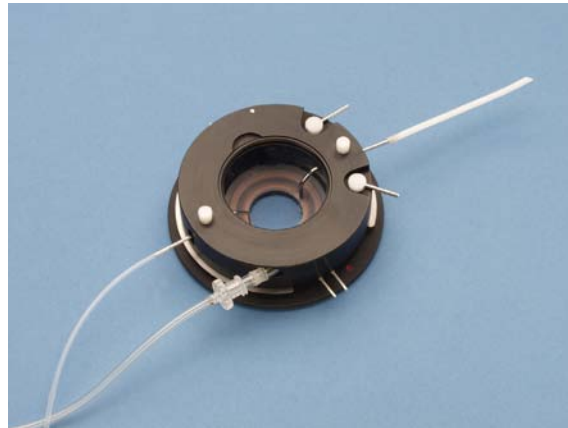


Warner Instruments, Inc.
Micro-Incubation Platform
For RC-40 Chambers
Model DH-40i



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The **DH-40i** Micro-Incubation Chamber from Warner Instruments is designed to provide complete environmental control over **RC-40** Series Chambers and 35 mm culture dishes while maintaining the ability to make both short- and long-term imaging studies. This versatile system readily accommodates culture dishes from several manufacturers and will adapt to any microscope stage supported by Warner Instruments.

Features of the **DH-40i** include:

- ✓ Designed for Warner's **RC-40** Series glass bottom quick change imaging chambers
- ✓ Designed for 35 mm culture dishes. Adapts to dishes made by Willco Wells, Corning and Falcon
- ✓ Permits imaging, temperature, and gas atmosphere control
- ✓ Unique dish clamps permit either open or closed use
- ✓ Heated top coverslip (optional) eliminates condensation when used as a closed chamber
- ✓ Accommodates upright and inverted microscopes

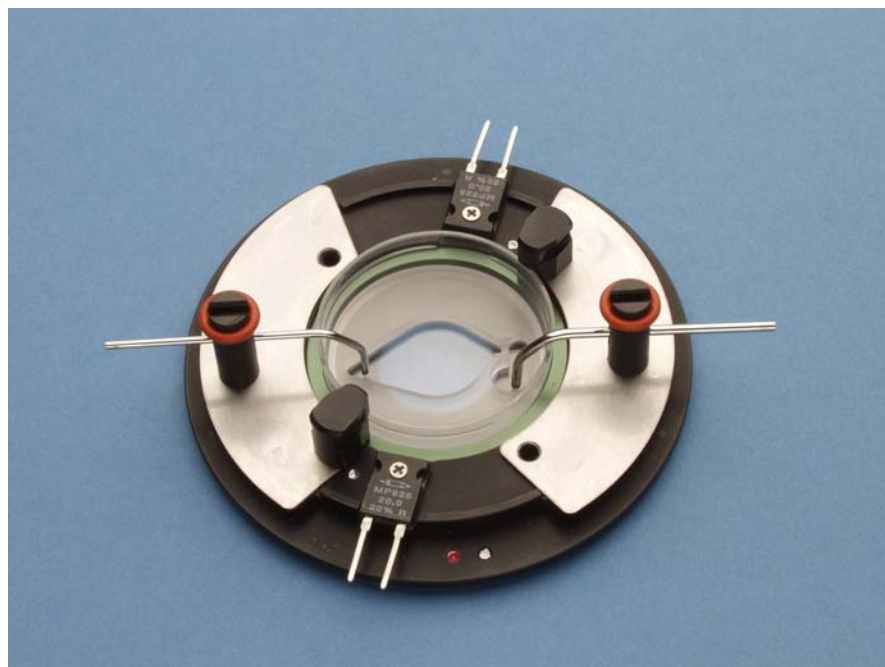
**THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS**

INTRODUCTION

The **DH-40i Culture Dish Incubator** is designed to facilitate the imaging of live cells in a **RC-40** Series chamber or cell culture dish. Cells can be imaged using either an open or closed dish format. The open dish format readily accommodates the use of microelectrodes or rapid throughput tools while the closed dish format can be used to perform long-term studies or to maintain pH, [CO₂], or other environmental factors. Temperature control is available in both open and closed formats.

RC-40 Series Glass Bottom Quick Change Chambers are small volume imaging chambers featuring rapid solution exchange, short working distances and an open bath. Since bath volumes are generally small, exchange times are measured in seconds even when flow rates are less than 1 ml/min.

