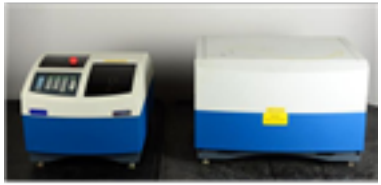
Pressure BioSciences NEP3229-002 Barocycler

Pressure Cycling Technology Sample Preparation System (PCT SPS)



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Barocycler™

Model NEP3229™
Operator's Manual
Version 3.0

PBI Pressure BioSciences Inc.

The Model NEP3229 Barocycler Pressure Cycling Technology system is covered by a limited warranty. A copy of the warranty is included with this manual. The analyst may be required to perform routine maintenance as described herein on a periodic basis to keep the warranty in effect. For routine maintenance procedures, refer to Chapter 5.

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The Model NEP3229 Barocycler Pressure Cycling Technology 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: 6,036,923; 6,111,096; 6,120,985; 6,127,534; 6,245,506; 6,258,534; 6,270,723; 6,274,726; 6,448,065; 6,569,672; 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 Model NEP3229 Barocycler Pressure Cycling Technology system.

1. **Maintain a Well-Ventilated Laboratory.**

   If volatile organic solvents are used, ensure that the laboratory is well ventilated so that a buildup of vaporized solvent cannot occur. Any waste material should be collected in a container that minimizes its escape into the atmosphere.

2. **Avoid Open Flames and Sparks.**

   If volatile organic solvents are used in the system, do not use an open flame in the laboratory and do not install any equipment that can cause sparks in the same room as the instrument.

3. **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.

4. **Treat all Samples and Reagents as if they may Contain Hazardous Substances or Transmit Disease.**

   The sample and/or reagents that are used in the system may contain compounds that may present a hazard to the operator. Take all precautions to ensure that they do not come into contact with the skin or eyes. A sink and eyewash should be located in the laboratory. In the event of an accident wash the affected part with copious quantities of water and seek medical assistance. If you are handling biological/clinical samples, treat them in accordance with the infectious disease control program of your institution.

5. **Ensure that the Instrument has been Disconnected from the Line before Removing any Components**

   Potentially hazardous currents and voltages may be present inside the unit.

6. **Wear Protective Eyewear.**

   Reagents used in the system can damage your eyes. Install an eyewash sink as close as possible to the system. If any solvents or chemicals splash on the skin or eyes, immediately rinse the affected parts in the sink. Obtain medical help as needed.

7. **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.

8. Maintain Good Laboratory Practices

All users of the system should observe good laboratory practices (GLP). Examples of GLP include:

a) Wearing of lab safety equipment, including gloves, eye protection and lab coat when preparing samples.

b) Do not pipette by mouth; do not eat or drink in areas where specimens are being handled.

c) Clean any spill immediately.

d) Disposing of all specimens, controls and materials used in testing as though they contain infectious agents.

e) Replacing the chamber fluid and cleaning the pressure chamber after working with potential infectious agents.

9. High Pressures are used in Various Parts of the System

High pressures are employed when the system is operating. Do not attempt to interfere with the system during operation. Make certain that all pressures have been safely relieved before unscrewing the cap.

Safety/Operating Symbols

The following symbols appearing on the instrument or in the manual are defined as follows:

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 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.

This symbol on the back of the instrument indicates a ground terminal.

WARNING The WARNING statement used throughout the manual presents dangers that could result in personal injury.

CAUTION The CAUTION statement used throughout the manual presents hazards on 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.

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Table of Contents

1 Introduction

1.1 Overview

The Pressure BioSciences Model NEP3229™ Barocycler™ Pressure Cycling Technology system (Figure 1-1) is used in the application of Pressure Cycling Technology (PCT) to disrupt intracellular and extracellular structures or to homogenize bacteria, plant tissue, animal tissue and other hard-to-lyse materials. The technique is based on the use of rapid cycles of hydrostatic pressure between ambient and ultra high levels. Typical applications involve the release of DNA, RNA, protein, and small molecules from samples so that they can be readily analyzed.

Figure 1-1: Pressure BioSciences Pressure Cycling Technology Barocycler Model NEP3229

The system allows for the overall extraction process to be performed automatically in a few minutes. The computer controlled extraction process can be customized to enhance the isolation of the desired material by maximizing yields and provide consistent, reproducible results.

The heart of the system is the PULSE™ tube (Figure 1-2) which transmits the pressure to the sample. As the pressure increases, the ram pushes the sample into the lysis disk. When the pressure falls, the sample is partially homogenized. This process is repeated rapidly to successfully extract the material of interest. A PULSE tube typically is charged with approximately 100 to 500 mg of a solid sample or approximately 1.5 mL of a liquid suspension.

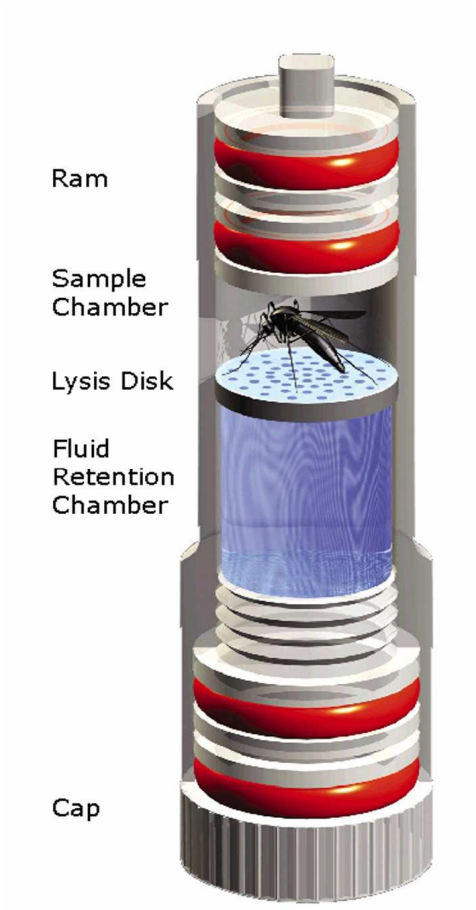

Figure 1-2: Cross Section of PULSE Tube

The basis of operation of the system is the protocol, which is an automated procedure that defines all activities for a given extraction. Up to 25 protocols can be loaded into the instrument and a protocol can be recalled via the numeric value with which it was stored.

