

# BRAINCUBATOR™

## User Manual



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## A MESSAGE TO OUR CUSTOMERS

Thank you for selecting to buy the Brincubator™. This is a unique device aiming to provide optimal conditions for the survival of tissue cells *in vitro* and aid you in your research. You will find the Brincubator™ easy to use and easy to maintain.

Because of its unique features we have developed this user manual. It contains valuable information on how to properly install, operate and maintain your new Brincubator™ for years of safe and efficient operation.

For your convenience, all product questions can be answered by a PAYO Scientific customer care representative at +61-468328301, or email: [Yossi@payoscientific.com](mailto:Yossi@payoscientific.com).

**NOTE:** Inspect the product to verify that there is no shipping damage. If any damage is detected, call the shipper and initiate a damage claim. PAYO Scientific Pty Ltd is not responsible for shipping damage.

DO NOT discard any packing material (box, pallet, straps) until the unit has been inspected.

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## **WARNING**

Before conducting any form of servicing, disconnect the Braincubator™ from the power source to prevent the risk of electric shock and further damaging the unit. The Ultraviolet light (UVC) produced by the lamp is harmful to the eyes and skin. **DO NOT LOOK DIRECTLY AT THE UV LIGHT PRODUCED BY THE LAMP.** The UVC Lamp operates at extremely high temperatures and incorrect handling may result in severe cuts and burns. To prevent injury, protective gloves and safety glasses must always be worn when handling the lamp. To ensure safety, allow the lamp to cool for several minutes before servicing. If the lamp is broken or damaged, refer to the lamp disposal section of this manual to ensure safe discarding practices are met.

To reduce the risk of fire, electric shock, or injury to persons, read the section on Safety Practices and Precautions before operating the Braincubator™. The Braincubator™ must only be used for its intended purpose as described in this User Guide.

## **1. Purpose of the Braincubator™**

The Braincubator™ is a novel incubation system capable of extending the lifespan of thin slice tissue, such as acute brain slices and wholemount retina. This is done by controlling the temperature and pH of the artificial cerebral spinal fluid (aCSF) in which the slices are incubated, while continuously passing the fluid through a UVC filtration system. This system is capable of maintaining extremely low bacterial levels and significantly extending the slice lifespan.

The Braincubator™ offers the investigator a method of maintaining thin slice tissue for many hours, which allows for stable intracellular or extracellular recording as well as imaging experiments. The solution conditions are recorded and saved so they can be viewed for further analysis and validation of slice viability.

The purpose of the Braincubator™ is also to set a gold standard requirement for acute slice tissue incubation. The use of the Braincubator™ will not only extend experimental time for those using acute slice tissue, but will reduce the number of animals required to complete experimental goals.

## 2. Safety practices and precautions

Quartz Germicidal Lamps are low-pressure lamps that generate most of their energy at 254nm (253,7nm - Mercury line) and some energy at 185nm. In medical terminology they are known as UVC lamps. The mechanical structure of germicidal lamps is the same as for a Miniature Cold Cathode Fluorescent Lamp but instead of glass the envelope is manufactured with quartz. At these wavelengths most bacteria, spores and viruses are completely destroyed. Since UV radiation is harmful to human eyes, it is mandatory to avoid direct exposure to UV radiation/light using proper mounting fixtures for these lamps.





### CAUTION!

Germicidal Cold Cathode Lamps are manufactured with quartz glass. The lamp is filled with gas and a very small amount of Mercury (less than 5mg per lamp). These lamps generate UV radiation at 254nm. There is also a small amount of Ozone generated by the residual radiation of 195nm and corona effect. The voltage supplied to activate the lamp is over 300V and is direct proportional with the lamp length.

Although the amount of UV and Ozone generation is very small (UV is Max. 5.0mW/cm<sup>2</sup>), we recommend all suitable precaution to avoid eye or skin exposure to UV radiation. It can produce serious damage to the eye, which may lead to temporary or permanent vision impairment. For this reason, the light produced by a germicidal lamp must be carefully shielded against both direct viewing, reflections and dispersed light that might be viewed. To make sure no exposure of the user to UVC light, please shut off the UV light before opening the lid of the Braincubator™, as illustrated in Figure 16.

Do not touch the glass/quartz with bare hands. Use appropriate protection, wires and insulated materials to avoid surge voltage from the lamp leads. Discard the lamps in accordance with safety code requirements for materials containing Mercury. Follow suitable requirements recommended by the government safety code to avoid prolonged inhalation of Ozone generated by the lamp.

**DO NOT OPEN THE BRAINCUBATOR™ LID BEFORE SHUTTING OFF THE UV LAMP!**

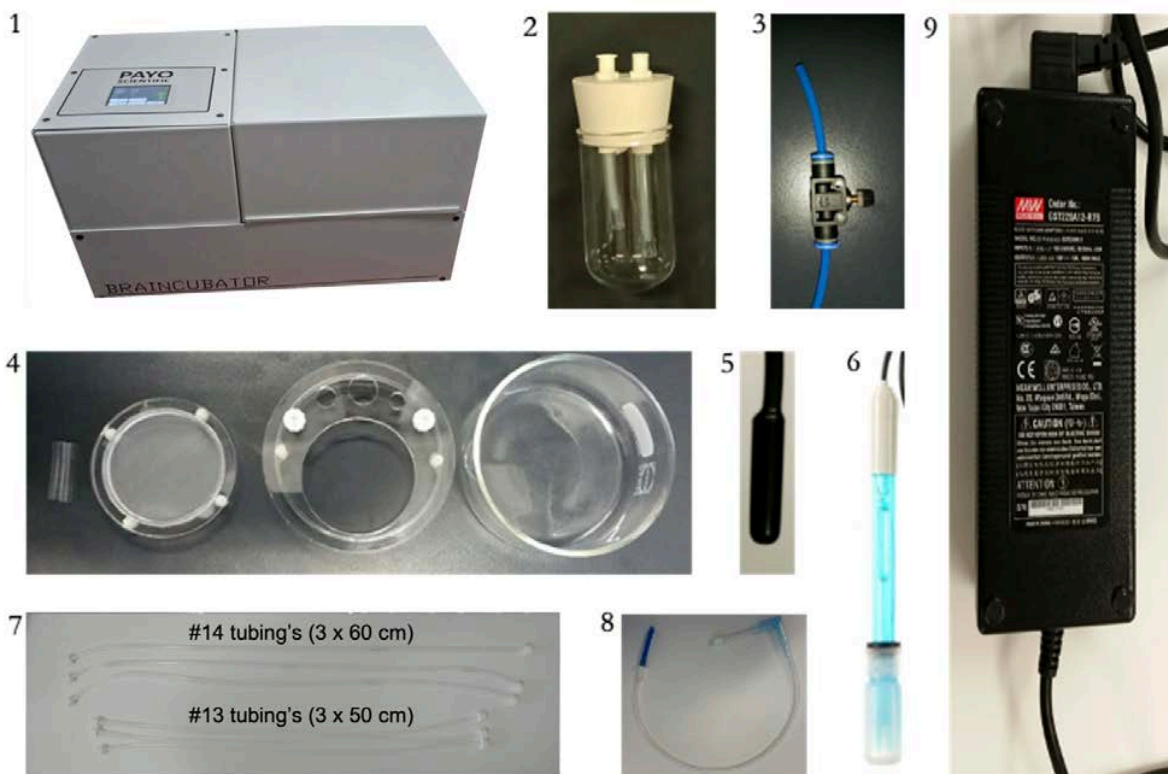
	Hazardous voltage is involved. Care must be taken to reduce risk of electric shock or electrocution.
	Moderate Health Hazard may be harmful if inhaled or contact with skin, causes eye irritation.
	Eye protection must be worn to perform the following tasks.
	Protective Gloves must be worn to perform the following tasks.