The **DH-40i** incubator is primarily designed to work with the RC-40 Series chambers, but will work equally well with 35 mm glass-bottom cell culture dishes from Willco Wells, Corning or Falcon. The incubator is machined in two interlocking sections and metal adapters are supplied to insure a secure fit for supported 35 mm culture dishes.

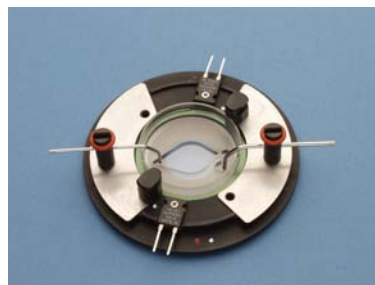


COMPONENTS

The completed **DH-40i** assembly is comprised of a platform, a **Series 40** chamber or 35 mm culture dish (with adapter ring, if necessary), and a cover. The assembly is mounted onto a microscope using a Warner microscope stage adapter. Thermal control is provided using a **CC-28** cable and temperature controller.

Platform

The platform directly accepts a **Series 40** chamber. Supported culture dishes are positioned using one of four supplied adapter rings (see **Accessories**). The platform also includes two resistive heating elements, a 19 mm diameter aperture, dish clamps, and a single hole for attaching a control thermistor (e.g. via Warner's **CC-28** cable). The periphery of the platform is framed with stainless steel plates for mounting magnetic holders (when the **DH-40i** is used in the open configuration).



Cover

The cover (shown inverted) is used to form an enclosed micro-incubation system. The cover window is sealed using a 40 mm round cover glass. The window cover glass is positioned via a recessed annular lip and is held in place using a rubber o-ring. When in place, the cover glass seals a 36 mm wide window useful for illumination purposes.



Accessories

Four colored-metal adapter rings are included to insure a close fit between the platform and a cell culture dish. Rings are tapered for a precise fit and should be mounted with the *notch facing down*. A special tool (i.e. a modified 18G hypodermic needle) is provided to assist in removing adapter rings from the Bottom Section. Color codes for the different adapter rings are shown below.

Adapter ring color codes	
Blue	Willco Wells: Models D3512 and D3522
Red	Falcon: 37 mm
Gold	Falcon: 38 mm
Green	Corning: 25000 series



SETUP

General Comments

The **DH-40i** is quickly and easily assembled. The platform may be used alone for open format applications, while the cover is required for closed format applications.

The cover contains several ports allowing entry of atmospheric and perfusion lines, as well as electrode and thermistor access. We recommend the use of the **RC-40 Series** quick-change chambers when using the **DH-40i** in closed format.

Open Format

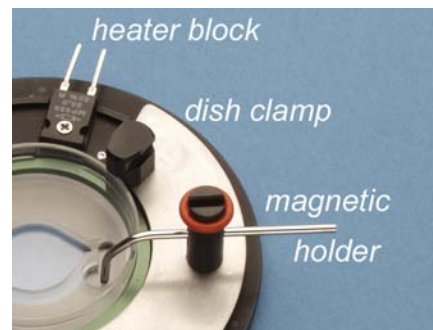
Chambers

If using an **RC-40 Series** chamber, begin by assembling the chamber. Use of an adapter ring is not necessary.

If using a cell culture dish, begin by selecting the appropriate adapter ring for your dish. (See adapter guide, page 5.) Place the adapter ring, notch side down, on a firm surface and insert the cell culture dish into the ring by gently pushing it into place.

NOTE: Care must be taken to evenly insert the dish into the ring so that the assembly seats properly within the platform.

The chamber is placed into the recess in the platform and is clamped into place. Dish clamps are provided and are placed into two small holes located on either side of the platform. These holes can be found directly adjacent to the heater blocks. (See the figure to the right for clamp positioning.)



Perfusion

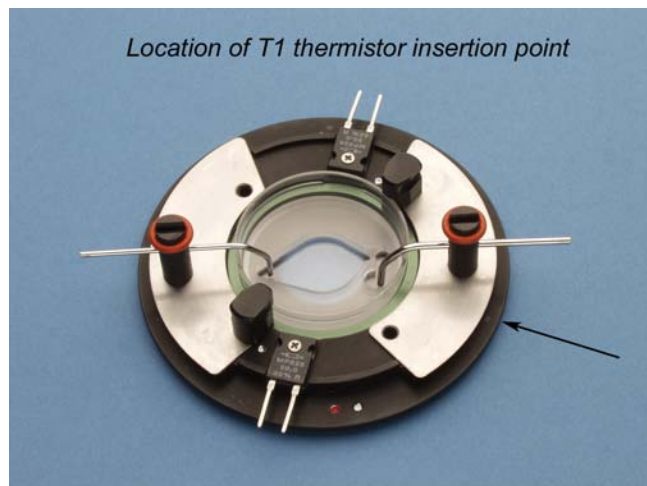
An input tube and an aspiration tube are provided with the **DH-40i** to facilitate perfusion within the chamber. These tubes can be found as part of the cover assembly. The aspiration tube can be identified by its larger diameter. It also has a plug at its tip.

