars fixed to the underside of the top plate. After removing the top plate, all tubing can be removed or changed as required. All parts should be cleaned using warm soapy water. Rinse well in clean water. Do not use any solvents for cleaning plastic parts or they may be damaged or destroyed. Acrylic parts must not be autoclaved.

Maintenance intervals

Service intervals will depend on usage and operating conditions.
With normal every-day usage Campden Instruments would recommend the following:

Replace Ag/AgCl reference electrodes every 3months or sooner if the signal to noise ratio deteriorates.
Replace perfusate tubing every 6 months or sooner if there is evidence of bacterial growth.
Replace chamber inserts every 12 months or sooner if marks and crevices become a source of infection.

A suitable chart is appended to this manual to log service operations as they are performed.
Replacement parts are listed on page 16

The life of the electrodes, perfusate tubing and chamber inserts can vary greatly depending on the operating conditions and the perfusate used.

Ag/AgCl Reference (ground) electrodes
Ag/AgCl electrodes are quite brittle and should be handled with care. The silver wire tail should not be bent repeatedly. Silver chloride is light sensitive and will darken if exposed to light. This darkening does not normally affect the performance of the electrode but may be removed if desired by rubbing with a fine grade carborundum paper.
Further details are included with each electrode.

Service

Should any problems arise that cannot be solved easily on site, the instrument may be returned to Campden Instruments for investigation and/or repair. Please note the following:

Instruments will not be accepted for service or repair unless the unit has been adequately and properly packaged. Additionally, instruments will not be accepted without prior authorisation and have been certified as being uncontaminated with any material that may be hazardous to the health of service personnel.

Returns Authorisation and Decontamination Certificate forms can be obtained by contacting Campden Instruments at the addresses given at the front of this manual.

6. Spare Parts, Accessories and Order codes

Replacement chambers, temperature probes, etc. or chambers to your own design can be obtained via your local agent or direct from Campden Instruments.

Order codes for In-vitro Slice Chambers for submerged and interface mode and replacement parts

<u>CI.7450 (B) series</u>
Chambers for biochemistry with High Resolution Temperature Controller

CI.7450-4P (B)	4 chamber system with p.t.f.e. inserts
CI.7450-4A (B)	4 chamber system with acrylic inserts
CI.7450-6P (B)	6 chamber system with p.t.f.e. inserts
CI.7450-6A (B)	6 chamber system with acrylic inserts

Chambers for biochemistry - <u>Top Plate Only</u> without heated water bath or Temperature Controller

CI.7450-2P (B) TPO	2 chamber top plate with p.t.f.e. inserts
CI.7450-2A (B) TPO	2 chamber top plate with acrylic inserts
CI.7450-4P (B) TPO	4 chamber top plate with p.t.f.e. inserts
CI.7450-4A (B) TPO	4 chamber top plate with acrylic inserts
CI.7450-6P (B) TPO	6 chamber top plate with p.t.f.e. inserts
CI.7450-6A (B) TPO	6 chamber top plate with acrylic inserts

<u>CI.7450 (E) series</u>
Chambers for electrophysiology with High Resolution Temperature Controller

CI.7450-2P (E)	2 chamber system with p.t.f.e. inserts
CI.7450-2A (E)	2 chamber system with acrylic inserts
CI.7450-4P (E)	4 chamber system with p.t.f.e. inserts
CI.7450-4A (E)	4 chamber system with acrylic inserts

Chambers for electrophysiology - <u>Top Plate Only</u> without heated water bath or Temperature Controller

CI.7450-2P (E) TPO	2 chamber top plate with p.t.f.e. inserts
CI.7450-2A (E) TPO	2 chamber top plate with acrylic inserts
CI.7450-4P (E) TPO	4 chamber top plate with p.t.f.e. inserts
CI.7450-4A (E) TPO	4 chamber top plate with acrylic inserts