### 3. Unpackaging and Installation

The package you have just received includes a complete Braincubator™ system, which contains:

1. Braincubator™ machine
2. UVC Chamber System (Quartz chamber with 3 holes rubber plug and rods)
3. Carbogen valve + 4mm blue tubing
4. Slice Chamber system (Glass chamber + probes holding plate with connectors, rods and a slice holder)
5. Temperature probe (already connected to the main unit)
6. pH probe (packed separately)
7. 2 sets of Tubing's with connectors (3 x #13 pump tubing (50 cm each); 3 x #14 pump tubing (60 cm each)
8. Carbogen diffuser with connector
9. Power supply with 240/110 adaptor

**Figure 1 – Components of a full Braincubator™ system**



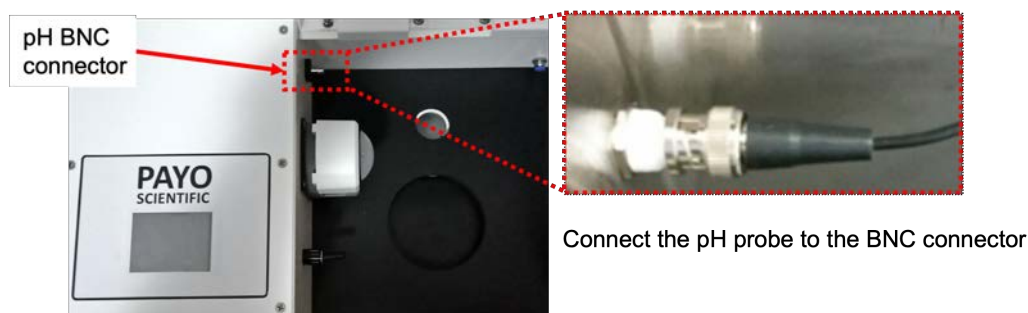
1) A Braincubator unit. 2) UVC chamber with rubber plug. 3) Carbogen valve (to be connected to the carbogen outlet at the back of the Braincubator). 4) Main chamber, probe plate, slice holding mesh and plastic rods. 5) Temperature probe (already connected to the Braincubator). 6) pH probe. 7) Pump tubing (#13 and #14). 8) Carbogen diffuser (To be connected to the carbogen inlet from within the Braincubator). 9) Power supply.

## Testing the Braincubator™ for the first time after shipment

The Braincubator™ has been assembled, calibrated and tested in our factory before shipment. Once you have opened the package, make sure all components are within a good condition and no damage occurred during shipping. To test the Braincubator after shipment, please follow the next steps:

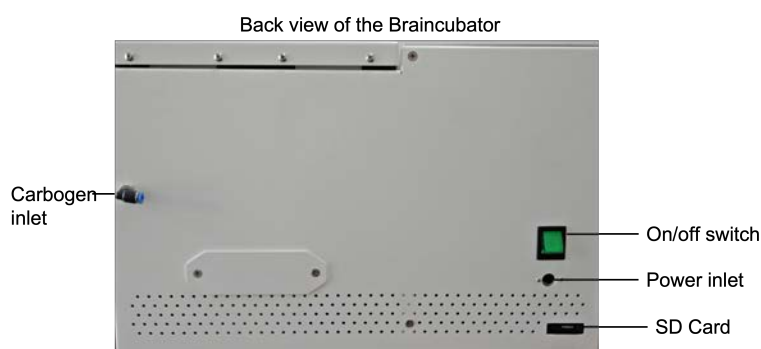
- Connect the pH probe into to proper socket as illustrated below in Fig 2a.

Figure 2a



- Connect the power adaptor to the power inlet at the back side of the Braincubator ⚠.
- Switch on the Braincubator™ from the On/Off button at the back side of the unit.

Figure 2b



- You should see a message on the LCD screen “BRAINCUBATOR V2.01” which will be replaced by the main display showing the control buttons as illustrated in Fig 2c.

Figure 2c



Note: The pump, UVC lamp, Heat/Cool plate and fans should turn on automatically.

- Let the Braincubator™ run for 1-2 minutes. Within that period the UVC lamp should turn on for 20 seconds and then turn off. Switch off the Braincubator™.

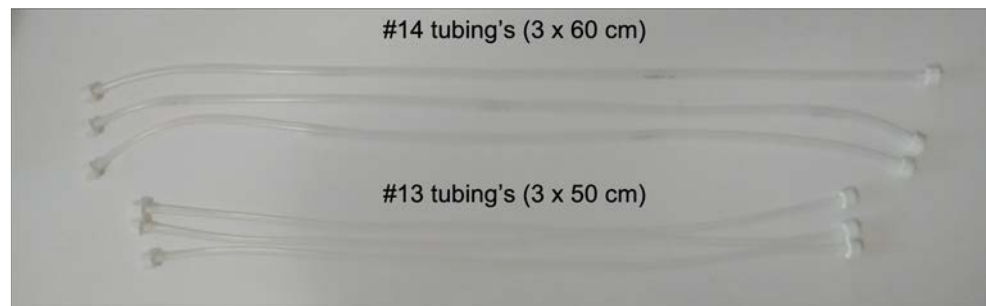


#### 4. First time use

- Place the **pump tubing's** in their place as illustrated in Figure 4. First place the #13 silicone tubing at the left side of the pump (most internal) and then put the #14 silicone tubing on the right slot of the pump.

**\*\*\*This is a very important step. It ensures that flow to the UVC chamber is always slower than the returning flow to the slice chamber. The #13 tubing internal diameter is 0.8 mm while the #14 tubing internal diameter is 1.6 mm.\*\*\***

Figure 3



- Assemble the UVC chamber. Insert the rubber plug into the UVC chamber and place the UVC chamber in its place (UVC chamber port), as shown in Figure 9B. You will notice that there are 2 rods going through the plug rubber. The long rod is for 'inlet aCSF' #13 tubing arriving from the left (internal) side the pump, and the short is for 'outlet aCSF' #14 tubing going through the right side of the pump, as illustrated in Figures 4 and 5.
- Assemble the probe holding plate as illustrated in Figure 5. The probe holding plate has 5 holes. The first hole has a connector and serves as the chamber outlet (aCSF goes from the main chamber towards the UVC chamber). The fifth hole also has a connector and serves as the chamber inlet (the circulating aCSF from the UVC chamber returning to the main chamber). Connect the two plastic rods to the 'inlet' and 'outlet' connectors, from the bottom side of the plate. Place the plate on top of the glass slice chamber.  
\*\*\*\*\* In some cases, the plate will come assembled from our factory\*\*\*\*\*
- Connect the tubing as illustrated in Figure 4. Open the pump lid by turning the pump knob counter clockwise. Insert the #13 tube into the left internal slit and the #14 tube into the right slit. Close the pump lid by turning the pump knob clockwise.
- Connect #13 tube inlet end to the 'outlet rod' of the probe holding plate, as illustrated in Figure 4. Connect the other end of the #13 tube to the 'inlet' (long rod) on the rubber plug of the UVC chamber, as illustrated in Figures 5.
- Rotate the #14 tubing, such that the inlet end is connected to the UVC chamber 'outlet' (short rod), and the other end is connected to the 'inlet' connector on the probe holding plate, as illustrated in Figures 4 and 5.

\*\* In some cases, the UVC chamber/main chambers/tubing's arrive assembled as illustrated in figure 5A.

**Figure 4**

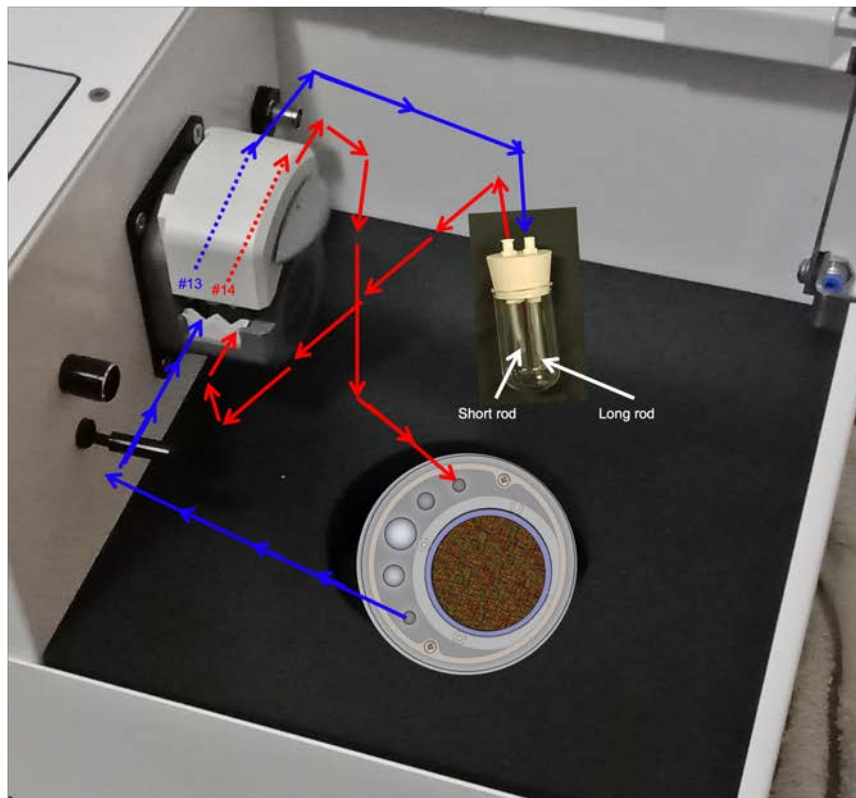
**Schematic diagram of the tubing's connectivity**

**A.** First install #13 (blue) tube on the left (internal) slit of the pump and the #14 (red) tube on the right slit of the pump

**B.** Close the lid of the pump by turning the pump knob counterclockwise

**C.** Connect the #13 tube's so the right side will connect to the long rod of the UVC chamber, and the left side will connect to the main chamber as illustrated by the blue arrows. This will allow the flow of aCSF from the main chamber to the UVC chamber.