The pressure environment is controlled in the sample chamber. The chamber can hold up to three FT-500 sample tubes. To operate the system, the tube is placed in the chamber, the chamber is capped and the protocol is initiated. The sample is pressurized to the programmed pressure, held for the pressure dwell time and the pressure is then released. The chamber is then held at the programmed dwell time at ambient pressure. The system is then sequenced for the next cycle and is repeated for the number of pressure cycles in the programmed protocol.

At the end of the protocol, the cap is unscrewed from the chamber and the sample(s) are removed for the next step in the process.

1.2 For Additional Information

A detailed list of publications and other information about Pressure Cycling Technology can be found on the internet at:

www.pressurebiosciences.com

1.3 Contents of this Manual

This manual includes the following:

- *Chapter 2*, **Installation**, describes how the instrument is to be installed.
- *Chapter 3*, **Operation**, discusses the use of the application program and PULSE tubes.
- *Chapter 4*, **Maintenance**, presents a list of activities that the user should perform on a routine basis to optimize system performance.
- *Chapter 5*, **Troubleshooting,** describes a series of activities that can be used to assist the user in the event that the system is not functioning in a proper manner.

A number of appendices are included which provide instrument specifications, a list of accessories and spare parts and decontamination procedures

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2 Installation

2.1 The Site Preparation Checklist

The Site Preparation Checklist, which is sent to the user before the system is shipped, includes the environmental requirements, space requirements and other requirements to properly install the NEP3229™ Barocycler™. This material is reproduced here for reference and can be used in the event that the system is to be moved to another facility.

2.1.1 Space Requirements of the System

The Model NEP3229 Barocycler consists of a control unit and a power unit which can be mounted on a bench or laboratory cart (Figure 1-1). If a chiller is included in the system, additional space must be provided.

a) The Control Unit

Height	45 cm (17.5 inches)
Width	48 cm (19.0 inches)
Depth	70 cm (27.5 inches) [a]
Weight	73 kg (160 lb)

b) The Power Unit

Height	46 cm (18.0 inches)
Width	72 cm (28.2 inches) [b]
Depth	55 cm (21.5 inches) [a]
Weight	95 kg (210 lb)

[a] Hydraulic hoses connect the two units. These hoses extend out from the rear panel and require an additional 10 cm (4.0 inches).

[b] An additional 20 cm (8.0 inches) should be provided so that the front panel of the power unit can be opened to allow access to the locking mechanism of the instrument.

2.1.2 Electrical Requirements

The NEP3229 requires a dedicated 120 VAC, 20 Amps, 60 Hz or 230 VAC, 10 Amps, 50 Hz electrical power line. The system is configured for the power that is employed at the end user site.

The chiller (optional) requires a second dedicated line for 120 VAC, 20 Amps, 60 Hz or 230 VAC, 10 Amps, 50 Hz. The chiller is configured for the power that is employed at the end user site.

2.1.3 Laboratory Environment

The Model NEP3229 Barocycler is to be operated in a normal laboratory environment as follows:

Temperature 15 to 35° C (59 to 95° F)
Relative Humidity (non-condensing)< 85%

The thermal output of the control unit is 25 watts during normal operation and the thermal output of the power unit is 1900 watts during normal operation.

2.2 Receiving the System

NOTE: The Model NEP3229 Barocycler is installed by service personnel from Pressure BioSciences Inc. The information in Sections 2.2 through 2.4 is provided in the event that the user must move or reinstall the system.

The Model NEP3229 Barocycler is shipped in two wooden crates mounted on a standard pallet.

NOTE: If any damage to the shipment is noted, contact the shipping company and Pressure BioSciences immediately in writing.

A shipping list is provided with the shipment. Refer to the list and ensure that all components have been delivered. If any parts are missing or damaged, contact the shipper and Pressure BioSciences in writing, indicating the nature of the problem.

It is recommended that the shipment be located as close to the area of use as possible before unpacking. In the procedure below, it is assumed that the crate will remain on the shipping pallet until it is ready to be installed. The crate can be removed from the pallet for ease of moving within your facility.

2.3 Unpacking the System and Locating it on the Bench-top

CAUTION: The two units of the instrument weigh approximately 70 kg and 90 kg (approximately 150 and 200 pounds). To manually lift these units onto a laboratory bench or table, a minimum of four people is recommended. Follow the procedure described and use proper lifting techniques. Failure to use proper lifting techniques may result in possible injury.

The wooden crates to which the units are mounted consist of a base to which the unit is mounted and a full cover which is attached to the base with wood screws. To remove the units from the crates, a reversible electrical screwdriver with a number 2 Phillips head is required.

NOTE: It is recommended that the user store the shipping crates in the event that it is necessary to return the unit to the factory. If it is necessary to return the instrument to the factory, please contact Pressure BioSciences for packaging and shipping instructions. The instrument must be properly packaged for transportation by a common carrier.

To remove the units from the wooden crates:

a) Remove and discard any strapping that is securing the crate to the shipping pallet. The crate can stay on the shipping pallet during this procedure.

b) Remove the bolts that are around the bottom of the crate which secure the top to the base.

c) Loosen the four latches securing the top of the crate to the base.

d) Lift the top of the crate. Two people are required for this step. Place the top aside and out of the way.

e) Locate the two 5/8-in aluminum lifting rods from the accessory kit and place them into the holes in the module. In the power module, the holes are parallel to the side edge, for the control unit, the holes are parallel to the front edge. Slide the rods through to the other side of the unit and position them so that approximately 10 cm (4 inches) of the rod is extended on either side.

f) Raise the unit from the crate to the laboratory bench and then remove the lifting rods.

NOTE: Four people are required for this step.

g) Repeat steps d and e for the second unit.

2.4 Setting up the Instrument

CAUTION: Before connecting the units, make certain that the power switch is turned off. The circuit breaker on the Power Unit should be opened and the Stop button on the Control Unit should be depressed, that is, in the DOWN position.

When the instrument is shipped from the factory, all pressures within the system have been relieved. Three different quick disconnect mechanisms are used to connect the hydraulics as shown in Figure 2-1 and 2-2.

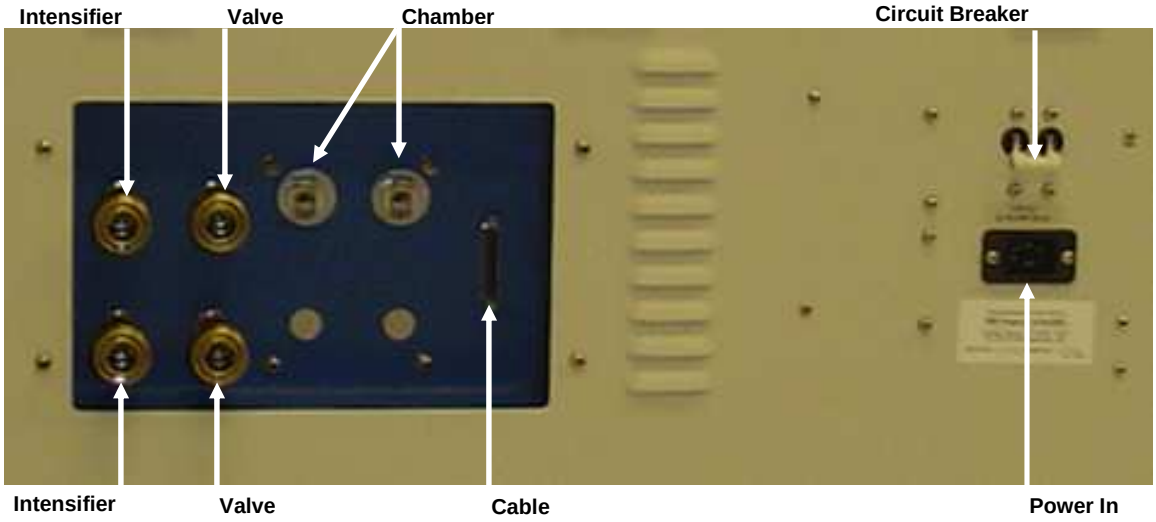

Figure 2-1: Rear Panel of Power Unit

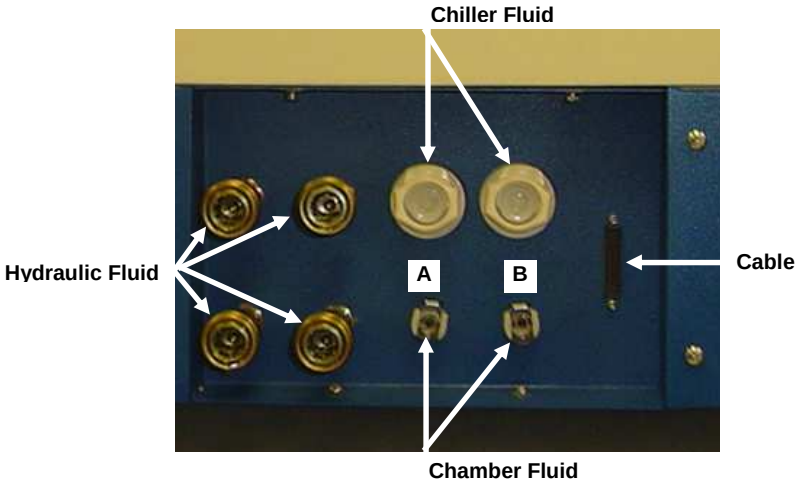

Figure 2-2 Rear Panel of Control Unit

2.4.1 Connect the Hydraulic Oil Hoses

CAUTION: Do not connect or disconnect hydraulic hoses if the instrument is running or if the system is pressurized. Doing so may cause hydraulic fluids leak from the system and potentially create a hazardous condition.

Hydraulic oil circulates through the black hoses with the brass fittings. Four identical hoses which contain the hydraulic fluid are provided. Connect each hose between the same location on each unit (e.g. connect a hose between the sockets labeled Intensifier A on the Control unit and Intensifier A on the Power unit).

The bulkhead fitting for making this connection contains an annular ring that slides parallel to the axis of the tubing.

To make the connection:

a) Slide the ring back towards the unit.

b) Insert and seat one of the connections from the hose in the fitting. When the ring is released, it will slide forward, capturing the hose connection.

c) When the hose is properly sealed, give it a gentle tug to insure that the connection is properly seated. The connection will disengage if the slide did not properly seat.

2.4.2 Connect the Chamber Fluid Hoses

Chamber fluid (Water) circulates through the white hoses with the small plastic connections. There are two hoses, one for fluid in and one for fluid out. One hose has a filter on it. This hose must be attached to the connectors labeled “chamber inlet”. The arrow on the filter should be pointing toward the top unit. The second hose is attached to the connectors labeled “chamber outlet”.

To connect the chamber fluid hoses, place the tube on the fitting and push down on the metal tab on the top of the fitting.

2.4.3 Connect the Chiller Fluid Hoses

The chiller fluid from the optional chiller circulates through the large plastic insulated tubing.

The coupling mechanism is similar to chamber fluid hoses. The hose and connector coupling to the Model NEP3229 Barocycler are included in the accessory box. The coupling to the optional chiller will be provided with the chiller or from the vendor of the chiller that is being provided by the user.