Replacement inserts

CI.7450-P(B)	Replacement p.t.f.e. insert for biochemistry
CI.7450-A(B)	Replacement acrylic insert for biochemistry
CI.7450-P(E)	Replacement p.t.f.e. insert for electrophysiology
CI.7450-A(B)	Replacement acrylic. insert for electrophysiology

Chamber bases (heated water bath) only for both biochemistry and electrophysiology types

CI.745-2	Replacement 2 chamber heated water bath
CI.745-4	Replacement 4 chamber heated water bath

Slice Chamber Consumables and Spares

CI.745-1	P.I.D. Temperature Controller
CI.745-5	Adhesive and primer for fixing Lycra net to p.t.f.e. rings
CI.7450-6	Suction needle for perfusate extraction (pack of 5)
CI.7450-7	Adjustable mounting for perfusate extraction needle (c/w one needle)
CI.745-8	Replacement Chamber Temperature Probe
CI.745-10-2	Replacement p.t.f.e perfusate tubing for 2 channel Chamber
CI.745-10-4	Replacement p.t.f.e perfusate tubing for 4 channel Chamber
CI.745-9	Chamber clamping blocks (1 pair)
CI.745-11	Ag/AgCl reference electrode with sleeved lead
CI.745-13	Replacement water bath temperature probe#
CI.745-16	Submerged Chamber Rings for Nylon Net (inserts) Pack of 5
CI.7450-14	Perfusate inlet needle (pack of 5)
CI.7450-15	Replacement chamber cover (with sliding doors)
307416	Holding Chamber rings for nylon net (inserts) Pack of 5

7. Packing list

Interface chamber (2 or 4 well)

1. Chamber
2. Inner chamber Acrylic or p.t.f.e (2 or 4 off)
3. Holding chamber insert and 4 rings
4. Temperature probe
5. Heater controller
6. Mains lead
7. P.t.f.e & silicone tubing for perfusate

Submerged chamber (2 or 4 well)

1. Chamber
2. Inner chamber Acrylic or p.t.f.e (2 or 4 off)
3. Acrylic/p.t.f.e rings for mounting nylon mesh (2 or 4 off)
4. Holding chamber and 4 rings
5. Temperature probe
6. Heater controller
7. Mains lead
8. P.t.f.e & silicone tubing for perfusate

8. Chamber Specifications

	Outer chamber nominal size			Chamber size*	Bath volume		Minimum aeration	Heater wattage	Water bath	Maximium bath	Ag/AgCl electrode	PID Controller	
	L	W	H		litre		gas delivery	(max)	temperature stability	settable temperature	diameter x length	Voltage	Wattage
	mm	mm	mm		min	max	litre/min	W	°C	°C	mm x mm	V	W
											(electrophys only)		
				diameter x depth									
				mm x mm									
2 channel	300	150	110	17 x 3	1.1	1.3	2.5	85	+/- 0.1	50	2 x 4	115/230	300
4 channel	500	150	110	17 x 3	1.9	2.3	3.5	170	+/- 0.1	50	2 x 4	115/230	300
6 channel	500	150	110	17 x 3	1.9	2.3	3.5	170	+/- 0.1	50	n/a	115/230	300
*Chambers available in acrylic or p.t.f.e (Teflon®)													

Controller Specification

Voltage rating: 115/230V Switchable. Power Rating: 300W. Fused at T4A.

DC output when used with 745 chamber: 5V to 27VDC continually variable. 8A maximum. Ripple and noise <60mV @ full load

DC output when used with 3rd party heater mat: 5V to 27VDC continually variable or manually set. Connect only to a resistive load of not less than 5ohms.

Water Bath temperature probe accuracy +/- 0.25 °C. Hand held probe temperature accuracy +/- 0.1°C.

Service Record

745 Slice chamber & Temperature controller **Serial Number:…………………………….**

Service Operation	**Date performed**							
Date of first use								
Water bath washed & cleaned								
Replace Ag/AgCl electrode (recommended: every 3 months or as required)								
Replace perfusate tubing (recommended: every 6 months)								
Replace chamber inserts (recommended: every 12 months)								