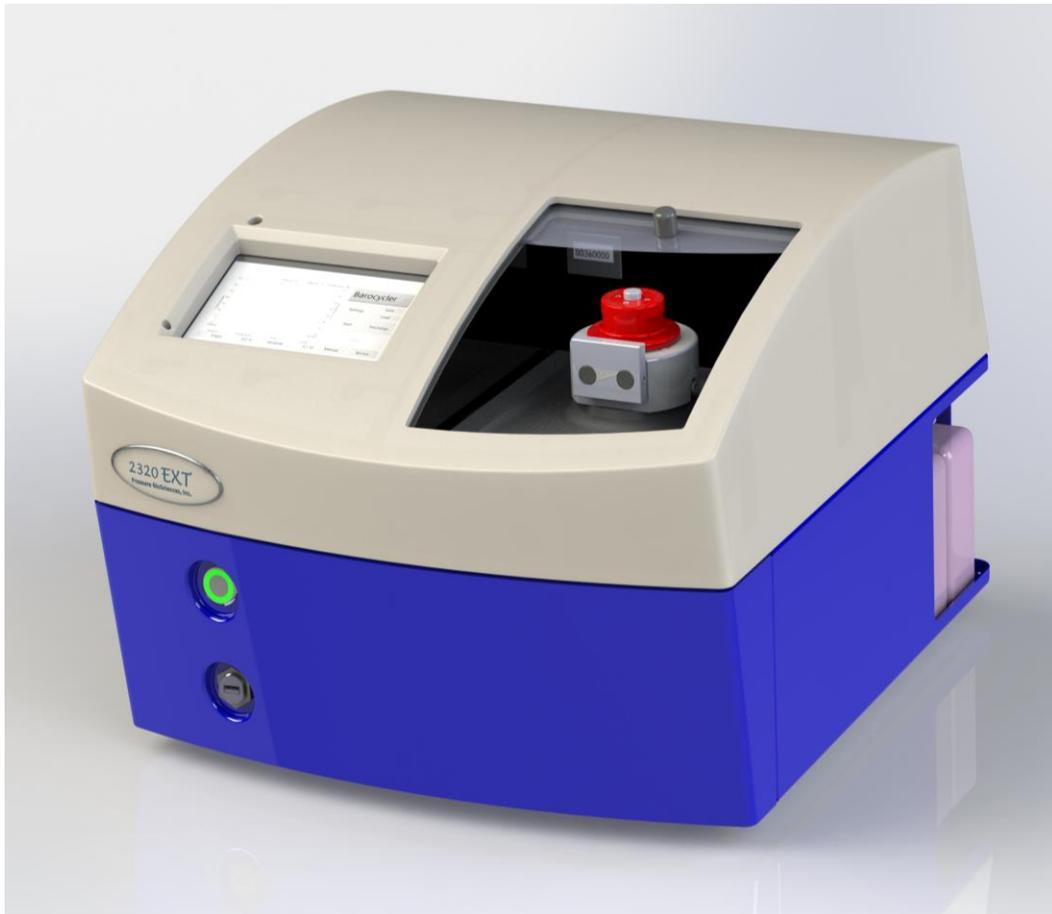


# **Barocycler 2320EXT**

## **Pressure Cycling Technology (PCT)**

### **Sample Preparation System**

## **Operator's Manual**



Operator's Manual Version: 2.3, Sept. 2016

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The Pressure Cycling Technology Sample Preparation System is designed for research use only and is not intended for use in diagnostic procedures.

The instrumentation in the system described herein is covered by the following patents: U.S. Patent Numbers: 6,111,096; 6,120,985; 6,127,534; 6,245,506; 6,258,534; 6,270,723; 6,274,726; 6,448,065; 6,635,469; 6,696,019; 6,753,169; European Patent Numbers EP 814900; EP 924991; EP1112091, Australian Patent AU 745925; additional patents pending.

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## Warnings and Safety Precautions

The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the Barocycler 2320EXT Pressure Cycling Technology Sample Preparation System.

Always Follow Good Laboratory Practices.

Use the System in a Proper Manner. Do not use the system and/or its accessories in a manner not specified by Pressure BioSciences, Inc. If you do so, the protection provided by safety equipment may be impaired.

The System Must be Plugged into a Grounded Power Line. Ensure that all parts of the system are properly grounded. It is strongly recommended that all parts of the system are connected to a common ground. Do not attempt to bypass the earth ground connection. A serious shock hazard could result. Potentially hazardous currents and voltages may be present inside the unit.

The pressure vessel hardware may be hot when operating at high temperatures. Appropriate personal protection (insulating gloves or glove liners) must be used to protect skin exposure to hot surfaces.

The 24V DC power adapter supplied with the system is sealed and not intended to be serviced by the user. Do not attempt to disassemble, service, or modify the power adapter. Contact Pressure BioSciences support in order to get a replacement part if power adapter malfunctions.

There are no user serviceable parts inside the Barocycler 2320EXT housing. No user access is permitted beyond the sliding door of the instrument. Do not attempt to open or disassemble the unit. Any service beyond routine maintenance has to be performed by the manufacturer or other authorized service facilities. Contact Pressure BioSciences Customer Service at **1-(508) 230-1828** or on-line at [www.pressurebiosciences.com](http://www.pressurebiosciences.com) to obtain a list of authorized service facilities near you.

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# 1 *General Information*

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## 1.1 **Warning Symbols**

Notes, cautions and warnings are used to highlight certain operating procedures and recommendations.

A note indicates a special procedure, an exception to normal operation, or something else of specific interest to the reader.

The following symbols describe the warning and cautions used in the operation of this instrument.



This symbol on the instrument indicates that the user should refer to the operating manual before attempting to connect the power/interface cables and operate the instrument.



This symbol on the instrument states that high voltage or high current may be present when panels/covers are removed. Any adjustment, maintenance, and repair of the opened apparatus under voltage should be avoided and, if inevitable, must be carried out only by a skilled person who is aware of the hazard involved.



**WARNING**

The WARNING statement used throughout the manual presents dangers that could result in personal injury, or in conditions that could cause damage to the instrument or the reporting of erroneous results.



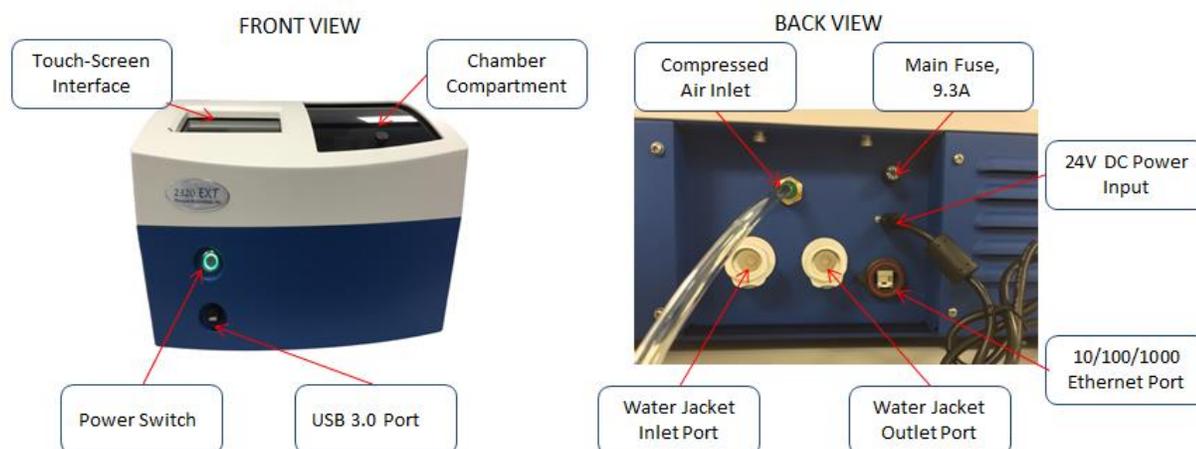
**NOTE**

The NOTE statement used throughout the manual highlights important information about the instrument and its use.

## 1.2 Overview and Theory of Operation

The Barocyler 2320EXT is a bench-top instrument designed for high pressure applications such as PCT-HD and accelerated enzymatic digestion of proteins. The pressure chamber can accommodate up to 16 samples at a time in PCT MicroTubes, or one sample at a time in PCT PULSE Tubes.