**D.** Rotate the #14 tube's so the right side will connect to the main chamber and the left side will connect to the short rod of the UVC chamber as illustrated by the red arrows. **This is a crucial step** to insure the flow of aCSF from the UVC chamber to the main chamber.



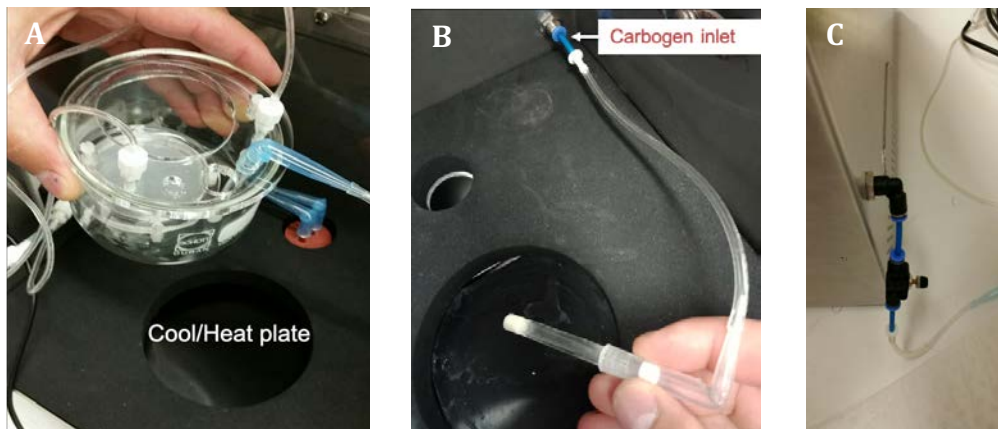
\* To ensure proper operation of the Braincubator, please make sure to install pipes correctly. The #13 pipe internal diameter (0.8 mm) is deliberately smaller than the #14 pipe (1.6 mm), to make sure that the UVC chamber won't overflow.

**Figure 5**



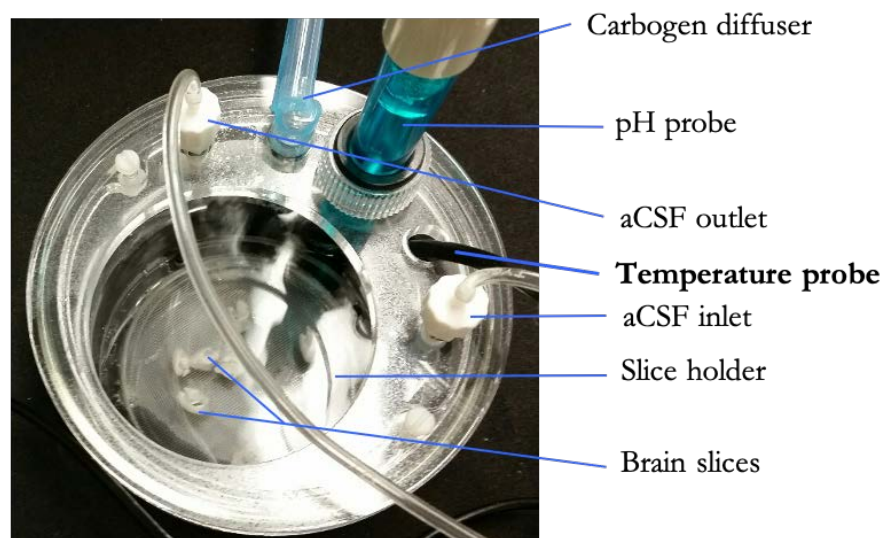
- For first use, fill the slice chamber with 200 ml of distilled water, put the probe holding plate on top of the slice chamber and place the chamber on the cool/heat plate as illustrated in figure 6 below.

**Figure 6**



- Insert the temperature probe into the main chamber, as illustrated in Figure 7 ⚠.
- Connect the pH probe to the BNC connector (inside the Braincubator, see Figure 2a) and insert the pH probe into the main chamber through the probe holding plate as illustrated in Figure 7.
- Connect the carbogen diffuser to the carbogen inlet (from within the Braincubator, as illustrated in Figure 6B) and the carbogen valve to the carbogen outlet (from outside the Braincubator, see Figure 6C). Then connect the carbogen valve to the main carbogen supply in your lab. See carbogen flow in page 13.
- Insert the carbogen diffuser into the main chamber through the probe holding plate, as illustrated in Figure 7.

**Figure 7**




A photograph of assembled slice chamber

- Switch “on” the Braincubator™ from the main switch at the back.

- Let the Braincubator™ run for an hour. During this time familiarize yourself with the Braincubator™ controls. First, set the target temperature to 35°C for 10 min, as described in the operation section. After reaching the desired temperature, set it to 15°C for 20 min. The temperature and pH are recorded to the SD card at the back of the Braincubator at one-minute intervals.
- The Braincubator™ is ready to use. Before shutting it off, please refer to the cleaning section to assure proper cleaning and drying for next use.
- We are here to help! Please contact us at [yossi@payoscientific.com](mailto:yossi@payoscientific.com) if these instructions are not clear enough, or you have any issue.

## 5. Operation

Button	Action
Up (+)	Increase target temperature by 0.5 C <sup>0</sup>
Down (-)	Decrease target temperature by 0.5 C <sup>0</sup>
	Enable/Disable pump
	Enable/Disable UVC Lamp
	Enable/Disable Heat/Cooling Plate
Right (>)	Getting into pH calibration mode
Left (<)	Set date and time mode
SAVE	Save data entered for pH or Time/date

- Before operating the Braincubator™, refer to the section above for proper installation.
- **Make sure that all pipes, UVC and slice chambers are clean and dry before use, as this can impact the tissue viability.**
- Fill the main chamber with 150-250ml of aCSF, insert the slice holder into the glass chamber and make sure it is well submerged.
- Turn on the Braincubator™. The LCD display will turn on indicating “BRAINCUBATOR V2.01” which will be replaced by the main display showing the control buttons as illustrated in Fig 2c. The display will state “pH calibrated”, the name of the file where all temperature and pH measurements are saved and lastly the home screen with all measurements displayed as illustrated in Figure 2c. You will notice that the pump and fans are working. The LCD display is a touch-screen in which all control buttons are displayed.
- Set the desired temperature, as detailed below, usually for brain slices the initial temp is 30-32°C for 10 min and then 15-16°C until use, as illustrated in Figure 19.
- The UVC lamp is cycled on and off, so may be on at any given time. ⚠ Before opening the lid, disable the UVC lamp by pressing the UVC lamp button on the touch-screen (on depressing the button it will turn from green to red).
- Insert the temperature probe, carbogen inlet, outlet and inlet tubing's and pH probe in



- their place on the probe holding plate, as illustrated in Figure 7.
- Adjust the carbogen flow to reach pH 7.2-7.4. Over-bubbling can cause physical damage to the slices and should be avoided.
  - Once the temperature reached the target temperature, begin your slicing procedure.
  - Following slicing, place slices in the Braincubator™ for 10-30 min at 30-32°C.
  - Once all slices are in the Braincubator™, close the Braincubator™ lid and enable the UVC lamp, by pressing the UVC button (it will turn from red to green).
  - Reduce the temperature to 15°C, as illustrated in Figure 15.
  - Slices can be kept in the Braincubator™ until use.

