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ACD HybEZ™ II Hybridization System and EZ-Batch™ Slide Processing System

User Manual

Document Number 321710-USM

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Citing HybEZ[™] in Publications

When describing a procedure for publication using these product, please refer to them as the ACD HybEZ[™] II Hybridization System (Cat. No. 321710 and Cat. No. 321720).

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Chapter 1. Product Information



Before using this product, read and understand the following information regarding safety when using the unit.

IMPORTANT! We recommend reading the entire user manual before beginning any protocols.

Safety

The following symbols marked on the equipment mean:



CAUTION: Read these operating instructions fully before use and pay particular attention to sections containing this symbol.

ATTENTION: Suivre attentivement les instructions avant l'usage et prêtez une attention particulière aux sections comportant ce symbole.



CAUTION: Surfaces can become hot during use.

ATTENTION: Les surfaces peuvent devenir brûlantes pendant l'usage.



CAUTION: Risk of electric shock. Before attempting any service to this unit remove power cord from the rear of the unit.

ATTENTION: Risque électrique! Débrancher la prise arrière de réparer l'appareil.

Always observe the following safety precautions:

- Read this entire manual before using the slide hybridization oven.
- Use only approved accessories. Do not modify system or components. Any alterations or modifications to your incubator may be dangerous, and will void the warranty.
- Do not position the equipment so that it is difficult to operate the disconnecting device.
- Use only as specified by the operating instructions or the intrinsic protection may be impaired.
- After transport or storage in humid conditions, dry out the unit for 48 hours before connecting it to the supply voltage. During the drying out period, the intrinsic protection may be impaired.
- Connect only to a power supply that provides a safety ground terminal.
- Do not check temperature by touch. Use the temperature display or a thermometer.
- Do not touch surfaces that become hot.
- Ensure that the power supply cord plug is easily accessible during use.
- Do not block or restrict ventilation slots. Allow at least 3" clearance around the entire unit.

- If liquid is spilled inside the unit, disconnect it from the power supply and have it checked by a trained person.
 - This product must be used with a power supply cord that is rated for a minimum temperature of 90°C, and that complies with national and local certification requirements.
 - Do not use with flammable, corrosive, or hazardous material.
 - Never leave the unit unattended.
 - Do not mount equipment on a surface of flammable material due to a hazard that could be caused by hot items falling from the equipment when the door is opened.
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About this guide

This user manual describes how to use the ACD HybEZ™ II Hybridization System.

Product description

Background

Many staining assays that detect molecular markers in microscope-mounted biological specimens involve repeated operational cycles. Repeated operations include incubation, where slides are held in a horizontal position that allow a small volume of reagents to incubate with the specimen; and washing, where slides are submerged and agitated in bulk reagents. During incubation, special measures have to be taken to prevent loss of reagent due to evaporation. These processes often require separate slide handling devices. When many slides are processed in a batch, transferring slides repeatedly between two different devices becomes tedious, labor intensive, and error prone.

Intended Use

The ACD HybEZ™ II Hybridization System is a new generation, evaporation controlled incubator designed to conduct the hybridization and incubation steps in Advanced Cell Diagnostic's proprietary RNAscope® and BaseScope™ assays. The HybEZ™ II Humidity Control Tray, EZ-Batch™ Slide Holder, and EZ-Batch™ Wash Tray included in the system allow batches of slides to be processed at the same time. The ACD HybEZ™ II Hybridization System is compatible with other hybridization based assays or any assay step where a condition of high humidity with an elevated temperature is required.

Components

The ACD HybEZ™ II Hybridization System contains the following components:

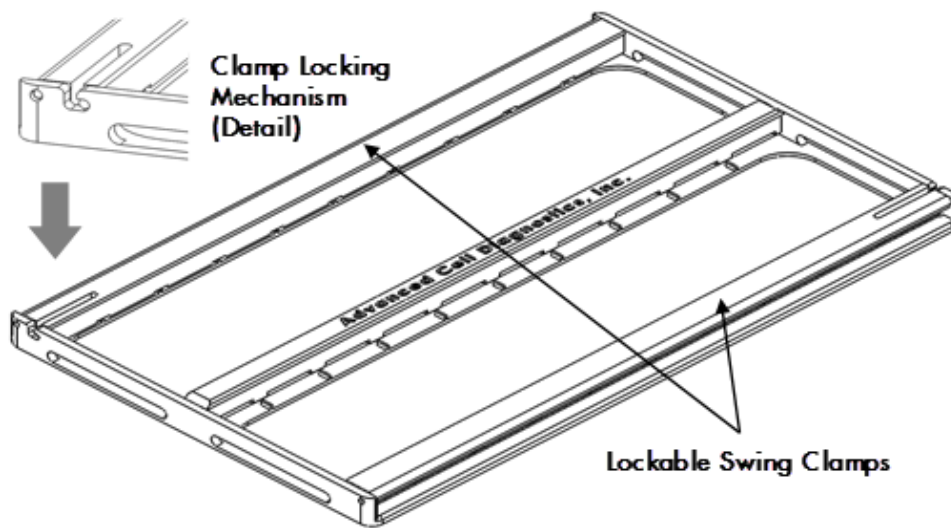
- HybEZ™ II Oven (110 VAC, Cat. No. 321719; 220 VAC, Cat. No. 321720)
- HybEZ™ Humidity Control Tray with lid (Cat. No. 310012)
- EZ-Batch™ Slide Holder (Cat. No. 310017)
- EZ-Batch™ Wash Tray (Cat. No. 310019)
- Two sheets of HybEZ™ Humidifying Paper (Cat. No. 310025)

The system can hold up to twenty standard microscope slides on the slide rack. The unit has an advanced microprocessor temperature controller that provides stable and accurate chamber

temperatures from ambient plus 10°C to 75°C. The unit uses a sealed tray to create a lasting humid environment for overnight runs or weekend runs, depending on the operating temperature.

EZ-Batch™ Slide Holder

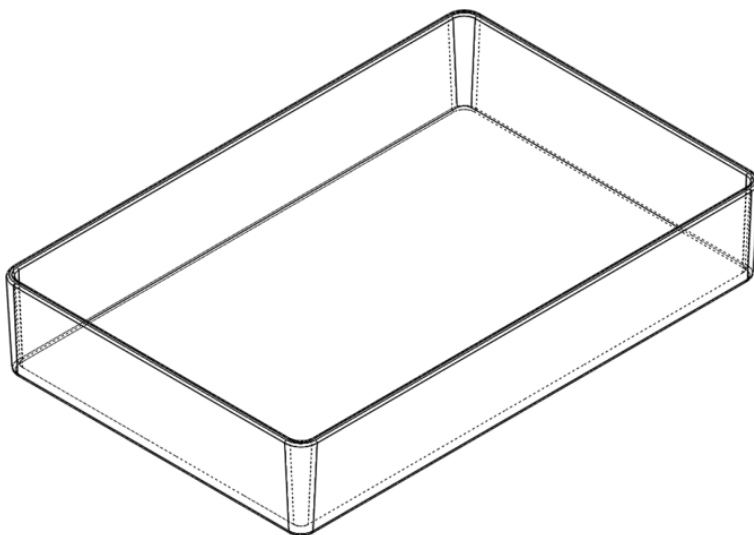
The EZ-Batch™ Slide Holder (Cat. No. 310019) can hold up to 20 standard microscope slides in secure, lock-down positions arranged in two parallel rows. Lock-down is achieved by two lockable swing clamps, one per row, along both sides of the slide holder. Clamp locking mechanisms are located at the slots found at one end of each clamp.



EZ-Wash Tray

Batch™

The following is a diagram of the EZ-Batch™ Wash Tray (Cat. No. 310019):



IMPORTANT! The ACD EZ-Batch™ Slide Processing System is not compatible with xylene and acetone. Do NOT submerge the Slide Holder or Wash Tray in reagents containing these materials.

Product specifications

General

This equipment is intended for indoor use and will meet performance specifications within the temperature range of Ambient +10°C to 75°C, with maximum relative humidity of 80% (non-condensing). Installation Category II (transient voltages). Pollution Degree 2 in accordance with IEC 664. Suitable for operation at altitudes of up to 6500 feet.

Technical Specifications

Temperature Range:	(Ambient +10°C) to 75°C
Setting Range:	20°C to 75°C
Stability:	±0.5°C
Overall Accuracy:	±0.5°C at 40°C
Temperature Display Resolution:	0.1°C
Alarms:	Yes in cases of temperature deviation, step complete, or program complete
Stackable:	Yes
Supply Voltage Range:	115 V +/- 10%, 50/60 Hz 230 V +/- 10%, 50/60 Hz
Power Rating:	155 W
Heating Rate:	Ambient to 40°C within 20 minutes
Dimensions (W x D x H):	11.00" x 15.96" x 8.09"
Weight:	21.00 lbs
Safety and Electrical Certification:	UL, CE, and CSA

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Chapter 2. Assembly

Unpacking

Remove packing materials carefully, and retain for future shipment or storage of the unit. Inspect all materials for damage.

IMPORTANT! Report all shipping damage to the carrier immediately. Shipping damage is covered by the carrier, and repair/replacement for shipping damages must be coordinated through the carrier.

Packs should contain:

- HybEZ™ II Oven
- Power Line Cord
- HybEZ™ II Hybridization System User Manual
- HybEZ™ Humidity Control Tray (includes a tray with gasket installed and lid)
- HybEZ™ Humidifying Paper
- EZ-Batch™ Slide Holder*
- EZ-Batch™ Wash Tray*

*May be sent in a different package.

Installation

To install the system:

1. Place the HybEZ™ II Oven on a flat and stable surface, preferably away from drafts.
2. Fit the power line cord into the IEC power socket on the rear of the unit.
3. Plug power cord into a power supply that matches the voltage listed on the serial number label on the rear of the unit.
4. Turn on the Power Switch, which is near the inlet of the power cord on the rear of the unit.

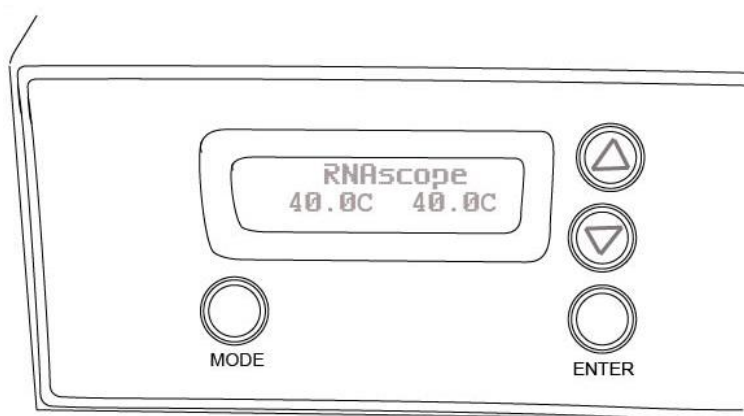
Controls and screen display

The oven automatically starts in the RNAscope mode:

- "RNAscope" is displayed on the first line of the screen
- The second line of the screen displays the set temperature (40°C) on the left, and the current temperature (flashing) on the right.
- The HybEZ™ II Oven defaults to the RNAscope mode each time the unit is turned on.
- If you are using or have used the HybEZ™ II Oven for functions other than running RNAscope® assays, return to the RNAscope mode by pressing the **Mode** then **Enter** buttons on the front panel (see **Appendix B. Other Functions** on page 17 for details).

Note: The oven will produce an audible alarm if the actual chamber temperature has deviated more than $\pm 2.5^{\circ}\text{C}$ of the set (40°C) temperature.

Figure 1. Screen display in the RNAscope mode

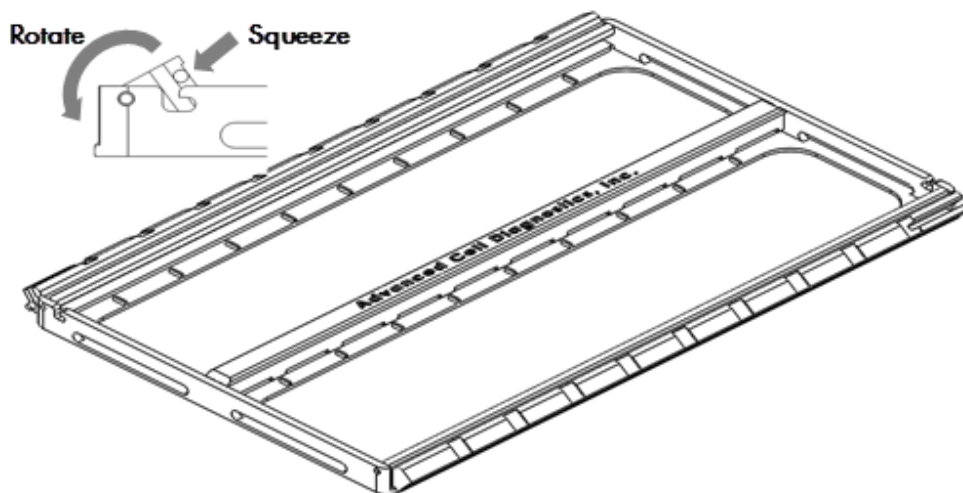


Preparing the HybEZ™ II Humidity Control Tray

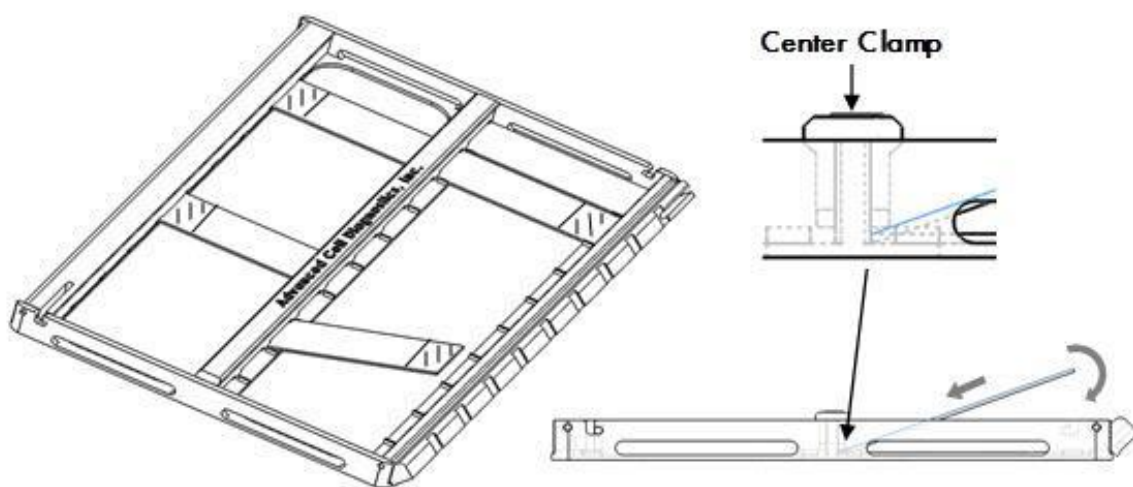
1. Place the HybEZ™ Humidifying Paper in the center of the bottom of the tray
2. Add approximately 50 mL of distilled water or current incubation solution to the paper.

Loading slides into the EZ-Batch™ Slide Holder

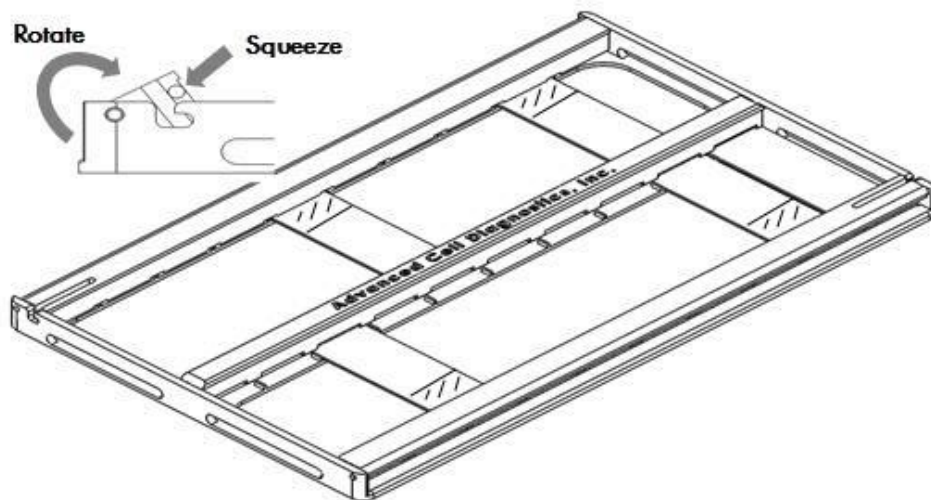
1. Open swing clamps one at a time by simultaneously squeezing the slotted portion of each clamp, and rotating it as shown in the following figure.