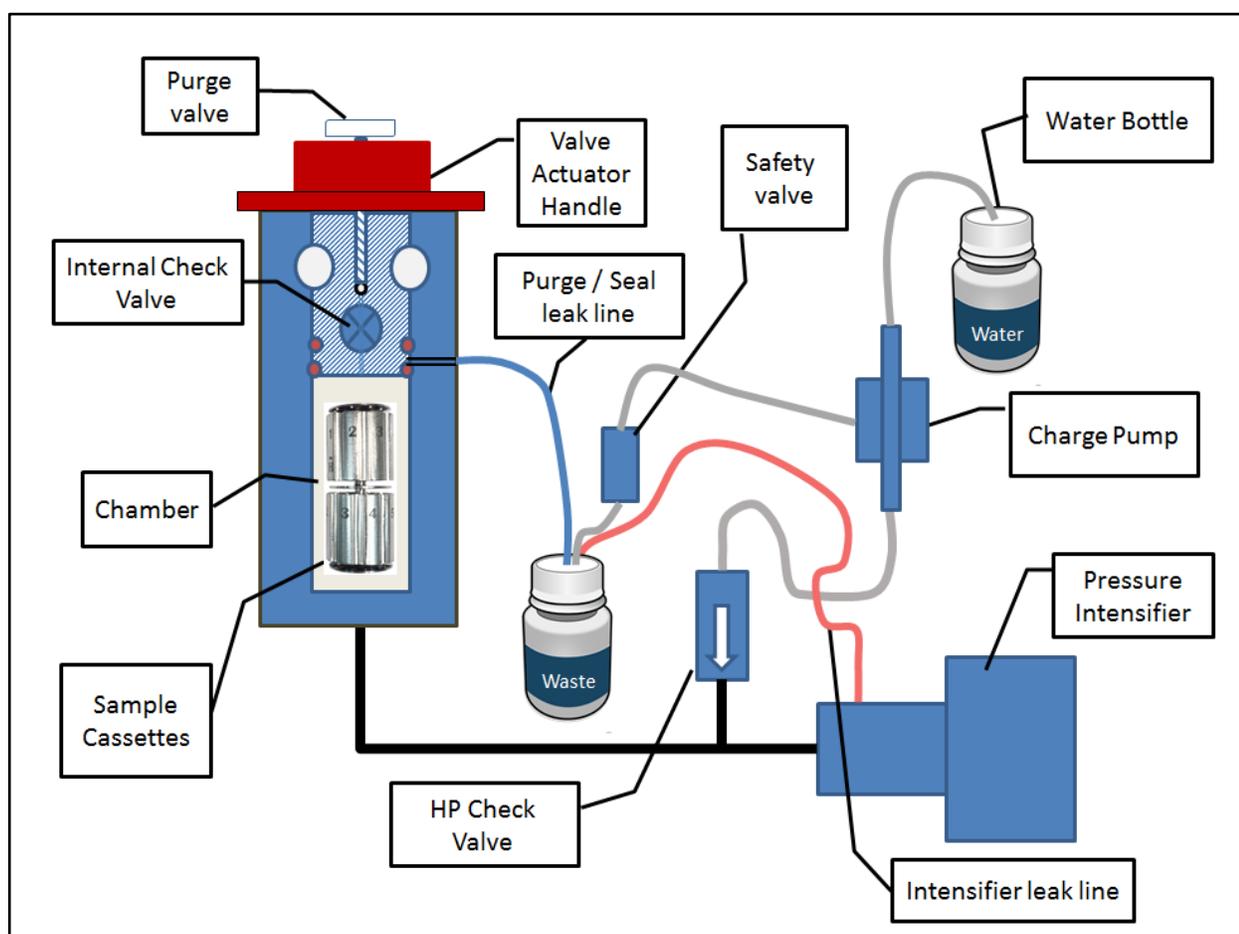
The Barocyler 2320EXT is capable of generating pressure up to 45,000 psi (45 kpsi). The instrument's intensifier amplifies air pressure to generate high hydrostatic (water) pressure. The air-water intensifier has an amplification ratio of 450:1. Thus, every 1 psi of air pressure will produce approximately 450 psi of hydrostatic pressure in the chamber. Water is supplied to the system from the Fill Bottle. A high pressure check valve (CV) separates the low pressure section from the high pressure section of the system. System pressure and chamber temperature are displayed on the software front panel. Pressure is read directly by means of a high pressure transducer. Temperature is measured via a thermocouple inserted into the wall of the chamber. Certain troubleshooting and diagnostic functions are performed by the controller and error codes are displayed, should the controller detect a malfunction. For detailed description of those functions and diagnostic error messages see the troubleshooting guide (Section 5). The chamber closure features an internal check valve that is used to allow air or residual pressure to be purged from the chamber.



Barocyler 2320EXT Sample Preparation System, front and back panels. Note that units equipped with electrical heating option have plugs in place of Water Jacket ports.

The Barocyler 2320EXT is equipped with a built-in touch-screen panel computer running Microsoft® Windows® 7.0 Embedded Standard operating system and Barocyler 2320EXT software. The instrument software allows users to input pressure (up to 45,000psi), pressure profile (ramping, waveform, or Barocyler mode), number of cycles and temperature (see below for unit temperature control options). The software is touch-screen friendly, but can also be controlled with an external USB mouse. USB 3.0 peripherals can be plugged into the front panel USB port. Most low power USB peripherals are fully supported when plugged into the USB port; however, peripherals consuming high charging current (e.g., some Apple products, 3.5" external hard drives, etc.) require the use of a powered USB hub and should not be plugged in or removed during instrument operation, to avoid undesirable problems with data acquisition. The use of older (pre-USB 2.0) USB peripherals is not supported. Screen cleaning can be performed by appropriate computer screen cleaning reagents when the instrument is powered down.

Two models of the Barocyler 2320EXT are available. The Barocyler 2320EXT-EH is equipped with an electric heating jacket for temperature control of the pressure chamber. Heating and cooling of the Barocyler 2320EXT-WJ requires the unit to be connected to an external circulating water bath. Chamber temperature in the Barocyler 2320EXT-EH is controlled via the software interface. Note that units equipped with an electric heater do not have a cooling function. These units can be run at temperatures from ambient to 95°C. When the electrical heating option is installed, the -EH suffix is appended to the Model Number and two circular metal plugs can be seen on the back panel. Units equipped with a water jacket have Model Number suffix -WJ and feature hose connectors at these locations for attaching a circulating water bath. Heating and cooling of units connected to a water bath is achieved via the water bath controller and cannot be set via the 2320EXT software. When connected to a circulating water bath, the 2320EXT-WJ chamber can be heated up to 95°C or cooled down to as low as 0°C. Interconversion between these two configurations is technically possible, but must be performed by specially trained personnel. Please contact your local distributor or PBI Customer Service to learn more about the features and limitations of either configuration and to arrange a conversion, if desired.



Barocyler 2320EXT Fluid Schematics. Water from the Water Bottle is pumped by the precharge pump through the check valve to fill the system and displace air. The pressure intensifier extends and retracts to pressurize and depressurize the system. A check valve inside the chamber closure is used to purge the chamber of residual air at the start of each run, and to release residual pressure at the end of each run. Leak indicator tubes are attached to the intensifier and the chamber to help troubleshoot potential critical leak points.

## 2 *Installation and Operation*

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WARNING: Improper operation of the instrument may cause serious injury to the user.



WARNING: Lifting and moving the Barocyler 2320EXT instrument requires two people!



NOTE: The Barocyler 2320EXT must be installed by a PBI-trained professional. If the unit must be moved after installation, please contact PBI for moving instructions. Any internal modifications to the unit must to be performed by PBI-certified service personnel. Such modifications include repairs and conversion between electrical and water bath-based temperature control options.

### 2.1 *Powering up the System*

- Barocyler 2320EXT utilizes external 24V DC 9.3A switching power adapter that supports power input between 100 and 240 VAC, 50 or 60 Hz. Appropriate power adapter is supplied with the instrument. The use of any other power adapters may present a risk of personal injury and will void instrument warranty!
- A PBI field engineer or your local distributor will supply an appropriate AC power cord to match your local electrical code requirements during the initial installation visit. Make sure the unit is plugged in to an appropriate power outlet.
- Press the Power button on the front panel of the Barocyler. The Button has a built-in Green LED light that turns on to indicate that the system is powered up.
- Wait until the system boots and logs in automatically into Microsoft Windows 7 Embedded.



NOTE: Default Auto Logon can be disabled if enterprise IT security policy prohibits this feature. Please call your local network administrator to configure Windows logon features accordingly. Enabling user-level security on the built-in computer will not affect functionality of the Barocyler 2320EXT software. However, please be advised that PBI personnel will NOT be able to recover the Windows user password once it has been enabled or changed – *it is the customer's responsibility to maintain a record of the system password.*

- Open 2320EXT software by double-clicking on the desktop Barocyler icon or clicking the corresponding shortcut on the Taskbar.

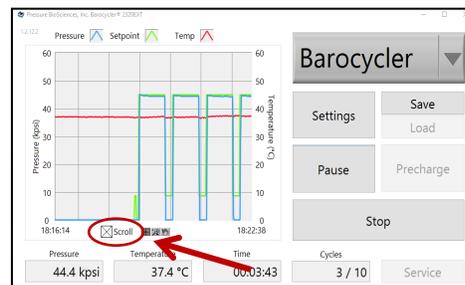


NOTE: Always shut down the built-in computer before powering down the Barocyler. Avoid powering down the system without shutting down built-in computer first to prevent operating systems issues arising from an improper shutdown. See Section 2.4 below for more information on the proper system shutdown procedure.

## 2.2 Preheat the 2320-EXT Chamber

Set unit to desired temperature and allow chamber to preheat.

- If using a circulating water bath, set desired temperature, make sure circulation is turned on, and allow unit to reach the desired temperature.
- If using a unit equipped with electric heating, press the Settings button on the software main screen to open the settings screen and set desired temperature. Return to Main screen to initiate the heating. If desired, activate the **Scroll** function (red arrow in Figure on the right) to monitor chamber temperature on the graph during instrument pre-heat or idle operation.
- While heating the chamber, insert the closure **but NOT the closure pins** so that the chamber is closed, but not sealed. Heating the chamber to high temperature while it is sealed may result in a significant pressure build-up inside the chamber due to water expansion, which can make it difficult or impossible to remove the closure pins until the chamber is cooled back to ambient temperature.



