Agilent

Agilent Seahorse XF Pro Analyzer

Operating Manual

Notices

Copyright

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CAUTION

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WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

About This Manual

This manual contains information for operating and maintaining the Agilent Seahorse XF Pro Analyzer.

1 **“Introduction"**

Chapter 1 provides an introduction to the Agilent Seahorse XF Pro Analyzer.

2 **“Installation"**

Chapter 2 provides unpacking and installation instructions for the Agilent Seahorse XF Pro Analyzer.

3 **“Basic Operation"**

Chapter 3 provides basic operating procedures for the Agilent Seahorse XF Pro Analyzer.

4 **“Maintenance"**

Chapter 4 provides routine maintenance, troubleshooting, contact, and additional resource information for the Agilent Seahorse XF Pro Analyzer.

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Contents

1 Introduction

This chapter provides an introduction to the Agilent Seahorse XF Pro Analyzer.

General Information

This manual covers all models of Agilent Seahorse XF Pro Analyzer:

Instrument type	Part Number(s)
Seahorse XF Pro	S7850A (manufacturing part reference #S7850-64000)

Safety Considerations

The XF Pro has been carefully designed so that when used properly the instrument is accurate, fast, flexible, and safe.

Information on safety practices is provided with the instrument and operation manuals. Before using the instrument or accessories, thoroughly read these safety practices.

WARNING

Observe all relevant safety practices at all times.

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired. Unskilled, improper, or careless use of this instrument can create shock hazards, fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property.

Electrical hazards

WARNING

The XF Pro contains electrical circuits, devices, and components operating at dangerous voltages. Contact with these circuits, devices, and components can cause death, serious injury, or painful electric shock.

Panels or covers that are retained by fasteners which require the use of a tool for removal may be opened only by Agilent-trained, -qualified, or authorized service engineers. Consult the manuals or product labels supplied with the XF Pro to determine which parts are operator-accessible.

Instrument must always be turned off and disconnected from all power before removing any outer covers. Replace all covers prior to reconnecting power.

WARNING

Connection of the instrument to an incorrectly wired supply outlet, or lack of proper electrical grounding can create a fire hazard or a potentially serious shock hazard and could seriously damage the instrument and any attached ancillary equipment.

Always use a three-wire outlet with ground connection which is adequately rated for the load. The installation must comply with local safety regulations.

Always use the supplied power cords. Use of power cord with inadequate ratings can create a fire hazard or cause damage to the instrument.

Safety Labels

The following table lists the common safety labels you might find on the Agilent Seahorse XF Pro

Symbol	Description
	Indicates pinch, crush, or cut hazard.

Electromagnetic Compatibility (EMC) Information

This product conforms to:

Emission

EN 55011/CISPR 11: Group 1, Class A

Group 1 ISM equipment contains all industrial, scientific and medical (ISM) equipment in which there is intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment itself.

Class A equipment is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network that supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

1. Relocate the radio or antenna.
2. Move the device away from the radio or television.
3. Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
4. Make sure that all peripheral devices are also certified.
5. Make sure that appropriate cables are used to connect the device to peripheral equipment.
6. Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.

Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

Immunity

IEC 61326-1/EN IEC 61326-1.

This product is intended to be used in a basic electromagnetic environment with the following test requirements applied:

Test Items	Basic Standards	Test Limits	Performance Criteria
Electrostatic discharge immunity	IEC 61000-4-2	4 kV Contact Discharge; 8 kV Air Discharge	B
Radiated frequency immunity	IEC 61000-4-3	3 V/m (80 MHz to 1 GHz); 3 V/m (1.4 GHz to 6.0 GHz)	A
Electrical fast transient/burst immunity	IEC 61000-4-4	1 kV (AC, 5k Hz or 100 kHz); 0.5 kV (I/O, 5k Hz or 100 kHz)	B
Surge immunity	IEC 61000-4-5	±2 kV (Line to ground); ±1 kV (Line to line)	B
Conducted immunity	IEC 61000-4-6	3 V (150 kHz to 80 MHz)	A
Magnetic field immunity	IEC 61000-4-8	3 A/m (50 Hz, 60 Hz)	A
Voltage dips, short interruptions, and voltage variations immunity	IEC 61000-4-11	0% Half-cycle; 0% Full-cycle; 70% 25/30 Cycles; 0% 250/300 Cycles	B B C C

CAUTION **The shielding and length of USB and other ports cables are critical to electromagnetic compatibility performance, only use the cables provided from Agilent.**

Instrument Overview and Intended Use

The Agilent Seahorse XF Pro analyzer measures the rate of change of dissolved oxygen and pH in the media immediately surrounding living cells cultured in a microplate. Changes in the extracellular media are caused by the consumption or production of analytes by the cells. Therefore, a sensitive measurement of the media flux can be used to determine rates of cellular metabolism with great sensitivity and in a totally noninvasive, label-free manner.