### Setting up the chambers (UV and main chamber)

To set up the slice chamber, insert the slice holding mesh into the chamber. Then place the probe holding plate on top of the chamber, as illustrated in Figure 7. Assemble the probe holding plate, as illustrated in Figure 7. The probe holding plate has 5 holes. The first hole has a connector and serves as the chamber outlet. The fifth hole also has a connector and serves as the chamber inlet. Connect the two plastic rods to the 'inlet' and 'outlet' connectors, from the bottom side of the plate, as illustrated in Figure 18. Place the chamber on the heat/cool plate, **insert the temperature probe**, pH probe, aCSF inlet tubing's, aCSF outlet tubing's and carbogen diffuser into their place, as illustrated in Figure 7. The chamber is now ready for operation.

### Carbogen flow

Carbogen (mixture of 95% Oxygen and 5% CO<sub>2</sub>) is essential to the viability of the tissue, as well as to tune and maintain the proper pH. To assure the flow of carbogen into the slice chamber, connect the diffuser into the carbogen inlet inside the Braincubator™ using the supplied valve and 4mm blue tubing, as illustrated in Figure 6. Your system includes a flow valve, which can be attached to the back of the Braincubator™, as illustrated in Figure 18E, to allow adjustment of carbogen flow. Connect the carbogen valve to the Braincubator™ from one end and to the carbogen source in your lab on the other end.

Please note that the carbogen valve supplied with the Braincubator™ is designed to work on pressure levels below 10 Bar.

Alterations of the carbogen flow will result in changes of the pH. The optimal pH for brain slices is in the range of 7.2-7.4. To increase the pH, decrease carbogen flow and vice versa.

### Touchscreen LCD display

The LCD home screen will show the time, date, target temperature, current solution temperature, cooling/heating plate temperature, pH and whether the Braincubator™ is currently heating, cooling or neutral mode. On the right side of the display, there are 3 buttons to enable/disable the pump, UVC lamp and heating/cooling plate.



### Adjusting the date and time (already pre-set in our factory)

- From the Home Screen, press the '<' button once to access the 'set time & save' mode.



- Press the + or - buttons to increase or decrease the time by 1 minute. You will notice that the time display will turn red. NOTE: The time is set to 24 hour format.
- Press the save button once, the screen will display "time saved".
- Once the desired time has been chosen press the '>' button to resume normal mode.

### Adjust the Date

1. From the Home Screen, press the '<' button twice to access the 'set day' mode, press it three times to access the 'set month' mode or four times to set the 'set year' mode as shown below. You will notice that the date display will turn red.




2. The date is set to DAY/MONTH/YEAR format; each 'set mode' allows you to adjust either the day, month, or year only.
3. Press + or - buttons to increase or decrease the day/month/year.
4. To save and exit press the '>' button to resume normal mode.


### Setting the target temperature

- You can change the target temperature using the + or - buttons.


### Turning the pump off/on

From the home screen, press the  button to turn the pump off. A second press on the button will turn the pump on.

### Enable/Disable the UVC lamp

From the home screen, press the  button to disable the UVC lamp. A second press on the button will enable the lamp, as illustrated in Figure 16.

### Turning the Heat/Cool plate on/off

Pressing the  button will disable the Heat/Cool function. A second press on the button will enable the Heat/Cool plate functionality (Figure 17).

### pH calibration

This Braincubator™ has been calibrated and tested in our factory. However, you should re-calibrate the pH periodically to assure accurate measurements.

- From home screen, press the > button once. The display will indicate “pH calibration” and the solution needed for calibration, as illustrated in Figure 14.
- Adjust the pH calibration point to pH 4 using the – button. The screen will display ‘Calibrate pH 4’ in blue. Insert the pH probe in a calibration solution with pH 4 for 1 min.
- Press the ‘save’ button to start calibration and store information. The display will show ‘calibrating pH 4’ for several seconds and will go back to the “Calibrate pH 4” window.
- Change the pH calibration point to pH 7 using the + button. Insert the pH probe into a calibration solution with pH 7 for 1 min.
- Press the ‘save’ button to start calibration and store information. The display will show ‘calibrating pH 7’ for several seconds and will go back to the “Calibrate pH 7” window.
- Change the pH calibration point to pH 10 using the + button. Insert the pH probe into a calibration solution with pH 10 for 1 min.
- Press the ‘save’ button to start calibration and store information. The display will show ‘calibrating pH 10’ for several seconds and will go back to the “Calibrate pH 10” window.
- Press the < button once to finalise calibration and resume normal mode.

## 6. Cleaning

For efficient operation of the Braincubator™, the unit must be cleaned after each use with distilled water as detailed below. Water or aCSF should not be allowed to remain in the perfusion lines or chambers as this promotes bacterial growth. Removing the water by a gentle vacuum will help to remove all solution from the pipes.



Personal Protective Equipment (PPE), such as gloves and protective glasses should be worn throughout the cleaning process to reduce the risk of injury.

Further cleaning is needed on a weekly basis, passing 200 ml of 1N HCl through both the slice chamber and UV chamber being used. Immediately after the acid wash, 200 ml or more of distilled water should be run through the system to assure removal of all acid. This should be followed by draining the pipes.

Step-by-step cleaning instructions:

1. Disable the heat/cool plate and the UVC lamp.
2. Empty the pipes by elevating the UV rubber plug and the probe holding plate.
3. Once the pipes are empty, switch the Braincubator™ off via the power switch.
4. Remove Slice chamber and UVC chamber, discarding all tissue safely.
5. Rinse chambers thoroughly with distilled water.
6. Reconnect the chambers in their correct positions.
7. Fill the slice chamber with 200 ml of distilled water.
8. Switch on the Braincubator™, ensuring the UVC lamp and cool/heat plate have been disabled, and allow the unit to run for 1 hour.
9. Repeat steps 2-3 to dry both chambers and pipes till next use.
10. Ensure Braincubator™ has been correctly switched off and stored away safely for next use.

A detailed cleaning is required weekly to ensure correct maintenance is being performed. Previous cleaning instruction steps should be followed with the use of 200 ml of 1N HCl being passed through both the slice chamber and the UVC chamber for 30 minutes during step 7. Immediately after the acid wash, 200 ml or more of distilled water should be run through the system to assure the removal of all acid for an additional 30 minutes. Once internal cleaning has been completed and the Braincubator™ has been switched off, wipe down the machine using a sanitized cloth to remove any buildup of surface grease and bacteria taking special care of rubber seals and hinges.

- DO NOT allow the HCl solution to remain in the pipes.
- DO NOT use organic solvents, including ethanol, to clean the chamber as these substances can damage components and seals.
- 3 sets of tubing pipes are provided. One set of tubing is required to use the Braincubator™ while a second receives enhanced cleaning. The third set is included as a reserve in case of damage. If you require additional tubing sets, these can be purchased separately for an additional cost.



## 7. Care and Maintenance



Before conducting any form of care and maintenance, please ensure the Braincubator™ has been switched off and disconnected from any power supply. Personal Protective Equipment (PPE), such as gloves and protective glasses should be worn throughout and in the event of injury first aid should be administered immediately.

### Replacing tubing

To reduce bacterial growth within the Braincubator™, tubing pipes should always be kept clean, sanitized and dry prior to each use. For this reason, three sets of tubing have been provided. While using the first set of tubing, the second set should be thoroughly cleaned with Pyroneg solution or sterilized in autoclave. The remaining third set should be stored for replacement in the case a pipe is damaged or worn. If you required additional tubing, you may purchase these separately.

The Braincubator™ is supplied with 2 types of tubing, as illustrated in Figure 11:

- 4 mm blue tubing to connect the carbogen valve to the carbogen inlet.
- Silicone #14 and #13 tubing's to connect the slice chamber to the UVC chamber (through the pump). Please note that there are 2 diameters of this tubing. Tubing# 14 is longer (60 cm) with outer diameter of 4.8mm (inside diameter of 1.6 mm). The shorter silicone tubing #13 has an internal diameter of 0.8mm (inner diameter of 2 mm) and should always be placed on the left (most internal) slot of the pump. The #14 tubing must be rotated and placed according to the schematic diagram in figure 4. This configuration is of great importance to advantage outflow of aCSF from the UVC chamber. **Placing the tubing in the opposite order will result in overflow of the UVC chamber and might lead to leakage and serious damage to your system.**

### Replacing Interior UVC Bulb

- a) Ensure the Braincubator™ unit has been switched off and disconnected from any power supply.
- b) Using a hex key, open the service window at the back of the unit.
- c) Remove the UVC bulb with the housing and disconnect the power lines.
- d) Replace the UVC bulb, tighten into the housing and reconnect the power lines.
- e) Mount the bulb with the housing in place.
- f) Close the service window.
- g) Discard the removed interior lamp according to the relevant waste disposal guidelines.