NOTE: Heating the chamber to high temperature while it is sealed may result in a significant pressure build-up inside the chamber due to water expansion and steam, which can make it difficult or impossible to remove the closure pins until the chamber is cooled back to ambient temperature.

- On units supplied with the electric heating option, temperature can be set in the range between room temperature and 95°C. Note that the 2320-EXT is not equipped with a built-in chiller. Setting temperature to below ambient will not result in chamber cooling. Once the chamber is heated to the desired temperature, it can take several hours for it to cool down. If more rapid cooling is desired, fill the chamber with cold water. Replace water as needed until desired temperature is reached.
  - Use appropriate personal protection when working with equipment or samples heated to high temperatures. Protective eyewear and insulated gloves are recommended.
- On units supplied with circulating water bath hook-ups, temperature can be set in the range between 0°C and 95°C (depending on model of water bath being used).



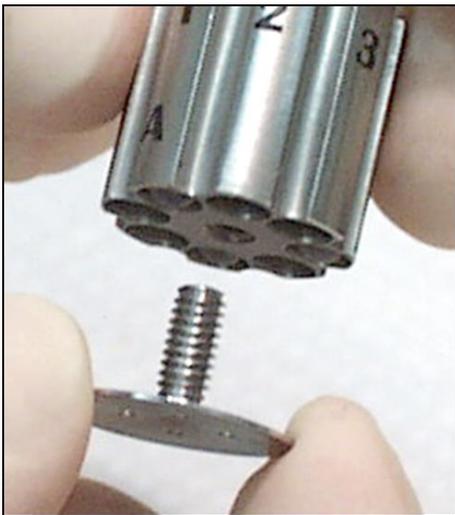
NOTE: Avoid exceeding the specified temperature range of 0°C to 95°C when using external temperature controlled option. Water freezing or boiling inside the instrument may lead to component damage and will void the instrument warranty. Moreover, overheated water in the pressure chamber may result in a rapid discharge of steam and personal injury.

## 2.3 Load the Sample Cartridges and Pressure Chamber, and Start the Run



NOTE: Only PCT MicroTubes or PCT PULSE Tubes from Pressure BioSciences should be used in the Barocyler 2320EXT. Use of unapproved sample containers may result in tubes breaking and/or leaking under pressure. In addition, fragments of broken sample containers may enter the fluid lines and cause malfunction of the check valves that would require instrument disassembly and service.

- Load samples into MicroTubes as described in MicroTube Product Insert. Close MicroTubes using either PCT MicroPestles (for tissue homogenization) or PCT MicroCaps.
  - For homogenization with PCT MicroPestle, place up to 3mg of solid tissue per MicroTube. Add 30µl lysis reagent, and cap with a MicroPestle.
  - For liquid and suspension samples, place 50, 100, or 150µl of sample per MicroTube and cap with MicroCap of appropriate length. Longer caps are used with smaller sample volumes to displace excess air from MicroTubes prior to pressure cycling. Presence of a large volume of air will result in tube denting under pressure.
- Load MicroTubes into Cartridges as shown below. Space tubes evenly in Cartridges.
  - If running 8 samples or fewer, place all samples into one cartridge, leaving the second cartridge empty.
  - If running fewer than 4 samples in a cartridge, use blanks (be sure to fill blank tubes with water) in order to distribute force evenly on the connector screw. Avoid using fewer than 4 tubes per cartridge as this may result in bending of the connector screw.
- Assemble Cartridges Head-to-Head (as shown below). Tighten the cartridge assembly so that the connector is applying even pressure to all tubes (finger tight is sufficient, do not over-tighten!).
- Make sure Cartridge End-caps are in place before dropping assembled Cartridge stack into pressure chamber.
- *If necessary*, pipet excess water out of chamber before inserting cartridges.
  - If water level in chamber is high (e.g., if chamber was previously run without Cartridges), the water may overflow when the samples are inserted. This could lead to an unsafe condition due to wetting of the nearby electrical or computer components.
- Ensure that chamber has been preheated and has reached the desired temperature.
  - Use appropriate personal protection when working with equipment or samples heated to high temperatures. Protective eyewear and insulated gloves are recommended.
- Insert assembled cartridges into the preheated 2320EXT Chamber.



Screw End-caps onto cartridges, one per cartridge.



Place sample tubes into cartridge. Space samples evenly.



Insert connector piece into first cartridge.



Connect cartridges head-to-head.

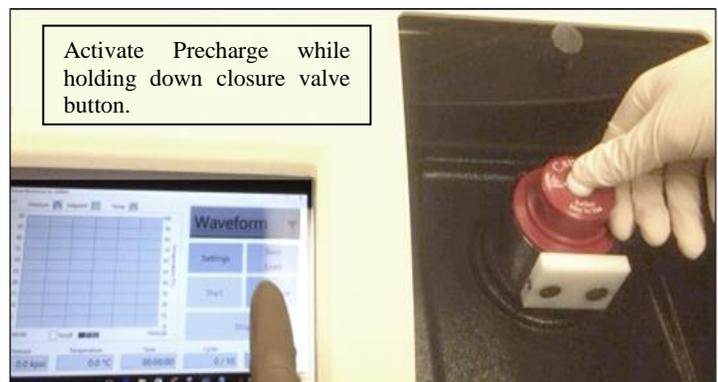
When samples are ready and Cassettes are assembled and inserted into the preheated pressure chamber, insert the closure.

- Make sure closure and chamber holes are lined up.

- It may be necessary to press down on the closure valve button in order to fully insert closure into the chamber.
- Insert Closure Locking Pins.
- Check water level in the Water Supply Bottle and refill if necessary. Tap or deionized water may be used in the Fill Bottle.
  - If water bottle is allowed to run empty, air will be drawn into the tubing. In this event, it may be necessary to prime the water line. See section 5.2 for instructions on priming the line.
- Empty Waste Water Bottle if necessary.
  - If biohazard contamination is a concern, a disinfectant solution may be added to the fill and/or waste bottle to disinfect water coming out of the pressure chamber.
- Purge excess air from the Chamber by pressing the **Precharge** software button on the main screen while pressing down on the closure valve button. The Precharge button turns on the charge pump for 4 seconds. It may be necessary to repeat the Precharge operation until all air is purged from the chamber, as indicated by the lack of air bubbles emerging from the chamber into the blue tubing.
- Slide the top cover toward you over the pressure chamber compartment until its full stop.



NOTE: The instrument will not run while the cover is open. If the “Cover not in place” pop-up window is on the screen, check the position of the cover, and then close this warning window to proceed.



- Select desired operating mode (Barocycler, Ramp or Waveform) from the pull-down menu.
- Press the **Settings** button on the software main screen to open the settings screen and set pressure cycling parameters. Note that contents of the settings screen will vary depending on the selected operating mode.

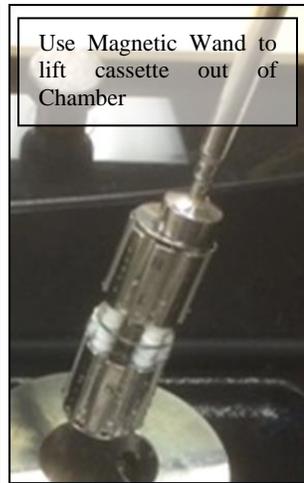


NOTE: Altering settings in one of the operating modes will neither alter currently selected settings of any other modes, nor change the current parameters if a program is already running.

- To retrieve a previously saved program, press **Load**. Once the parameters are set they can be saved using the **Save** key. A Save window will open. The default directory for saved parameter files is “My Documents” (C:\Users\USERNAME\My Documents). Previously saved parameters can be loaded using the Load button. All parameter files have the extension *.pbi*, although parameter files are instrument mode-specific. For instance, loading a parameter file saved in Barocycler mode will change the instrument mode to Barocycler and load the selected parameters into its Settings.
- To run the pressure program, press the **Run** key on the software main screen. A Save File window will open allowing the user to rename the system-generated pressure and temperature log file name (see Run Logging below).
- During the pressure run the **Run** key will become the **Pause** key and the **Stop** key will no longer be grayed out. When the Run stops, The Pause key will once again become the Run key and the Stop key will be grayed out.
  - A run in progress can be stopped at any time by pressing the Stop button on the screen. Pressing the Stop button will stop the pressure cycling, depressurize the chamber, and reset the cycle counter and timer to zero. Pressing the Stop button will NOT turn off the chamber heating.



Release residual pressure by pressing the closure vent button



Use Magnetic Wand to lift cassette out of Chamber



Use Spanner to loosen End-caps and Connector

- A run in progress can be paused at any time by pressing the **Pause** button. This will pause the run timer and keep all of the parameters (pressure, temperature) at their respective levels at the time the Pause button was pressed until the Pause button is pressed again. The run will continue where it left off. Note that if the run was paused during the high pressure step, the chamber will remain pressurized and, therefore, locked. The Pause feature should not be used to access the pressure chamber contents at any time.
- When the run is complete a window will pop up with the message “Run completed successfully!”
- To open the chamber, first release the residual pressure in the chamber by pressing the closure vent button.
- **Note that the closure locking pins will not come out easily if the valve has not been opened, due to a low level of residual pressure remaining in the chamber.**
- Remove the Locking Pins and Closure.
- Retrieve the sample cassette from the chamber using the magnetic wand.
- To disassemble cartridges, use the spanner tool to loosen the end caps and the connector.

## ***2.4 Shutdown and Emergency Stopping of the Instrument***

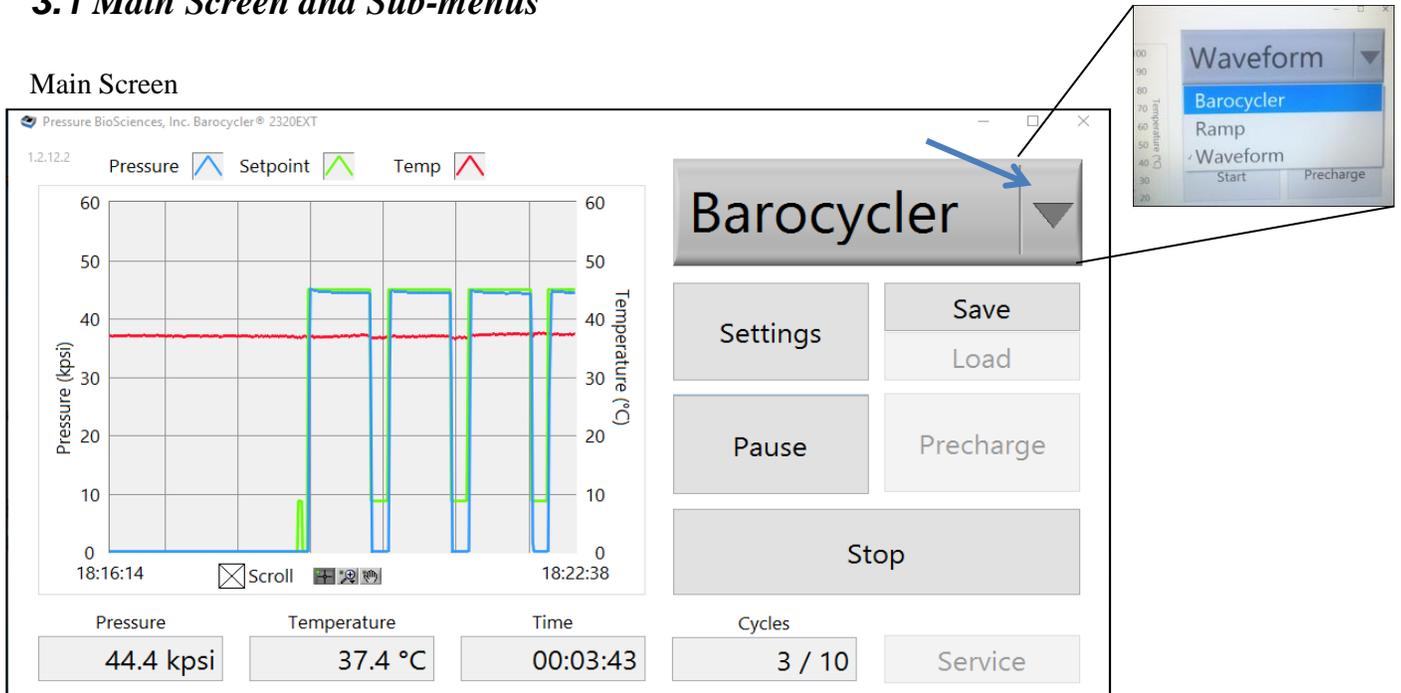
- During operation, it may be necessary to manually stop the instrument. Possible reasons for stopping the instrument include:
  - The system has to be transported to a new location.
  - Power loss is expected due to Building maintenance or inclement weather conditions.
  - The operator believes that the instrument is operating in a dangerous manner.
  - Unusual noise is heard from the instrument.
  - A large leak is observed from the instrument (e.g. fluid is observed on the laboratory bench or the floor).
  - An unrelated emergency on site (e.g., fire alarm) requiring immediate evacuation of the facility.
- To stop operation of the system and shut it down:
  - Press the STOP button on the software main screen. Wait until the system is depressurized and STOP button is greyed out.
  - Press Windows Start Button and select Power → Shutdown. Wait until Windows shuts down and the rectangular power indicator LED (visible through the small hole near the bottom left corner of the touchscreen) turns from green to orange.
  - Depress the main Power button on the front panel of the 2320EXT to power down the system. Unplug the external 24V DC Power adapter from the wall outlet.



**WARNING: THIS INSTRUMENT IS CONTROLLED BY A MICROSOFT WINDOWS COMPUTER. AVOID POWERING DOWN THE INSTRUMENT BEFORE SHUTTING DOWN WINDOWS TO AVOID POSSIBILITY OF FILE CORRUPTION!**

## 3 Barocyler 2320EXT software

### 3.1 Main Screen and Sub-menus



The Main Screen consists of the following controls and displays:

- **Operating Mode Selector (blue arrow):** Select Barocyler Mode, Waveform Mode or Ramp Mode.
- **Pressure:** Displays the current pressure in the chamber.
- **Temperature:** Displays the current temperature in the chamber.
  - Note that chamber should be preheated to desired temperature for at least 30 minutes before setting up digestion reactions. Longer temperature equilibration time may be necessary for systems equipped with an external temperature control option, i.e., circulating water bath.
- **Time:** Total time elapsed from the beginning of run.
- **Cycles:** Number of cycles completed out of total number of cycles set in the program.
- **Scroll:** Selecting the Scroll function will continuously display chart values, even if there is no run in progress.
- **Pressure/Temperature Graph:** Graph of Pressure and Temperature in chamber versus elapsed time.
  - **Red trace:** Temperature (scale on right)
  - **Green trace:** Set pressure (scale on left)
  - **Blue trace:** Actual pressure (scale on left)
- **Adjusting the X and Y-axes** (temperature or pressure scales). The scale of the X- and Y-axes can be adjusted manually by clicking on one of the values and typing in a different value. For example, in the Figure above, Clicking on the number 60 on the pressure axis (left-side Y-axis) and changing it to 40, will cause the Y-axis to change from a 0-60 scale to a 0-40 scale (after changing the number, press enter or click anywhere on the chart. This will adjust the axis to the new scale). Similarly, the time scale on the X-axis can be expanded or contracted. Selecting Autoscale will adjust the scale of the axis as the run proceeds; so that the entire run will

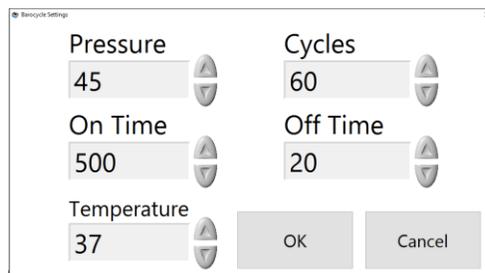
be shown on the graph. Right-click on each chart axis to access the auto-scale feature which can be toggled ON or OFF. Note: Autoscale feature must be turned off for manual scaling of that axis to work.