The input and aspiration tubes can be easily removed from the cover and inserted into the provided magnetic holders for placement onto the steel shelves of the platform. Attach perfusion lines to the input and aspiration tubes and place into position.

Heating

If heating with Warner's **TC-324B** or **TC-344B** temperature controllers, slip the white colored T1 feedback thermistor on the **CC-28** cable into the milled hole in the side of the platform. (See figure on top of next page for thermistor placement.) A small amount of mineral oil can facilitate good thermal contact between the feedback thermistor and the metal of the platform.

Slip the blue colored leads over the two resistive heating elements and then plug the **CC-28** cable into the temperature controller. Consult the temperature controller user's manual for methods of maintaining the temperature of the clamped culture dish.



Placement

If imaging, place the fully assembled **DH-40i** into your microscope's **DH-40i Stage Adapter** and mount onto the microscope.

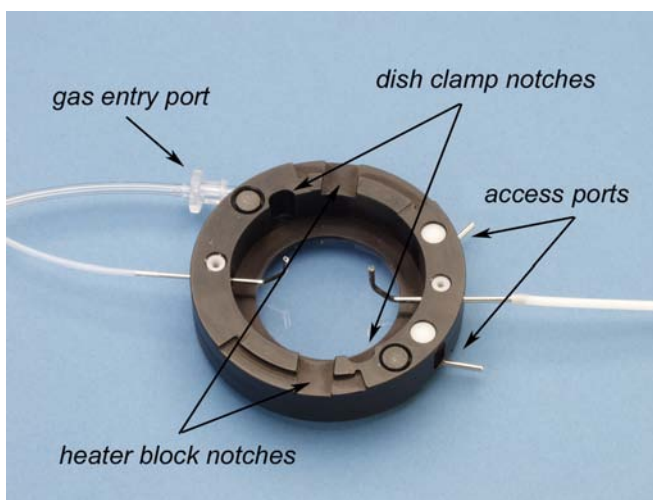
Closed Format

Chambers

Chambers are assembled into the platform as described on page 6.

Cover components

The **DH-40i** cover has a gas entry port, notches for the dish clamps and heater blocks, two access ports, and two perfusion tubes for solution exchange. An inverted cover is shown to the right.



The gas entry port connects to your atmospheric control system. Gas vents into the chamber around the window which provides good distribution while eliminating window condensation.

The dish clamp and heater block notches are provided for the obvious reasons. They also serve to orient the cover onto the platform.

The access ports can be used to provide a vent for waste gasses or to provide easy entry for special lines of your choosing. Each entry port swivels open and closed and has a handle to simplify manipulation.

Two perfusion tubes (input and aspiration) are incorporated into the platform cover and represent the input and output flow lines for the chamber. The input tube has a smaller diameter and is open at the tip. The aspiration tube can be identified by its larger

diameter and has a plugged tip. Both tubes are fully adjustable, removable, are bent to aid in positioning, and will maintain their orientation when the cover is removed and replaced.

Finally, there are two magnets embedded into cover to provide a secure attachment between the cover and the platform base.