### Pump Maintenance

When the pump is idle, we recommend releasing pressure from the tubing. This helps to protect the tubing from unnecessary strain and prolongs its service life.

Keep rollers clean and dry, this will prolong the service life of the tubing and pump head.

The surface of the drive and the pump head are not resistant to organic solvent and aggressive liquids, please pay attention when using.

## 8. Troubleshooting

This Braincubator has been tested for quality assurance before leaving the factory. In case of malfunction, which is not due to the delivery, see table below:

Leakage	Check connectors and pipes.
Data not saved	Check the SD card is inserted properly. Try another SD card. If data is still not saved please contact PAYO Scientific.
Pump not working	Make sure the pump speed knob is not at minimum. If still not working, contact PAYO Scientific.
UVC light not working	Replace lamp as illustrated in Figure 17.
Bacteria are accumulated	Refer to the cleaning and maintenance section. If pipes cannot be cleaned, replace them with new pipes.

## 9. Warranty

### Serial Numbers

All inquiries concerning our product should refer to the serial number of the unit(s).

PAYO Scientific warrants the instrument(s) for a period of one year from date of purchase. At its option, PAYO Scientific will repair or replace the unit(s) if it is found to be defective due to workmanship or material.

This warranty does not extend to damage resulting from misuse, neglect or abuse, normal wear and tear, or accident.

This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL PAYO SCIENTIFIC BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.

If a defect arises within the one-year warranty period, promptly contact PAYO Scientific Pty Ltd, through our website ([www.braincubator.com.au](http://www.braincubator.com.au)) or direct email to [Yossi@payoscientific.com](mailto:Yossi@payoscientific.com). Our office is located at S1 Werrington Park Corp, Western Sydney University, Kingswood 2747, NSW, Australia

Goods will not be accepted for return unless an RMA (returned materials authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device. This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

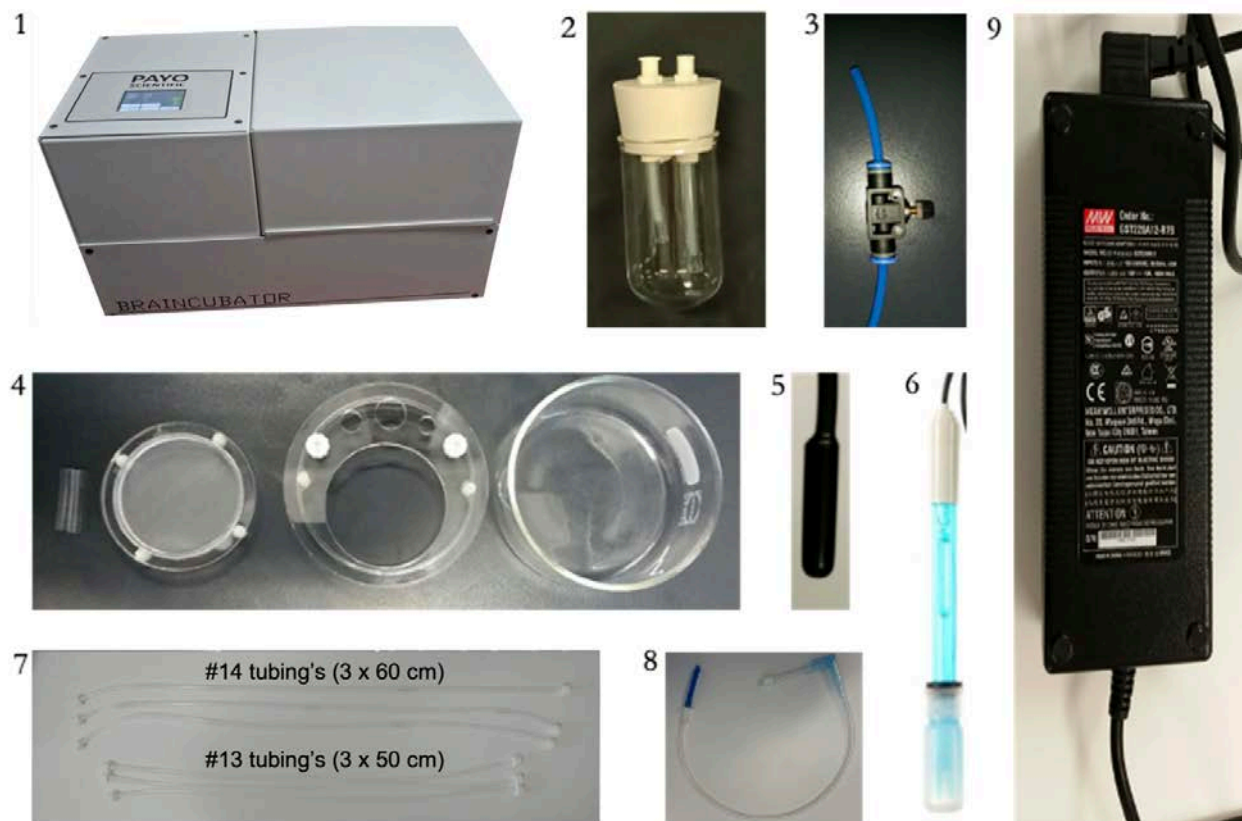
### Repair Facilities and Parts

PAYO Scientific Pty Ltd stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We also offer a complete reconditioning service.