A unique feature of the Seahorse XF technology is its ability to make accurate and repeatable measurements in as little as five minutes. The instrument, working with a sensor cartridge, isolates a few µL of media above the cell monolayer. Cellular metabolism causes rapid, easily measured changes to the "microenvironment" in this small volume.

An XF sensor cartridge is required to run an assay. The cartridge has 96 probes, and each probe has a single multifluor sensor spot that is sensitive to both oxygen and proton concentration. The system measures the concentration of each analyte over time and automatically calculates the oxygen consumption rate (OCR) and proton efflux rate (PER) - which is a quantitative measure of extracellular acidification rate (ECAR) - simultaneously in every well of the microplate.

Typically, a measurement cycle is performed for 6 minutes. The media is gently mixed, the probe is positioned 200 µm above the well bottom, and the analyte levels are measured.

Baseline metabolic rates are typically measured three to four times and are reported in pmol/min for OCR, mpH/min for ECAR, and pmol/min for PER. Compound is added to the media and mixed, and then the post-treatment OCR and ECAR measurements are made and repeated. As cells shift metabolic pathways, the relationship between OCR and ECAR/PER changes.

The XF Pro system, comprising a bench top analyzer and touch screen controller, is driven by Agilent Seahorse Wave Pro software. This software enables all aspects of Seahorse XF assays including assay setup, instrument control, and data analysis.

Consumables are sold separately and include Agilent Seahorse XF FluxPaks (comprising sensor cartridges, cell plates, and calibrant) as well as a variety of assay kits, reagents, and media. XF sensor cartridges are specific for the instrument type and can be purchased exclusively from Agilent.

Technical Specifications

Model Number	S7850A
Controller Dimensions:	Width × height × depth 21" × 18" × 12" 53.34 cm × 46 cm × 30.48 cm
Analyzer Dimensions:	15.25" × 23" × 16" 38.74 cm × 58.42 cm × 45.72 cm
Weight	Analyzer: 49 lbs/22.2 kg Controller: 22 lbs/10 kg
Power requirements	100 to 240 V AC 50/60Hz Analyzer: 300W Controller: 3.2 A
Power cord rating	Three-wire (grounded) AC power cord rated 10 A or
Power Fuse ratings	250 V/10 A time delay fuses
Environmental conditions	"Normal" environmental conditions- indoor use, altitude to 2,000 m
Room temperature range	+40 °F to 86 °F (+4 °C to 30 °C) No direct sunlight Do not place directly under air conditioning vents. 20 to 80% relative humidity
Sample temperature and environment	Controlled to user-selected temperature between 16 °C and 42°C, but at least 8 °C above ambient temp No gas or humidity control
Software OS	Windows 10 LTSC, 64-bit
Data interface	RS-232c 64-bit barcode reader (internal) TCP/IP (external) USB Type B
Equipment class	Class 1 (PE connected)
Pollution degree	2
Installation (overvoltage) category	II
Mains supply voltage fluctuations	±10%

2 Installation

This chapter provides unpacking and installation instructions for the Agilent Seahorse XF Pro Analyzer.

Unpacking and Component Identification

The XF Pro Analyzer system is packaged in two boxes.

Upon receipt, immediately check each box for damage. Shipping damage must be reported to the transportation company and Agilent. See **"Contact Information"** on page 40.

WARNING

The XF Pro Analyzer requires two people to lift and handle

Each person should firmly grasp the base of the unit at opposite ends to each other. Use Occupational Safety and Health Administration standards for lifting techniques.

CAUTION

XF Pro instruments must be installed by trained Agilent personnel.

The analyzer is shipped with protection components that must be removed prior to use. Agilent recommends these components be removed by Agilent personnel during installation.

To prevent damage during shipping, the instrument is shipped with a cartridge loaded onto the probe head and lowered onto a plate on the tray. These items must be removed prior to running the first assay. Agilent personnel will remove these shipping protection components from the XF Pro Analyzer during installation.

Instrument/ Component	Quantity	Image
XF Pro Instrument	1	
XF Controller 64-bit	1	

Instrument/ Component	Quantity	Image
Power cord (instrument)	1	
Power cord	1	
Power supply (controller)	1	
RS232 cable	1	

Instrument/ Component	Quantity	Image
External barcode reader (optional)	1	
USB cable	1	
USB extension cable	1	

Installation Procedure

The following items are included in an XF Pro system:

- XF Pro Analyzer - The analyzer is a temperature-controlled instrument containing all optical and electronic measurement components to measure oxygen and proton flux of cells grown in XF cell culture plates. The analyzer is used in conjunction with XF sensor cartridges.
- XF Controller - Operation of the Analyzer is done through a high-resolution color LCD touch screen with stand, that may be installed in front of or beside the XF Pro. The controller communicates with the analyzer using a single serial cable and a single USB A-B cable.