2.4.4 Install the Electrical Connection between the Two Units

The electrical connection between the two units is made with the 2-meter, 25-pin “D” connector cable (see Figures 2-1 and 2-2). The cable is polarized such that the Power Unit will accept the male connections from the cable and the Control Unit will accept the female connections. This connection method is required for electrical safety.

2.4.5 Connect the System to the Mains

Connect the instrument to a 120 VAC, 20-amp (in most countries of The European Union - a 240 VAC 10-amp) dedicated line receptacle via the power input module on the Power unit (Figure 2-3). The instrument is configured during manufacturing to conform to the power requirements of the location to which it is to be shipped.

The instrument is now properly connected and is ready for operation.

3 Operation

3.1 Overview

This section discusses how the Model NEP3229™ Barocycler™ is employed and describes the operating system. In addition; it explains the use of the PULSE™ tube that is used to contain the sample. The general format is to present a series of step-by-step procedures that explain exactly how to perform common operations.

While the instrument is designed with a number of critical safety features, each user should be properly trained in the proper operation and safety requirements including:

- The operator should be properly trained in general safety practices for laboratories.
- The operator should be trained in the safe operation of the instrument.
- The operator should be aware of the composition and proper handling of the samples and reagents and handling that are used with this instrument. MSDS data sheets should be reviewed, where available.
- Waste materials should be discarded in accordance with local environmental regulations.

WARNING Improper operation of the instrument may cause injury, including death, to the user.

3.2 Powering up the System

To Power the System Up:

a) Insure that the hydraulic hoses, electrical cables and earth ground are securely connected.

b) Insure that the chamber cap is in place and secured.

c) Check that the red STOP button on the front panel is pulled out.

d) Verify that the circuit breaker switch which is located on the right side of the rear panel of the power unit is in the UP (I) position (Figure 2-1).

e) Press the power ON/OFF switch located on the right side panel of the power unit to the “I” position.

f) When the instrument is powered ON it will go through an initialization routine. Upon completion of the initialization routine, the instrument will be in the Automatic mode.

3.3 Modes of Operation

There are three modes of operation of the Model NEP3229 Barocycler, which are accessed via the MODE button.

- **Manual –** instrument functions can be performed on an ad hoc basis (i.e. there is no need to generate a protocol). Manual Mode is described in Section 3.6.
- **Automatic –** the instrument operates via a protocol that is generated and stored by the user. Automatic mode is described in Section 3.5.
- **Calibrate** – accesses features that are used by the service engineer for calibration and servicing the instrument.

NOTE: The user should not edit any parameters in the calibrate mode.

3.4 The Front Panel of the Instrument

The front panel of the NEP3229 (Figure 3-1) consists of the following displays and controls.

Figure 3-1: Front Panel of the Barocycler

A 2-line by 20-character display provides visual prompts and feedback to the operator.

A keypad provides for alphanumeric inputs and control of the instrument.

Three Light Emitting Diode (LED) meters which display the Hydraulic Pressure (in psi), Chamber Pressure (in x10 psi) and the external Chamber Temperature Meter (in ^{o}C) are present on the panel directly above the keypad/display module.

The following keys are present on the keypad:

- **Dump –** Relieves the pressure (manual mode only).
- **Hi Reg –** Turns the high pressure regulator on, setting the hydraulic pressure to approximately 2250 psi (manual mode only).
- **Low Reg –** Turns the low pressure regulator on, setting the hydraulic pressure to approximately 650 psi (manual mode only).
- **Intensifier –** Activates the Intensifier hydraulics (manual mode only).
- **Motor –** Turns the hydraulic motor on (manual mode only).
- **Mode** – Used to select the desired mode of operation. The present mode will be indicated on the lower right side of the LCD display.
- **Select** – Used to indicate the location where a protocol is to be stored.
- **Save –** Used to store a protocol.
- **Edit** – Initiates the generation of a protocol.
- **Run –** Initiates operation of the system.
- **Yes/No –** Used to provide information by the operator. When Intensifier is active, Yes turns On/Off increase chamber pressure and On/Off for decrease chamber pressure.
- **Enter** – Accepts the information that was keyed in.
- **Chamber** – Activates chamber fluid pump (manual mode).

NOTE: Some keys may have a different function based on the mode that the instrument is in.

3.5 Automatic Mode of Operation

3.5.1 Creating a Protocol

A protocol is a program that describes the desired operation for the Model NEP3229 Barocycler. It can be stored and retrieved as desired.

To create a protocol:

a) Press the **MODE** button to present A (for Automatic mode)

b) Choose **SELECT** on the keypad

c) Enter the location number where you want to store the protocol and press **ENTER** (25 locations are provided).

NOTE: When the Model NEP3229 Barocycler is shipped, a common default program is placed into all the memory locations. These parameters will be stored in these locations until changed. There is no recommendation regarding the utility of this particular protocol.

d) Press **EDIT t**o initiate the generation of the protocol in the selected location.

e) The name of the protocol selected is presented in the upper left of the display. To accept the indicated name, press ENTER. If you want to change the name:

i) Select the key containing the first letter of the name. In this example, we will name the protocol SEEDS, so the key to be pressed is the **7STU** key.

ii) Press the **7STU** key to place the character 7 on the display.

iii) Press the **7STU** key again to replace the 7 with the character S.

NOTE: If you pass the "S" and a "T" appears, keep depressing the key until the "S" reappears.

iv) Press the **YES** key and then press the **2DEF** key as it contains the second letter of the name (as an alternative, you can simply press the 2 DEF key).

v) Press the **2DEF** key three times to place the character E on the display (SE); then press **YES** to access the next character.

