Instruction manual

THERMAL GRADIENT RING

ugobasile.com

Cat. No 35550

Pain and Inflammation

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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 opened instrument under voltage should be avoided as much as possible and, when inevitable, 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.

35550
Instruction Manual
dated May 2019 Revision 0

TGR Thermal Gradient Ring

for Mice

Cat. No. 35550

General

The TGR is a novel device for THERMAL PREFERENCE PHENOTYPING in mice, ac-cording to the method devised by Dr. Katharina Zimmermann.

A NOVEL DEVICE FOR
THERMAL PREFERENCE PHENOTYPING IN MICE
ACCODING TO ZIMMERMANN'S METHOD

Main Features

- New circular design, ID 45cm, OD 57cm: duplicate values, no border effects, no spatial cues
- Two heating devices on opposite sides, to establish a symmetric gradient
- Exact temperature gradient measured in real time by embedded thermocouples
- Thermal Insulated Ring-shaped Aluminum Runway
- 12 zones per side (specular), 40cm^2 ea.
- Test results automatically recorded via dedicated camera (included) & ANYmaze video-tracking soft-ware
- Including a set of 4 dual (visible/I.R.) lights

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CHECK-LIST

35550 TGR Thermal Gradient Ring

CLIENTE / CUSTOMER_____

Ordine No. / Order No. _____ **Data / Date**____/_____/_____

UB code	*CAT.No.*		√	*DESCRIPTION*		*DESCRIZIONE*
	35550-001	1		TGR runway and thermal device assembly		Assieme corridoio circolare ed elementi termici TGR
	35550-050	1		Light and camera support		Assieme sostegno videocamera e luci
	35550-035	1		USB Camera for TGR (assembled on the 35550-050 support)		Videocamera USB (assemblata su support 35550-050)
M-LM 1229-F	**35550-320**	1		External enclosure ring		Anello di contenimento esterno
M-LM 1231	**35550-321**	1		Internal enclosure ring (IR Transparent acrylic)		Anello di contenimento interno (acrilico IR trasparente)
M-LI 338	**35550-322**	1		Anti-glare disc		Disco anti-riflesso
	50104	1		USB Hub		
E-AU 055		1		Hub power supply		Alimentatore per Hub
E-AU 041 USB pen-drive	**35550-302**	1		Instruction Manual, on pen drive		Manuale d'istruzione, su chiavetta
E-WP 008		3		MAINS CABLE	EUROPE	
E-WP 008-1		3			U.S.A.	
E-FT 016		2		FUSE (T5A 6X32)		FUSIBILE (T5A 6X32)

DATE / /	**Serial No.**	**IMBALLATO DA / PACKED BY**

Universal input 85-264 VAC, 50-60Hz

IMPORTANT/IMPORTANTE:

Check the shipment for completeness immediately after receipt: should you find any discrepancy, please fill in the following part and transmit it to our fax no. **+39 0332 745488**

Al ricevimento della merce controllate che la spedizione sia completa: in caso di discrepanza, completate il formulario di seguito riportato ed **inviatelo** al nostro fax no. **0332 745488**

FROM: Name	Company/Institution
DATE	REF.

NOTE

CONTENTS

FIGURE INDEX

TGR Thermal Gradient Ring

Cat. 35550

1 GENERAL

The TGR is a novel device for **THERMAL PREFERENCE PHENOTYPING** in mice, according to the method devised by Dr. Katharina Zimmermann.

Current devices such as linear temperature gradients do not allow to discriminate between exploration-driven behaviour and thermal-driven behaviour. The limitations are especially severe when it comes to quantifying normal cold sensitivity, cold hypersensitivity and cold hyperalgesia knock-out and double knock-out mice cold transducers such as TRMP8-, TRPA1- and TRPM(/A1- mice (Touska et al. 2016). Beyond these specific transducers a very large number of Pain research areas do require enhanced sensitivity and robustness versus confounding factors such as exploration and thigmotaxis.

With these objectives and limitations of currently available devices in mind, Dr. Zimmermann (University of Erlangen-Nuernberg, see BIBLIOGRAPHY, paragraph 8.1-Method Papers) had a brilliant idea and created a home-made fully working prototype, which Ugo Basile ten transformed and validated into a reliable, industrialized device for scientists benefit worldwide.