**CAUTION: Not for clinical use on human patients.**

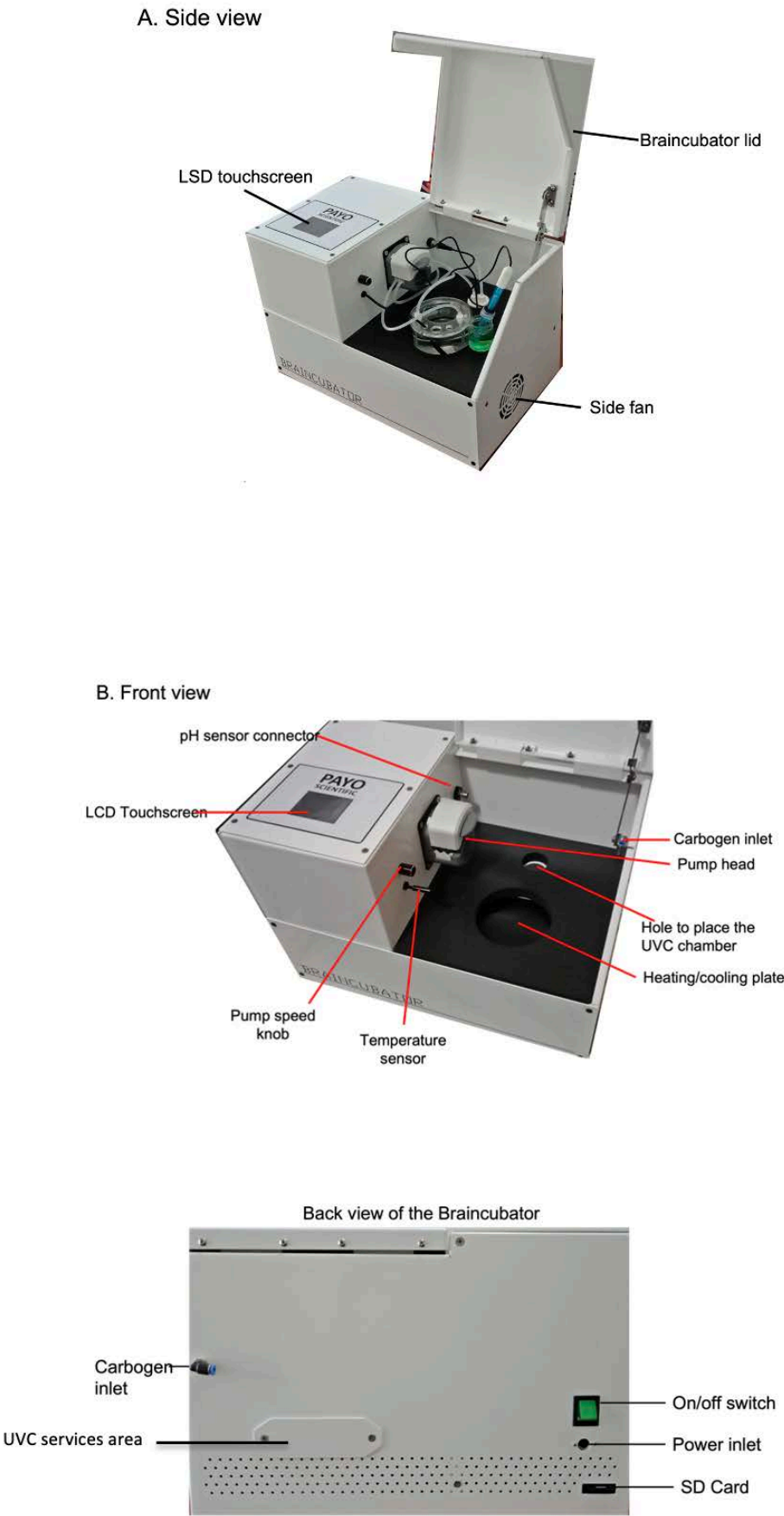
## 10. List of Figures

**Figure 8 – Components of a full Braincubator™ system**

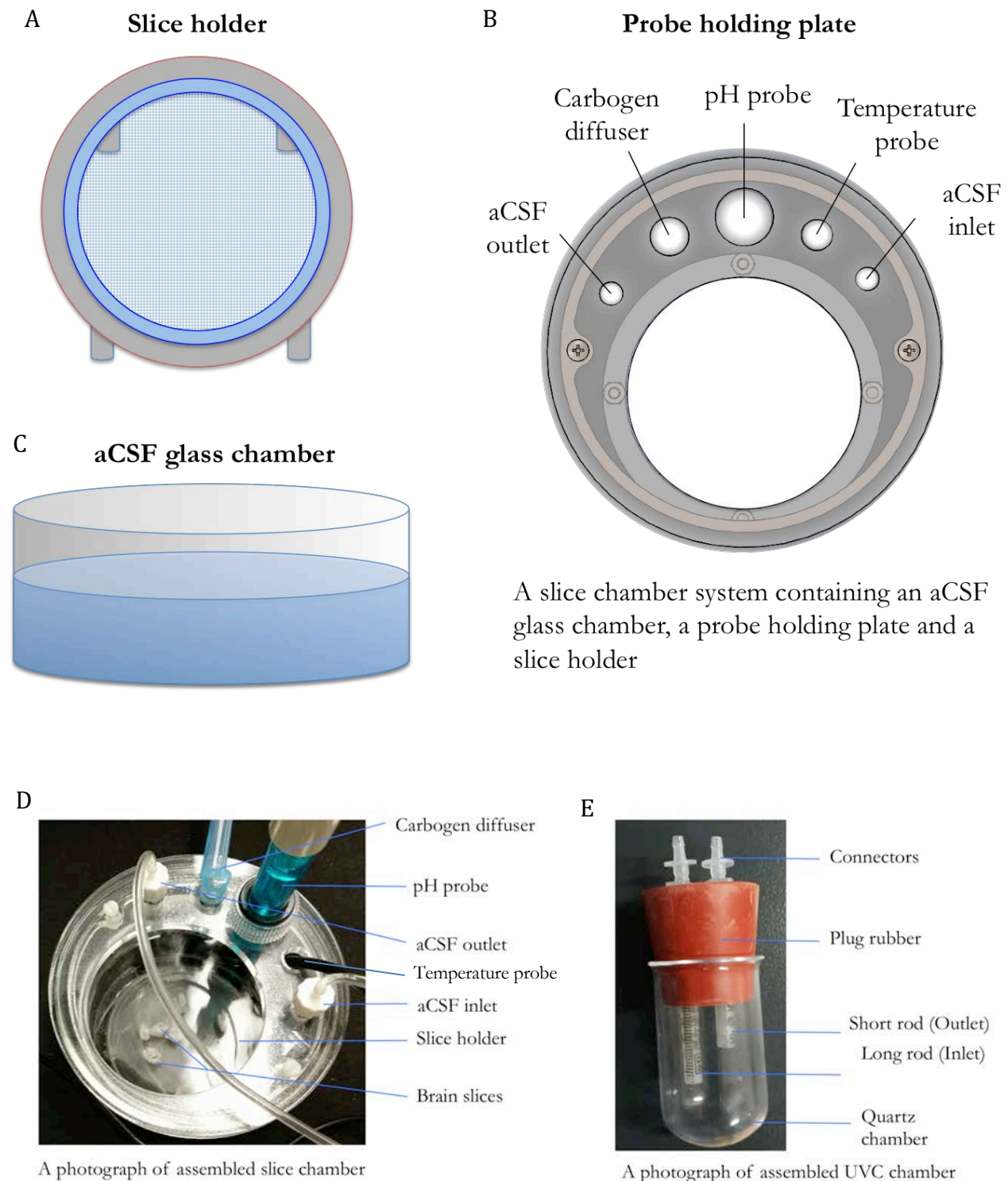


1. Braincubator
2. UVC chamber with rubber plug
3. Carbogen valve (to be connected to the carbogen outlet at the back of the Braincubator)
4. Main chamber, probe plate, slice holding mesh and plastic rods
5. Temperature probe (already connected to the Braincubator)
6. pH probe
7. Pump tubing's (top and bottom)
8. Carbogen diffuser (To be connected to the carbogen inlet from within the Braincubator)
9. Power supply

Figure 9 – Diagram of the Brincubator™

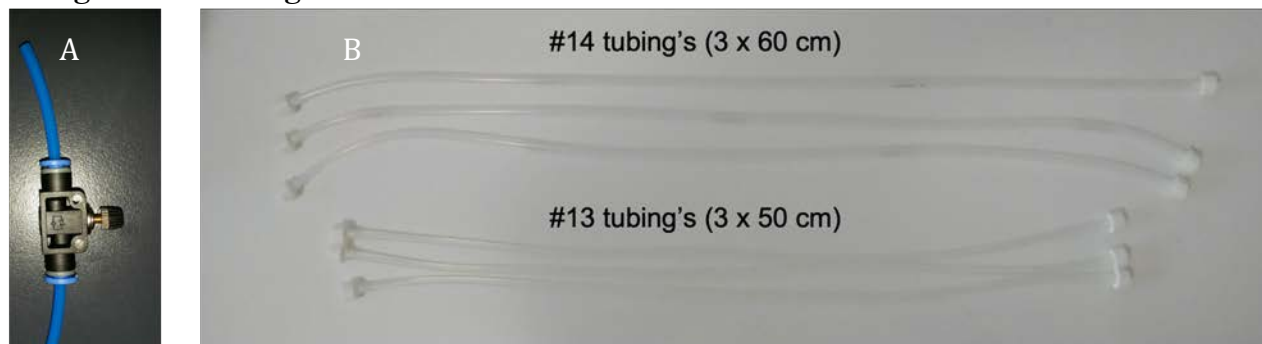


**Figure 10 – Assembly of UVC and slice chambers**



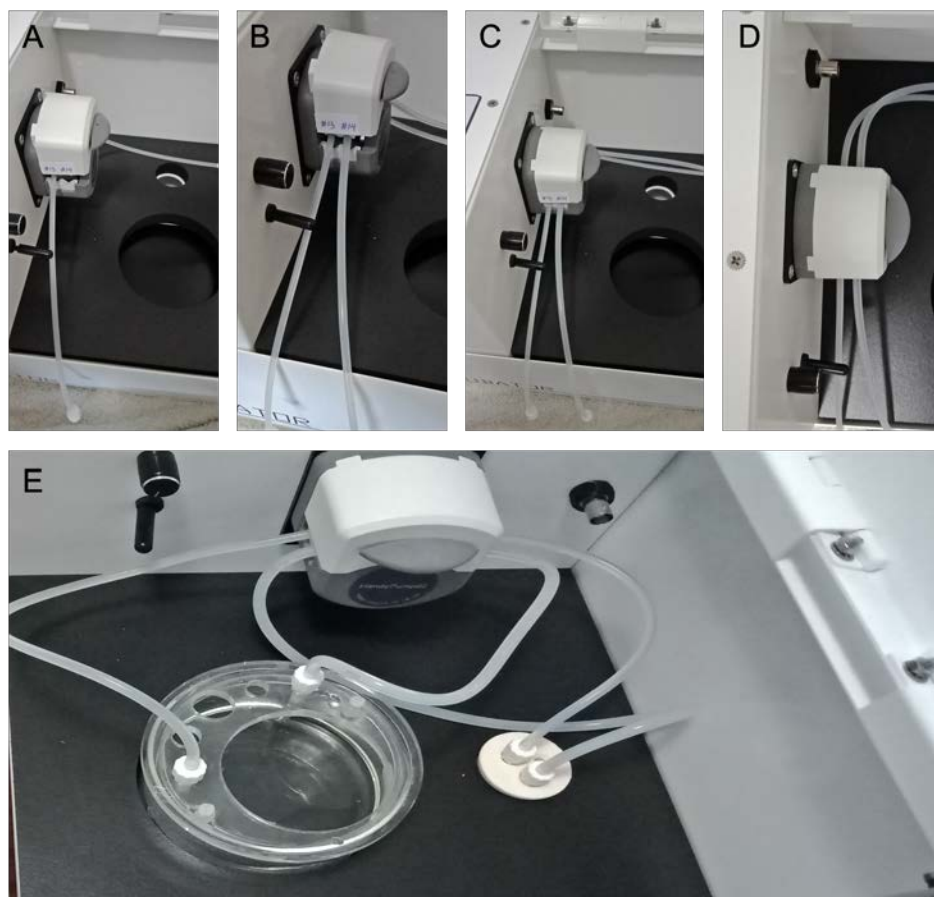


**Figure 11 – Tubing**



Tubing set: A) Carbogen valve with 4 mm blue tubing. B) Silicone #13 and #14 tubing's to connect the main slice chamber with the UVC chamber. These tubing's go through the pump and must be placed properly for the Braincubator to work. See schematic diagram in figure 4.

**Figure 12- Loading tubing fittings into pump head**



A) Open the pump head by turning the knob counterclockwise and place the #13 tubing on the left slot of the pump. B) Place the #14 tubing on the right slot of the pump, make sure that the tubing's are aligned along the slots. C) Close the pump head by turning the knob clockwise. Make sure that the tubing's are not mixed and aligned along the slots, as can be seen in D. E) connect the tubing's to the main and UVC chambers as seen in E. To ensure proper operation of the Braincubator™, please make sure to install pipes correctly. The #14 pipe internal diameter (1.6 mm) is deliberately larger than the #13 pipe (0.8 mm), to make sure that the UVC chamber won't overflow.

Figure 13 - LCD touchscreen display

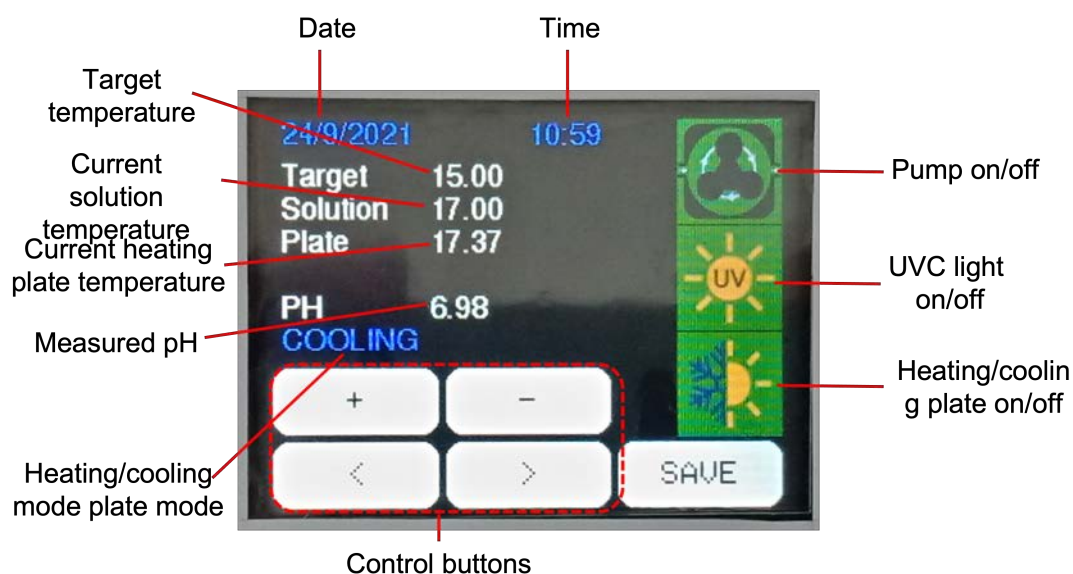


Figure 14 - pH Calibration



**A)** Photograph of the main display. Pressing the '>' button once will open the pH calibration window. **B)** At the calibration mode, use the '+' or '-' buttons to toggle between the three calibration points (pH-4, pH-7 and pH-10). **C)** Place the pH probe into pH 4 solution and let it rest for 1 minute. Press the 'save' button. The display will say "Calibrating pH 4..." and then pH 4 calibrated. Clean the pH probe with distilled water and place the probe into a pH 7 solution. Press the '+' button to toggle to the "calibrate pH 7" mode. Let the probe to set for 1 minute and then press the 'save' button. The display will say "Calibrating pH 7..." and then to 'pH 7 calibrated'. Clean the pH probe with distilled water and place the probe into a pH 10 solution. Press the '+' button to toggle to the "calibrate pH 10" mode. Let the probe to set for 1 minute and then press the 'save' button. The display will say "Calibrating pH 10..." and then to 'pH 10 calibrated'. **D)** Press the '<' button to resume to normal mode.