NOTE: If you want to edit a character which has already been accepted by the YES key, press the < key.

vi) Continue with this process until the complete name of the protocol has been spelled out; then press **ENTER** to accept the name.

vii) The display will present PRESSURE (5..35) and the operator can enter the desired pressure value via the numerical keypad. The range is from 5 to 40 psi. When the desired value is entered, press **ENTER**.

viii) The display will present TIME1 (1..99). TIME1 refers to the dwell time (in seconds) at the desired pressure. The range is from 3 to 99 seconds. When the desired value is entered, press **ENTER**.

ix) The display will present TIME2 (1..99). TIME2 refers to the dwell time (in seconds) at the ambient pressure. The range is from 3 to 99 seconds. When the desired value is entered, press **ENTER**.

x) The display will present CYCLES (1..99). CYCLES refers to the number of pressure iterations to be employed. The range is from 1-99 cycles. The range is from 3 to 99 seconds. When the desired value is entered, press **ENTER**.

xi) The display will now present STORE PROTOCOL ? YES/NO.

If you press the YES key, the protocol will be saved.

If you press the NO key, the protocol will remain in the working register and can be run, as described below.

If another protocol is selected or the system is turned off, the protocol will be erased and the protocol that was previously in the register will be saved.

3.5.2 Preparing the PULSE Tube

Prepare your samples before loading the PULSE™ Tubes. If you are working with solid tissue samples, use about 100 to 500 mg of tissue. If you are working with a liquid suspension such as *E. coli*, yeast or *C. elegans*, insert a ram and then load up to 1.5 mL of the liquid suspension into the Fluid Retention Chamber.

NOTE: If your work requires larger samples, it may be necessary to split the sample into two PULSE Tubes or run some tests to establish how small a sample will provide your desired results.

The PULSE Tube is show in Figure 3-2 and includes the following components:

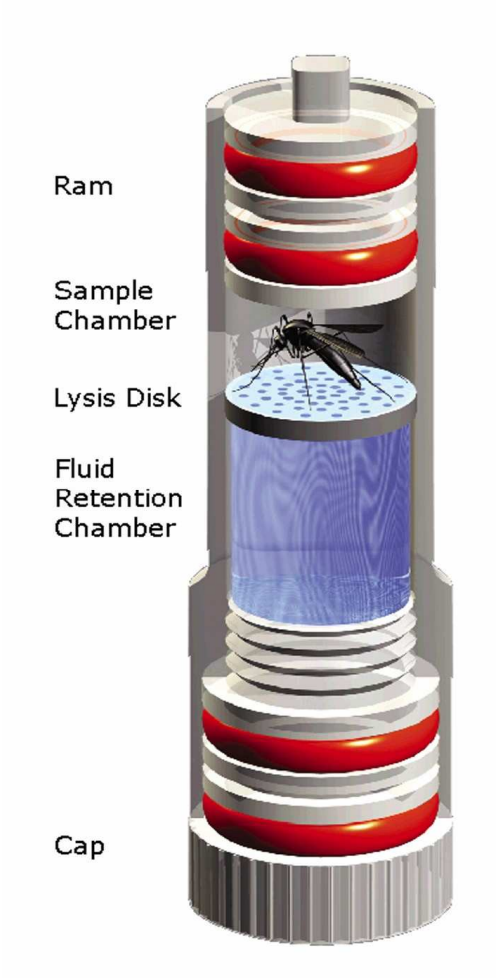

Figure 3-2: PULSE Tube

- **Ram** – It moves up and down during the pressure cycling process.
- **Sample Chamber** – This is the space between the Lysis Disk and the Ram. Samples must fit into this space and not prevent the Ram from being set.
- **Lysis Disk** – A disk with holes which separates the Sample and Fluid Retention Chambers.
- **Fluid Retention Chamber –** Holds buffers, reagents or liquid suspension samples. After processing, extracted samples are withdrawn from this chamber.
- **Cap –** Closes Fluid Retention Chamber and provides access to extraction products after cycling.

To load the FT500 PULSE tube:

a) Place the sample in the sample chamber (the “Ram” side of tube) as shown in Figure 3-3. The sample chamber has a volume of 1.3 - 1.5 mL and should be filled. As an example if you use a 500 mg sample of tissue, add 0.0 - 1.0 mL of buffer.

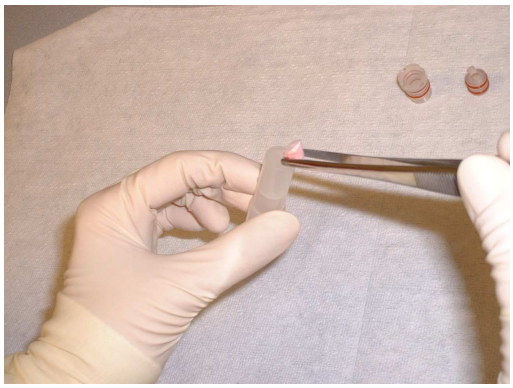

Figure 3-3: Place the Sample in the Sample Chamber

b) With the tool, push the ram down until the tool is flush with the top edge of the tube. Turn tool 90^{o} to push the ram into tube as far as possible as shown in Figure 3-4. The “ram” is now set.

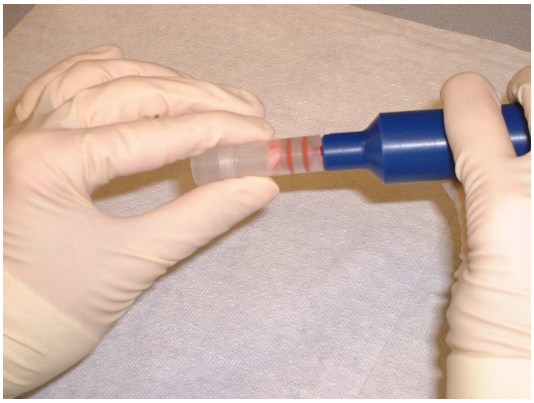

Figure 3-4: The Ram is Set in Position

c) Use a pipette or dispenser to place 1.2 mL of buffer in the Fluid Retention Chamber (“cap” side of the tube) (Figure 3-5).