Cover assembly

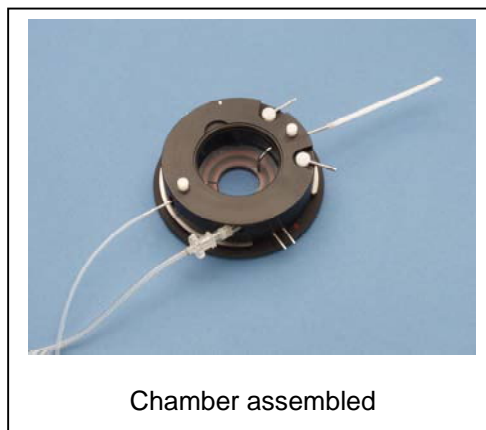
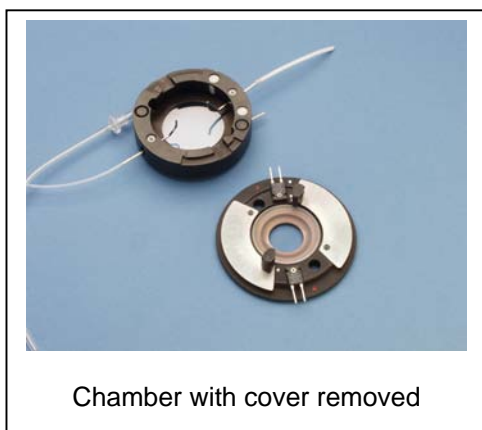
1. Begin by placing a clean 40 mm round coverslip into the recess on the cover. Secure the coverslip using the supplied o-ring.
2. Attach the gas line to the gas input port on the cover.
3. Attach the inflow and outflow perfusion lines to their respective perfusion tubes.
4. If needed, carefully route any accessory lines through the miscellaneous entry ports on the cover.

Heating

Follow the heating instructions for the open bath configuration detailed on page 6.

Placement

1. Place your chamber into the platform and secure the cover into position over the chamber.
2. With the cover in place, adjust the position of the perfusion tubes into the chamber. Begin solution flow. Make adjustments to (1) the position of the perfusion tubes and (2) the perfusion flow to achieve the desired flow characteristics. Stop solution flow until the **DH-40i** is placed onto the microscope.
3. If imaging, place the assembled **DH-40i** into your microscope's **DH-40i Stage Adapter** and mount onto the microscope.
4. Begin gas and solution delivery.
5. Adjust the entry ports for gas venting if necessary.



OPERATION

Open Format Applications

Only the platform, together with one of the four colored adapter rings and the dish clamps is required for open format use. The open format enables easy access for microelectrodes, perfusion lines and aspiration lines. Cell culture dishes can be used with and without covers, and dish perfusion can be simplified by use of Warner's **RC-40 Series** Perfusion Chambers.

The open format permits imaging using either upright or inverted microscopes. Warner **Series 40 Stage Adapters** are used to mount the **DH-40i** onto most commonly used microscope stages. Contact our Tech Support Department for custom stage adapters.

Resistive heating of the **DH-40i** is achieved using Warner's **TC-324B** or **TC-344B** Temperature Controller and a **CC-28** cable. The two blue **CC-28** connectors attach directly to each resistive heating element. Feedback control is provided by thermistor (i.e. T1) which is inserted into the platform's metal chassis via a special hole.

Note: We recommend using a drop of mineral oil to insure good thermal contact between the white T1 thermistor and the metal chassis.

The **CC-28** cable's bath thermistor (i.e. T2) can be placed directly in the bath to check solution temperature. The temperature control unit regulates the heating current by sensing the temperature of the incubator via the T1 control thermistor in the **CC-28** cable. Heating of perfusate can be achieved using our **SH-27B** or **SF-28** In-Line solution heaters, which are also controlled via our **TC-324B** and **TC-344B** Temperature Controllers.

If using 35 mm culture dishes in the open format, Warner Instruments' **RC-33** perfusion chamber inserts are available for Corning dishes and can be used together with the **DH-40i**. These inserts incorporate our diamond fluidics design to maintain laminar perfusate flow.

Closed Format Applications

In the closed format, the platform and windowed cover section are used together to maintain an enclosed environment. Gas ports (inlet and outlet) in the cover enable the user to control the cell's pH, [CO₂], and [O₂]. The windowed cover is easily removed allowing quick replacement of cell culture dishes during replicate trials. The cover secures onto the platform via two magnetic posts. Access ports are provided in the cover to permit insertion of accessory lines (such as a monitor thermistor) into the enclosed chamber. In the closed format, resistive heating is achieved in the same manner as described for the open format.

APPENDIX

Cleaning and maintenance

The **DH-40i** is constructed of anodized aluminum. It can be easily cleaned with common laboratory detergents or with ethyl alcohol.

Warranty and service

Service

We recommend that all questions regarding service be referred to our Technical Support Department. Normal business hours are 8:30 AM to 5:00 PM (EST), Monday through Friday.

Our offices are located at:

Warner Instruments
1125 Dixwell Avenue
Hamden, CT 06514

(800) 599-4203 / (203) 776-0664
(203) 776-1278 – fax

support@warneronline.com
<http://www.warneronline.com/contact.html>

Warranty

The **DH-40i** is covered by our Warranty to be free from defects in materials and workmanship for a period of one year from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse.

In the event that repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments.