Instruction manual Hot/Cold Plate

Revision 1.5.1 - July Y24





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

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We hereby declare that

Instrument. HOT/COLD PLATE

Catalog number 35300

is manufactured in compliance with the following European Union Directives and relevant harmonized standards

- 2006/42/CE on machinery
- 2014/35/UE relating to electrical equipment designed for use within certain voltage limits
- 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

Nome / Name

20th April 2022

Date Firma / Signature

MOD. 13 Rev. 1

Product features and general information

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)

Novelties of this model:

 External keypad for scoring animal behaviour, included as standard. (Cabled)

Completely re-design firmware plenty of new functions:

- · Real time experiment view.
- · New electronic platform.
- Real silent mode to avoid animals distractions.
- Improved temperature measurement precision.
- Fahrenheit and Celsius degrees option.
- LAN Connection for data retrieving and file loading (password protected)
- · via Web Browser.

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

What's in the box and optionals

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 height 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@uqobasile.com

Hot/Cold Plate Support QR code:



1.2 Safety instruction

The following guide lines must be followed to ensure safe operation:

DO NOT attempt to open or perform any service work before having contacted Ugo Basile support team

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

Depart operate in hazardays applicable processible

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 analgesic drugs on the animal by the hot plate test.

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 a paw as the temperature of the surface becomes uncomfortable: the animal is then immediately removed from the apparatus by the operator.

The dependent variable in this test is the latency time, defined as the time period between the 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 to avoid thermal pain; the operator stops the counter via the pedal switch, thus recording the current temperature and the reaction time.

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 inadvertent harm.

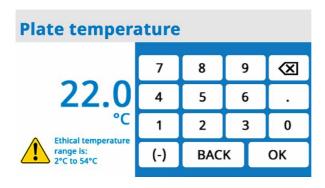
When an investigator initially begins using the hot plate test, and when first assessing a new strain of animal, care must be taken to identify the appropriate amount of stimulus (i.e., temperature of surface) that will produce the desired response (i.e., paw lick or jumping).

When operating at constant temperature, a cut-off time, generally 30 sec must be established to minimize risk of an animal sustaining tissue injury from prolonged exposure to the heated surface.

One should also assess the effects of a reference analgesic 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 when operating at temperature extremes and with extremely quick temperature ramps, to avoid irreversible damages on the animals on test (in particular when testing mice).

While setting the test temperature a warning advise the operator to not exceed the range of $\pm 2/\pm 54$ °C as a best practice for ethical reasons.



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.



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.

When using the Ramp mode (linear Ramp or Custom Ramp) both the X-Pad software and the device itself, advise the researcher about the time limit needed to reach the desired temperature.

As an example if You ask the X-Pad software or the device to perform a ramp of temperature starting from 10°C up to 48°C and the total ramp time has to be 10", the device and the software will tell the researcher that the requested time it is not possible due to physical thermal dynamics of the aluminium and advise a minimum time for the requested ramp.

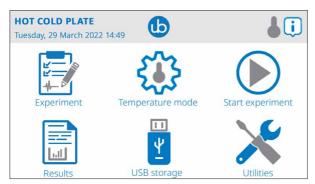
This minimum time is calculated based on test we made in the factory placing the Hot/Cold Plate in a temperature controlled room (23°C)

If Your Hot/Cold plate is placed on a different environment temperature this times may vary.

For this reason please consider the times suggested as "indication".

2.3 Touch-screen command/display

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



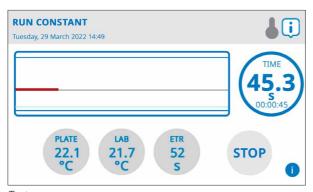
Home menu

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.

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

The 1 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:

1. 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):for LAN connection, provide communication with a PC to load experiment from the Ugo Basile X-Pad software and for experimental data retriving, with out the need of a USB pen use.



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 push the connector in.

Is there a correct position to interlocking 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

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.

3.6 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

3.7 LAN connection

You may want to communicate from a standard PC to the Hot/Cold plate via Local Area Network.

This connection make possible to load experiment data created with the Ugo Basile X-Pad software (Windows app.) and the experimental data retrieving from the Hot/Cold plate to the PC without the need of use the USB pen.

You do have two ways to cable the Hot/Cold plate to a PC:

- Connect the Hot/Cold plate directly to a PC Ethernet port by an Ethernet standard cable.
- 2. Connect the Hot/Cold plate to a LAN Switch/(wall LAN port) as part of a private or a Lab/Company network.

