Instruction manual Hot/Cold plate

Revision 1.4 - September Y22





SKU: 35300



SAFETY CONSIDERATIONS

Although this instrument has been designed with international safety standard, this manual contains information, cautions and warnings which must be followed to ensure safe operation and to retain the instrument in safe conditions.

Service and adjustments should be carried out by qualified personnel, authorized by Ugo Basile organization. Any adjustment, maintenance and repair of the powered instrument should be avoided.

If inevitable, it should be carried out by a skilled person who is aware of the hazard involved.

Capacitors inside the instrument may still be charged even if the instrument has been disconnected from its source of supply.



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	CE CONFORMITY STATEMENT			
Manufacturer	UGO BASILE srl			
Address	Via G. di Vittorio, 2 – 21036 Gemonio, VA, ITALY			
Phone n.	+39 0332 744574			
Fax n.	+39 0332 745488			
We hereby declare that				
Instrument.	HOT/COLD PLATE			
Catalog number	35300			
is manufac	is manufactured in compliance with the following European Union Directives and relevant harmonized standards			
• 2006/42/CE on	machinery			
• 2014/35/UE rela	• 2014/35/UE relating to electrical equipment designed for use within certain voltage limits			
• 2014/30/UE rela	2014/30/UE relating to electromagnetic compatibility			
• 2011/65/UE and 2015/863/UE on the restriction of the use of certain hazardous substances in electrical and electronic equipment				
Account Manager	Mauro Uboldi			
	Nome / Name			
20 th April 2022				
Date	Firma / Signature			
MOD. 13 Rev. 1				

The 35300 Hot/Cold plate:

Screens thermal hyperalgesia and allodynia, conditions often observed in patients with neuropathic pain or inflammation. Also used for testing analgesic and anti-inflammatory drugs.

Fixed or increasing temperature (RAMP) modes for dynamic experiments. Reaches user-defined temperatures within minutes. Touch screen or X-PAD software for setting up and monitoring tests, including complex temperature ramps.

This device can be used for both species; mice and rats.

New features of this model

(This model SKU 35300 substitute the previous model SKU 35150 which is not available anymore)

- External keypad for scoring animal behaviour, included as standard.
- Completely redesigned firmware plenty of new functions.
- · Real time experiment view.
- New electronic platform.
- Increased silent mode to avoid animals distractions.
- Improved ambient temperature
- measurement precision.
- · Fahrenheit and Celsius degrees option

Other features:

- 4.3" Touch screen for all setting and results.
- Embedded USB pen drive port for data export and acquisition.
- TTL input and output signals for other devices synchronisation.
- Firmware update via USB key



SKU: 35300 package content:

- 1 Hot/cold plate control unit.
- 1 Hot/cold plate cylindrical animal enclosure, perspex made. (For mouse and rat)
- 1 Hot/cold plate grey plastic lid.
- 1 Hot/cold plate pedal switch.
- 1 Hot/cold plate USB external numeric keypad. (Cabled as standard, wireless as optional)
- 1 Power cord according to your country outlet.
- 1 USB pen drive containing:
 - This instruction manual.
 - The quality control and warranty certificate.

Optional items ordering informations:

SKU	Item description
35150-002	Additional Hot Plate
35170	Combo package 35350 and 35150-002
35250-003	Set of Tubes/Catwalk for 35350
35350/35360	Thermal Place Preference
35300-005	Wireless numeric keypad

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1 Background

Classic thermal pain test of animal's sensitivity to pain caused by exposure to hot or cold temperatures, cause nociceptive responses (hind paw licking, flinch or jump).

Used for precise, rapid screening tests for analgesic drugs (such as opioid), according to the N.B. Eddy and D. Leinbach hot plate test.

Useful to study cold receptors and thermal allodynia and hypersensitivity, a phenomenon often observed among persons with chronic pain.

Wide temperature range, fast and reliable.

Wide operating temperature range suited to animal use from -5° C to 65° C. with adjustment steps of 0.1° C with a precision of 0.5° C.

Modes of operation for dynamic experiments:

- · Constant temperature.
- Increasing/decreasing temperature (RAMP)
- Exponential temperature (FAST RAMP)
- Complex ramps (configured using the X-pad software)

Quickly reaches set temperatures. Reliably maintains temperatures.

Touch-screen to set and monitor the test in real time.

Clear acrylic wall for easy observation (standard and custom heights available).

Transfer data with the USB memory key

Lid reduces humidity condensation on the plate at low temperatures.

User friendly X-PAD software for setting up the experiment and creating custom ramps.

Optional "auxiliary" Hot Plate 35150-002 is useful in the training and acclimation phase and for high throughput tests.

The instrument is provided as standard with a USB cabled numeric keypad to score animal behaviour. (a wireless numeric keypad is available as optional).

1.1 Notes on the instruction manual

This instruction manual included in the box is necessary for a correct installation of the instrument.

Please save this manual, keep it ready to be consulted by the qualified personnel using the instrument.

