Agilent

Seahorse XFe Analyzer

Operating Manual

Notices

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About This Manual

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

1 “Introduction”

Chapter 1 provides an introduction to the Agilent Seahorse XFe Analyzer.

2 “Installation”

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

3 “Basic Operation”

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

4 “Maintenance”

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

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This chapter provides an introduction to the Agilent Seahorse XFe Analyzer.

General Information

This manual covers several models of Agilent Seahorse XFe Analyzer:

Instrument type	Model numbers (32-bit)	Model numbers (64-bit)
Seahorse XFe96	101991-100, S7800A	S7800B
Seahorse XFe24	102238-100, S7801A	S7801B

Safety Considerations

The XFe has been carefully designed so that when used properly you have an accurate, fast, flexible, and safe instrument.

Information on safety practices is provided with your instrument and operation manuals. Before using your instrument or accessories, you must 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 XFe 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, Agilent-qualified, or Agilent-authorized service engineers. Consult the manuals or product labels supplied with the XFe to determine which parts are operator-accessible.

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.

Instrument Overview and Intended Use

The Agilent Seahorse XFe 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 non-invasive, label-free manner.

A unique feature of the Agilent 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 XFe **sensor cartridge** is required to run an assay. The cartridge has 24 or 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 extracellular acidification rate (ECAR) simultaneously in every well of the microplate.

Typically, a measurement cycle is performed for 5-8 minutes. The media is gently mixed, the probe is positioned 200 µm above the well bottom, and the analyte levels are measured until the oxygen concentration drops approximately 20-30% and media pH declines approximately 0.1-0.2 pH units.

Baseline metabolic rates are typically measured 3-4 times, and are reported in pmol/min for OCR and in mpH/min for ECAR, 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 changes.

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

Consumables are sold separately and include FluxPaks (comprising sensor cartridges, cell plates, and calibrant) as well as a variety of Assay Kits, Reagents, and Media. XFe Sensor Cartridges are specific for the instrument type and can be purchased exclusively from Agilent.

Technical Specifications

Model number	**101991-100, S7800A (XFe96)** **102238-100, S7801A (XFe24)**	**S7800B (XFe96)** **S7801B (XFe24)**
Part number	**101991-100 (XFe96)** **102238-100 (XFe24)**	
Dimensions Controller: Analyzer:	Width × height × depth 19" × 17" × 12" 48 cm × 43 cm × 30 cm 16" × 24" × 17" 41 cm × 61 cm × 43 cm	
Weight	Analyzer: 45 lbs/20 kg Controller: 22 lbs/9 kg	
Power requirements	100-240 V AC 50/60Hz Analyzer: 6 A Controller: 3.2 A	
Power cord rating	3- wire (grounded) AC power cord rated 10 A or greater	
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 - 86 °F (+4 °C - 30 °C) No direct sunlight Humidity less than 80%	
Sample temperature and environment	Controlled to user-selected temperature between 16 °C and 42 °C, but at least 12 °C above ambient temp No gas or humidity control	
Software OS	Windows 7, 32 bit	Windows 10, 64 bit
Data interface	RS232c (internal) TCP/IP (external) USB Type B	64 bit barcode reader

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

Unpacking and Component Identification

The XFe 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 36.

WARNING

The XFe Analyzer requires two people to lift and handle safely.

Each person should firmly grasp the base of the unit at opposite ends to each other. Use OSHA standards for lifting techniques.

CAUTION

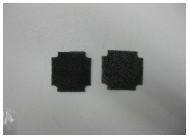
XFe Instruments must be installed by trained Agilent personnel ONLY.

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 XFe Analyzer During Installation.

Instrument/Component	Quantity	Image
XFe Instrument	1	

Instrument/Component	Quantity	Image
XFe Controller 32 bit OR	1	
XFe Controller 64 bit	1	
Power cord (instrument)	1	
Power cord (controller)	1	
Power supply (controller)	1	
RS232 cable	1	

Instrument/Component	Quantity	Image
USB cable	1	
USB extension cable	1	
Extra fan filters	2	

Installation Procedure

The following items are included in an XFe system:

- **XFe 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 XFe sensor cartridges.
- **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 XFe. The controller communicates with the analyzer using a single serial cable and a single USB A-B cable.

Suitable locations for the XFe system

XFe 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.

Tray temperature control performance can be monitored using the status display on the right side of the analyzer.