Despite the cabling You choose You need to consider:

LAN connection is based on TCP/IP protocol that need on unique IP number to be assigned to the Hot/Cold plate as well as the appropriate Subnet mask number.

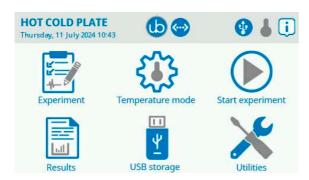
In case You decide to cable the Hot/Cold plate directly to a PC You will create a small LAN outside the company network and thus You will not need to have the IP number from Your IT administrator.

In case You want to cable the Hot/Cold plate into the company LAN You will need to ask Your IT administrator an IP number and a subnet mask to avoid conflict on the LAN

Note that Default gateway number and DNS number are not necessary for this purpose.

Scenario 1 - Hot/Cold plate directly connected to a PC:

- You need to have an Ethernet cable (UTP Cat 5 minimum) of the necessary length to go from the Hot/Cold plate to the PC (not more than 100 mt.)
 Connect one end (Rj45 connector) into the Ethernet Hot/Cold plate port labelled "Eth" and the other end of the cable to Your PC Ethernet port.
 Be aware the not all PCs can be connected by a standard Ethernet cable, some old PCs needs a special Ethernet cable called "Ethernet cross cable", if You find miss-connection using a standard cable You may need to try with a cross one.
- Switch the Hot/Cold plate on and from the main menu press the Utility button and then Device Setup and then Network, to enter into the Network configuration page.



Temp. Offset



Network

Set the IP Assignment to Manual and assign an IP address pressing on the number at the of the text "IP Address", delete the inserted number and digit 10.0.0.1, then press OK (this number will be the ID to reach the Hot/Cold plate)

Factory Reset

Now press right the text "Subnet mask", delete the inserted number and digit 255.255.0.0, then press OK.

Gateway, Primary DNS and Secondary DNS does not need to be configured.



Exit the Network page pressing the rounder arrow on top of the screen.

On Your PC (depending of the operating system You are running, Mac OSX or Windows or Linux) go to Your Ethernet card settings (the one You cable to the Hot/Cold Plate)

You may have multiple Ethernet card on Your PC, be sure to choose the right one

and remember to switch off the WiFi to avoid confusion.

Configure the appropriate LAN card with the following data in manual mode:

IP address: 10.0.0.10 Subnet Mask: 255.255.0.0

Default Gateway, Primary DNS (DNS1) and Secondary DNS (DNS2) does not need

to be configured.

Close the LAN card set-up

Open a Web browser on Your PC (Google Chrome is advisable to use but You may use as well Safari, Edge or Firefox or others)

On the browser address field digit the Hot/Cold plate IP address: http://10.0.0.1 The Login page will appear

Enter the default Login password (You may want to change it later) which is: UgoBasile.

You are now logged into the Hot/Cold plate.

Scenario 2 - Hot/Cold plate connected to an Ethernet Switch (Lab LAN)

- Use a standard Ethernet cable (UTP Cat 5 minimum) to connect the Hot/Cold Plate to the Lab LAN
- Go to the Network page into the Hot/Cold plate and set the IP Assignment as Automatic (DHCP) and go back to the previous page using the top screen arrow button
- Go to the main page of the Hot/Cold plate and press the top screen arrow to open the info page; You will see the assigned IP address of the device, take note of this number (E.G.: 192.168.1.26)
- Be sure Your PCs is connected at the same LAN (via cable or via WiFi and configured using a DHCP.
- Open a web browser and digit http:// followed by the Hot/Cold Plate IP number:

E.G. http://192.168.1.26

- The login page will appear
- Enter the default password (UgoBasile)
- You are logged in the Hot/Cold plate

4 Operation

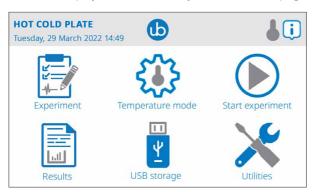
4.1 Icons, button and pages on the display.

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 pages instate of this text in this position, you will find name of the page you are currently in.

Tuesday, 29 March 2022 14:49

Here is where the instrument shows the current date and time set into the Utili-Page: 20 < Hot/Cold plate instruction manual 4 Operation ties/Date and Time.



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

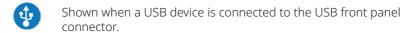
Use the button to return to the Main page (this is the back button on all the device pages).

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



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 and the Trial and an Animal ID.

You can fills this field up using the X-Pad Windows application on You PC, which result easier to manage and import this data to the Hot/Cold plate using the provided USB key.



