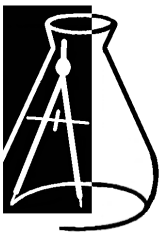

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Innovative Engineering for Science

TISSUE CHAMBER

TCKG

TISSUE CHAMBER

CAUTION !

YOUR TISSUE CHAMBER IS A PRECISION ENGINEERED TOOL FOR SCIENTIFIC INVESTIGATIONS. PLEASE TAKE A FEW MINUTES TO FAMILIARISE YOURSELF WITH THE CHAMBER AND READ THROUGH THIS SHORT MANUAL BEFORE ATTEMPTING TO USE THE SYSTEM.

DO NOT UNDER ANY CIRCUMSTANCES OPERATE THE PTC03 TEMPERATURE CONTROLLER AND TISSUE CHAMBER WITHOUT ADEQUATE WATER IN THE LOWER CHAMBER OR WITH THE SENSOR PROBE REMOVED FROM THE CHAMBER END. THIS CAN CAUSE OVER-HEATING OF THE HEATER ELEMENT. A THERMAL FUSE IS LOCATED IN THE TISSUE CHAMBER TO PREVENT WATER TEMPERATURE RISING ABOVE 70°C. DO NOT LEAVE THE CHAMBER RUNNING UNATTENTED FOR EXTENDED PERIODS OF TIME - PLEASE CONTACT US FIRST FOR DETAILS IF YOUR EXPERIMENTS NEED TO RUN OVERNIGHT AND UNATTENDED.

DO NOT USE ALCOHOL OR SIMILAR SOLVENTS IN ANY CONCENTRATION ON ANY PART OF THE CHAMBER SINCE AS WITH MOST ACRYLICS, ™PERSPEX MAY FRAGMENT OR DEVELOP HAIR-LINE CRACKS.

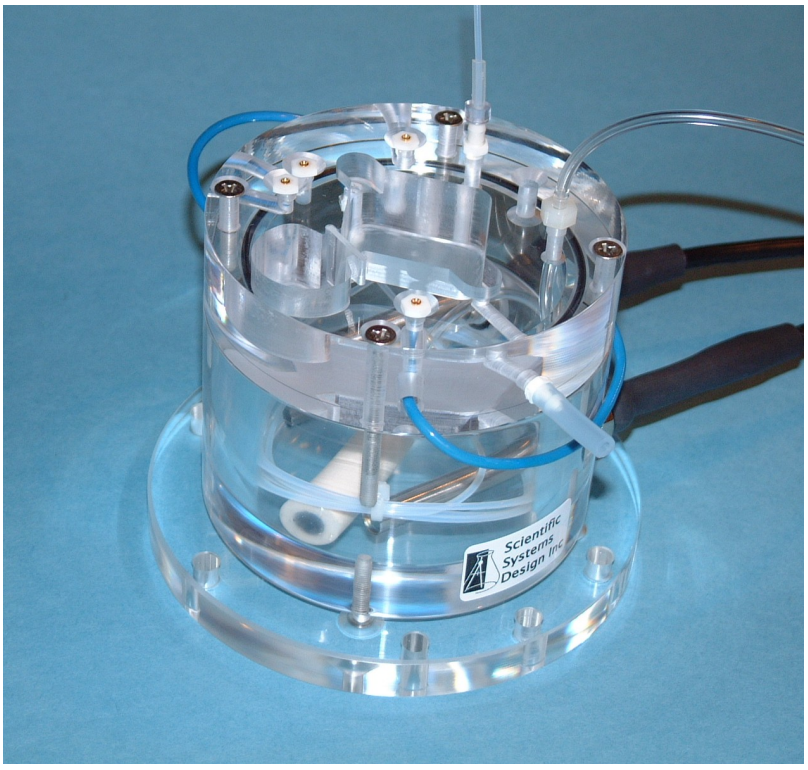
CONTENTS

The TCKG Tissue chamber has been designed to support various types of preparations in experimental test media and allow recordings to be made from nerves arranged across a grease gap, facilitated by a removable partition between the nerve and associated preparation.