The XFe 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 XFe is the primary disconnect for the instrument. The XFe 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 XFe 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**.)

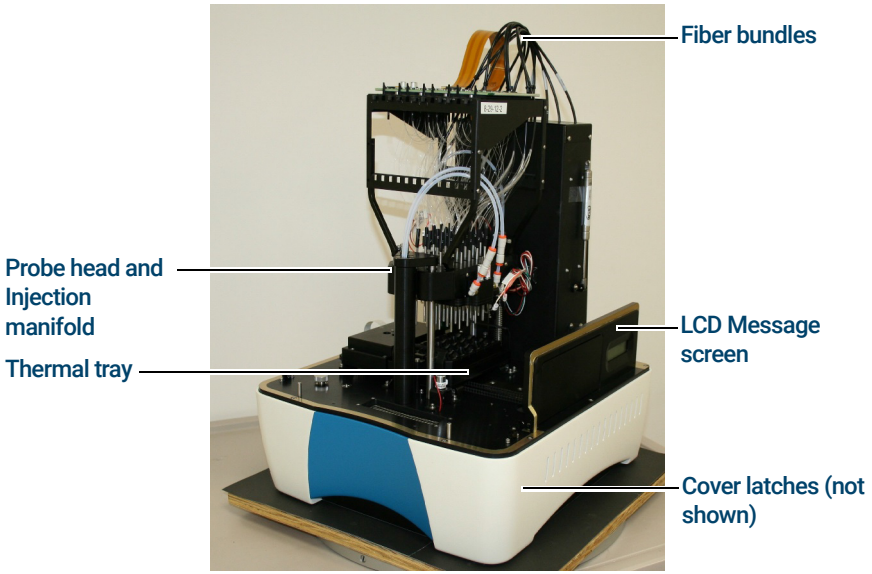


Figure 1. XFe Front/Side view. Base color may vary.

- **LCD Message screen** - Displays current instrument action and Thermal tray 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.
- **Probe head and injection manifold** - The probe head consists of 24 or 96 “light pipes” 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.

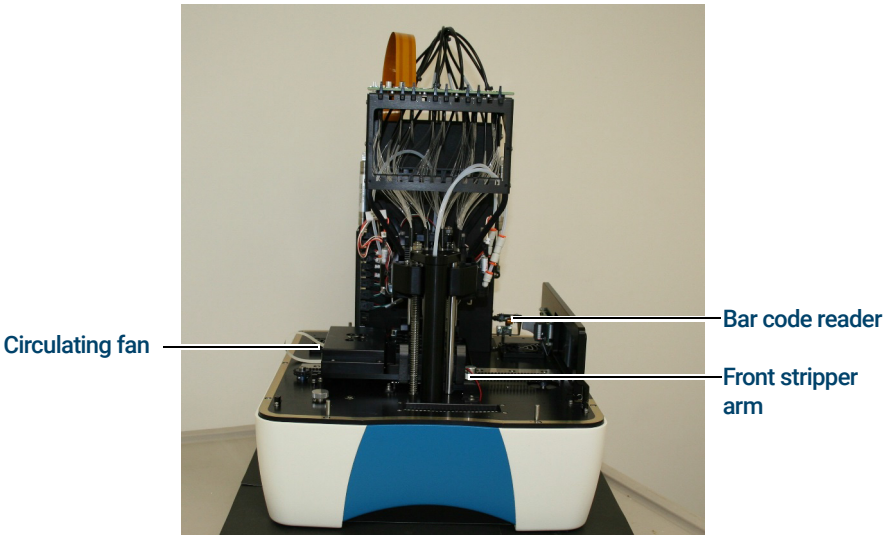


Figure 2. XFe Front view

- **Front stripper arm** - Used for Sensor Cartridge removal at the end of assay runs.
- **Bar code reader** - Reads bar code on sensor cartridge and cell plate.
- **Circulating fan** - Helps to stabilize environment inside XFe Analyzer.

Setup and interconnects: cable installation

The XFe 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 14 to identify each cord, and refer to the figures below 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**.)