Suitable locations for the XF Pro system

XF Pro Analyzers are designed for laboratory use. The internal environment of the analyzer is controlled to a preset temperature by the user; therefore, laboratory room temperature must be maintained within the range listed in the specification table.

Sample temperature control performance can be monitored using the status display on the right side of the analyzer or on the Wave application display.

The XF Pro uses optical detection technology to measure extremely low levels of fluorescent emission from analyte sensors. While the analyzer has been designed to shield room light, excessive light (such as direct sunlight) should be avoided.

WARNING **The electrical connection at the back of the XF Pro is the primary disconnect for the instrument. The XF Pro should be positioned to allow accessibility to the power cord for easy disconnection.**

CAUTION **Avoid drafty areas, as well as areas experiencing significant vibration (such as a centrifuge).**

Internal components of the XF Pro Analyzer

Removing the side doors reveals the measurement chamber in which the assay is conducted. The electro-optics hardware is enclosed in a card cage in the rear chamber, and this is connected to the probe head through a set of fiber optic cable bundles. The base of the enclosure contains the primary controller board and heater assembly. (See **Figure 1** and **Figure 2**.)

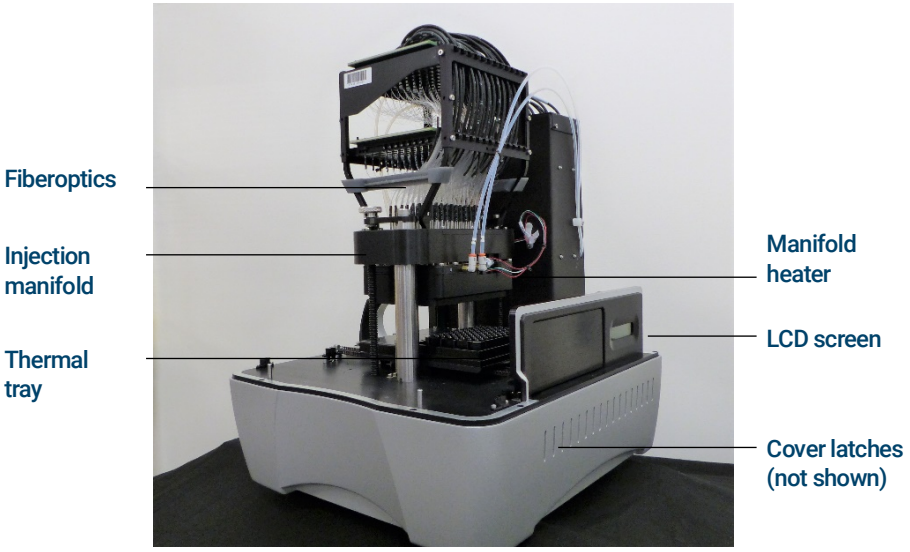


Figure 1 XF Pro front/side view. Base color may vary.

- LCD message screen - Displays current instrument action and setpoint temperature, and sample temperature.
- Cover latches - Pull on indented hand-holds molded into the side doors (not shown) to lift them up, exposing the internal components of the instrument. Magnets hold in place.
- Probe head and injection manifold - The probe head consists of 96 "light guides" to carry the optical signals to and from the sensors. The injection manifold uses compressed air to inject compounds loaded into sensor cartridge ports into the assay wells.

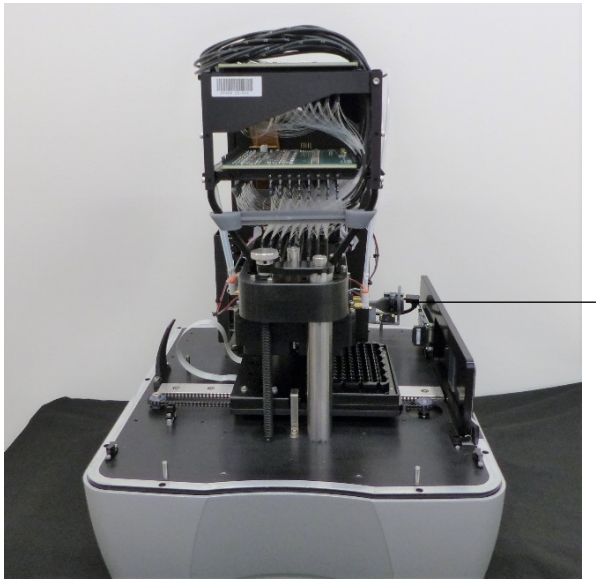


Figure 2 XF Pro front view.