In practice, the double hot/cold plate or the corridor thermal gradient approaches (which unavoidably exposes mice to instinctive tendency to explore and prefer the edges and walls of the device) were replaced by a thermally calibrated ring, where the animal faces no edge and which has no start/end points, being completely symmetrically, spatially and thermally.

1.1 The technology behind the Thermal Gradient Ring

With more than 2,600 papers published with the Ugo Basile Hot-Cold Plate, the industrialization of Dr. Zimmermann prototype started with ensuring that the ring could be set at the desired temperature and that by setting a high and a low temperature at two extremes of the ring the temperature over the metal corridor would correspond to the desired gradient.

Special sensors and conductive material was used to ensure that not only the 15-40° temperatures Dr. Zimmerman most frequently uses would translate in predictable “temperature slices”.

The second step was the automation of the mouse behaviour and the automated calculation and display of the thermal preference data. This was achieved using an IR sensitive camera, visible and IR lights above the arena, an inner IR-transparent corridor, a special paint for the corridor to maximize contrast and the use of the best in class video-tracking

software ANYmaze. To make it even simpler for the user, a special ANYmaze module was developed, so that the system is literally plug and play.

Only 1 USB connection from the TGR to your PC and ANYmaze will do the rest for you!

1.2 Features and Benefits

- Discriminate thermal-driven behaviour from exploratory/motory/emotional-driven behaviour
- Boost sensitivity thanks to the discrimination above and to the accurate thermal gradient with duplicate thermal zones, resulting in a thermally symmetrical arena, whose temperature can be adjusted according to the experimental needs in duplicate “slices” of temperature zones
- Sturdy and seamless to use with very broad ranges of temperatures for the most diverse supraspinal pain studies, yet avoiding exploration, anxiety and other non-thermally driven disturbances
- Ready-made and comprehensive results collected and analysed by video-tracking software across temperature and time dimensions (e.g. preferred temperature, time spent in each zone, number of entries, thermal preference in different time windows of the experiment, etc.)

2 INSTRUMENT DESCRIPTION

The TGR consists of a circular running track, which provides a thermal gradient between the two extremes of a colder and a hotter zone in which the mouse is free to move.

The Thermal Insulated ring-shaped aluminium runway has an ID of 45cm and an OD of 57cm.

Figure 1, “TGR Set-Up”

A heating and a heating/cooling device (based on the technology employed in UB Hot/Cold Plate), placed at the opposite sides of the ring, create a symmetric thermal gra-

dient, controlled by 4 embedded thermocouples, measuring the temperature gradient in real time.

2.1 TGR Features

- New circular design, ID 45cm, OD 57cm: duplicate values, no border effects, no spatial cues
- Two heating/cooling devices on opposite sides, to establish a symmetric gradient
- Exact temperature gradient measured in real time by four embedded thermocouples
- Thermal Insulated ring-shaped aluminum runway
- 12 zones per side (specular)
- Test results automatically recorded via dedicated camera (included) & ANYmaze video-tracking software
- Including a set of 4 dual- lights (visible/I.R.)

2.2 Heating Units

The heating and heating/cooling devices are based on the well-tested technology employed in UB Hot/Cold Plate.

2.2.1 Heating Unit

The heating unit can be set in a temperature range from 20°C (or room temperature) to 65°C.

The temperature is set via a precision digital thermoregulator, driven by a PID controller.

This guarantees that the temperature is continuously regulated with a threshold less than ±0.5°C.

Figure 2, "Heating Unit"

The unit is also provided with two potentiometers, labelled "IR Light" and "Visible Light", to set the brightness of the lamp set.

See paragraph 4-OPERATION for operating instructions.

2.2.2 Heating/Cooling Unit

Similarly to the heating unit, the temperature in the Heating/Cooling device is set via a precision thermoregulator, in the range 4-35°C.

See paragraph 4-OPERATION for operating instructions.

Figure 3, "Heating/Cooling Unit"

2.2.3 Important Notice on Temperature Range

The declared temperature range of both thermal units can be reached provided the room temperature remains in the interval 18-25°C.

2.2.4 Considerations Over Animal Welfare

In any analgesia test, great care must be taken to prevent the animals from inadvertent harm.

However, as in the TGR the animal is free to move in the temperature are it considers comfortable, a real risk of harming the animal does not exist.

2.3 Circular Running Track

The TGR consists of a circular running track in which the mouse is free to move, enclosed by an inner and an outer ring, both 24cm tall.

The advantages brought about by the circular design are duplicate values, no border effects and no spatial cues, guaranteeing bias-free, reproducible data.