Figure 15 - Turning on the Braincubator™ and setting the target temperature

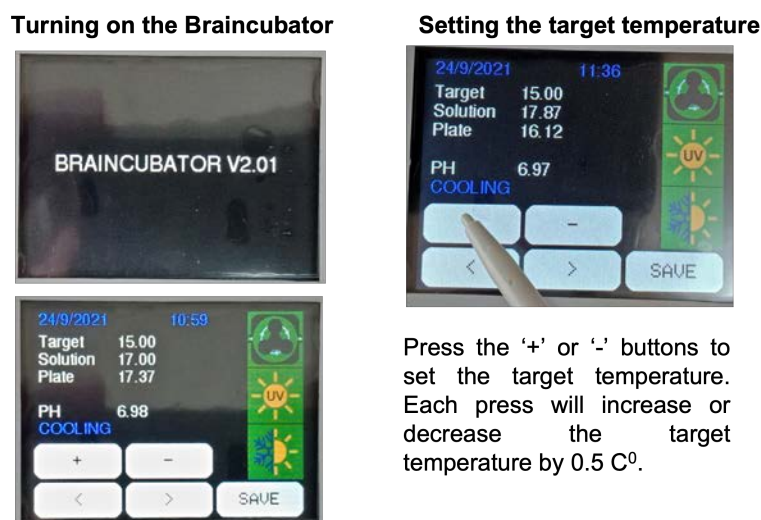
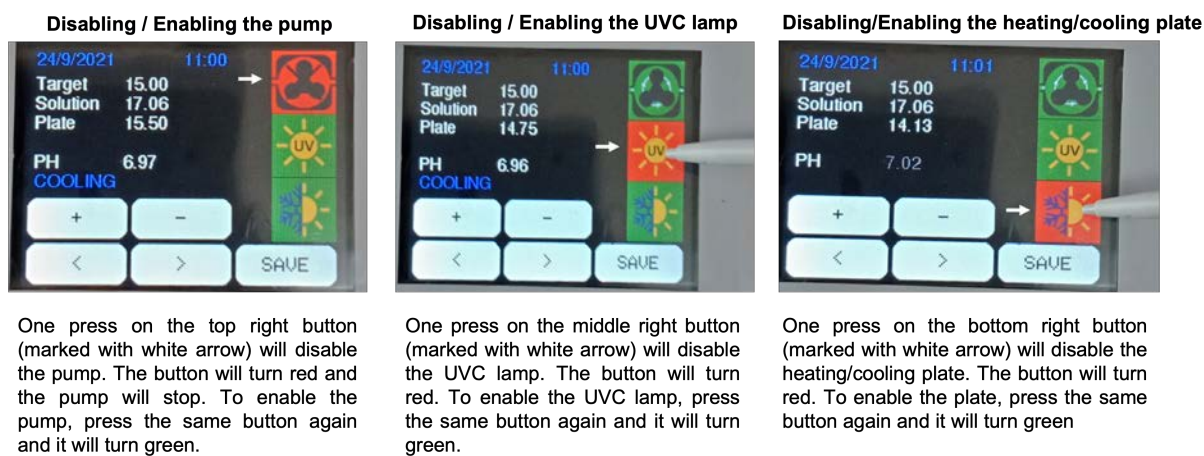
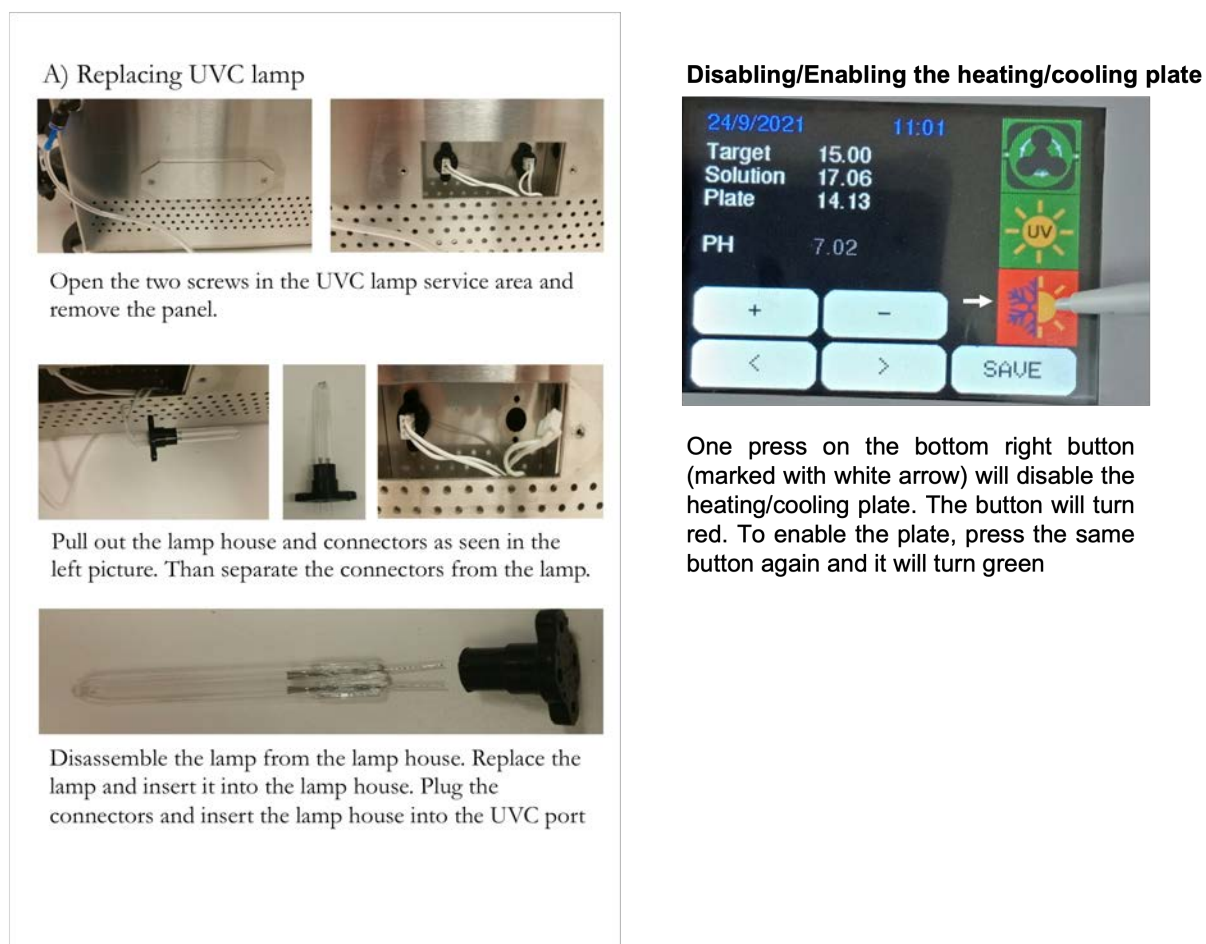


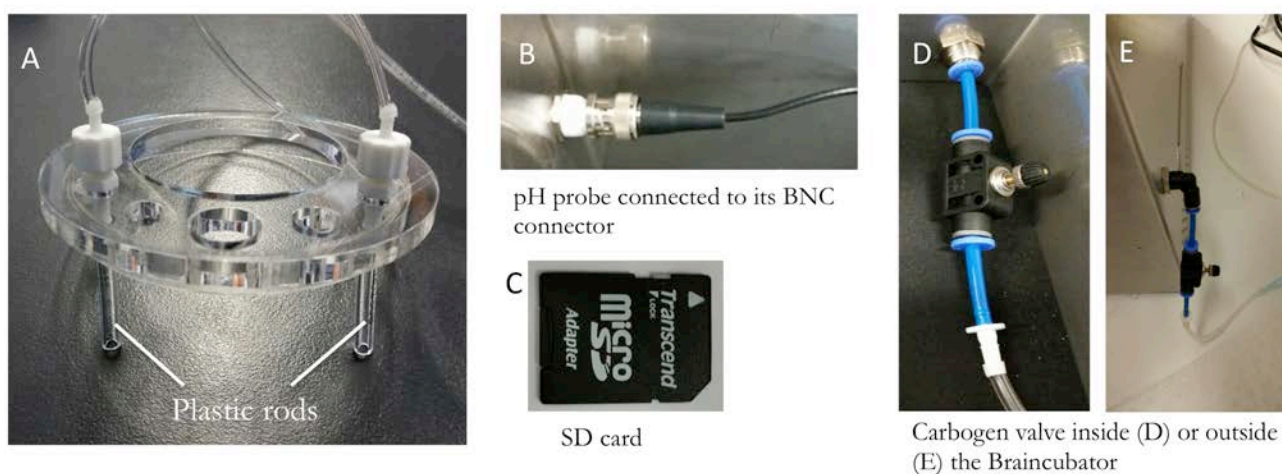
Figure 16 - UVC lamp, pump and LCD display



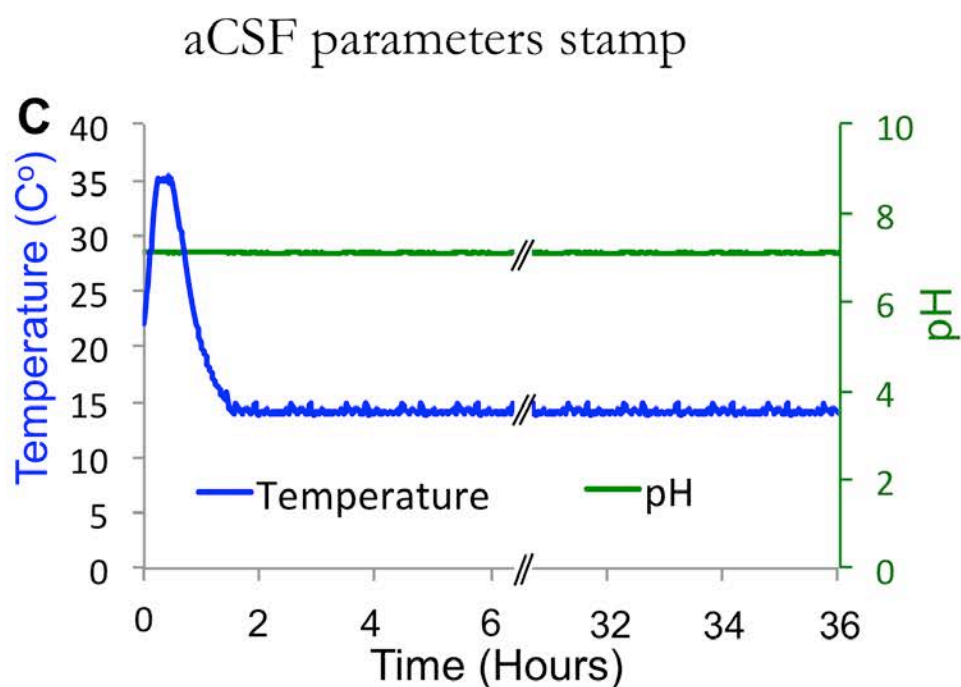
**Figure 17 - replacing the UVC lamp and turning off the Heat/cool plate**



**Figure 18 - Braincubator™ accessories**



**Figure 19 - Plotting the aCSF parameters stamp**



Eject the SD card from the back panel of the Braincubator by pushing it once. Insert the SD card to your computer and open the files. The data is stored as CSV file and can be view with Excel software. Plot the aCSF parameters stamp (APS) as above

## 11. References

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