- Barcode reader - Reads barcode on sensor cartridge and cell plate.

Setup and interconnects: cable installation

The XF Pro Analyzer is operated from a touch screen computer monitor mounted to a stand, referred to as a controller. One RS232 cable and one USB cable handle the communication of commands and data between the instrument and the controller.

The controller may be connected to an external network, through the ports on the underside.

See **"Unpacking and Component Identification"** on page 16 to identify each cord, and refer to Figures **3** and **4** to identify the connectors.

1 Connect power cords - One power cord is used to connect the instrument to a grounded AC (mains) outlet. A second power cord is used to connect the controller power supply module to the AC supply. The power supply module is

then connected to the socket at the bottom of the controller. (See **Figure 3**.)

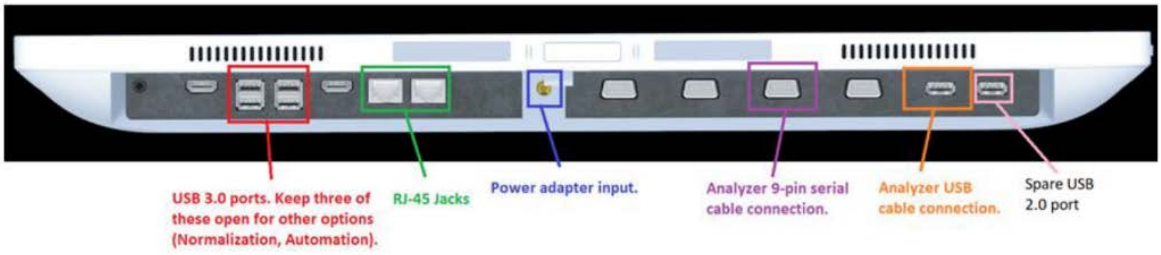


Figure 3 Controller ports (underside).

2 Connect the data cables to the analyzer. One RS-232 cable connects the controller serial port to the analyzer socket labeled "COM". (See **Figure 4**.)

Figure 4 XF Pro rear panel - USB and serial ports.

3 A second cable (USB) connects the analyzer socket labeled "USB" to the USB port on the controller. This port must be used for proper functioning of the instrument and barcode reader. (See **Figure 3**.)

4 Connect external network cables. The controller may be networked via the Ethernet port on the controller.

5 Connect the AC power cord to the AC input on the XFe rear panel and then switch the power switch to the on position. (See **Figure 5**.)

Figure 5 XF Pro rear panel - AC input and power switch.

WARNING **The door opens automatically when the tray is extended, allowing the operator to insert or remove the well plate/cartridge consumables. The operator must exercise caution during the loading of the well plate/cartridge to avoid the possibility of a pinch hazard. After the well plate/cartridge is securely on the tray, the operator's hand must be removed from the area of the tray before continuing the assay. After the command is given to continue the assay via the controller, the tray will move slowly back into the instrument and the door will close.**

CAUTION **Safe operation of the instrument requires that the cover be securely attached and plate tray door is closed. This also prevents heat loss and system cooling which can affect data quality.**

6 Securely attach the cover and close the tray door.
When the cover is securely attached and tray door is closed, optical switches are engaged to monitor the system. An optical sensor is also used to determine the status of the door.

The XF Pro has a heater that maintains a stable internal system temperature. Typically, the temperature will be maintained at 37 °C, as monitored by temperature sensors and controllers embedded in the tray and above the tray. A thermal fuse will disable the sample tray heater should it reach an abnormally high temperature. The manifold heater will not be disabled if this situation occurs, and the user should power off the instrument and contact technical support. In this situation the user should *not* attempt to open the instrument covers.

3 Basic Operation

This chapter provides basic operating procedures for the Agilent Seahorse XF Pro Analyzer.

Power and Warm Up

To power on the touch screen controller, press the power switch on the front of the controller. To prevent accidental power down of the controller, the switch may be disengaged in the **Power Options** menu of the Windows OS control panel under the **Advanced** tab. Turn on the instrument using the power switch on the rear panel.

On the right side of the instrument near the access door, there is an LCD message screen. When the instrument is powered up the LCD message screen will show **WAITING FOR WAVE**. (See **Figure 6**.)

Figure 6 LCD message screen (idle).

Instrument and Software Operation

Launch Wave

Seahorse XF Pro analyzer is controlled and operated through the Agilent Seahorse XF Pro Controller software. When it is launched on the controller, the LCD message screen will update and show the following display in **Figure 7**. For more information about the Seahorse XF Pro Controller software, visit the software website:

https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-software/seahorse-xf-pro-controller-software-2007524.

Figure 7 LCD message screen (ready).

NOTE

Allow at least 1 to 2 hours (or overnight) for the instrument to fully warm and equilibrate to the set temperature. If starting ambient temperature conditions are <0 °C, allow the instrument to equilibrate to room temperature for 24 hours.