The **thermal insulated ring-shaped aluminium runway** has an ID of 45cm and 57cm OD.

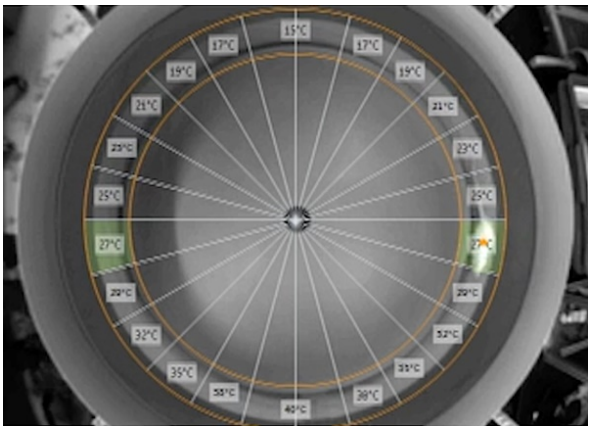
Figure 4, "Circular Running Track"

The running surface has a special glare-free finishing, resilient and durable, which is ideal for videotracking, and has a “smooth” feeling comfortable for the animal on test.

Speaking about the enclosing rings, the inner one is made of an IR transparent material; this feature limits the animal view of the surrounding area, but will not limit the camera view of the testing are.

The temperature is proportionally distributed over the runway: 12 symmetrical areas on each side are virtually, but precisely identified via ANYmaze software

In the picture an example of how the 12+12 virtual areas are identified and displayed.



For example, in the protocol described in the method paper, where the 2 preset temperatures are respectively 15°C and 40°C, each sector represents an increment of 2.27°C.

The **black plastic ring** which holds the inner and outer walls in place, has the essential feature of insulating the runway.

The hardware can be completely **disassembled** for a thorough **cleaning**, see paragraph 5.2-Cleaning.

2.4 Stand, Camera and Lights

A stand positioned over the device holds the B/W USB camera in the correct position.

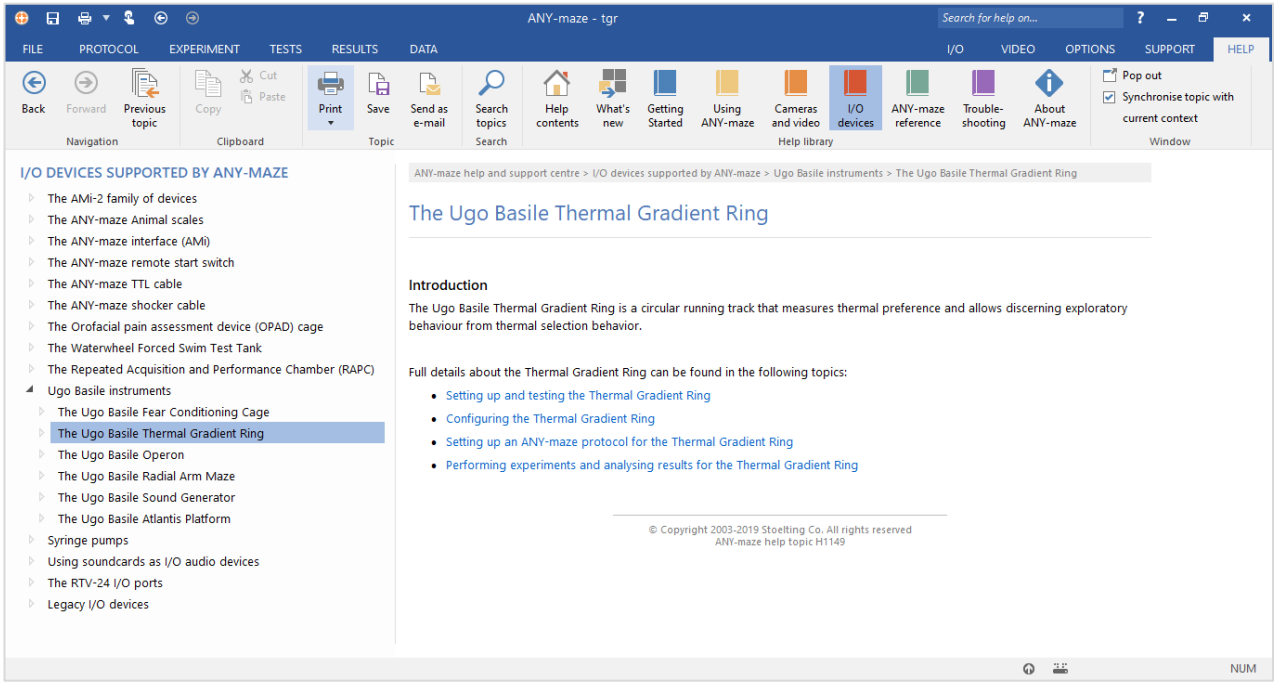
The assembly also encompasses 4 dual (visible/I.R. lights), which intensity is regulated on the Heating Device front panel, see paragraph 2.2.1.