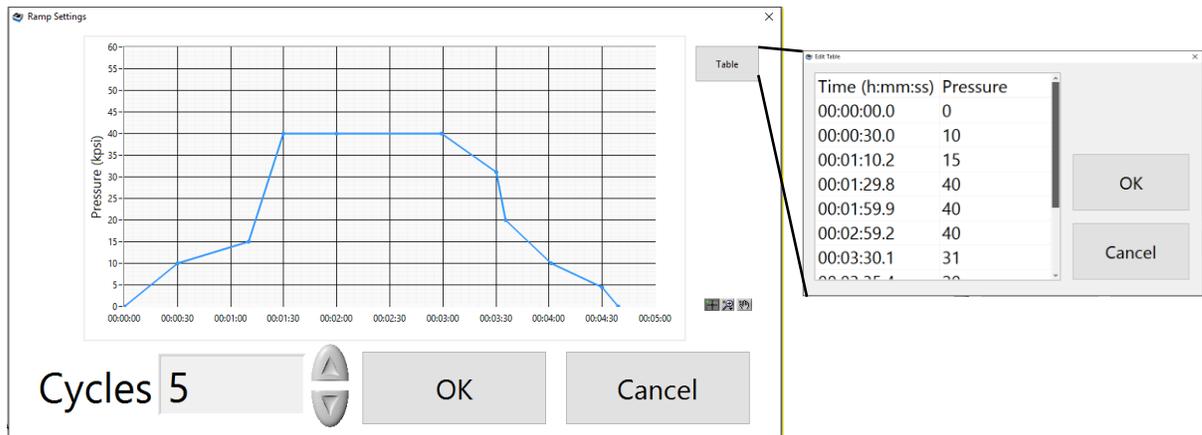
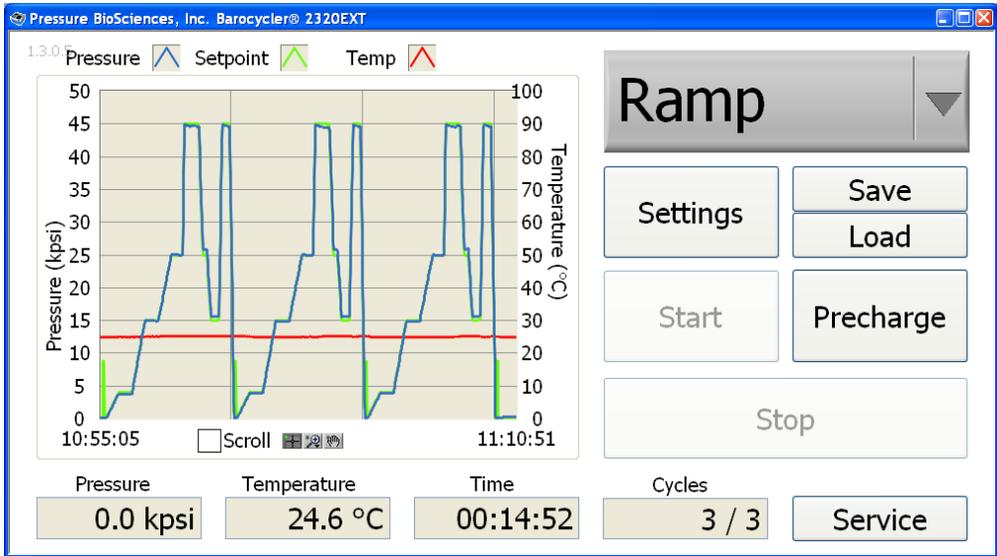
- **Start and Pause Button:** Pressing the **Start** button starts the pressure run. During the pressure run the **Start** button becomes the **Pause** button. Pressing the **Pause** button pauses the run and maintains parameters (pressure, temperature) that were current at the moment this button was pressed until the **Pause** button is toggled once again to restart the run.
- **Stop:** Stops the pressure run and returns chamber back to atmospheric pressure (0 kpsi).
- **Load** – loads previously saved protocol.
- **Save** – saves protocol settings (mode, pressure levels, number of cycles, ramp rate, etc.).
- **Precharge:** Turns on water flow from water source bottle for 4 seconds. In some cases, it may be necessary to press the **Precharge** button more than once to fully purge the air from the chamber.
- **Service:** Opens Service Screen.
  - **About** – leads to an information screen that reports the software version installed on the system.
  - **Contact** - Clicking this button will take you to the Pressure BioSciences website. Enter “Product Information” in the subject line. Please provide your name and email address, as well as a brief description of your question or problem.
  - The Service screen also has several password-protected functions, including user management features and system-wide settings, such as changing the pressure units, adjustment of thermocouple parameters, and calibration of the pressure transducer output and pressure display. Some of the features that can be accessed from the Service Screen are described in more detail in section 3.2.
  - **Manual** – For manual control of the pressure intensifier, see Advanced Features of the 2320EXT Software (section 3.2).

**The Settings screen is used to set the pressure run parameters such as the pressure level, number of cycles, temperature, etc. The Settings sub-menu is dynamically changed depending on the instrument Mode selected.**

➤ **Barocycler Mode Settings:** In Barocycler mode the pressure profile is a square wave. The high pressure level can be set between 5 kpsi and 45 kpsi. Between pressurizations, the chamber pressure will drop to “0 kpsi” (ie, ambient pressure).

- **Temperature** – set the desired temperature for the pressure run. Allow unit at least 30 minutes to reach the desired temperature. (Accessible only on units with built-in electric heating option).
- **Pressure** – high pressure level (5-45 kpsi).
- **On Time** – the time (in seconds) system maintains high pressure during each pressure cycle.
- **Off Time** – the time (in seconds) system maintains atmospheric pressure during each pressure cycle.
- **Cycles** – the number of pressure cycles per run.
- **Ramp Mode Settings:** Ramp Mode is a flexible application that gives users fine-tuned control of chamber pressure over time using a table or graph interface.



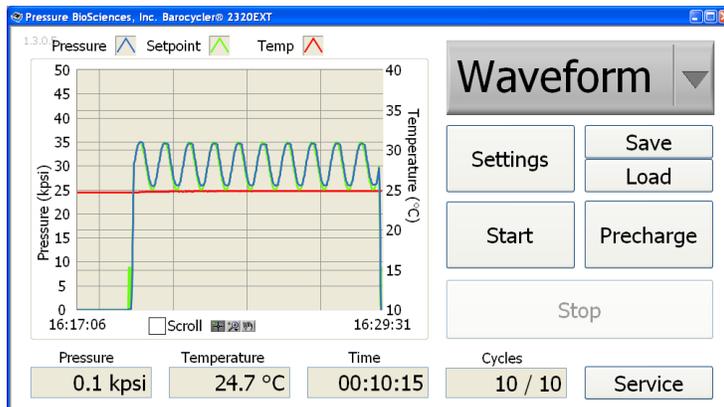


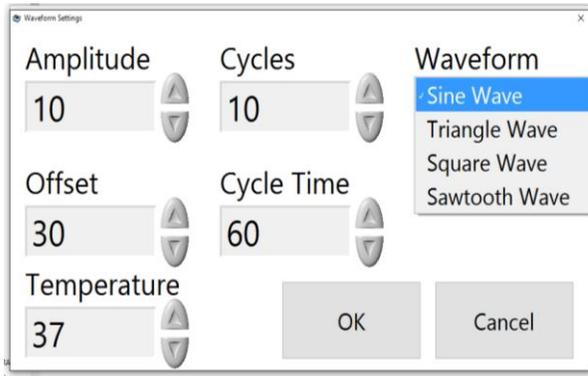
- **Graph:** Graphical editor offers an ability to draw the pressure profile on the touch screen. Inflection points can be placed on the graph by tapping the screen at appropriate places. Software will interpret the coordinates of the taps approximately. If more accuracy is desired, values for each point in the graph can be edited using the Table feature accessible via the button in the top right corner of the graph.
- **Table:** To design the ramp pressure profile, set the time and pressure conditions by typing the values into the table.
  - **Time (h:mm:ss) and pressure**  
Set the time to reach the desired pressure. In the example below, the chamber is set to ramp from 0 kpsi to 20 kpsi in 1 minute (ie, the ramp is 20,000 psi per minute). Note that the time values in the table also show tenths of a second. The tenths are optional, but can also be entered into a program using a decimal point as a separator. If no separators are used, the integer values are interpreted as seconds and parsed into a hh:mm:ss format, i.e., 312 = 00:05:12.0

- Duplicate time values are not allowed in the Ramp mode program. The time values entered are additive. The software will warn you if you have entered an invalid ramp pattern and you will be unable to save the pattern until corrected.
- Multiple ramp steps can be programmed during a single Ramp Mode run. Set the ramp conditions (as described above) to reach a first pressure, then set conditions to ramp from the first pressure to a second pressure, third pressure, etc. At each step, after the desired pressure is reached, it can be held steady for the desired amount of time (see example below).

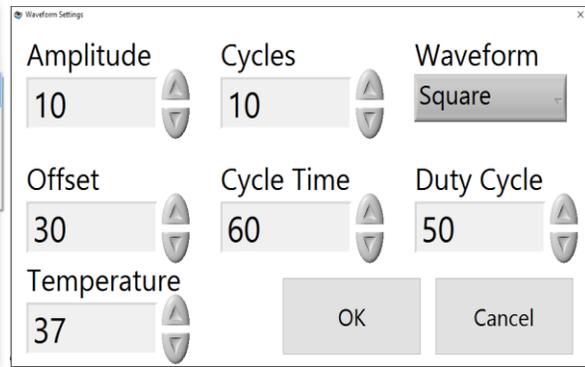
Time (h:mm:ss)	Pressure (kpsi)	Explanation of step
0:00:00	0	This is the starting condition with no pressure in the chamber at time zero
0:01:00	20	1 minute ramp from ambient pressure to 20 kpsi
0:05:00	20	4 minute hold at 20 kpsi
0:06:00	30	1 minute ramp from 20kpsi to 30 kpsi
0:26:00	30	20 minute hold at 30 kpsi
0:27:30	0	1.5 minute ramp down from 30 kpsi to ambient pressure

- **Temperature** – set point for instrument temperature control (accessible only on units with built-in electrical heating option).
- **Cycles** – the number of times the pressure ramping profile is repeated.
- **Waveform Mode Settings:** In Waveform mode the pressure profile can be set to oscillate around an offset value. The amplitude and period of the oscillation can be controlled (as long as the maximum pressure does not exceed 45kpsi). Unlike Barocycler mode, in Waveform mode the pressure in the chamber does not have to drop to ambient between cycles.