Manage Software Licenses

The **Options** > **Licenses** view is where software licenses can be managed for the XF Pro Controller.

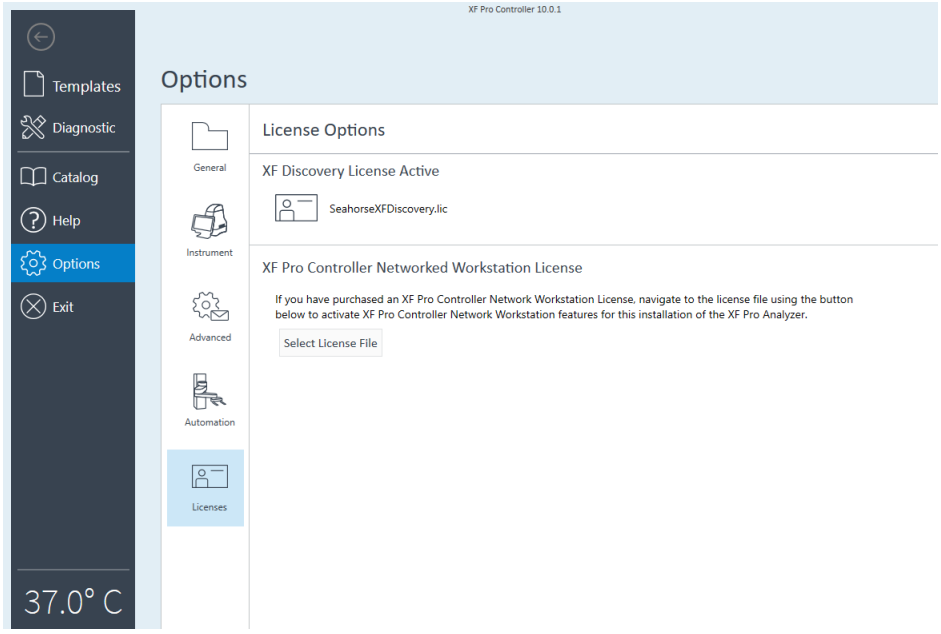


Figure 8 Options > Licenses view

The XF Discovery license is a single-use node license installed within the XF Pro Controller software application. Data files generated by an XF Pro Analyzer with the XF Discovery license will have access to valuable data analysis features in Wave Pro software and Seahorse Analytics, such as data plotting in a dose response curve, analysis of multiple result files, and more. Seahorse XF Pro Analyzers with the XF Discovery license also will have access to the automation service, enabling automation of the XF assay workflow, from template selection to swapping plates and executing an assay.

For more information on how to claim and activate the XF Discovery license, please read the **Agilent Seahorse Wave Pro & XF Pro Controller software user manual** in chapter 3, "Claiming your Agilent Seahorse XF Discovery License using XF Pro Controller software" on page 60.

Performing XF assays

Information and protocols for preparing medium associated with XF assays, experimental design, running XF assays and analyzing XF data may be found online at **www.agilent.com/en/product/cell-analysis/how-to-run-an-assay.**

XF Pro status indicator

During an assay, the Status Indicator light on the top of the XF Pro Analyzer will change from green to amber if a task requires user interaction, such as:

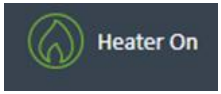
- Waiting for Wave (not connected to Wave or controller).
- To load a sensor cartridge or cell plate.
- To remove a used sensor cartridge or cell plate.
- To accept or cancel an assay if one or more wells did not calibrate properly after calibration.
- User interaction timeout.
- Cover not properly attached or tray door left open.

The Status Indicator light will change from green to red if an error has occurred, such as:

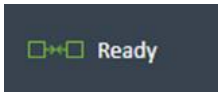
- Any errors that can occur during the run, such as barcode read errors for the sensor cartridge, cell plate, or a protocol error.
- Cannot connect to the barcode.
- Motion error/motor stall.
- Other instrument errors.

Wave Controller widgets

The widget icons are located on the lower left side of the XF Pro Controller software and display the status of the XF Pro Analyzer and current temperature.

Temperature widget: Current tray temperature and heater status display.	

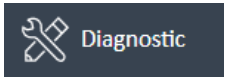
Status widget: Connection status between the XF Pro Controller (computer), Wave Pro Controller (software), and the XF Pro Analyzer.



XF Pro maintenance

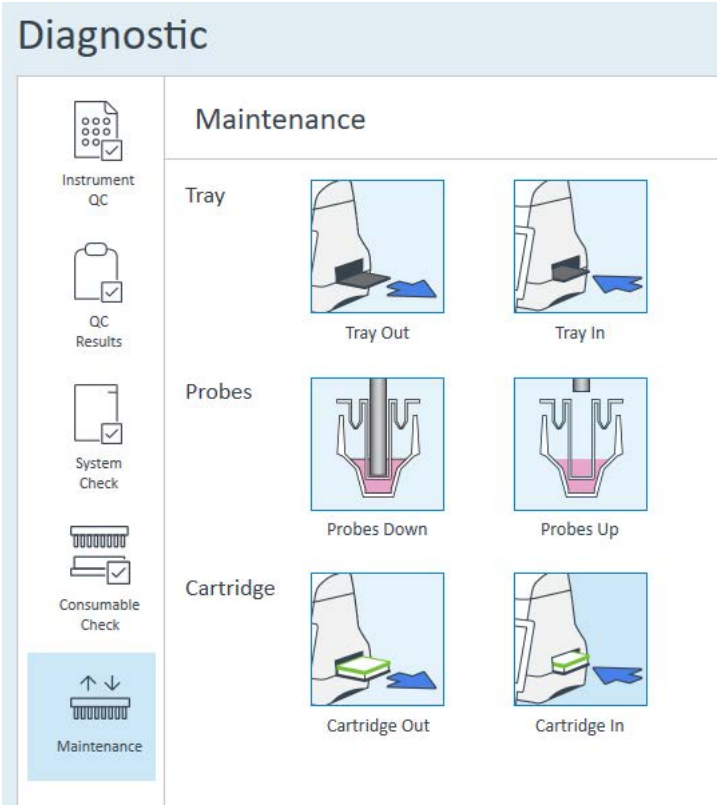
The **maintenance** options (in the **Diagnostic** menu) allow for ejection/insertion of the instrument tray, and to raise/lower the probes.

1 Click the **Diagnostic** menu at the top left of the main ribbon.



2 Select **Maintenance**, the last option in the list.

- **Tray control**: Manually eject or insert tray, with or without a utility plate or cell culture plate.
- **Probe control**: Manually raise/lower the probes of the XF Pro Analyzer.
- **Cartridge control**: Manually load or unload a sensor cartridge.


Diagnostic
Instrument QC
QC Results
System Check
Consumable Check
Maintenance
Maintenance
Tray
Tray Out
Tray In
Probes
Probes Down
Probes Up
Cartridge
Cartridge Out
Cartridge In

XF Pro Assays at Non-37 °C Temperatures

Seahorse XF Pro Analyzers have been validated to deliver desired target temperatures in the range of 16 to 42 °C, provided the ambient room temperature is 8 to 20 °C below the target temperature, and in the validated operational room temperature range of 4 to 30 °C. To understand the relationship between the desired sample temperature and required ambient temperature, see the temperature chart in **Figure 9**.

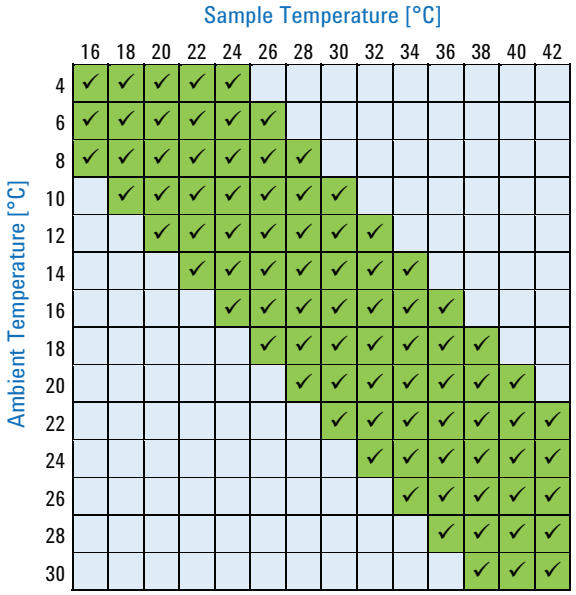


Figure 9 Temperature chart.

Operational and assay guidelines for non-37 °C assays

- For all non-37 °C operation, the XF Pro Analyzer must equilibrate overnight in the required ambient temperature.

- If it is required to set up the XF Pro Analyzer in a cold room, avoid direct fan sources.
- For all non-37 °C operation, the tray heater must remain On. Do not turn the tray heater Off.
- For assay temperatures below 30 °C, hydrate the sensor cartridge in the dark at room temperature.
- Prior to starting an assay, an additional 30 minutes of precalibration equilibration time has been added to ensure temperature stability.

To adjust the **Target Temperature** (set point) using the up/down arrows, do the following:

1 Click the temperature widget. (See **Figure 10**.)

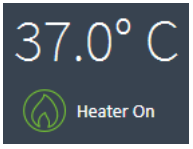


Figure 10 Temperature widget.