FEATURES

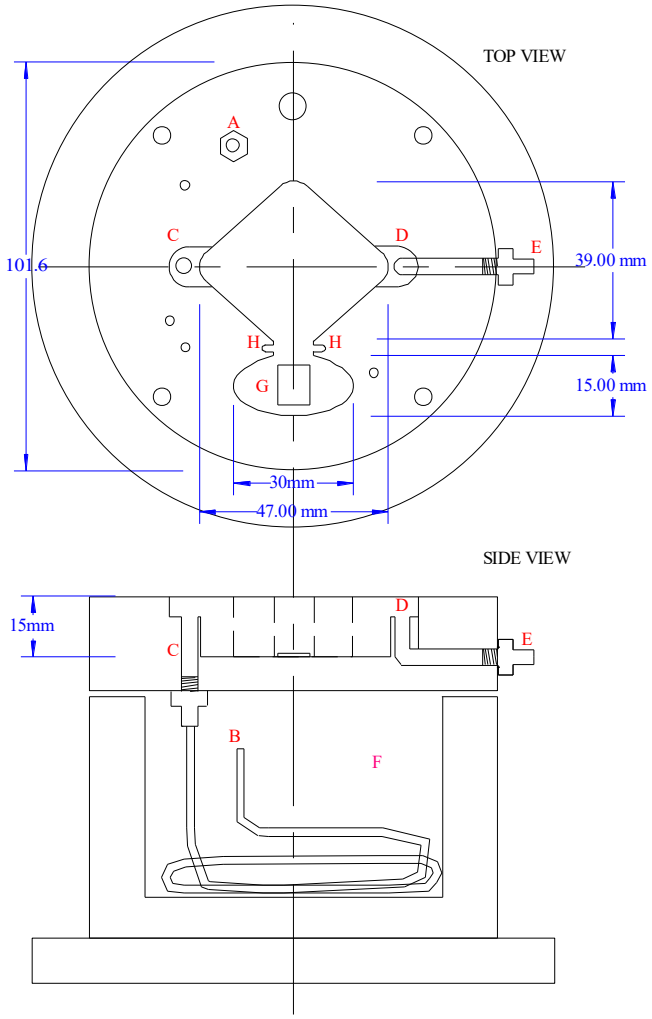
* ***Preparations maintained for many hours in flowing incubating media***
* ***Large diamond-shaped flow cell for maintaining preparations on a custom substrate***
* ***Grease-gap across a removable acrylic partition***
* ***Temperature controlled base heats incoming media flowing across preparation***
* ***Humidity and gas environment can be controlled within diamond-shaped flow cell***

The chamber consists of two main cells, one is a large diamond-shape for containing the preparation and the other is a smaller oval-shaped cell where nerve ends are arranged for making electrical recordings. These two cells are separated by a removable acrylic partition. The bottom edge of this partition can be fashioned to leave a small arch through which nerve bundles can pass through for grease gap recording methods. The larger cell is normally pre-filled with an inert self-curing silicone rubber substrate to allow various types of preparations to be pinned in place. The smaller oval cell is supplied with a small pedestal on which a small pane of glass such as cover-glass is used as a substrate to separate nerves from a nerve bundle with the aid of a dissecting microscope arranged over the cell. Hook-wire electrodes are arranged from nearby 1mm connectors for recording and stimulating functions as are ground electrodes for both nerve and preparation areas. Perfusion fluid flows into the chamber via a Teflon tube which first spirals in the base of the chamber. This base contains temperature control heating and sensor elements connected to a separately available proportional temperature controller **PTC04** or the low noise version **PTC03**. The warmed perfusion solution in the spiral then flows over the preparation in the diamond-shaped area and exits at the opposite end where it can be sucked off from a tube fitting. In addition there is provision for the diamond-shaped cell to maintain high humidity and control the gaseous environment for experimental anoxia or other gas combinations by using the supplied lid.



PARTS DESCRIPTION

Your parcel should contain both the Tissue Chamber and optionally the PTC03 Temperature Controller. Once all packing material has been removed, please take some time to examine the construction of the chamber. Remove and identify: two removable 'window' inserts for the grease gap, two pedestals to mount cover-glass, one lid, a pack of 1mm connector pins for electrode connections.



Feed solution enters through a plastic screw fitting **A** located on the top surface that connects to approx 30 inches of PTFE tubing to point **B** (connection not shown). The PTFE tubing spirals in the chamber base **F** which contains distilled water heated by a heater cartridge and connected to a temperature controller (**PTC03**). Warmed feed solution enters plastic fitting at **C** raised 5mm above main diamond shaped trough into which appropriate depth of Sylgard* or other silicone substrate will be set by the end user. The Sylgard will not spill into the in / out ports as they are raised 5mm above the base. Solution is made to wick across the diamond Sylgard base with filter paper and reaches exit port **D** and then on to an exit plastic screw fitting **E** connected to a house waste vacuum line.
Nerves are routed through a barrier located into grooves **H-H** (not shown) with a hole in it at the bottom. The nerve bundles are arranged on a raised mirror located in an oval area **G** and wire electrodes arranged from pin sockets on the surface.

**Sylgard is a registered trade name and trade mark of Dow Corning Corporation, USA*

OPERATION

Once secured on a table, fill the lower chamber with approx 110 mls of distilled water using a syringe with tubing attached that can be pushed down through the vent hole loosely. Make a note of the fill level which should be seen to completely immerse the heating element visible in the lower chamber and be 2 to 4 mm below the junction between the upper and lower sections. Check this level routinely on a daily basis before switching on the power to the system. Once a week at the end of the day switch off the power and use a fast vacuum line to suck out the distilled water in the lower section, rinse and refill with fresh distilled water as before to the correct level before switching on power. This operation prevents the growth of foreign matter.

In operation, do not block the vent at the top of the chamber - this allows the release of gas from the bubbler and from expansion of gas with heater operation. A groove from this vent leads to the large diamond cell, placing the lid supplied with the chamber allows the humidified oxygen to be directed across to the diamond area, to enhance viability of the living preparation contained here.

Depending on the requirement of the preparation, the ceramic air stone can be fed from a regulated house air supply or from an aquarium pump or from an O2/CO2 cylinder supply which has a regulator fitted to it. The bubbler additionally serves to stir the distilled water in the base of the chamber in order to provide rapid feedback from the heater to sensor elements. Gentle bubbling provides adequate stirring: fast bubbling will take away too much heat, lead to unstable temperature control and increase evaporation from the distilled water reservoir.

CONNECTION TO TEMPERATURE CONTROLLER PTC04 OR LOW NOISE OPTION PTC03

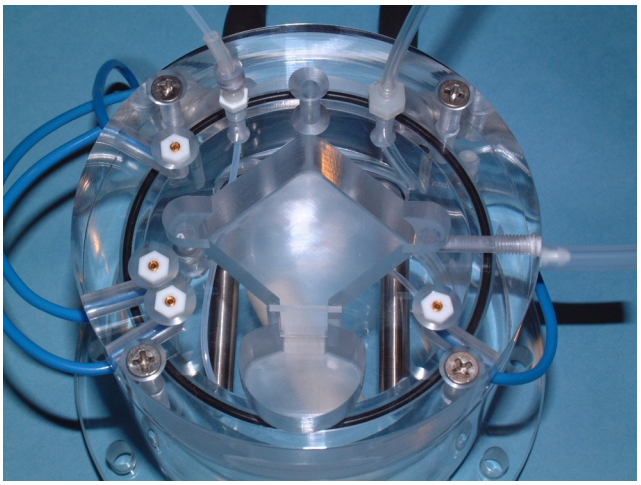
Check that the SENSOR probe is inserted into its hole and that the plug end is connected to the PTC03 Temperature Controller SENSOR socket. Connect the heater power cable from the chamber to the HEATER socket on the PTC03 Temperature Controller. Connect the mains power lead to a suitable socket WHICH MUST HAVE AN EARTH CONNECTION for safety and low noise operation. Turn on the power switch located on the rear of the PTC03. On the front panel the 'LINE ON' light should now be on. Move the selector switch to 'SET', a light above the temperature adjustment knob will turn on to indicate 'SET' mode. Adjust the knob and read the LCD display to set to a desired temperature in ^{o}C. Once set move the selector switch to 'CONTROL'. Assuming you have selected a temperature above ambient, the 'HEATER ON' light will glow brightly or dimly depending on how close the lower chamber temperature is to the set temperature.