Sine Wave

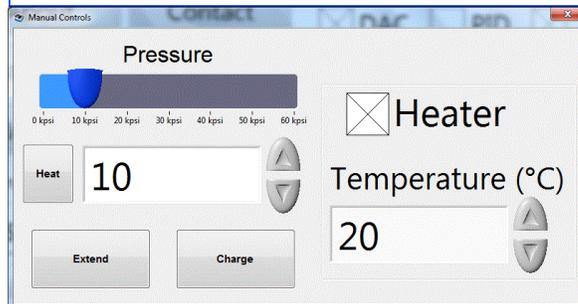
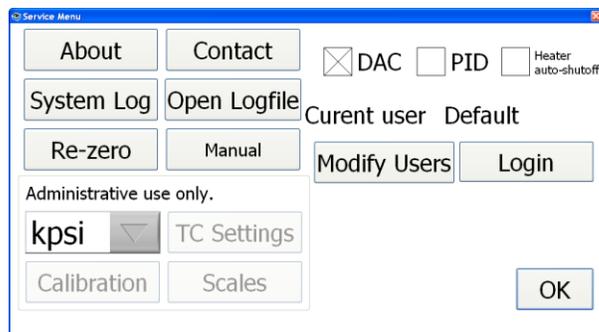


Square Wave

- **Waveform** – Select square wave, sine wave, sawtooth wave, or triangular wave.
- **Temperature** – set the desired temperature for the pressure run. Allow unit at least 30 minutes to reach the desired temperature. (Accessible only on units equipped with electrical heating option).
- **Offset** – Set the pressure level around which the waveform will oscillate.
- **Amplitude** – Set the total amplitude of the pressure waveform (example: If the amplitude is 20 kpsi, the waveform will oscillate between 10 kpsi above the offset value and 10 kpsi below the offset value).
- **Cycle Time** – Set the total time (in seconds) to complete each wave cycle (i.e., wave period).
- **Duty cycle (square wave only)** – Set the percent of each cycle that is spent at the highest pressure level (example: If the waveform period is 60 seconds and the duty cycle is 50%, the pressure will remain at the higher level for 30 seconds and at the lower level for 30 seconds).
- **Cycles** – the number of pressure cycles per run.

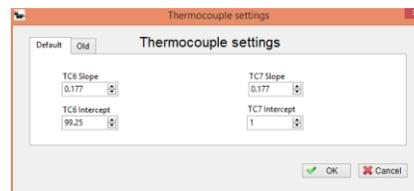
## 3.2 Advanced Features of the 2320EXT Software

The **Service** screen offers several functions, some that are accessible to all users, and others that are password-protected. Password-protected functions include system-wide changes, such as changing the pressure units, adjustment of thermocouple parameters, as well as calibration of the pressure transducer output and pressure display. Some of the features that can be accessed from the Service Screen are described in more detail below.



- **Manual** control mode offers direct, real-time control of the instrument by the operator
  - **Extend** – Toggles the direction of the intensifier piston to pressurize or depressurize water in the chamber. The button caption automatically changes to “RETRACT” when the intensifier is Extended.
  - **Heat** – Turns ON the chamber temperature control. Note: unchecking this box will shut off the heater systemwide.
  - **Charge** – Turns the precharge pump on to purge air from the chamber. The chamber closure check valve has to be depressed during priming operation to allow unrestricted flow through the chamber (see section 2.3). Note that in manual mode, the pump will *remain on* until the prime button is toggled again.
- **Login:** select your user name from the pull-down menu and enter the corresponding password. USER NAMES AND PASSWORDS ARE CASE SENSITIVE! Default user with administrative rights is *Admin*, default password is *password*. **See additional information on user management features in Section 3.3 below.**
- **Modify Users** allows to create or modify new users and passwords with two levels of privileges. Any user can change another user’s password if the appropriate old password is entered. In order to ensure user-level security, please keep passwords for users with administrative privileges confidential.
- **Run logging** is done automatically by the software in a standard comma-delimited format that can be loaded directly into Microsoft Excel® or compatible spreadsheet software. A unique file name, containing the date and time stamp of the run is generated by the system for each run and appears as the default file name at the beginning of each run. By default, log files are saved in the My Documents folder (C:\Users\USERNAME\My Documents\\*.csv). The file name and location can be modified by the user at the start of the run. After completion of the run, this file can be opened in Excel for viewing and graphing. *See also section 3.3 below pertaining to GLP/GMP and CFR21 Part 11-compliant features of the 2320EXT software.*
- **Enabling/disabling the DAC.** The Data Acquisition and Control module (DAC) must be enabled for the 2320EXT to function. Under some circumstances (e.g., if computer was restarted or USB cable was unplugged for any reason) the DAC may lose contact with the 2320EXT. If this occurs, contact can be reestablished by clicking on the **DAC** button, provided that the power and/or USB connection to the computer has been re-established.
- **Pressure PID.** The PID (proportional-integral-derivative) algorithm is used for maintaining pressure at the Setpoint value. Enabling PID allows the software to make small adjustments with the intensifier to maintain pressure at the setpoint in situations when small fluctuations in pressure can occur. The PID is not required for normal pressure cycling runs, but may be helpful when the chamber needs to be held at a constant pressure for an extended period.

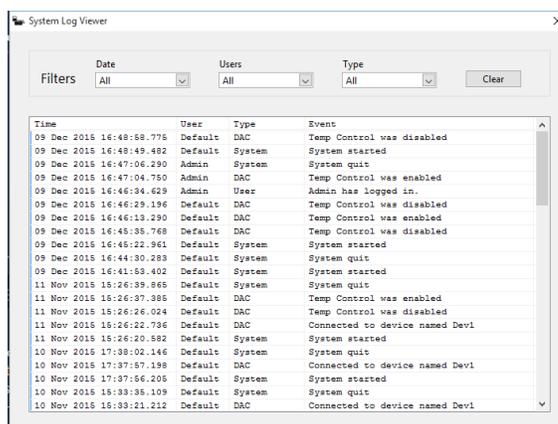
- **Thermocouple settings.** The 2320EXT is equipped with a built-in thermocouple and is configured to provide temperature logging within  $\pm 0.5^{\circ}\text{C}$ . Fine-tuning of the temperature reading may require changing the calibration of the thermocouple channels. Please contact PBI support if you believe that the chamber temperature reading is inaccurate and needs to be adjusted.



- Changing **pressure units** (kpsi, MPa, Bar). The 2320EXT software can display pressure in kpsi (default setting) as well as in MPa or Bar. The selected pressure units will be used in all functions of the software, including the main screen and the logged data files. The selected units will remain persistent in the software until the units are changed again. Units can NOT be changed once a run is in progress.



- **System Log** is a separate list of system events that, in conjunction with the run logs, is useful for troubleshooting system performance issues. The software maintains a log of every system-wide event, such as user logon and logoff, software shutdowns, power loss, temperature control configuration changes and interactions with the data acquisition hardware. Filters are provided for real-time selection of events of a particular type, occurring on a particular date(s) or related to specific system users. Any filter selection removes unrelated events from view. Pressing the Clear button restores default display of all logged events.



### 3.3 User Management, GLP/GMP and Electronic Records Compliance.

The Barocyler provides built-in OS-independent user management that offers two levels of user access to the program features. The login prompt is accessible from the *Service* window, as shown above (pg. 17). User *Admin* comes with a default password *password* that can be changed using the *Modify users* option. Upon logging in, the *Login* prompt changes to *Logout*. *Modify users* option also allows admin-level user to add new users and change their passwords. **BOTH user names and passwords are CASE SENSITIVE!** Each new user can be assigned one of the two levels of privileges. Basic level offers the ability to create, save, load and run Barocyler programs, switch between instrument modes, re-zero the pressure transducer, and start/stop the run in every instrument mode. Admin privilege level offers access to additional settings – changing pressure units, calibrating pressure and temperature signals as well as optimize PID parameters for both pressure and temperature controls. Additional instrument configuration features are available at the Superuser level, accessible only by authorized PBI representatives.

2320EXT system log contains time-stamped information about user login and any changes to the system setup or the experimental protocol. Run logs capture numerical data on pressure and temperature during operation in a standard comma-delimited file format readable in Microsoft Excel or similar spreadsheet software applications. In addition to saving unencrypted .CSV run log files, the software also archives them into password-protected .zip files with identical file names. The password is system-generated and not available for end-users. The 2320EXT software keeps track of the encrypted password internally and offers the user the option to extract the archived reference run logs when necessary. The encrypted files are duplicates of the editable .CSV files and act as read-only, tamper-evident archives of the actual run log files, to provide compliance with electronic laboratory records

guidelines. Note, while the archived files cannot be manually altered, they can still be deleted by someone with administrative access to the Windows file system. PBI recommends that users save run log archives onto backed-up network drives where they can be labeled as read-only to protect them from accidental or intentional deletion.

## 4 Maintenance

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The 2320EXT is designed to require only a minimum amount of maintenance to keep the system in optimum operating condition.

	<b>WARNING:</b> Only authorized persons should service the electrical and pressure generating components of the instrument. Improper service may lead to system damage and could cause personal injury.
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### 4.1 General Maintenance

The following maintenance should be performed as indicated.

Table 1. 2320EXT Maintenance Schedule

Task	Daily	Weekly	Monthly
Clean Surfaces	X		
Fill Water Supply Bottle	X		
Empty Waste Bottle	X		
Inspect for leaks in tubing and under unit	X		
Clean closure pins (lubricate if necessary)		X	
Clean and lubricate closure O-ring		X	
Replace closure O-Ring			X

This schedule is based on typical equipment usage of approximately 2,000 pressure cycles per month. Greater usage may require more frequent maintenance.

Table 2. Maintenance Schedule for optionally provided air compressor

Task	Weekly	Monthly	Annually
Check oil levels in all heads and refill if necessary.	X		
Clean compressor with soft cloth	X		
Drain water collected in air outlet filter		X	
Remove collected condensate from air tank		X	
Check connectors for air leaks and tighten if necessary.		X	
Check air filter and replace if necessary.		Every 3 months	
Drain and replace all oil.			X

For more detailed information regarding maintenance of the compressor refer to the manufacturer's instruction manual that came with your compressor.

## 4.2 *Confirm quality of compressed air*

Failure to properly maintain the air compressor will lead to accumulation of condensation in the lines and potential water damage to the 2320EXT. The compressor should be maintained according to the compressor's instruction manual. If using house air, or any air supply not provided by Pressure BioSciences, make sure that the air supply is clean and at a minimum inlet pressure of 110 psi. Compressed air source capable of continuous supply of air at 110-135psi is recommended for optimum performance.

## 4.3 *Replacement and lubrication of chamber closure O-rings*

It is important to keep the surfaces around the O-rings on the chamber closure clean and free of debris. Inspect O-rings before each pressure run. If O-ring shows wear, or appears damaged in any way, replace it.

The rubber O-ring should be lubricated once per week, and changed once per month. If the closure becomes difficult to insert into the chamber, replace the O-ring and clean the back-up ring.

If chamber is failing to reach pressure and water is flowing from the blue tubing on the right side of the chamber during pressurization (see Leak Indicator Tubing, section 5.2), change the closure O-ring. If this does not stop the leak, inspect and clean the closure valve (Section 4.4).

### **Replacing the rubber O-ring:**

Step 1. Pull the used rubber O-ring (orange arrow, color of O-ring may vary) up and over the metal retaining ring (green arrow).

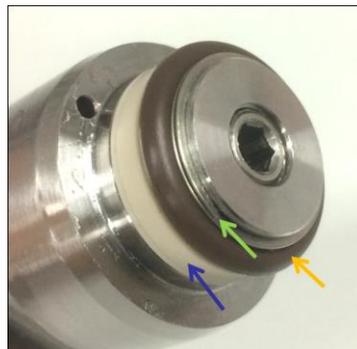
Step 2. Inspect the area under the O-ring, retaining ring and back-up ring (blue arrow) for debris or O-ring particulates. Wipe with a Kimwipe or other lintless wipe.

Step 3. Check to make sure the back-up ring is not wedged in place. A wedged back-up ring indicates that O-ring debris has gotten under it. The back-up ring should be able to slide and turn with only moderate resistance. If back-up ring appears stuck follow directions below for cleaning back-up ring. If it does not, proceed to step 4.

Step 4. Place a new rubber O-ring onto the closure by pulling it over the metal retaining ring.

- Several extra O-rings are provided in the 2320EXT accessories kit. Additional O-rings can be purchased from PBI (cat# UC-90144).

Step 5. Apply a small amount of O-ring lubricant (provided in 2320EXT accessories kit) to the new rubber O-ring. Spread the lubricant around the O-ring in a thin layer and wipe away the excess. Only a small amount of lubricant is required.



### **Cleaning the Back-up Ring:**

Step 1. Pull the rubber O-ring up and over the metal retaining ring.

Step 2. Take off the metal retaining ring. This may require a small screw driver to help lever the retaining ring out of its groove (Figure at right).

Step 3. Remove the backup ring.



Use a small flat screwdriver, or your fingernail, to lever the retaining ring out of its groove.

- Note that the backup ring is composed of **2 parts**. Their orientation as pictured is critical for normal performance of the closure seal.

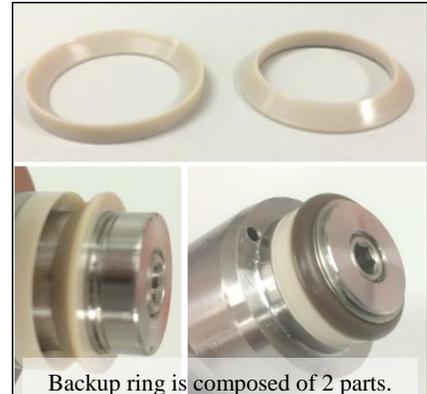
Step 4. Wipe all the rings and clean off any debris that may have been caught underneath them.

Step 5. Place the two halves of the backup ring back onto the closure as shown. Place the concave ring onto the closure with the flat side against the back. Place the convex ring onto the closure so that it fits onto the first ring. Push the two rings together so that they form a single backup ring.

Step 6. Place the cleaned or new rubber O-rings back onto the closure.

Step 7. Place the retaining ring onto the closure, into the groove.

Step 8. Apply a small amount of O-ring lubricant (provided in 2320EXT accessories kit) to the rubber O-ring. Spread the lubricant around the O-ring in a thin layer and wipe away the excess. Only a small amount of lubricant is required.



#### 4.4 *Cleaning and reassembly of chamber closure valve.*

If there is a leak through the closure valve, the unit will fail to reach pressure. If unit is failing to reach pressure, check for water flow in the blue tubing running from the chamber to the waste bottle (see section 5.2). If water is flowing from the blue tubing while the chamber is trying to reach pressure, replace closure O-ring (see instructions above). If water continues to flow from this tubing, there is an issue with the Closure Check Valve. Clean check valve (see below). If this does not solve the problem, call PBI.

##### **To clean the closure check valve and reassemble the closure:**

Step 1. Loosen the bottom set screw using the Allen wrench provided in the 2320EXT accessories kit.

Step 2. Remove the set screw, ball, washer and spring from the closure. Take care to not let the ball drop out and roll away.

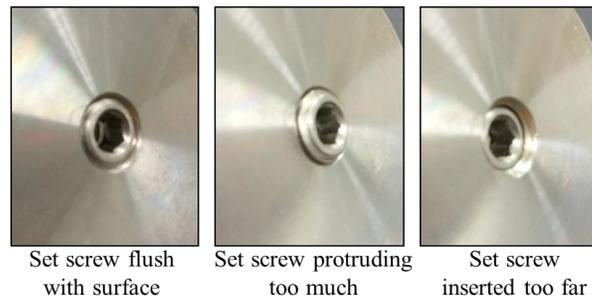
Step 3. Inspect ball for any damage or nicks.

Step 4. Clean the ball with a lintless wipe. Clean the inside of the closure by squirting it with distilled water. Avoid using wipes or swabs, as these can leave fibers inside the closure valve.

 *Take care to avoid getting any lint or dust into the closure, as this can lead to clogging of the valve.*

Step 5. Replace ball, spring and set screw. Gently drop the ball in, and insert the spring. If a washer is present, insert it between the ball and spring. Use the set screw to push the spring into the closure, then tighten the set screw using the Allen wrench.

Step 6. Tighten the set screw until it is flush with the closure surface. If chamber screw is not inserted far enough, the valve will not shut fully during pressurization, resulting in fluid flowing from the blue tubing during pressurization. If this occurs, tighten the set screw slightly. Do not overtighten.



Set screw flush with surface

Set screw protruding too much

Set screw inserted too far

## **4.5            *Contacting PBI support.***

To reach PBI Technical Support by email, send your questions to:

TechnicalSupport@pressurebiosciences.com

Or you can call us at: **(508) 230-1828 (select option 2).**

You can also visit our website at pressurebiosciences.com. Click on Contact Us at the top of the page. Please provide your name, contact information and question(s).

To Order more MicroTubes, MicroCaps, MicroPestles, or other Barocycler accessories, please contact our Sales Department by phone or email. Phone: (508) 230-1828 (option 3).

[PurchaseOrders@pressurebiosciences.com](mailto:PurchaseOrders@pressurebiosciences.com)

Website: [www.pressurebiosciences.com](http://www.pressurebiosciences.com)

# 5 Troubleshooting Guide

## 5.1 Software Error Messages and Remedies

Error	Likely Cause(s)	Solution/Repair
Screen is blank	Computer in sleep mode.	Tap on screen once.
	Unit not plugged in or not powered on.	Make sure unit is plugged into a power outlet. Press Power button on front panel.
“The 2320E reached End Of Stroke (EOS)”	Pressure leak through the closure valve.	During pressurization check for water flow in the blue tubing running from the chamber to the waste bottle. If water is flowing there is an issue with the Closure O-ring or Closure Check Valve. See sections 4.3 and 4.4.
	Pressure leak through intensifier.	During pressurization check the red tubing running into the WASTE bottle. If water is flowing through this tube there is an intensifier seal leak. Call PBI Support.
	Check Valve or other Leak	Check for visible leaks of water under the Chamber Module. Call PBI Support.
“The 2320EXT appears to be powered off.”	2320EXT is powered off	Make sure unit is plugged in. Press power button on front panel.
Error -88709	DAC disconnected	Go to Service Screen and enable DAC (an X will appear in the box).
“Data acquisition and control (DAC) was disabled. The program has been paused. Please re-enable the DAC to continue.”	Loss of communication between computer and data acquisition hardware.	Go to Service screen and enable the DAC. If Service button is grayed out, press stop button to stop the paused run. This will allow access to the Service screen. If that does not fix the problem, call PBI Support.
“The 2320EXT was not detected. Please ensure the USB cable is connected and try again.”		
Start and Precharge buttons are grayed out.	The chamber compartment door is open.	Close the door by sliding it all the way toward the front of the instrument. Note: the door error message is not displayed if the door was open at the time software was started. Opening the door after software has initialized will generate the on-screen prompt.
“Precharge pressure not detected (40psi). Please ensure the chamber is full of water and the pressure is reading 0.0 when the chamber button is depressed. If you continue to see this error, please contact PBI.”	Chamber was not precharged	Press Precharge on screen while pressing down closure vent button. Make sure Fill bottle is full.
	Pressure transducer zero drift results in negative reading at atmospheric pressure.	Fill and prime the pressure chamber. Go to Service screen. Press “re-zero” software button while holding the white purge valve button on top of the closure. Touch OK to confirm. Repeat the attempt to start the run after it has been re-zeroed.
Missing features on service menu	Insufficient user privileges	Log in as a user with administrative rights
Loading the .pbi method file changes instrument mode	Parameter file was saved under different instrument mode.	Return to the desired instrument mode and save the parameter file from that window.
Automatic calibration fails	Chamber closure leak or insufficient air pressure during calibration run.	Fix the problems and repeat the calibration.
“The cover is not in place”	Chamber compartment cover is not fully closed	Make sure cover is fully in place. Close error message to continue.
Temperature button in Settings screen is grayed out	Electric heater not enabled.	Go to Manual mode in Service Screen and make sure Heating function is on (click an X in the box). Unchecking this box will disable heating in all pressure modes. See page 17.
	Unit not equipped with electric heater	Unit is equipped with waterbath hookups. Use temperature controls on waterbath.

## 5.2 Hardware Troubleshooting

Problem	Likely cause	Solution
No power up, no green power light around power button.	Main fuse blown; external power adapter unplugged.	Ensure that external power adapter is plugged in into the power outlet and to the connector at the back of the instrument. Check the main 8A 250V fuse and replace with equivalent part.
Main power light is green, Computer power indicator stays orange, computer does not boot.	Computer was improperly shutdown. Possibly incorrect BIOS configuration.	Plug external USB keyboard into the front USB port and restart the instrument. Hit “Delete” button several times while computer attempts to initialize. Enter BIOS setup. Ensure that the computer BIOS setting “Restore AC Power Loss” is enabled. This feature can be found under “Chipset” menu in the BIOS setup screen.
Pins cannot be pulled out after run.	Residual pressure in chamber.	Press down on closure vent button to release residual pressure. Allow chamber to cool to room temperature if the vent button is stuck. Avoid pre-heating the chamber with closure pins in place
Unit is not pre-charging, although the vibration and sound indicates that the pump turns on.	Air in water inlet line.	Prime the water inlet line. See below.
Constant buzzing noise. Note: Occasional buzzing is normal.	Insufficient air pressure from air compressor or air/gas supply line.	Make sure compressor is on. Check output pressure (should be 110-135 psi). Check that switch on the air filter/moisture trap is in the Open position.
The Chamber is not heating (for units with electric heating option only).	Heater fuse blown; Control software malfunction	The chamber will not heat without a confirmation (watch dog) signal from the control system. If the control software malfunctions or freezes for any reason, the confirmation signal will be lost and the heater will be disabled. Contact PBI Customer support.
The unit fails to start when the Run button is pressed. Or A run in progress has stopped, but the “Run completed Successfully” message is not displayed. Or A run has stopped but the full number of cycles was not completed.	No air output from Compressor	Make sure the air compressor is on. Make sure there is at least 110psi in the compressor tank. Make sure the compressor’s air regulator is set to 110psi.
	Air Valve on Moisture Trap assembly is in the closed position	Make sure the valve is in the open position.
	Slow pressure leak through the closure valve.	During pressurization check for water flow in the blue tubing running from the chamber to the waste bottle. If water is flowing through the blue tubing while the chamber is pressurized, there is an issue with the Closure O-ring or Closure Check Valve. See sections 4.3 and 4.4.

### Priming the water inlet line:

During the initial installation, and in the event that the water FILL bottle has run empty and the water inlet line has drawn in air, the line will need to be primed. To prime the line, detach the pressure relief valve (see Figure below) from the clear tube inside the WASTE bottle and set it aside. To disconnect the PTC (push to connect) fitting, push down on the black plastic ring while pulling out the tubing. To reconnect, push the tubing in until it stops. Insert clear tube back into the WASTE bottle. Make sure the FILL bottle is full of water, and the pressure chamber is securely closed with its cap and locking pins. Press the Precharge button on the Main screen. Repeat the priming step until water is flowing steadily through the clear tubing into the WASTE bottle. Re-install the pressure relief valve and insert tubing back into the WASTE bottle.



**Indicator Tubing.** The 2320EXT has three color-coded leak indicator tubes running to the Waste bottle.

- The blue tube allows water to be purged from the chamber during the Precharge step. This tubing normally has fluid in it, but leakage of fluid *during pressurization or while at pressure* through the blue tube may indicate a problem with the closure seal or purge valve on the chamber closure. See Sections 4.3 and 4.4.
- The clear tubing is attached to the pressure relief valve. The purpose of this valve is to let high pressure out of the water lines in case of the high pressure check valve failure. The clear tube is also used to bypass the system plumbing for priming of the charge pump. Therefore, there will always be some water in this tube during normal operation, but a rapid water discharge through the attached valve would indicate that instrument service is required. Contact PBI support if this happens.
- The red tubing is connected to the intensifier seal. Leakage of fluid from the red tubing indicates an intensifier seal leak. Contact PBI support if fluid is leaking from the red tubing.



### 5.3 Sample Container Troubleshooting

Problem	Likely Cause	Solution
MicroTubes are dented and/or wedged into cartridges after pressure run.	Tubes were underloaded during pressure cycling.	Make sure the correct mCaps are used for the sample volume (50,100 or 150ul mCaps). (Dented tubes are not at risk of leaking, if dented tubes are acceptable, smaller volumes can be used).
Tubes are cracked or leaking after pressure run.	Inappropriate tubes used.	Use only PCT MicroTubes or PCT PULSE Tubes from PBI

For more information about use and troubleshooting of MicroTubes, MicroCaps, MicroPestles or PULSE Tubes, please refer to the respective documentation supplied with those consumables.

## **Appendix A: Installation Requirements:**

### **Space Requirements:**

The Barocyler can be placed on a bench or a laboratory cart.

Height 36.8 cm (14.5 inches)

Width 48.2 cm (19.0 inches)

Depth 54.6 cm (21.5 inches)

Weight 34 kg (75 pounds).

For ventilation and access to fill/waste bottles an additional 5 inches of space is required at the rear and sides of the Barocyler.

### **Electrical Requirements:**

North America/ Japan configuration: 100- 120 VAC (50/60 Hz)

Europe configuration: 200-240VAC (50/60Hz).

Equipment Rating: 24VDC, 9.3A

### **Laboratory Environment:**

The Barocyler is to be operated in a normal indoor laboratory environment as follows:

Temperature 5 to 40° C (59 to 86° F)

Maximum relative humidity 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity at 40 °C

Altitude up to 2000m

## **Appendix B: Pressure BioSciences Limited Warranty Statement**

Pressure BioSciences, Inc. (PBI) warrants that the Barocycler 2320EXT, under normal use and service, and operated according to the manufacturer's instructions, shall be free from defects in material and workmanship for a period of 90 days following the date that the instrument was installed at the customer's site. In addition, all electrical and electronic parts including circuit boards and indicators will be warranted against defects in material and workmanship for a period of 1 year from the same date. Specifically excluded from this warranty are consumable items such as hydraulic seals and disposable items such as O-rings. During the Warranty Period, PBI shall repair or replace, at its sole option, the system or any part therein, in which the defect has been isolated. The customer has the responsibility to advise PBI Service of any aberrant operation in a timely manner and to discontinue use of the system if advised by PBI Service personnel or if the customer can reasonably observe that continued use of the system could potentially cause additional damage to the instrument or compromise the safety of the operator. Only authorized parts and supplies should be used with the system. The use of unauthorized tubes, parts or supplies will void the Warranty.

Prior to an on-site visit by PBI Service personnel, the customer must allow PBI service personnel to perform reasonable diagnostic tasks to either "repair" the instrument over the telephone or to help isolate the issue to a particular component. The benefit to the customer is a faster time-to-repair interval, and possibly, a lower cost repair as well.

The customer must provide access for the repair of the instrument during normal working hours along with reasonable off-time hours including weekends. If the customer wants the instrument to be repaired during non-business hours, arrangements must be made prior to the visit or non-warranty service charges will be assessed.

This Warranty applies only to systems that have been installed and made operational at the customer site and with operators trained by PBI Service personnel.

This Warranty shall apply to those components that have been repaired or replaced by PBI Service personnel but only for the period of the original 12-month warranty or 20,000 cycles, whichever comes first. Unless PBI expressly consents in writing, the Warranty Period shall not be extended by reason of any defect, or any period of time during which the system is not available to the customer because of defects or repairs. Transfer of the system by the customer to any third party shall not extend the Warranty Period.

The stated Warranty is exclusive and in lieu of all other warranties. Except for such Warranty, PBI makes no warranties, expressed or implied, with respect to the system. No representation or statement not expressly contained in the agreement shall be binding upon PBI as warranty or otherwise.

PBI shall not be liable to the extent allowed by local law for loss, expense, or damage to customer or third party, for incidental or consequential damages. The obligation of PBI under this Warranty shall be to repair or replace the product, as set herein.

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