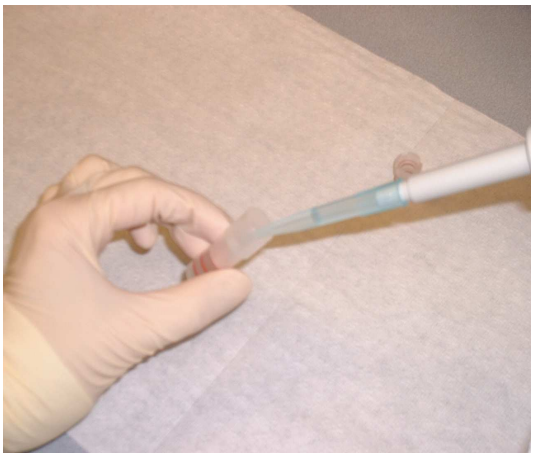

Figure 3-5: Place the Buffer in the Chamber

d) Use the tool to screw the cap onto the tube and stop when the cap is flush with the tube. Do not over-tighten the cap.

e) Place three tubes in the tube holder “ram” end down (Figure 3-6). If you are running less than three samples, use tube spacers in place of PULSE Tubes.

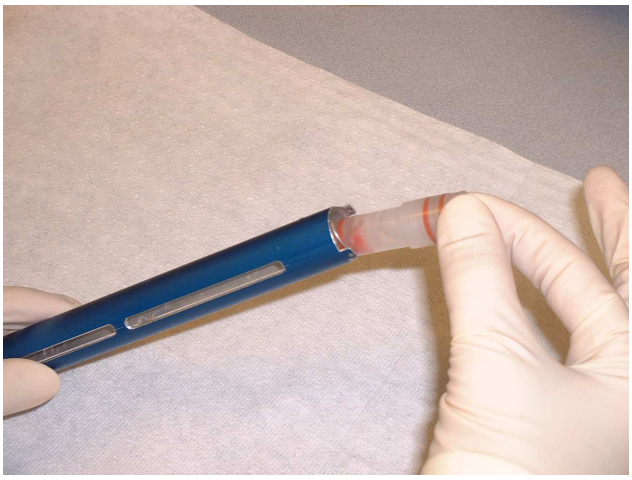

Figure 3-6: Placing Tubes in the Tube Holder

3.5.3 Running a Protocol

To run a Protocol:

a) Verify that the chamber fluid is being circulated. The fluid bottles are located behind the front panel of the control unit, which can be removed with the hex key that is provided.

b) Make certain that there is sufficient fluid in the chamber fluid reservoir bottle.

CAUTION: If the Chamber Fluid Reservoir Bottle is allowed to completely empty, the proper operating Chamber Pressure will not be achievable and the test in process will be automatically terminated. The results of the in process test will be compromised or lost.

c) Press **SELECT** on the keypad.

d) Choose a protocol number and press **ENTER**.

e) Remove the cap from the pressure chamber.

f) Place the tube holder in the pressure chamber (Figure 3-7).

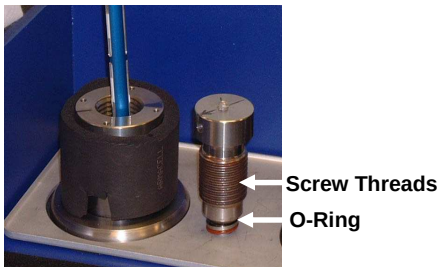

Figure 3-7: Placing the Tube Holder in the Pressure Chamber

g) Make sure the cap o-ring and retaining ring are seated properly on the cap.

h) Place the cap over the pressure chamber and tighten with the Ram Cap Installation and Removal Tool. The cap should be made as tight as possible and then eased off about 5^{o}.

i) Close the Chamber Door.

j) Press the **RUN** key. The instrument will go into the *Auto Fill* mode after which the protocol will be performed.

NOTE: If the chamber door is opened during the run, the instrument will go into a controlled shut down and the run will be terminated.

When the protocol is completed, the chamber door can be opened. The chamber cap can then be removed and the sample can be taken from the chamber.

3.5.4 Stopping the Instrument during Operation

During operation, it may be necessary or desirable to terminate operation of the instrument. Possible reasons for stopping the instrument include:

a) The operator believes that the instrument is operating in a dangerous manner.

b) A sharp, loud noise is heard from the instrument

c) A screeching or grinding noise is heard from the instrument

d) The operator performed some operation that he believes may be a hazard such as neglecting to put the caps on the chambers prior to starting a run.

e) A leak is observed from the instrument (e.g. fluid is observed on the floor).

f) An emergency in the site requiring immediate evacuation of the facility.

To stop operation of the system:

a) Depress the STOP button (the Red button adjacent to the keypad) and the instrument will be turned OFF.

WARNING: If there is pressure in the chamber or the system, this method of stopping the instrument will not release the pressure. Do not attempt to work on the hydraulics of the instruments after this type of shut down.

To relieve the pressure in the system prior to opening the cap:

a) Pull out the STOP button when it appears safe to return power to the system .The instrument be in the Manual Mode of operation.

b) Press the DUMP key. The instrument will automatically relieve the pressure in the chamber.

c) Put the instrument back in Automatic mode.

The chamber pressure should be close to zero. The cap can now be easily unscrewed from the chamber.

3.6 Manual Mode of Operation

When the instrument is in the Manual Mode, the operation of the keys on the keyboard is similar to that of an on-off two position push button switch. When a switch is pressed, the indicated action is performed. Pressing the switch again turns the indicated action off. As an example, if you press the Intensifier button, the Intensifier is powered up and the Intensifier LED will be illuminated; when the button is pressed again, the Intensifier is powered down and the LED will be turned off.

NOTE: In Manual mode, the pressure rise time is less than three seconds and it may be difficult to control the pressure accurately.

If you press the YES key for an instant when the intensifier is activated will cause the chamber pressure to increase; pressing the key again will halt the increase.

Prepare the sample and load the PULSE tube as described in Section 3.5.2.

3.6.1 Setting Manual Mode Conditions

To operate in Manual Mode:

a) Depress the MODE key until “M1” appears in the LCD Display.

b) Press the MOTOR key, located above the MODE key on the keypad. This will turn the hydraulic motor on and the MOTOR LED will illuminate.

c) Depress the LOW REG key. The hydraulic pressure is set to approximately to 650 psi and the LED next to the key is illuminated. If you depress the key again, the low-pressure regulator will be turned off.

d) Press the HI REG key to turn the high-pressure regulator ON. The pressure will be set to approximately 2050 psi. Pressing the key again will turn OFF the regulator.

NOTE: If both regulators are activated at the same time, the system pressure will remain below 100 psi

e) Press the INTENSIFIER key to activate the intensifier hydraulics. To increase the chamber pressure one of the regulators must be activated and the UP key pressed. To stop the increase of pressure, press the UP key again.

f) Press the DUMP key to release the chamber pressure. The system will automatically sequence the pressure to LOW REG and activate the dump valve and illuminate the dump LED. To close the dump valve, press the DUMP key. The Dump LED will turn off.

g) To activate the Chamber Fluid Pump, press the CHAMBER key.

h) To charge the intensifier with chamber fluid, press the CHAMBER key to activate the chamber fluid motor, activate LOW REG and the INTENSIFIER and the press the DOWN key. When the chamber is withdrawn, press the CHAMBER, DOWN, and LOW REG keys

3.6.2 To Exit Manual Mode

To exit manual Mode:

a) Press the Motor key to stop the hydraulic pump.

b) Depress the MODE key once. The instrument will be in Automatic Mode.

3.6.3 To Stop the Instrument in the Middle of a Manual Run

To stop operation of the Model NEP3229 Barocycler during a manual operation:

a) Press the red **STOP** button located on the instrument panel. This will cut power to the controller and the hydraulics.

b) When power is removed from the instruments in this manner, the system stops and mechanically maintains the state existing at the time of shutdown. There is no automated attempt to release any pressures within the system.

WARNING: If there is pressure in the chamber or the system, this method of stopping the instrument will not release the pressure. Do not attempt to work on the hydraulics of the instruments after this type of shut down.

c) When it is safe to return power to the instrument, pull out the red **STOP** button. The instrument will come on in the Manual Mode.

d) Press the RUN key and the instrument will automatically relieve the pressure in the chamber.

e) When the instrument completes the cycle it will then go into the Automatic Mode.

f) The chamber pressure should read approximately zero. The cap can now be easily unscrewed from the chamber.

3.6.4 Powering Off

To turn off the instrument power, press the power off button on the right side of the power unit.

4 Maintenance

4.1 Maintenance Schedules

The Model NEP3229™ Barocycler™ is designed to require a minimum amount of maintenance to keep the system in optimum operating condition.

The activities that the operator should perform are indicated in Table 4-1 and Table 4-2. The suggested frequency is based on normal operation of the system with typical samples and buffers and assumes that approximately 2,000 pressure cycles are performed per month. It is likely that the frequency for each activity may vary depending on the nature and number of cycles. The user should monitor these activities and adjust the frequency to optimize system performance.

In addition, a variety of service related activities should be performed by an authorized Pressure BioSciences service engineer on a periodic basis. Please contact Pressure BioSciences for additional information.

Table 4-1: Maintenance Schedule - Model NEP 3229 Barocycler

Task	Daily	Weekly	Monthly	6 Monthly
Clean Surfaces	x			
Check Chamber Fluid Reservoir		x		
Check Intensifier Drain Reservoir			x	
Check for Leaks		x		
Chamber Cap O-Ring			x	
Chamber Cap Back-up Ring				x

Table 4-2: Chiller Maintenance

Task	Daily	Weekly	Monthly	Quarterly
Clean Surfaces	x			
Check Chiller Reservoir	x			
Replace Chiller Reservoir Fluid	x			
Check Chiller Hoses				x

4.2 Daily Maintenance

On a daily basis:

a) Dispose of fluids as required.

b) Clean surfaces with a water moistened soft cloth or paper towel.

c) Perform any site-specific cleaning or disinfectant routines.

d) Check for any indication of leaks.

4.3 Weekly Maintenance

On a weekly basis:

a) Wipe down the external surfaces with a damp cloth.

b) Open the front panel of the Power Unit by opening the latches on both sides using the 5/16" hex key and check the chamber fluid reservoir. Dispose of the fluid as appropriate.

c) Check for leaks of chamber fluid or hydraulic oil. Call for service if there are signs of leakage.

WARNING: Only authorized persons should service the hydraulics. Failure to understand the methods of assembling the high-pressure components in a proper manner may lead to failure of the system at high pressure which could cause serious injury.

4.4 Monthly Maintenance

On a monthly basis, change the o-ring on the cap by removing the top spring, removing the old ring and replacing the top spring. While you replace the o-ring, inspect the backup ring and apply a thin coating of anti-seize compound to the cap screw threads.

5 Troubleshooting

Troubleshooting involves the determination of the cause of a fault in the system.

Although the system consists of several components, troubleshooting can be simplified by a consideration of the following guidelines:

- In almost all cases, there is one proximate cause for the problem.
- A fundamental knowledge of the role of each component of the system is extremely useful in diagnosing the problem.
- The availability of critical spare parts to substitute into the system is extremely useful. A listing of spare parts and supplies is provided as Appendix 2.
- If any aspect of the analytical conditions are to be changed, run a "before and after" to ensure that the effect of the change is understood. Do not consider any change as "trivial". As an example, if you change the supplier of the buffer salt, verify that the change has no effect on the separation.
- Run standards as part of the troubleshooting process.

The following error messages may be displayed in the touchpad screen:

DOOR OPEN

This error message will occur if the chamber door is opened at anytime during operation of the instrument. Press any key to erase the error message.

TIMED OUT

This error message will occur if the programmed pressure is not reached within 10 seconds. See Problem #2 in the troubleshooting table below. Press any key to erase the error message.

BM: (Followed by a series of numbers)

The BM error message followed by numbers indicates the intensifier piston reached its bottoming out position before the programmed pressure was attained. See Problem Number 2 and 3 in the troubleshooting table below.

Please note the numbers in the error message should a service call be required. Press any key to erase the error message.

Problem	Possible Cause	Possible Remedy
1. No power	Mains Circuit Breaker off (down position)	Turn on Mains Circuit Breaker (up position)
	Emergency Red Stop Button depressed	Pull up Emergency Red Stop Button
	Interconnect Cable disconnected	Connect Cable
2. Chamber not pressurizing or holding pressure.	Air in the system.	Program a protocol for 10 kpsi, 5 second hold times and 20 cycles. Run program. If the unit is still not reaching pressure, see below.
	Chamber cap o-ring worn	Replace orange silicone o-ring on chamber cap.
	Water not entering chamber.	See Problem 3 below.
	Intensifier seal failure.	Check for fluid in the intensifier overflow bottle. Presence of fluid indicates intensifier seal failure. Call Service.
	Dump Valve failure	Check for fluid emptying through chamber output hose while unit is trying to build pressure. If yes, possible dump valve failure. Call Service.
	Check Valve Failure	Call Service.
3. Water not entering chamber	Chamber Fluid reservoir empty	Fill reservoir with distilled water.
	Intake hose inside fluid reservoir disconnected	Reconnect intake hose.
	Chamber input/output hoses between units disconnected or improperly connected.	Connect hoses
	Stuck Check Valve	Call Service
4. Water leaking from vent port on chamber.	Chamber is overfilled with fluid when chamber cap is screwed in.	Ensure that water is not above the level of the tube holder before screwing chamber cap in place.
5. Chamber does not drain after operation.	Chamber input/output hoses between units disconnected or improperly connected.	Connect hoses.
	Build up of foreign material in chamber output hoses.	Check for build up of material in the chamber output hoses near the connectors on both the top and bottom units.
	Broken Dump Valve Needle	Check decompression rate of chamber. Normally this is virtually instantaneous. If the chamber decompresses slowly this is an indicator of a broken dump valve needle. Call Service.

Appendix A Specifications

PRESSURE	Maximum allowed Pressure:	35K PSI
	Working Pressure Range	5K to 40K PSI
	Recommended Pressure Range	10K to 40K PSI
	Stability at Set Point	+1K/-2K PSI (1-Minute)
RAMP TIME	Up	~10K PSI/Second
	Down	<0.3-second

INSTRUMENT PROTOCOL SETTINGS

Settings	Maximum Pressure
	Pressure Hold Time Duration
	Ambient Pressure Hold Time Duration
	Cycles
	Protocol Number to Save

TEMPERATURE CONTROL - Manual (External re-circulation Bath)

Temperature Ranges	Minimum Temperature:	3.0°C
	Maximum Temperature:	40.0°C
	Stability:	+/- 2.0°C

CHAMBERS

Number	1-Chamber
PULSE Tubes per Chamber	3-FT-500's

INSTRUMENT CHARACTERISTICS

Controller Unit

Dimensions	Width	48 cm (19.0")
	Height	44 cm (17.5")
	Depth	70 cm (27.5")
	Weight	71 kg (157 lb)

Power Unit

Dimensions	Width	72 cm (28.2")
	Height	46 cm (18.0")
	Depth	55 cm (21.5")
	Weight	93 kg (207 lb)
Electrical(a)	Voltage	120+/-10 (240+/-10) VAC
	Current	16 (8) Amp
	Frequency	50/60 Hertz

(a) The system is configured for the power supplied at the customer's facility.

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Appendix B Barocycler Accessories

Table B-1 lists accessories and consumables that are provided with the initial installation of the Model NEP3229™ Barocycler™. Table B-2 contains a list of consumable items and indicates the suggested quantity that should be on hand for normal operation.

Table B-1: Accessories and Consumables Supplied with the Model NEP3229 Barocycler

Part Number	Description	Quantity Supplied
1.	Four Prong Auto Pick Up Tool (to remove debris in chamber)	110705-001
2.	Chiller (optional)	
3.	Chiller Hose Connection	
4.	Ram/Cap Installation And Removal Tool	110733-001
5.	Carrier, FT 500 Tube Chamber	110545-001
6.	Tool, Cap Removal	110735-001
7.	Tweezers, Long	110740-001
8.	O-Rings, Chamber Cap	110584-003
9.	Tool, Chamber Carrier Removal	110707-001
10.	Pulse Tube, Blanks	110644-001
11.	Ring, Back Up Chamber	110741-001
12.	Anti-Seize Grease	110710-001
13.	Door Hex Key 5/16”	111153-001

Table B-2: Consumables for Operation of the Model NEP3229 Barocycler

Part Number	Description	Suggested Quantity
8.	O-Rings, Chamber Cap	110584-003
11.	Ring, Back Up Chamber	110741-001
12.	Anti-Seize Grease	110710-001

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Appendix C Decontaminating the Model NEP3229 Barocycler

The following procedure should be employed if it becomes necessary to decontaminate the system:

a) Make sure the chamber is empty.

b) Make sure that the waste reservoir is empty.

c) Enter Manual Mode and activate the Hydraulic Pump.

d) Activate the Low Pressure Regulator and close the Dump valve.

e) Fill the chamber with disinfectant and use as recommended by manufacturer.

f) Open the dump valve. Allow the chambers to completely drain.

g) Repeat steps d through f two more times.

h) Repeat steps d to f with distilled water.

i) Fill with ethylene glycol.

j) Exit Manual Mode. Dispose of waste in a manner that is consistent with local environmental regulations.

WARNING: NEVER USE BLEACH TO DECONTAMINATE THE PRESSURE CHAMBER

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Appendix D Model NEP3229 Barocycler Warranty

Pressure BioSciences Limited Warranty Statement

Pressure BioSciences, Inc. (PBI) warrants that Model NEP3229 Barocycler™, 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 displays 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 by 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 quite 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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