Our manuals are available as free download on our web site *www.ugobasile.com* For any additional information and/or assistance, you are welcome to contact our service department, first of all by specifying the serial number of your instrument at *service@ugobasile.com*

Hot/Cold Plate support QR code:



1.2 Safety instruction

The following guidelines must be followed to ensure safe operation:

- DO NOT attempt to open or perform any service work before having contacted Ugo Basile support team
- DO NOT use the device on human subjects

1.3 Intended use

The device is intended for investigation use on laboratory animal only. DO NOT USE THIS DEVICE ON HUMANS

1.4 Additional safety consideration

Use original accessories and spare parts only Immediately disconnect and replace damaged main cord. Do not operate in hazardous environment or outside prescribed environmental limitation. Do not spray any liquid on the connectors, or other parts.

Ugo Basile cannot in any way and form be held responsible for damage caused to things and people and warranty will be void, due to:

- Incorrect electrical supply
- Incorrect installation procedure
- Incorrect or improper use or, in any case, not in accordance with the purpose for which the instrument has been designed and the warnings stated in the instruction manual supplied with the instrument
- Replacement of original component, accessories or parts with others not approved by the manufacturer
- · Servicing carried out by unauthorized personnel

1.5 Principle of Operation

While in the original model, hot plate temperature was obtained by thermoregulated water circulation, commercially available models were later designed, including heating elements, precisely regulated, to perform rapid and precise screening of nociceptive behaviour and analgesic drugs in rodents.

The test is performed by placing the animal on a heated (or cooled) surface; a transparent glass cylinder is used to keep the animal on the surface of the plate. After a brief period, typically several seconds, an animal will lift and lick its paws as the temperature of the surface becomes uncomfortable: the animal is then immediately removed from the apparatus by the operator and the latency time scored by pressing the pedal.

The endpoint in this test is most frequently the latency time, defined as the time period between "time zero" point, when the animal is placed on the plate surface, and the time when the animal shows stereotypical response to pain/strong discomfort, such as paw licking, or jumps, or vocalizations. Also frequency of licking over a certain time window is used as an endpoint in several experiments. The device records the current temperature and the reaction time and the number of licks can be scored via the keypad.

The provided external USB keypad allow researcher to score animal behaviour during the test; the software provide a standard keys assignment that can be easily modified. The long USB cable allow the operator to stay far away the device during the test to avoid animal distraction.

1.6 Animal Welfare

In any analgesia test, great care must be taken to prevent the animals from harm. When an investigator initially begins using the hot/cold plate test, and when first assesses a new strain of animals, care must be taken to identify the appropriate amount of stimulus (i.e., temperature of surface) that will produce the desired response (i.e., paw licking, jumping, vocalization).

When operating at constant temperature, a cut-off time, should be established to minimize risk of an animal's tissue injury from prolonged exposure to the heated surface.

One should also assess the effects of a reference analgesic drug, such as morphine to determine that the planned hot plate temperature will result in the desired sensitivity (J.N. Crawley "Current protocols in Neuroscience" J Wiley, 2001).

The 35300 is very quick in temperature changes: when operating in "ramp" mode, please pay attention to the final set temperature extremes, especially with extremely quick temperature ramps, to avoid animal harm during the test (in particular when testing mice). While setting the test temperature a warning advising the operator to not exceed the range of $+2/+54^{\circ}$ C as a best practice for ethical reasons, is shown on the display.



2 Instrument Description

The Instrument is composed by the following elements:

- 1. The main unit of original design, incorporating the aluminium plate and the touch screen command/display.
- 2. The pedal switch.
- 3. The external USB cabled numeric keypad.
- 4. The Perspex animal restrainer with the gray plastic lid.

The Hot/Cold Plate combines the same functionality of the previous version, higher performances, greater stability and uniformity, more precise and quicker temperature control, and much easier experimental organization and data management.

Operating modes will allow to work with constant temperature or ramp (timed or fast), defining the initial and final temperature to set an upward or a downward ramp.

The plate temperature is monitored by a precise temperature sensor embedded in the plate, in 0.1°C steps and reaction time is recorded in 0.1s increments. Also ambient temperature is monitored.



2.1 Main Unit

The plate is milled from a thick aluminium slab and is mated to high surface heating/cooling elements which provide homogeneous temperature to the entire test area. A solid-state temperature regulator senses the plate temperature via a digital thermometer and powers the heating/cooling elements through a proportional circuit to minimize temperature overshoot..

2.2 Important Notice on Temperature Range

The plate temperature can be set by the operator in the range -5 to 65° C (in steps of 0.1° C). The extremes of this wide range can be reached, provided the room temperature remains in the interval 18-25°C.

2.3 Touch-screen command/display

The 35300 incorporates a 4"3 touch-screen, for all settings and result view, via an intuitive panel. This panel can be used both with or with out wearing nitrile gloves.



Home menu

During the test (figure below), the touch-screen display shows with a diagram the running experiment where the horizontal gray thin line is the set temperature, the red line is the temp executed by the device.