2. Insert up to 10 slides one at a time into one row of the holder. The non-label end of each slide should be aligned toward the center of the holder, and inserted under the fixed clamp as shown. Rotate the rest of the slide down into the holder.



3. Close and lock the swing clamp of the row by simultaneously squeezing the slotted portion of each clamp, and rotating it in the direction opposite to the direction used to open the clamp.



4. Repeat steps 2 and 3 with the other row of the holder.

Note: Once all slides are locked into the holder, you can handle them as an integrated unit.

Applying reagents to the slides

1. Use your standard protocol to add reagents to each slide.

Note: To prevent samples from drying out when applying reagents, quickly add one drop of reagent to each sample. Then add enough reagent volume to completely cover the samples.

2. Place the loaded Slide Holder into the Humidity Control Tray.

Loading the tray into the oven

To insert the HybEZ™ Humidity Control Tray into the HybEZ™ II Oven:

1. Open the main door of the oven.
2. Insert the tray into the slot all the way.
3. Close the oven door.

Removing the tray from the oven

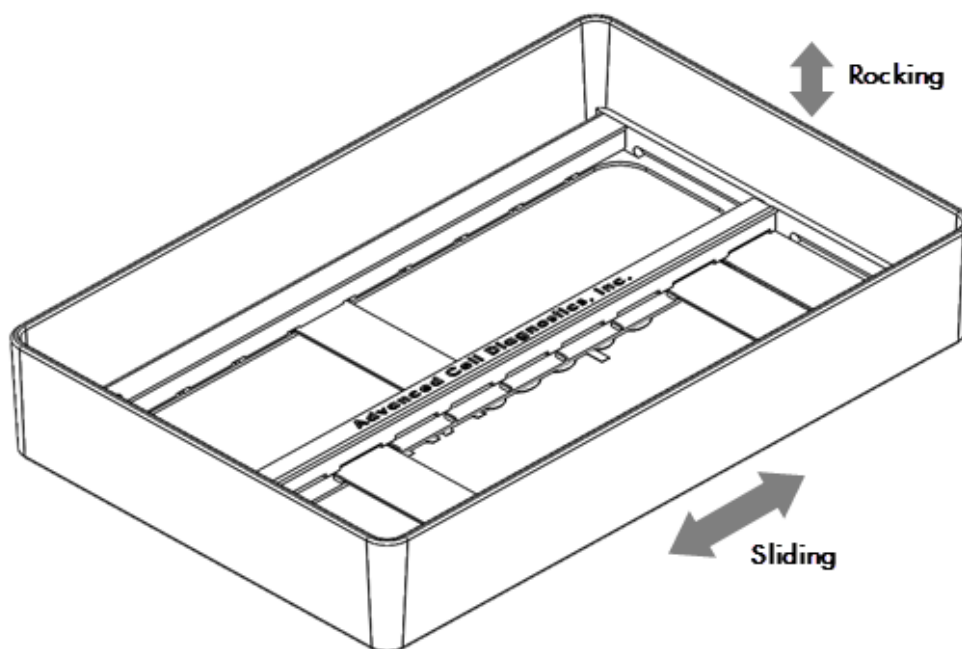
IMPORTANT! Extreme care must be used when removing the tray due to potentially high temperatures. The use of gloves may be necessary.

To remove the HybEZ™ Humidity Control Tray from the HybEZ™ II Oven:

1. Open the main door of the oven.
2. Slide the tray out.
3. Close the oven door.

Washing the slides

1. Pour 300 mL 1X Wash Buffer into the EZ-Batch™ Wash Tray.
2. Place the EZ-Batch™ Slide Holder into the Wash Tray. Make sure all slides are submerged in solution.
3. Incubate the slides for **2 MIN** at **RT**. Agitate slides by sliding or rocking the Wash Tray gently.



4. Decant the 1X Wash Buffer, refill with 300 mL fresh 1X Wash Buffer, and repeat the proceeding step.
5. Take the EZ-Batch™ Slide Holder out of the Wash Tray, and shake it or tap the Holder on a tissue to remove the residual Wash Buffer.

Note: Minimize residual Wash Buffer on the slides, but do not shake the slides out of their locked positions.

Troubleshooting

Issue	Possible cause	Recommended action
Unit does not operate	<ol style="list-style-type: none"> a. Unit not turned on b. Unit not plugged into power supply c. Fuses blown d. Power supply failure 	<ol style="list-style-type: none"> a. Push the Power Switch to turn on the unit b. Plug in and turn on c. Replace fuses (see Appendix A. Maintenance) d. Check that other electrical appliances on the same circuit are working
Chamber temperature does not rise when expected	<ol style="list-style-type: none"> a. Ambient temperature is higher than set temperature b. Temperature control circuit fault 	<ol style="list-style-type: none"> a. Check set temperature b. Have unit checked by expert
Temperature continues to rise when not expected	<ol style="list-style-type: none"> a. Actual temperature is lower than set temperature b. Temperature control circuit fault 	<ol style="list-style-type: none"> a. Check set temperature b. Have unit checked by expert
Slides dry out during heating cycle	<ol style="list-style-type: none"> a. Gasket not sealing 	<ol style="list-style-type: none"> a. Replace Tray and Gasket Assembly

Issue	Possible cause	Recommended action
	<ul style="list-style-type: none"> b. Lid bent c. Insufficient buffer placed in the bottom of tray 	<ul style="list-style-type: none"> b. Replace the lid c. Place more buffer in the bottom of tray



Appendix A. Maintenance for HybEZ™ II Ovens

The HybEZ™ II Oven is designed to comply with IEC1010-1. The system is highly reliable and requires minimal maintenance.

Cleaning the HybEZ™ II Oven

If the HybEZ™ II Oven requires cleaning, first disengage the power cord:

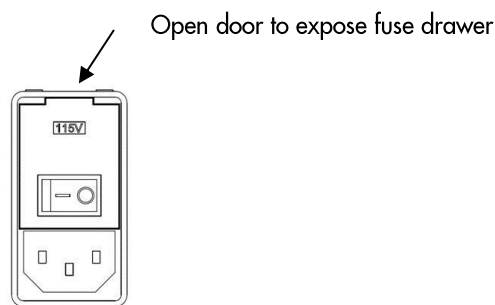
- For spills, use the appropriate radiation, chemical, or biohazard clean up procedures.
- Clean the outer casing with water and a damp cloth.
- Do not submerge or immerse the HybEZ™ II Oven in water.
- Before using any cleaning or decontamination method, except those recommended by the manufacturer, check that the proposed method will not damage equipment.

Replacing the fuses

Two supply fuses are located in the fuse drawer just above the Power Switch. To replace the fuses:

1. Disconnect the unit from the power supply.
2. Remove the plug from the socket in the back of the unit.
3. Open the door of the power entry module to expose the fuse drawer (Figure 3).

Figure 3. Power Entry Module



4. Pull out the fuse drawer.
5. Check fuses and replace if necessary. Replacement fuses must be 5 mm x 20 mm quick acting, rated 250 V.
 - 110 V model: 2A Fast acting
 - 220 V model: 1A Fast acting
6. Push the fuse drawer back in. Reconnect the unit to the power supply.

B

Appendix B. Other Functions

In addition to running RNAscope® and BaseScope™ assays, the HybEZ™ II Oven can also be used to perform other functions commonly needed in tissue or cell based assays.

Operating modes

The HybEZ™ II Oven is designed to operate in four different modes:

- **RNAscope** – Use this mode to run the RNAscope® and BaseScope™ assays. In this mode, the oven temperature remains at 40°C until a different mode is selected.
- **Bake** – Use this mode to bake slides mounted with tissue samples, and improve tissue adherence to the slide surface during an assay. In this mode, the oven temperature remains at 60°C for one hour.
- **Hold-T** – Use this mode to incubate slides at elevated temperatures other than 40°C. In this mode, the temperature remains at a user defined temperature (ambient + 10°C to 75°C) until a different mode is selected.
- **P-Run** – Use this mode to run one of ten stored temperature programs once. Each program comprises three different temperature stages at user defined temperatures and durations.

To enter a different mode

- Press the **Mode** button on the front panel to display the four modes on the screen.
- Use the **▲** or **▼** buttons to select the desired mode. The text of the selected mode will flash on the screen.
- Press the **Enter** button to enter the selected mode.