Figure 5, “Stand, Camera and Lights”

2.5 ANYmaze

The TGR test is managed by ANYmaze.

A specific Ugo Basile TGR mode is preconfigured to ease the protocol set-up.



Recording and analysis of thermal preference behavior is also accomplished by ANYmaze. Data output include:

- Preference Temperature time course ± SD
- Time lag to cover zones above a defined temperature (time course)
- Zone histogram

3 INSTALLATION

3.1 Unpacking & Preliminary Check

Check the contents of the shipment for completeness, packing list to hand, and visually inspect the instrument as soon you take it out of the packaging. Use the ***Check List***.

If the instrument is damaged, inform the carrier immediately, notifying our company. If after having tested it, the instrument fails to meet rated performances, please contact our after sales service, see paragraph 5.4-Customer Support.

Protect the environment!

Dispose of packaging properly, according to existing and applicable waste management rules and regulations.

3.2 Notes on the Instruction Manual

The 35550 Instruction Manual included in the package (on the USB pen drive) is necessary for the correct installation and operation of the instrument.

We recommend reading the manual with attention, as it is essential for the correct installation and operation of the instrument. Please save the manual, ready to be consulted by the qualified personnel who use the instrument. Print only if necessary.

Our Instruction Manuals are available as free download on our web. For any additional information and/or assistance, you are welcome to contact our Service Department (see paragraph 5.4-Customer Support), specifying the serial number of your instrument.

3.3 General Safety Instructions

The following guidelines must be followed to ensure safe operation.

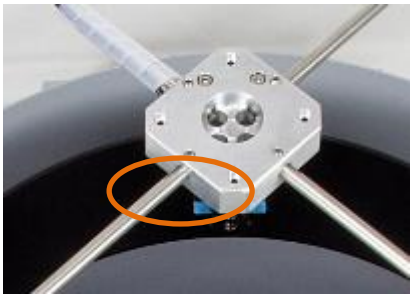
- ! **DO NOT** attempt to open or perform any service work
- ! **DO NOT** connect up human subjects

3.4 Assembling the Instrument

The TGR is delivered assembled, except for the light supporting structure. Put the instrument on a stable and flat bench or table surface.

One of the light supporting arms is provided with the camera support: position this arm first, on the corresponding hole on the plastic insulating ring, following the indication on the camera (toward heating/cooling unit).

Position the other three arms, and fix them on the camera support, tightening the related hex. screw by the allen wrench provided.



3.5 Before Applying Power

Consider the Power Module on the back of both heating and heating/cooling units, see paragraph 3.8-Connections, which includes:

- the inlet connector of the mains cord
- the mains switch
- the fuse holder

3.5.1 Mains Switch

This two-pole toggle switch, which complies with international safety standards, is lighted when the instrument is **ON**.

3.5.2 Fuse Holder

The fuse holder comprises two fuses, one on the live, and the other on the neutral. Use (T5A) timed fuses for operation at both 115 or 230 Volts. For fuse replacement, please refer to paragraph 5.1-Electrical.

3.5.3 Mains Cord

It is a standard cable, Cat. # E-WP008. Make sure your power outtake is provided with a reliable ground connection.

3.6 Intended Use

The TGR Thermal Gradient Ring is intended for investigation use on **laboratory animals only**.

3.7 Additional Safety Consideration

1) Use original accessories and spare parts only, see also paragraph 7-ORDERING INFORMATION.
2) immediately disconnect and replace damaged mains cord.
3) do not obstruct a comfortable access to the power module.
4) do not obstruct the grids of the cooling fans on the sides of the cabinets
5) do not operate in hazardous environments or outside prescribed environmental limitations (i.e. +10c° / +40c°, 95% max. relative humidity, non-condensing)
6) do not spray any liquid on the connectors and on the geared motor

UGO BASILE DOES NOT ACCEPT ANY RESPONSIBILITY FOR PROBLEMS OR HARM CAUSED TO THINGS OR PERSONS, ARISING FROM:

- 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 components, accessories or parts with others not approved by the manufacturer;
- servicing carried out by unauthorized personnel.

See also paragraphs 2.2.2 and 2.2.4

3.8 Connections

Connect the mains cord from both thermal devices to a power outtake, ***provided with a reliable earth connection***.

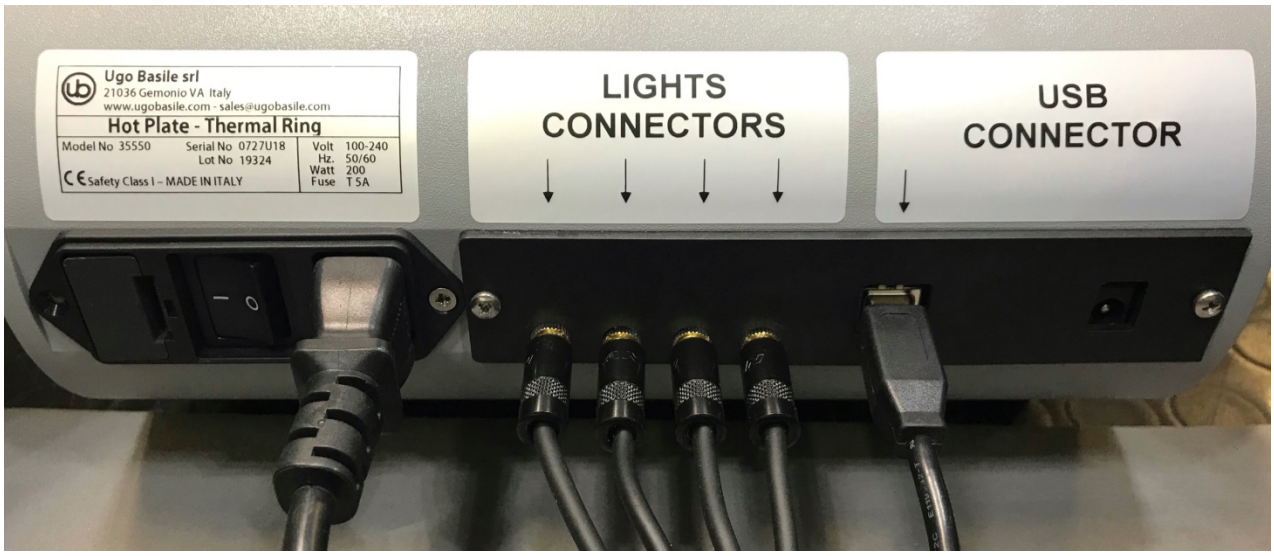


Figure 6, "UB-Hub"

On the back of the Heating Unit, branch the jack connectors from the 4 lights onto the 4 related sockets, see picture above.

Attach the unit to the Hub via the USB cable. The TGR icon on the hub (**3**, see figure 7 below) will indicate the correct USB port.

Connect the USB cable from the camera to the related USB port (**4**).

Attach the power supply to the USB (**1**).

Finally connect the Hub to PC, via cable (**2**).

Figure 7, "UB-Hub"

4 OPERATION

You're only a few steps away from operating your TGR:

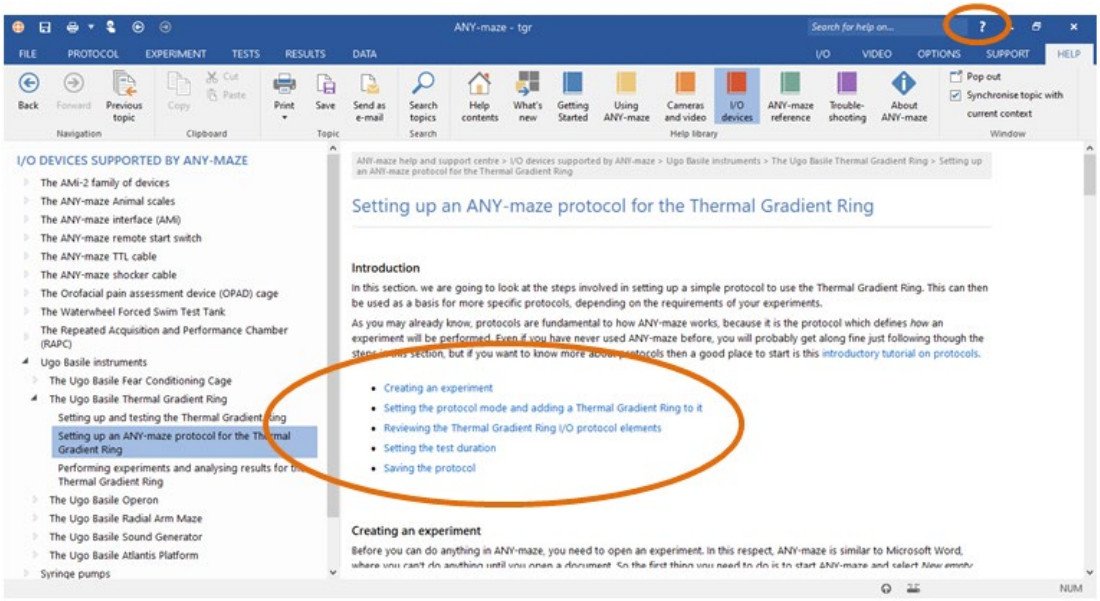
1) Set the desired temperature on the heating/cooling device: press (**P**), increase or decrease the temperature by the UP or DOWN arrow. Press (**P**) to confirm.

2) Set the desired temperature on the heating device: press (**P**), increase or decrease the temperature by the UP or DOWN arrow. Press (**P**) to confirm.

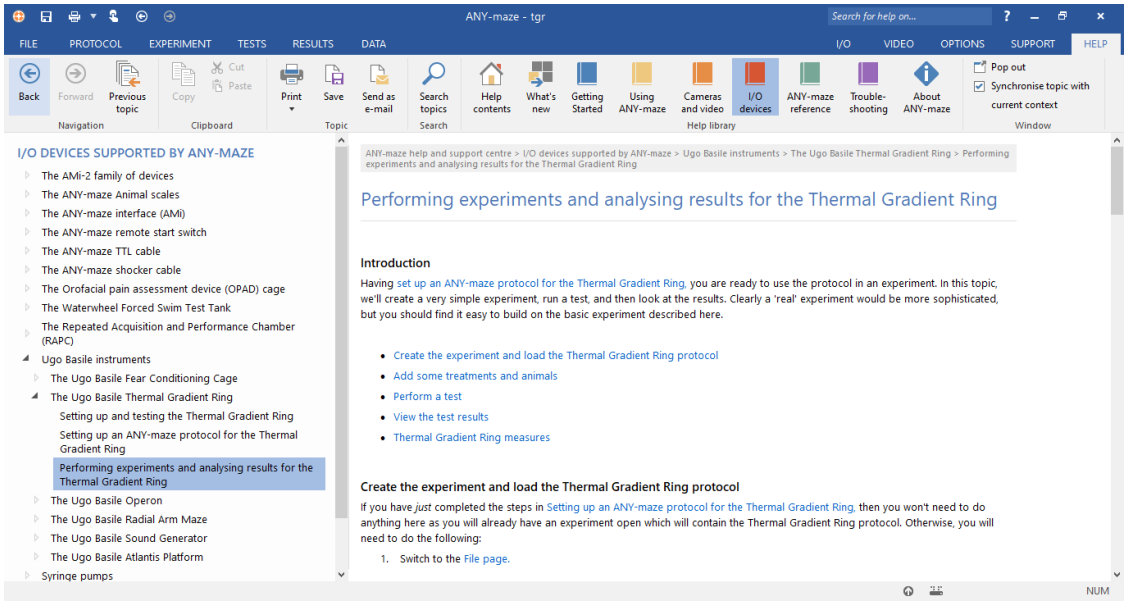
3) Set the light brightness by acting on the relate potentiometer: I.R: light on the left, visible light on the right.

Allow at least one hour for the temperature to reach a steady condition over the whole runway.

4) While your machine gets ready for the test, open ANYmaze software and configure your TGR protocol, following the wizard. Please refer to the help desk for guidance



5) When the device is ready and the protocol configured, you're ready to perform your experiment. ANYmaze will guide you through the necessary steps:



6) Manage and analyse data via ANYmaze.

5 MAINTENANCE

While any service of the instrument ought 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 MAINS CORD BEFORE CARRYING OUT ANY MAINTENANCE JOB!