The system shows the experiment time in seconds, the current plate temperature in degrees Celsius or Fahrenheit, and the Estimated Time to Reach the end of the experiment (ETR).

The **i** icon on the upper right corner, gives access to the integrated help giving the user information about the current page functions.

The **(i**) icon on the bottom right shows the current experiment settings.



Test page

2.4 Notes on Resistive Touch-Screens

The Ugo Basile Hot/Cold plate touch screen uses resistive technology and can be used with fingers, gloves or (recommended) a display pencil.

Resistive touch-screens (differently from commercial capacitive screens) are a better choice for laboratory applications because of:

- High resistance to dust and water
- Better use with gloved hand or stylus

2.5 Connection front panel

The connection module on the front panel (Figure below) encompasses the following connectors, from left to right:

- USB port: enable data export to a PC (via a USB pen drive), and allow firmware upgrade. Moreover, experiment created with the X-Pad software (see specific manual for details) can be uploaded into the device by using a simple USB pen drive. This port also connect the provided USB external numeric key-pad The lower USB connector is covered and has not to be used. DO NOT REMOVE THE USB CONNECTOR COVER
- 2. TTL I/O: 15pins D-SUB connector, provides TTL input and output for start/stop command and mode settings.
- 3. COM: Reserved for maintenance and service purpose, not to be used.
- 4. Ethernet connector (RJ45): only for support reason at the factory, not to be used.



Connection front panel

3 Installation

3.1 Unpacking & preliminary check

Check the content of the shipment for completeness and visually inspect the instrument as soon as you take it out from the packaging.

If the box looks damaged, inform the carrier and provide a conditional signature (not a full signature).

Once unpacking, if the instrument is damaged, notify our company, by writing an email to support@ugobasile.com

If after a test, the instrument fails to meet the expected behaviour and performance, please contact our after sales service at sales@ugobasile.com.

Protect the environment:

- Dispose of packaging properly, according to existing and applicable waste management rules and regulation.
- Open the packaging box and take the instrument out the box; make sure to remove all the packaging material form the apparatus before placing it on the experiment Lab room.

3.2 Assembling the instrument

Place the device on a stable and flat surface and do not block the lateral ventilation grids. To guarantee the rated performance, a free area should be left around the instrument of at least 20 cm left, right and rear.

Choose a place where with sufficient space to hold the Hot/Cold plate and al the other instruments/equipments you will need during the experiment.

Then, mount the clear plastic tube animal restrainer on top of the aluminium plate and place the gray PVC lid on top of the restrainer.

Connect the pedal switch to the main unit rear connector and pay attention to avoid strongly pushing the connector in.

There is a little pin outside of the connector showing the correct position to plug the connector in with a minimum force.

Find the correct connector position before pushing the connector in.

Connect the external USB numeric key-pad to the USB ports.

The Hot/Cold plate device does not need to be levelled, base foots does not have regulation, just ensure the table holding the apparatus it is in a stable and levelled position.



The Hot/Cold Plate finally mounted

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3.3 Before Applying Power

The Power Module (see figure below) is positioned on the right side of the rear panel and incorporates, from left to right (rear view), the fuse holder, the ON/OFF switch, the inlet connection for the power cord.



Power module

The fuse compartment holds two fuses. Use (T2.0A) timed fuses for operation at both 115 or 230V, for fuse replacement.

Make sure your power out-take is provided with a reliable ground connection.

3.4 Intended Use

The Hot/Cold plate is intended for **investigation use on laboratory animal only.** This device **MUST NOT** be used on humans.

3.5 Additional Safety Consideration

- Use original accessories and spare parts only.
- Immediately disconnect and replace damaged main cord.
- · Do not obstruct access to the power module.
- · Do not obstruct the ventilation grids of the cabinet.
- Do not operate in hazardous environment or outside prescribed environmental limitation.
- Do not spray any liquid on the connectors, display, or other parts; during cleaning spray the disinfection fluid on a cloth and not directly on any parts of the apparatus.

Ugo Basile cannot in any way and form be held responsible for damage caused to things and people and warranty will be void, due to:

- · Incorrect electrical supply.
- Incorrect installation procedure.
- Incorrect or improper use or, in any case, not in accordance with the purpose for which the
 instrument has been designed and the warnings stated in the instruction manual supplied
 with the instrument.
- Replacement of original component, accessories or parts with others not approved by the manufacturer.
- · Servicing carried out by unauthorized personnel.
- · Warranty seal broken.

3.6 Power Connections

Connect the mains cord between the power socket of the Hot/Cold plate and the power outlet with a reliable earth connection.



Rear view of the Hot/Cold plate

4 Operation

4.1 Icons, button and pages on the display.

To start using the Ugo Basile Hot/Cold plate you first need to power the unit up; use the rear ON/OFF switch to power the unit ON.

A splash screen window will appear on the touch-screen for 4" indicating the device name:



Then the display will automatically show the main page:



Most of the icons in this page are buttons that can be used for setting up the device, here is an explanation of the Main page buttons and icons from the upper left to the right:

HOT COLD PLATE

This is the instrument name, this element is not interactive; in other screens, instead of this text in this position, you will find the title of the screen you are currently in.