Always use the button to return to the previous page.



Temperature mode

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 forever.
- 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 °/min
- Fast ramp; to make a ramp experiment at the maximum 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, 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.



Start experiment

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



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



USB storage

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 applications)

Load experiment by a USB key which contains data created with the X-Pad Ugo Basile Windows application (note that this is not a custom ramp loading procedure, 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 other 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, where when is ON at the end of linear or Fast ramp mode the device stops the experiment or not if OFF

4.2 Performing experiments (general)

All experiment done with animals needs to be done after an habituation phase; refer to your lab director for the correct habituation procedure provided by your Lab standard for Hit/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 https://bio-protocol.org/e4079?sid=ee47bba2-8dfe-4e13-9420-fafffe8c4c47&sousouid=BP1662381181851395&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 https://www.ineurosci.org/content/32/30/10136.short

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:

Inputting the data into the field directly from the Hot/Cold plate touch display. Using the provided X-Pad3 windows application.

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 page press the button "Experiment" and inside the next page You can digit, by the electronic keyboard the experiment data, which are the following: (remember that this data will appear on the result data file, use text thinking on a reading the result.

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.

In addition You can transfer the data-file via web browser to the Hot/Cold plate if the device is LAN connected (see paragraph 3.7 LAN Connection)

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 you need to insert the Temperature value (in degrees °C of °F (depending if you set the device in the "**Utilities**" "**Measurement unit**") and the Experiment duration time in seconds.

To set the constant temperature press "Plate Temperature"

For ethical reason we advise you to set this value not out the range from 2 to 54 $^{\circ}$ C (35 to 129 $^{\circ}$ F) even if the device can reach a minimum of -5 $^{\circ}$ C and a maximum of +65 $^{\circ}$ C

Write the temperature you need and press "OK"

With the button "Experiment duration" you can set the time the experiment will last for, in seconds.

Please note if you want a never lasting test set the time to 0 (zero)

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 did 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 start to 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.

You need to wait until the "Reach" button, that during the reaching time is "STOP" to eventually let's you stopping the procedure, to become "START" winch 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 only to reduce foggy in the restrainer at very low temperature) 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 booth by the pedal switch or the STOP button on the display.

Generally the experiment has to be stopped while the animal shows feeling the pain, jumping or leaking the paw.

For ethical reason DO NOT EXPOSE ANIMAL AT LONGER TEMPERATURE STIM-ULUS

REMOVE THE ANIMAL IMMEDIATELY AFTER HE SHOWS FEELING PAIN.

During the experiment time you are able to visually score some animal behaviour and registering them into the result data file, using the USB keyboard pressing the keys.

Any time you press a mapped key the related behavioural test will be recorder

into the result data file.

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	
9	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"

In addition, if Your Hot/Cold plate is LAN connected (see paragraph 3.7 LAN Connection) You can export experiment data directly to the PC via web browser. Log in into the Hot/Cold plate via the web browser, by the menu go to the Result page and press the Download CSV file.

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.

If You ask the device to perform a ramp with a total time impossible to reach, the display will advise You about the minimum time to reach the requested temperature

Consider the advise a suggestion while the value is calculated based on test we made with the device in a temperature controlled environment at 23°C. If Your Hot/Cold Plate is placed in a a room having a ambient temperature different of 23°C the time needed to perform the ramp may vary.

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.

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 are not possible to generate 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 you need to insert the USB key where you wrote the ramp file and press "LOAD RAMP".

You will see the list of the founded 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 just described procedure.

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)

While your experiment is finish 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.

In addition, if Your Hot/Cold plate is LAN connected (see paragraph 3.7 LAN Connection) You can export experiment data directly to the PC via web browser. Log in into the Hot/Cold plate via the web browser, by the menu go to the Result page and press the Download CSV file.

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)

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 mapped to Enter key	
62	Keypad event mapped to 1 key	
63	Keypad event mapped to 2 key	
64	Keypad event mapped to 3 key	
58	Keypad event mapped to 4 key	
59	Keypad event mapped to 5 key	
60	Keypad event mapped to 6 key	
54	Keypad event mapped to 7 key	

Back to content

Event code	Event description	
55	Keypad event mapped to 8 key	
56	Keypad event 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)

Result (If OK the event occurred before the Ramp or Experiment end time, OVER if the event occurred after reaching the final temperature)

Temperature (Is the plate temperature when the event occurred)

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

Mode (Is the temperature mode chosen for the experiment)

Offset (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)

RampSpeed (in degrees per minutes for linear Ramp, for Custom Ramps it is the ramp

segment duration in seconds)

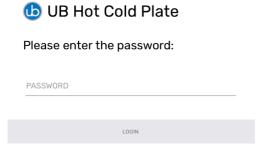
4.11Using LAN connection

If Your Hot/Cold plate is LAN connected (see paragraph 3.7 LAN Connection) You can load experiment parameters file generated by the Ugo Basile X-Pad app into the Hot/Cold plate and retrieve experimental data result without the need to use the USB pen as a data bridge.