5.1 Electrical

To inspect and/or replace the fuses, **disconnect the mains cable first!** Insert a miniature screwdriver in the slot indentation, see paragraph 3.5.2, and snap out the slide which houses the fuses. Use T5A fuses for operation at both 230 or 115 Volts.

Snap in the fuse slide: the mechanical "*click*" ensures that it is locked.

5.2 Cleaning

The TGR 35550 does not require any maintenance apart from normal cleaning. The unit can be completely and easily disassembled for a thorough cleaning.

Do not use organic solutions, on the internal wall and insulating ring, as they are liable to impair the surface of the acrylic components.

Cotton wool and water can be used for cleaning purposes. For disinfection, use a non-alcoholic disinfectant, or H_2O_2.

5.3 Long Inactivity

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

5.4 Customer Support

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

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Phone : +39 0332 744574

service@ugobasile.com
logistics@ugobasile.com
sales@ugobasile.com

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.

6 35550 SPECIFICATIONS

General	
Commands	Positioned on the front panel of the thermal units
Temperature Read-out	On LCD display on the front panel of the thermal units
Power Requirement	Universal input 85-264 VAC, 50-60Hz, 40W max.
Sound Level	< 60 dB (A)
Operating Temperature	18° to 25°C see paragraph 2.2.3
Operation	
Temperature Range	Heating Plate: from 20°C (or room temperature) to 65°C Heating/cooling Plate : from 4°C to 35°C
Precision	0.5°C
Temperature feedback:	By 4 thermocouples monitored in real time by ANYmaze
Light Intensity (I.R.)	Set by potentiometer with graded scale
Light Intensity (visible)	Set by potentiometer with graded scale

Data Collection & Management	Via ANY maze specific protocol for TGR
Animal position detection	Via ANYmaze videotracking software
Physical	
Aluminum runway	internal diameter 45cm outer diameter 57cm
External enclosing ring	57cm diameter, 24cm tall
Internal enclosing ring	45 diameter, 24cm tall, I.R. transparent acrylic
Total Weight	39Kg
Shipping Weight	57Kg
Dimensions	87(w)x64(d)x64(h)cm
Packing Dimensions	100x80x70(h)cm (wooden pallet)
Warranty	35550 is covered by a 24-month warranty

7 ORDERING INFORMATION

35550	**TGR Thermal Gradient Ring**, complete with following components:
35550-001	TGR runway and thermal device assembly, including: 35550-100 Heating Device 35550-150 Heating/Cooling Device
35550-050	Light and camera support, including 4 x 35550-325 Dual Lights (I.R. and Visible)
35550-035	USB Camera for TGR (assembled on the 35550-050 support)
35550-320	External enclosure ring
35550-321	Internal enclosure ring (IR Transparent acrylic)
35550-322	Anti-glare disc
50104	USB Hub
E-AU 055	Hub Power Supply
E-AU 041	USB pen drive, including: 35550-302 Instruction Manual
E-WP008	Mains Cord – Europe (or E-WP008-1 U.S.A. / E-WP008-2 U.K.)

Set of 2 fuses

60000	**ANYmaze** videotracking software, full version

8 BIBLIOGRAPHY

8.1 Method Papers

- F. Touska Z. Winter, A. Mueller, V. Vlachova, J. Larsen and Katharina Zimmermann: **"Comprehensive thermal preference phenotyping in mice using a novel automated circular gradient assay"** J.Temperature, Vol 3 (1) ***2016***
- Z. Winter, P. Gruschwitz, S. Eger, F. Touska and Katharina Zimmermann: **"Cold Temperature Encoding by Cutaneous TRPA1 and TRPM8-Carrying Fibers in the Mouse"** Front. Mol. Neurosci., ***2017***

We do the search for you: we weekly browse bibliography and link new papers to the bibliography section of each UB device.

Don't forget to check our web page periodically for updated bibliography!

Notes

Notes

Notes

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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

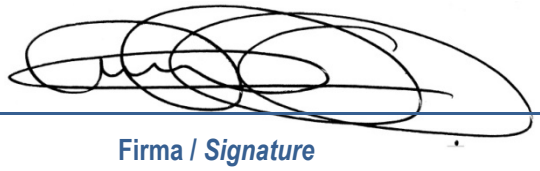
Instrument.	**THERMAL GRADIENT RING**
Catalog number	**35550**

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*



October 2018

Date — **Firma / *Signature***

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