Baking the slides

1. Load tissue slides into the EZ-Batch™ Slide Holder.
2. Place the Slide Holder into the Humidity Control Tray without the humidifying paper and the lid. Do not wet the tray.
3. Inset the tray into the oven chamber, and close the door.
4. Press the **Mode** button on the front panel.
5. Use the **▲** or **▼** buttons to select the **Bake** mode.
6. Press the **Enter** button.
7. The screen displays “Baking Slides” on the first line. On the second line, it displays the current chamber temperature on the left and the remaining time in mm:ss format on the right.
Note: The timer counts down from 60:00 to 00:00, which starts when chamber temperature reaches 60°C.
8. The oven beeps when the count-down finishes.
9. The oven chamber cools down to ambient, and the display returns to the mode selection screen.

Incubating slides at different temperatures

Many assays require the samples to be incubated at different temperatures. Use the following procedure to run these assays:

1. Press the **Mode** button.
2. Use the **▲** or **▼** buttons to select the **Hold-T** mode.
3. Press the **Enter** button.
4. The screen displays "Hold-Temp" on the first line. On the second line, it displays the target temperature on left and the chamber temperature (flashing) on the right.
5. Use the **▲** or **▼** buttons to adjust the target temperature.
6. Press the **Enter** button to set the target temperature.
7. Begin incubating your samples *only* after the oven temperature reaches the target temperature.
8. To end **Hold-T** mode, press the **Mode** button at any time.

Note: The oven will produce an audible alarm if the actual chamber temperature has deviated more than $\pm 2.5^{\circ}\text{C}$ of the set temperature.

Setting up a three stage temperature ramping program

1. Press the **Mode** button, and use the **▲** or **▼** buttons to select the **P-Run** mode.
2. Press the **Enter** button.
3. The screen displays "Programmed Run" on the first line. On the second line, it displays "Run" on the left and "Setup" on the right.
4. Use the **▲** or **▼** buttons to select **Setup**. The selected text flashes.
5. Press the **Enter** button.
6. The screen displays the target ID number of the program to be set as "Program # [n]". Use the **▲** or **▼** buttons to adjust to the desired ID number, and then press **Enter**.
7. The screen displays the first target temperature as "Temp 1: [xx.x]". Use the **▲** or **▼** buttons to adjust to the desired target temperature, and then press **Enter**.
8. The screen displays the target duration of Stage 1 as "Time 1: [mm:ss]". Use the **▲** or **▼** buttons to adjust to the desired target duration, and then press **Enter**.
9. Repeat steps 7 and 8 to set the target temperatures and times for Stages 2 and 3.

Running a pre-stored three stage temperature ramp program

1. Press the **Mode** button, and use the **▲** or **▼** buttons to select the **P-Run** mode.
2. The screen displays "Programmed Run" on the first line. On the second line, it displays "Run" on the left and "Setup" on the right.
3. Use the **▲** or **▼** buttons to select **Run**. The selected text flashes.
4. Press the **Enter** button.
5. The screen displays "Run" on the first line, and "Program # [n]" on the second line.
6. Use the **▲** or **▼** buttons to select one of ten (0-9) programs.
7. Press **Enter** to run the select program once.
8. When the run completes, the oven cools down to the ambient temperature and the display returns to the Run screen.
9. Push the **Mode** button at any time to terminate a run, and return to the mode selection screen.

Documentation and support

Obtaining support

For the latest services and support information, go to: <http://www.acdbio.com/technical-support/support-overview>.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, SDSs, application notes, citations, training videos, and other product support documents.
- Find out information about customer training events.

Contact information

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Orders: orders@acdbio.com
Support Email: support@acdbio.com

Warranty and Returns

Limited product warranty

Advanced Cell Diagnostics, Inc. and/or its affiliate(s) warrant their products as set forth in the ADC General Terms and Conditions of Sale found on the ADC website at <http://www.acdbio.com/store/terms>.

When used in laboratory conditions and according to this User Manual, Advanced Cell Diagnostics (ACD) warrants this product to be free of defective material and workmanship for a period of two years from the date of shipment. The liability of ACD for any defective equipment during the warranty period shall be limited to the repair of such equipment or replacement thereof without charge for parts or labor.

Returns

A Returned Goods Authorization (RGA) number must be obtained before any ACD products are returned for any reason. A Decontamination Notice must be completed, signed by the user, and returned to ACD prior to receiving the RGA number. Please be sure to mark the outside of the return goods package with this RGA number to ensure prompt handling.

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