Tuesday, 29 March 2022 14:49

Here is where the instrument shows the current date and time set into the Utilities/Date and Time.

This icon takes You to the general info page where You have several device information that can be useful for technical support.

Use the button to return to the Main page (this is the back button on all the device screens).

In the header part of the display there are several icons advising you about the device state and connections, that appear only in certain occasions or while external devices are connected:



This icon appears only in the main screen and will take you to the About screen with technical informations regarding your device.



This icon is in all pages except the main page and will take you to the previous page



This icon is shown when a USB key is connected to the front panel USB connector and it contains a valid firmware update; it is only used for firmware update.



Shown when a USB device is connected to the USB front panel connector.



This icon indicates a possible internal memory card problem.



This icon appears while the external numeric keypad is connected to the front panel USB connector regardless the type of keypad you use (wired or wireless).

This icon indicates the plate is not operating; next icons indicate the warming or cooling plate status.



This indicates the plate is warming up.



This indicates the plate is cooling down.



This icon will take you to the help page, explaining the features of the page you are in.



This button takes you to the Experiment page, where You can insert data regarding the experiment like, the Treatment name, the Protocol name, a name for the Stage, the Trial and Animal ID.

Note:

You can fill these fields using the X-Pad Windows application on You PC, which may be easier than from the touchscreen and then import the data into the Hot/Cold plate using the provided USB key and the import button.

Always use the 💙 button to return to the previous page.



Using this button you can select one of the possible operating modes available:

- **Constant**: to run a constant temperature experiment, at a set time or with an unlimited (type zero as duration).
- Linear Ramp: to run a ramp experiment where you can set the initial temperature, the final temperature, the ramp duration in seconds or the ramp speed in (C or F)^o/min.
- **Fast ramp**; to make a ramp experiment at the maximum (non-linear) speed where you set initial and final temperature (without setting any ramp speed or time duration)
- Custom ramp; to perform a test based on a custom ramp (made of different ramp segments, previously designed using the provided X-Pad windows application and imported into the Hot/Cold plate.

Note that with the X-Pad application is possible to create very complex custom ramps experiments.



This button will take you to the page for starting the experiment you previously chose in the temperature mode page. (By default this button take you to the constant experiment start page, or the last chosen temperature mode).



Results

Use this button to go to the Result page, where you can browse the various test data you have made.



This button takes you to a page where you can:

- Export result to a USB key (in .csv format to be used with data analyses apps like Excel)
- Load experiment by a USB key which contains data created with the X-Pad Ugo Basile Win- dows application (note that this is not a custom ramp loading procedure; it is used to load into the device data such as Treatment, Protocol, Stage, Trial and animal ID text).
- Unload Experiment: useful to clear up previous loaded experiment data.



Utilities

- This is the way to access the Utilities page where you can:
- Switch the Measurement Unit displayed by the device (Fahrenheit or Celsius)
- Set (or accept the default) **Keypad Event** text (to be added to the results) such as: Licking, Scratching, Jumping, Grooming and other, or whatever you prefer
- Set the device **Date and Time**.
- Update the device firmware and the system software (Device setup)
- Activate the **Diagnostic Cycle** (Device setup, only for service purpose)
- Erase the internal DB (Device setup, only for service purpose)
- Setting the **Temperature Offset** (Device setup, only for service purpose)
- Activate or de-activate the Ramp End Mode. When it is ON, at the end of linear or Fast ramp mode the device stops the experiment, while it keeps the temperature when it is on OFF

4.2 Performing experiments (general)

All experiments performed with animals require an habituation phase; refer to your lab manager, technician or to your own experience for the correct habituation procedure for the Hot/Cold plate experiment.

Sample hot/Cold plate experiment protocols can be found at the following links:

Josephine N. Massingham, Olga Baron and G. Gregory Neely, 2021, Evaluating Baseline and Sensitised Heat Nociception in Adult Drosophila, Bioprotocol <u>https://bio-protocol.org/e4079?sid=ee47bba2-8dfe-4e13-9420-fafffe8c4c47&sousouid=BP166</u> 2381181851395&ptype=0&px=1&intwzid=4079

DC Boorman, KA Keay, Sex differences in morphine sensitivity are associated with differential glial expression in the brainstem of rats with neuropathic pain, 2022, Journal of Neuroscience research

https://onlinelibrary.wiley.com/doi/full/10.1002/jnr.25103

M Möller, CV Möser, U Weiß, E Niederberger, The Role of AlphαSynuclein in Mouse Models of Acute, Inflammatory and Neuropathic Pain, 2022, Cells https://www.mdpi.com/2073-4409/11/12/1967

Li Chen, Wei Chen, Xiao Qian, Yun Fang & Naijun Zhu, 2014, Liquiritigenin alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. Scientific Reports (Nature) https://www.nature.com/articles/srep05676

Wiebke Kallenborn-Gerhardt et al., 2012, NADPH Oxidase-4 Maintains Neuropathic Pain after Peripheral Nerve Injury, The Journal of Neuroscience <u>https://www.ineurosci.org/content/32/30/10136.short</u>

A preliminary phase, before starting the test itself need to be done to have a correct result file at the experiment end.

This involves to populate some fields into the device and this operation can be done in two ways:

- 1. Inputting the data into the field directly from the Hot/Cold plate touch display.
- 2. Using the provided X-Pad3 windows application.
- 3. This second method implies the app installation in a computer (not provided) but gives the operator a easy way to populate the fields while using a computer keyboard and a computer mouse.

4.3 Inputting experiment data from the Hot/Cold plate touch panel.

From the main screen press the button "Experiment" and in the next screen digitthe experiment data, which are the following: (remember that these data will appear on the screen and on the CSV data file, once exported into Excel.

Treatment

The text inserted in the field "treatment" allows to place animals into groups, which differ in phenotype, drug concentration, or any other parameter or condition (age, sex, weight) you want to compare.

Protocol

The "protocol" field defines the name of how the experiment will be performed.

Stage

The "stage" field is used to define the sequence of tests you will perform in a experiment (e.g. week number, training, retention, extinction, habituation, etc.)

Trial

The "trial" field indicates how many times a test will be repeated within a stage (e.g., it would be 7 in case of a weekly stage with daily trials; Monday, Tuesday, Wednesday, etc.)

Animal ID

The "animal ID" field is used in case you do not want to refer to animals by a simple sequential number, like animal1, animal2, etc. Instead, the "animal ID" would be a code or a name you use to uniquely identify the animal to be tested.

Please note that using this inputting method you will have some limitation, not possible to use small letter.

The maximum number of character you can write is 20.

4.4 Inputting experiment data from the X-Pad windows App.

Please refer to the X-Pad3 Windows App instruction manual to understand how to install and use the software application.

Be informed that:

Using this Application you will not have the limitation you have using the Hot/Cold plate touch screen as inputting system and it will be easier to write the information into the Hot/Cold plate device, even more with the X-Pad3 App you will find automatic procedure to populate all the experiment data.

In general term the procedure is to create a file with the X-Pad application on a computer, write the data file into the provided USB key (using the X-Pad3 "Make File for device"), connect the USB key to the Hot/Cold plate and load the protocol into the device.

How to load a protocol made in the X-Pad application:

Please refer to the X-Pad instruction manual to understand how to do it. Once you have created the protocol and register the protocol file (.db) into the USB key, detach the USB key from the PC and connect it into the USB Hot/Cold plate connector.

(You need to prior detach the USB keypad to free the USB connector).

From the main page of the Hot/Cold plate display press the button "USB storage" and then press the button "Load Experiment", select the Protocol name you want to load from the list and press the "OK" button.

You will see the windows saying "Experiment has been loaded successfully, then press "OK" to close the windows.

Go back to the Main page and press the button "Experiment" you will notice that the Experiment data are loaded and you can browse with the upper left arrows the different animals.

4.5 Performing a constant experiment

The Constant temperature test is an experiment with a set temperature that will be maintained for all the time of the test.

Having the protocol data manually filled up or loaded from a X-PAD3 file you can start the experiment.

First go to the "**Experiment**" page and select the animal to test, eventually using the 2 upper left arrows to browse inside the protocol.

Go back to the main page

Press "Temperature mode" and choose the "Constant" button.

In the next page, type the Temperature value (in degrees °C of °F (depending on what you set in the "**Utilities**" "**Measurement unit**") and the Experiment duration time in seconds (type zero if you want an endless duration).

To set the constant temperature press "Plate Temperature"

For ethical reason we advise you to set this value within the range from 2 to 54 °C (35° to 129 ° F), even if the device can reach a minimum of -5°C and a maximum of +65°C. Type the temperature you need and press "OK"

With the button "**Experiment duration**" you can set the time the experiment will last for, in seconds. Then press "**Start Constant Experiment**"

Note that you can also select the animal in this page before starting the experiment.

This page is resuming the settings you input and in the lower part of the display you have some additional informations:

- Plate indicator is showing you the actual plate temperature.
- **Lab** is indicating the actual room temperature.
- **ETR** (Estimated Reaching Time) gives you an indication of the amount of time needed to reach the set temperature plate based on the actual temperatures.

The "i" at the bottom right, if pressed, will show you a summary of the protocol data you choose.

Press the "**Reach**" button will make the Hot/Cold plate starting reaching the set temperature to be ready for the test.

You will see the **ETR** value decreasing while approaching the set temperature.

During this time the upper thermometer icon can be red or blue indicating what the device is doing, heating or cooling.

Wait until the "**Reach**" button, that during the reaching time shows "STOP" to become "START", which means the plate is ready for the experiment.

Place your animal inside the clear perspex tube and if you like close the lid (lid is normally used to reduce foggy in the restrainer at very low temperatures), then start the experiment using the pedal switch or the display button.

In the experiment run page you will find:

- A red line in a graph, moving from left to right indicating the temperature variation during the experiment time
- Indication of:
- Time of the experiment from the time you start the experiment.
- The plate temperature
- The Lab temperature
- The ETE (Estimated time to End)

You can stop the experiment both by pressing the pedal switch or the STOP button on the display. Generally the experiment has to be stopped when the animal shows nociceptive responses, like paw leaking, jumping or vocalizing.

For ethical reason DO NOT EXPOSE ANIMAL AT LONGER TEMPERATURE STIMULUS. REMOVE THE ANIMAL IMMEDIATELY AFTER HE SHOWS PAIN.

During the experiment time you are able to visually score some animal behaviour and record them into the result data file, using the USB or bluetooth (optional) keypad.

Any time you press a mapped key of the related behaviour will be recorder into the result data file (CSV to be exported into the USB key and opened in Excel).

Each key is mapped to a particular behavioural comportment that can be set into the "Utilities"/"Key Pad events" page

Default mapping are the following:

Key	Behaviour text mapped		
Enter	Licking		
1	Scratching		
2	Jumping		
3	Grooming		
4	Vocalization		
5	Rearing		
6	Immobility		
7	Climbing		
8	Shivering		
<u>9</u>	Twitching		

You can also modify the mapped text just pressing the related key text.

When the experiment if finished the Hot/Cold plate return to the "**Start Constant**" page showing you the data of the last experiment performed and the plate is maintaining the set temperature to be ready for the next animal.

If you wish to stop the device to maintain the experiment set temperature just go back to one page and the plate will stop working.

To perform a second animal test:

- If you load a protocol experiment data from X-Pad3 you just need to switch to the second animal using the upper arrow.
- If you manually fill up the experiment data you need to revise the setting manually in the "Experiment" page.

Once a new animal is selected you can repeat the previous procedure test.

4.6 View and export result experiment data

In general, temperature mode wise at the end of an experiment you can review the result data going from the Main page to the result page pressing the "Result" button. In this page you have all the data and using the left up and down arrow browse all the animal you test..

In order exporting the result experiment data you need to:

- Got to the main page
- Press "USB Storage"
- Insert a proper USB key into the USB connector on the front panel
- Press "Export Result"

Result data are exported in .csv (Comma Separated Value) file (which can be imported/edited by several spread sheet application and data analysis software like Microsoft Excel®)

A confirmation windows will appear confirming the export procedure has been correctly done.

4.7 Performing a Linear Ramp experiment

Linear ramp is an experiment where you need to set:

- Initial ramp temperature.
- The final ramp temperature.
- The Ramp duration time in seconds or the Ramp speed in degrees/minute.

The experiment procedure is similar to the Constant temperature experiments with some exceptions:

When you set the Ramp duration time the Ramp speed will show you the calculated Ramp Speed; alternatively, when you set the Ram Speed the Ramp duration will show you the calculated duration of the test.

In this mode and in the Fast Ramp mode, when the function Ramp End Mode is ON, at the test end the plate will automatically return at the initial ramp temperature to be ready fro the next animal to test.

If End Ramp Mode function is OFF, the plate continue the ramp until you stop the test maintaining the final set temperature.

Note:

End Ramp Mode setting is available from Main page pressing" Utilities" "Ramp End Mode"

4.8 Performing a Fast Ramp experiment

Fast ramp is an experiment where you need to set:

- · Initial ramp temperature.
- The final ramp temperature.

The ramp duration is automatically calculated to make the experiment as fast as possible,

The experiment procedure is similar to the Constant temperature experiments with some exceptions:

In this mode and in the End Ramp mode, when the function Ramp End Mode is ON at the test end the plate will automatically returns at the initial ramp temperature to be ready for the next animal. If End Ramp Mode function is OFF, the plate continue the ramp until you stop the test maintaining the final set temperature. If End Ramp Mode function is OFF, the plate continue the ramp until you stop the test maintaining the final set temperature.

Note: End Ramp Mode setting is available from Main page pressing" Utilities" " Ramp End Mode"

4.9 Performing a Custom Ramp experiment

You can use the Ugo Basile X-Pad3 windows application to create complex custom ramps experiment, which can't be built from the Hot/Cold plate display.

Please refer to the Ugo Basile X-Pad3 instruction manual to learn how to use the software. In brief, once you have created the complex ramp using the H/C Plate tab in X-Pad3 you need to export to file the ramp using the "Make the Ramp File for Device" and write the file in a USB key (you can use the provided one or another one FAT32 formatted).

In order loading the ramp into the Hot/Cold Plate device:

From the main page of the Hot/Cold Plate device press "Temperature Mode" then choose "CUSTOM RAMP". To load the ramp simply insert the USB key where you saved the ramp file and press "LOAD RAMP".

You will see the list of the saved ramps into the key and with the up and down arrow you can select the one you need to load.

Once selected, press the OK button and you will have a confirmation message.

Please note that the last loaded ramp is in the memory and can be used several times; if you need to change it with another ramp repeat the procedure described above.

Now you have loaded you experiment ramp and to start the experiment you need to press the button "START CUSTOM RAMP EXPERIMENT"

4.10 Exporting result data (details)

When your experiment is finished you can review the saved experiment data from the Result page and exporting them to a USB key from the USB Storage page using the Export Result function. Please insert a valid (FAT32 formatted) USB key into the from USB connector before pressing the Export result button.

Experiment result data are saved into the USB key in .csv format, (Comma Separated Value) file, which can be imported/edited by several spread sheet application and data analysis software like Microsoft Excel®.

File name is having the following syntax:

HCP_Results_YYYY-MM-DD_HHMMSS.csv

Where YYYY-MM-DD represent the Year-Months-Day and HH-MM the hours and minutes when the result data are being saved.

Here is the list of the exported data:

Session (A progressive number of the experiment performed)

Date_Time (The date and the time the event occurred)

EventCode (An internal code to identify the event)

EventDescription (A text describing the event)

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Event code	Event description		
10	Start		
15	Stop		
16	Test complete		
18	Final temperature reached		
20	Custom Ramp end		
30	Start of a custom ramp segment at constant temperature		
32	Start of a custom ramp segment at non constant temperature		
65	Keypad event START mapped to Enter key		
62	Keypad event START mapped to 1 key		
63	Keypad event START mapped to 2 key		
64	Keypad event START mapped to 3 key		
58	Keypad event START mapped to 4 key		
59	Keypad event START mapped to 5 key		
60	Keypad event START mapped to 6 key		
54	Keypad event START mapped to 7 key		
55	Keypad event START mapped to 8 key		
56	Keypad event START mapped to 9 key		
165	Keypad event END mapped to Enter key		
162	Keypad event END mapped to 1 key		
163	Keypad event END mapped to 2 key		
164	Keypad event END mapped to 3 key		
158	Keypad event END mapped to 4 key		
159	Keypad event END mapped to 5 key		
160	Keypad event END mapped to 6 key		
154	Keypad event END mapped to 7 key		
155	Keypad event END mapped to 8 key		
156	Keypad event END mapped to 9 key		

Treatment (The text inserted manually or via X-PAD3 into the Treatment field in the Experiment page)

Protocol (The text inserted manually or via X-PAD3 into the Protocol field in the Experiment page)

Stage (The text inserted manually or via X-PAD3 into the Stage field in the Experiment page)

Trial (The text inserted manually or via X-PAD3 into the Trial field in the Experiment page)

ID (The text inserted manually or via X-PAD3 into the Animal ID field in the Experiment page)

Latency (Is the time in seconds between the Experiment start and the time the even occurred)

Temperature (Is the plate temperature when the event occurred)

RoomTemperature (Is the Lab room temperature when the event occurred)

Mode (It is the temperature mode chosen for the experiment)

Offset (It is the temperature offset value inserted)

MeasureUnit (Is the experiment measurement unit set for the experiment and can be °Celsius or ° Fahrenheit)

InitialTemperature (is the Ramp initial temperature)

FinalTemperature (is the Ramp final temperature or the target temperature if Constant mode has been used)

RampDuration (for Custom Ramps it is the ramp segment duration in seconds)

RampSpeed (in degrees per minutes for linear Ramp)

5 Connections

5.1 Communication port connection

The Hot/Cold Plate is provided with a D-sub (DA-15 female) TTL I/O port.

This port could be used to synchronize some events with external instruments or acquisition systems.

TTL Output signal are electrical isolated in order to guarantee an electrical barrier.

TTL signals are refereed to Power Ground (pin 14 and pin 15)

Refers to Figure and Table below for connector pin out



D-sub connector pins

DB-15 Pin#	Signal Name	Signal Type	Description
1	Start/Stop	TTL OUT	Start -> TTL High Level Stop -> TTL Low Level
2	Reserved	TTL OUT	Reserved
3	Reserved	TTL OUT	Reserved
4	Reserved	TTL OUT	Reserved
5	Reserved	TTL OUT	Reserved
6	Reserved	TTL OUT	Reserved
7	Temperature	TTL OUT	Reached -> TTL High Level Reaching -> TTL Low Level
8	Reserved	ANALOG OUT	Reserved
9	Reserved	TTL IN	Reserved
10	Reserved	TTL IN	Reserved
11	Reserved	TTL IN	Reserved
12	Reserved	TTL OUT	Reserved
13	Reserved	TTL OUT	Reserved
14	GND	POWER	Power Ground
15	GND	POWER	Power Ground

D-sub connector pin-out table

Page: 28 < Hot/Cold plate instruction manual

NOTE: TTL OUT is designed for connection with scientific instruments! DO NOT CONNECT ANY POWER DEVICE!

NOTE: DO NOT SINK a current more then 10mA from each TTL pin! DAMAGE WILL OCCUR.

7 Maintenance

While any service of the instrument have to be carried out by Ugo Basile personnel or by qualified personnel authorized by UGO BASILE organization, this manual section describes normal maintenance procedures which can be carried out at your facility.

UNPLUG THE MAIN CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB

The device does not require any particular maintenance.

7.1 Electrical

To inspect and/or replace the fuses, disconnect the mains cable first! Insert a miniature screwdriver in the slot indentation and snap out the slide which houses the fuses. Snap in the fuse slide: the mechanical "click" ensures that it is locked.

7.2 Cleaning/disinfection

To clean the aluminium plate we suggest You to use a cotton cloth sprayed of cold water or of ethanol. **DO NOT CLEAN THE PLATE SURFACE WHEN IT'S HOT**, wait unit it reaches the ambient temperature to avoid a permanent damage.

To clean the plastic parts of the case use the same suggestions above described.

- DO NOT use ethanol to clean the touch display.
- DO NOT use aggressive chemical agents to clean any part of the device.
- DO NOT use organic solutions. Cotton wool and water can be used for cleaning purposes.

Disinfection:

While the device can be sanitized using vaporized hydrogen peroxide at very low concentrations (e.g. 10%), keep particular attention during the sanitation process because H_2O_2 at high concentration and high temperatures for a long time can damage the aluminium and plastic device parts.

7.3 Long Inactivity

The instrument does not require any particular maintenance after long inactivity, except cleaning.

It is be possible that after years of inactivity the internal battery needs to be replaced.

7.4 Customer Support

For any further information you may desire, concerning the use and/or the maintenance of the device, please do not hesitate to contact our service department (or our local distributor) either directly of via our support page at the following link: *ugobasile.com/support/support-request*

Before sending any instrument to our factory for repair, please contact our logistics department to obtain a return authorization number (RMA) and shipping/packing instructions. We may not be held responsible for damages during transport due to poor packing; whenever possible, please use the original packing.

8 Specification

General			
Command Input	4,3 inches touch-screen (Resistive)		
Read-out	4,3 inches touch-screen (Resistive), PC		
Power Requirements	Universal input 100-240 VAC, 50-60Hz, 200W max		
Sound Level	70 dB		
Operating environment	10°C to 40°C; 5% to 95% RH (non-condensing)		
Detection	By pedal switch and via numeric key pad		
Operation			
Plate temperature range	-5.0°C to +65°C (in 0.1°C steps)		
Accuracy	+/- 0.2°C		
Mode	Fixed temperature, Ramp (increasing or decreasing), Silent, Fast Ramp, Custom Ramp		
Start	By START button or pedal switch		
Stop	By STOP button or pedal switch		
Experiment duration	Max 999.9 seconds		
Data Acquisition	Via X-PAD software (included)		
Data Portability	By USB flash drive (included)		
Data Input	Personalized protocol		
Data Output	Results table in .csv		
TTL Output	Temperature, Start, Stop		

Warranty

This device is covered by 12-month on-factory manufacturer warranty. An additional 12 month on-factory warranty period (total 2 years) is available for free after device registration.

7 Warranty

Your device is covered by 12 months on factory warranty period.

Registering the device on our registering web site page will give you a 12 months free warranty period.

To make the product registration.

- 1. Take a picture or a note of the device serial number which is written in a metallic label at the of the instrument case.
- 2. Browse the internet page: register.ugobasile.com
- 3. Fill out the form and you will receive the new warranty certificate

UB Care warranty extension

It is possible to buy a warranty period extension called UB Care 12 or UB CARE 24 respectively of additional 12 or 24 months.

UB CARE is available only in the first 12 months after the delivery date, not later.

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For other Ugo Basile products related to this device visit *ugobasile.com* web site for details



TGR - Thermal Gradient Ring (Zimmermann's method)



The original Plantar Test for thermal stimulation (Hargreaves Apparatus)





Dynamic Plantar Aesthesiometer (DPA) for mechanical stimulation



Plethysmometer, the 1st and original device for measuring paw volume & oedema

Thermal Place Preference (TPP Test) for Mice & Rats



Analgesy-Meter the 1st and original Randall-Selitto paw-pressure test

Ugo Basile SRL

Via Giuseppe Di Vittorio 2, 21036 Gemonio (VA) ITALY - Tel. +39 0332 744574 - sales@ugobasile.com



For other Ugo Basile products related to this device visit *ugobasile.com* web site for details





e-VF Handheld Electronic Von Frey of original design

I.R. Heat-Flux Radiometer for Tail Flick and Plantar Test



PAM Pressure Application Measurement (for joint pain)



Orofacial Stimulation Test (Fehrenbacher, Henry, Hargreaves method)



Tail-Flick Unit, thermal stimulation of the tail, according to D'Amour & Smith method

Ugo Basile SRL

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The 1st, original Mouse RotaRod for motory OPERON (Papaleo-Scheggia's method) for coordination studies

Attentional Set-Shifting Task





Fear Conditioning System - ANYmaze

Rodent Treadmill NG with interchangeable lane assembly for rats or mice



GSM Grip-Strength Meter for mice and rats

The Rota-Rod Family