LAN Connection is done by a standard web browser, while we do prefer to use Google Chrome, You can try to use Your preferred web browser.

To start a connection session make sure the LAN connection installation has been properly done (see paragraph 3.7 LAN Connection) and then open Your web browser

LAN connection to the Hot/Cold plate, for data security, is password protected; the factory password is UgoBasile, and You may want to change it.



To change the LAN connection password:

- 1. Log in to your Hot/Cold plate with the factory password (UgoBasile)
- 2. Go to the main menu (the 3 lines at the top left and select the command "Change Password"
- 3. You will asked to input the current password and input two times the new password.
 - Password need to be 8 or 15 characters, should contain at least a lower case letter, an upper-case letter, a number and a symbol.

It is advisable to secure store the password set for feature use, while if You loose it You do not have any chance to retrieve Your experiment data.

If You lost the password You can reset the password from the device touch panel, but be aware that the password reset will delete all the stored experiment result data.

To reset the password (and the stored result data):

On the device touch screen panel from the main page press the Utility button and then the Device Setup button, then press Factory reset and You will be asked for a reset confirmation.

Result data were deleted and the password has been set as UgoBasile.

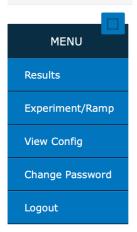
LAN Connection menu:





UGO Basile Hot Cold Plate

Clicking on the three white line at the top left on the browser windows You can open the main connection menu:



On the Result page You will find the experiment data result and the possibility to download the .csv file with the data on Your computer by pressing the Download CSV File button





UGO Basile Hot Cold Plate

Firmware version: V.2.0.0.3

Device clock: Wednesday, 17 July 2024 11:38

Number of records in DB: 0

Download CSV File

Records

Session	Date Time	Event Code	Event Description	Treatment	Protocol	Stage

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The Experiment/Ramp page will give You the ability to load into the device: Experiment data

Ramp experiment

Booth created by the Ugo Basile Windows app X-Pad which is included in Your Hot/Cold plate device.

Create the Experiment file and/or a Ramp file in X-Pad and safe it on Your PC. By the web browser connect to the Hot/Cold plate as described and select from the menu the function Experiment/Ram:



Scegli file nessun file selezionato Send to device X-Pad Ramp file (.rcr) Scegli file nessun file selezionato Send to device

X-Pad Experiment animal list

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Use the button Select File (is in Italian in the pics while this Windows is an Italian edition) to select on Your drive the Experiment or the Ramp file and then press the relative button Send to device.

You will receive a confirmation message.

The Experiment data and/or Ramp data will be loaded into the Hot/Cold plate.

The View Configuration page is intended for show the system data and can be required by our support team to have Your device information for service purpose.

The Logout command is to disconnect Your browser from the Hot/Cold plate and ending the working session.

5 Connections

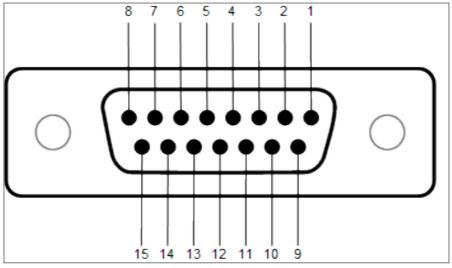
5.1 Communication port connection

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	Start/Stop IN	TTL IN	Like pedal switch signal
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

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 HO**T, 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, keep particular attention during the sanitation process while $\rm H_2O_2$ 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) Reading is calibrated at 22°C room temperature. 22°C (71.6° F) is the best room temperature to operate.
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
- B. 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.



For other Ugo Basile products related to this device visit *uqobasile.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



Thermal Place Preference (TPP Test) for Mice & Rats



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



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

Ugo Basile SRL



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



e-VF Handheld Electronic Von Frey of I.R. Heat-Flux Radiometer for Tail original design



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



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



The 1st, original Mouse RotaRod for motory coordination studies



OPERON (Papaleo-Scheggia's method) for 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