NOTE. The temperature shown on the LCD display will be the temperature of the lower chamber distilled water. The temperature achieved in the upper chamber at the location of the preparation depends on a number of factors principally:-

1) Whether the preparation is exposed to the atmosphere or submerged with perfusion solution
2) Ambient temperature
3) Incoming gas mixture flow rate
4) Perfusion fluid flow rate and initial temperature (e.g. from the refrigerator?)
5) Whether chamber lid is in position

Since the above factors are quite stable during the course of an experiment, there is a fixed temperature differential between the upper and lower sections of 3 to 4 degrees for interface and submerged modes. Given this differential, the PTC03 / PTC04 effectively controls the upper chamber temperature which should be monitored with an independent miniature (eg. thermo-couple type) temperature probe. Allow at least 10 to 15 minutes for the system to equilibrate, and approximately 5 minutes for a 5^{o}C temperature increase but 20 to 30 minutes for a 5^{o}C temperature decrease.

As part of our program of continual improvements, provision is already made on your PTC03 circuitry for a plug-in monitor temperature sensor (select MONITOR on switch). This sensor should be available in the future when a more compact version is designed and capable of insertion close to the



CONNECTION OF EXIT WELL

The exit port provides the outflow from the chamber. In the absence of a house vacuum line, typically a high pressure water vacuum adapter is used, peristaltic pumps are equally effective. A bleed valve is recommended when utilising powerful electric pumps to allow adjustments of the level of vacuum, excessive or inadequate levels will cause problems. The correct vacuum level will be found by trial and error, depending on perfusion flow rates. Try pouring a few mls of perfusion fluid into the centre of the upper chamber to see how the fluid behaves with your selected vacuum line.

CONNECTION OF THE PERFUSION FLUID SOURCE

Having connected the exit well it should now be possible to connect a source of perfusion fluid to the inlet line which is a PTFE tube that enters the base of the chamber and emerges at the opposite side of the exit port . Typically the simplest, cheapest and most stable system is gravity fed such as a raised blood-drip set filled with the desired perfusion fluid, pre-gassed with 95% oxygen / 5% carbon dioxide gas mixture or a suitable bottle raised and bubbled constantly with the above gas mixture. A blood-drip set has the advantage of allowing the flow rate to be monitored from the drip rate, in addition the flow adjustment clip is usually easy to operate.

ELECTRODE CONNECTIONS

An earth reference electrode such as a silver/silver chloride pellet connected to the 1mm pin supplied may be placed into the larger diamond area for the preparation and a similar one for the nerve endings in the smaller oval area for grounding for recording and stimulating electrodes. A pair of connectors for positive / negative electrodes are provided for making hook electrode connections to the nerve preparation past the grease gap window in the oval area of the chamber.

Noise problems usually arise from external high voltage sources such as mains power cords, computer monitors, oscilloscopes and fluorescent lights. Relocation of these potential sources may be necessary and/or shielding may be required around the recording electrode to avoid these noise problems.

The heating element in the chamber is driven by a low voltage, low noise direct current power source. If it is found that on switching off the power to the PTC03 (whilst the mains plug is still in the power socket) that noise is eliminated, check the earth connection at the mains plug and socket.

Peristaltic pumps will sometimes also generate very sharp transients due to static discharges along the silicone rubber tubing within the pump mechanism. This may be eliminated by piercing a section of connecting silicone rubber tubing (at a suitable point close to the chamber) with a piece of chlorided silver wire and grounding this to the central grounding point of the recording apparatus.

MAINTENANCE

Alcohol should never be used on the slice chamber for cleaning purposes even at low concentrations because it de-hydrates and produces hair-line cracks in acrylic. A laboratory detergent which completely rinses out should be used. Heavy deposits of salts should be washed out with distilled water overnight and carbonate salts treated with mild acids such as citric acid. The most common contaminant is fungal growth in the upper section tubes and cavities. This can be avoided by agitated washing i.e. suck out plenty of distilled water intermittently with air bubbles through the tubes and holes of the chamber by use of a powerful vacuum line at the end of each experiment. Continue to dry out by using the vacuum line around all the tubes and also below the removable insert. Leaving the chamber dry will prevent the growth of foreign matter. Cover the chamber with a sheet of clean medical wipes to prevent dust settling on the surfaces. Before the start of each experiment rinse with perfusion fluid.