The **Tray Heater** dialog box is displayed. (See **Figure 11**.)

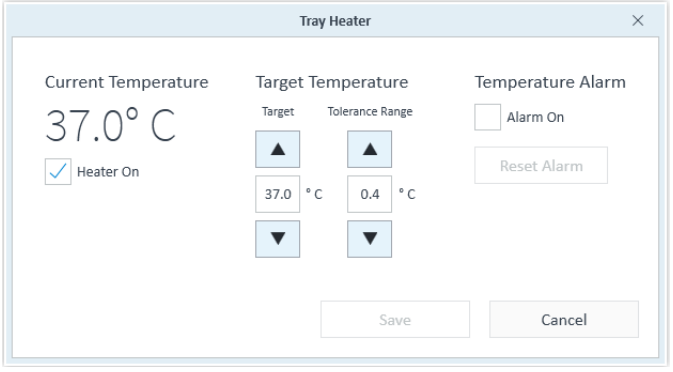


Figure 11 Edit temperature settings (Tray Heater dialog box).

2 Ensure the ambient conditions support the desired target temperature (8 to 20 °C above ambient). (See the temperature chart shown in **Figure 9** on page 32.)

NOTE **Changing the Target Temperature requires overnight equilibration to the new set point.**

3 Other temperature widget functions are:
- Turn the heater On/Off.
- Set the tolerance range for temperature fluctuation. If the temperature is above or below the acceptable tolerance range from the temperature set point, the temperature widget will change color, and the Status Indicator light (top of the XF Pro Analyzer) will change from green to amber. For networked XF Pro controllers, Wave Pro Controller software automatically sends an email notification to specified recipients.

4 To save any changes on the **Tray Temperature** window, click **Save**.

Set alarm (temperature tolerance range)

To set the alarm:

1 Select the **Alarm On** check box in the **Tray Temperature** window. (See **Figure 11** on page 33.)

2 Click **Save**.

To disable the alarm, clear the **Alarm On** check box, then click **Save**.

If the **Tray Temperature** exceeds the **Tolerance Range** and the alarm is activated, click **Reset Alarm** to acknowledge and reset the **Tray Temperature** alarm.

To ensure the **Tray Temperature** starts within the **Tolerance Range**, check the current temperature of the XF Pro Analyzer before beginning an assay. For any suspected temperature issues or unexpected temperature fluctuations, contact Technical Support. (See **"Contact Information"** on page 40.)

4 Maintenance

This chapter provides routine maintenance, troubleshooting, contact, and additional resource information for the Agilent Seahorse XF Pro Analyzer.

Cleaning and Routine Maintenance

The XF Pro instrument is designed for minimal cleaning, and user maintenance is not required. All consumables are disposable and none of the instrument components contact the cell plate or reagents during routine use, preventing cross-contamination of biological or chemical materials.

Agilent strongly recommends an annual service contract with Preventative Maintenance to keep the system in good working order.

CAUTION **Contact Technical Support if there is a spill of any reagents or liquids into the sample tray or system. Do not attempt to open the instrument unless specifically instructed to do so by an Agilent technical support representative.**

Troubleshooting

Barcode errors

The XF Pro Analyzer reads and records the cell plate and sensor cartridge barcodes before beginning an assay. A Barcode Read error is displayed on the rare occasion the barcode cannot be read. Contact Agilent Seahorse Technical Support to assist with resolving this error, and to start the assay.

Cartridge barcode read failure

For any sensor cartridge barcode read errors, Wave Pro Controller displays a dialog box and a choice of three corrective actions. (See **Figure 12**.)

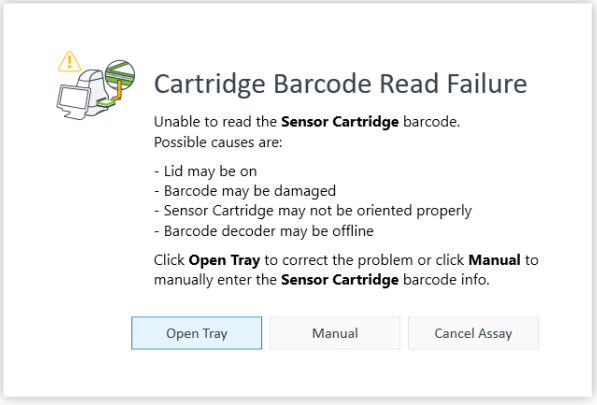


Figure 12 Cartridge Barcode Read Failure dialog box.

- **Open Tray**: Eject the sensor cartridge to inspect barcode quality or to reverse the sensor cartridge.
- **Manual**: Manually input the sensor cartridge barcode information. Contact Agilent Seahorse Technical Support for this step. (See **"Contact Information"** on page 40.).
- **Cancel Assay**: Cancel the assay.

Manually enter sensor cartridge barcode

1 To display the Cartridge Barcode Manual Entry dialog box, click **Manual**.

Use the telephone number to call for assistance with entering the sensor cartridge barcode information on the Cartridge Barcode Manual Entry dialog box. (See **Figure 13**.)

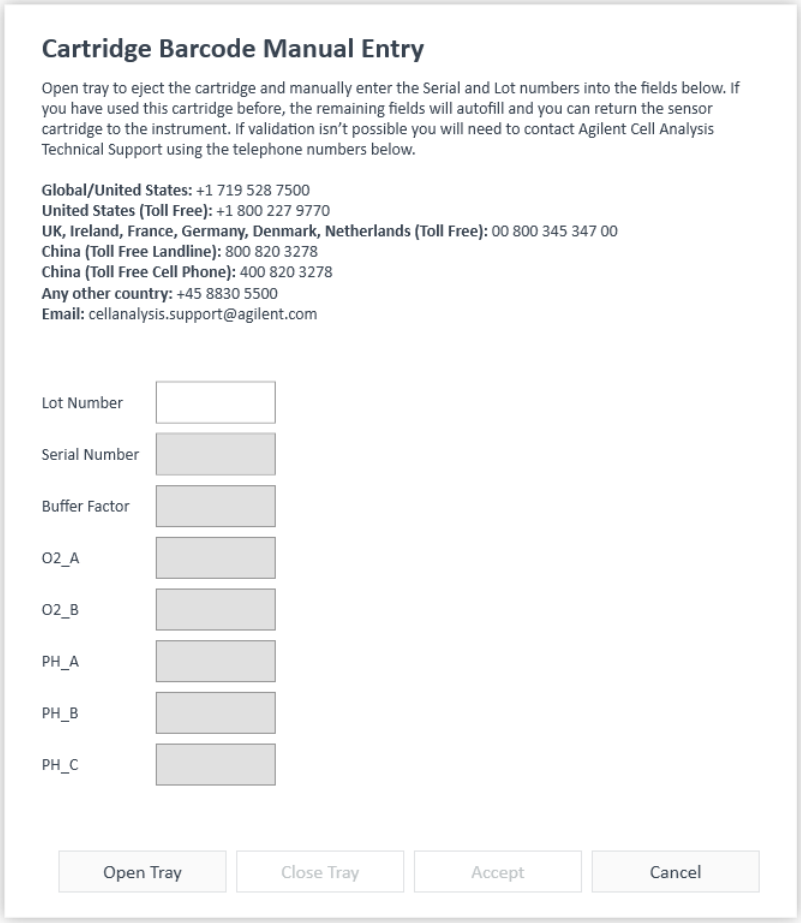


Figure 13 Cartridge Barcode Manual Entry dialog box.

Cell plate barcode read failure

For any Cell Plate barcode read errors, the XF Pro Wave Controller displays a dialog box and two corrective actions. (See **Figure 14**.)

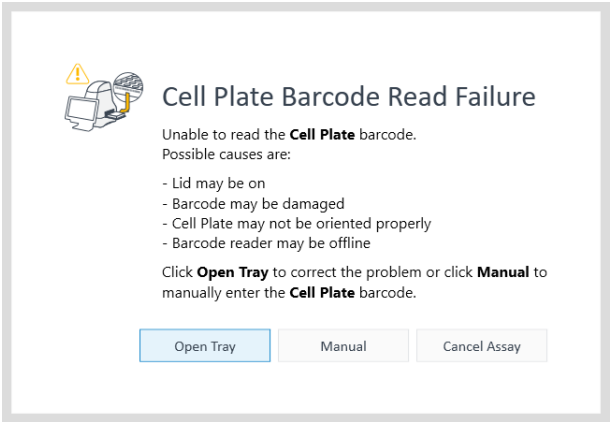


Figure 14 Cell Plate Barcode Read Failure dialog box.

- **Manual**: Manually input the cell plate barcode info.
- **Cancel Assay**: Cancel the assay.

Manually enter cell plate barcode information as follows:

1. Click the **Tray Widget**. Cartridge Barcode Read Failure dialog box appears. (See **Figure 12** on page 37.)
2. To eject the cell plate, click **Open Tray**.
3. The cell plate barcode is located on the side of the plate. Write down the barcode information.
4. Click **Close Tray**. The Cartridge Barcode Manual Entry dialog box appears. (See **Figure 13** on page 38.)
5. Enter the **Cell Plate** barcode, and click **Accept**.

Contact Information

Worldwide technical support

For questions about XF technology, the XF Pro Analyzer, XF experimental design, data analysis, troubleshooting and other information, contact Agilent Cell Analysis Technical Support:

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