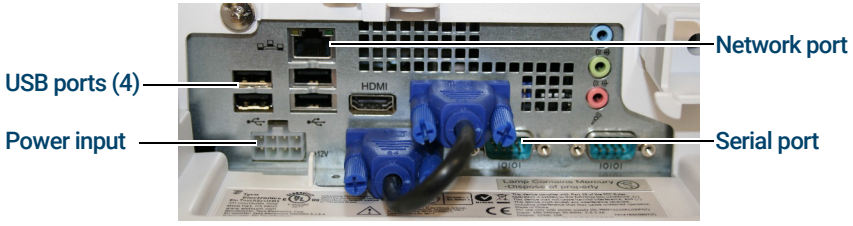


Figure 3. Controller ports (underside)

2 Connect the data cables to the analyzer. One RS232 cable connects the controller serial port to the analyzer socket labeled "COM." (See **Figure 4**.)

Figure 4. XFe rear panel - USB and serial ports

3 A second cable (USB) connects the analyzer socket labeled "USB" to the USB port on the controller directly adjacent to the network (Ethernet) port. 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. XFe rear panel - AC input, power switch, and serial port

WARNING **The door opens automatically when the tray is extended, allowing the operator to insert or remove the wellplate/cartridge consumables. The operator must exercise caution during the loading of the wellplate/cartridge to avoid the possibility of a pinch hazard. After the wellplate/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 XFe 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. Two fans circulate air through the plenum containing the heater. A thermal fuse will disable the heater should it reach an abnormally high temperature.

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

Power and Warm Up

Power 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 the instrument on 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 **IDLE: NO CONTROLLER**. (See **Figure 6**.)

Figure 6. LCD message screen (idle)

Launch Wave

When Wave software is launched on the controller, the LCD message screen will update and show the following display. (See **Figure 7**.)

Figure 7. LCD message screen (ready)

NOTE It is recommended that you allow at least 18 hours (overnight) for the instrument to fully warm and equilibrate to the set temperature.

Performing XF assays

For detailed instructions on using Wave, the software interface for operating the XFe Analyzer, please see document *S7894-10000, Wave User Guide*.

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/chem/discoverXF**.

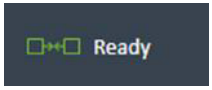
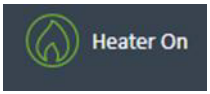
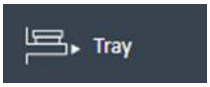
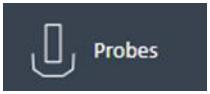
XFe status indicator

During an assay, the Status Indicator light on the top of the XFe Analyzer will change from Blue to Amber if a task requires user interaction or if an error has occurred, such as:

- 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
- Any errors that can occur during the run, such as barcode read errors for the Sensor Cartridge, Cell Plate, or a protocol error.

Wave Controller widgets

The Widget icons are located on the lower left side of Wave Controller software, and display the status of the XFe Analyzer, current temperature, and controls to eject/insert the tray and raise/lower probes.

Status widget: Connection status between the XFe Controller (computer), Wave Controller (software), and the XFe Analyzer.	
Temperature widget: Current tray temperature and heater status display.	
Tray widget: Manually eject or insert tray, with or without a Utility Plate or Cell Plate.	
Probes widget: Manually unload a Sensor Cartridge and raise/lower the probes.	

Tray widget

Use the Tray widget to manually eject a Utility Plate or a Cell Plate from the XFe Analyzer:

1 Click the Tray widget to display the Tray widget dialog box (See **Figure 8**).

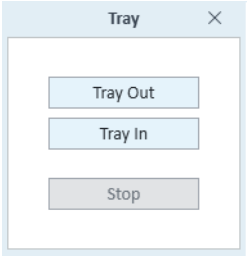


Figure 8. Tray widget dialog box

2 Click **Tray Out**, and remove the Utility Plate or Cell Plate.

3 To insert the tray and maintain the Target Temperature, click **Tray In**.

Probe control widget

Use the Probes widget to load/unload a Sensor Cartridge or raise/lower probes. To display options and select the appropriate action, click the Probes widget.

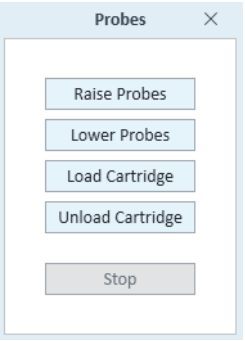


Figure 9. Probes widget

XFe Assays at Non-37 °C Temperatures

Agilent Seahorse XFe Analyzers have been validated to deliver desired target temperatures in the range of 16-42 °C, provided the ambient room temperature is 12-20 °C below the target temperature, and in the validated operational room temperature range of 4-30 °C. To understand the relationship between the desired sample temperature and required ambient temperature, see the temperature chart in **Figure 10**.

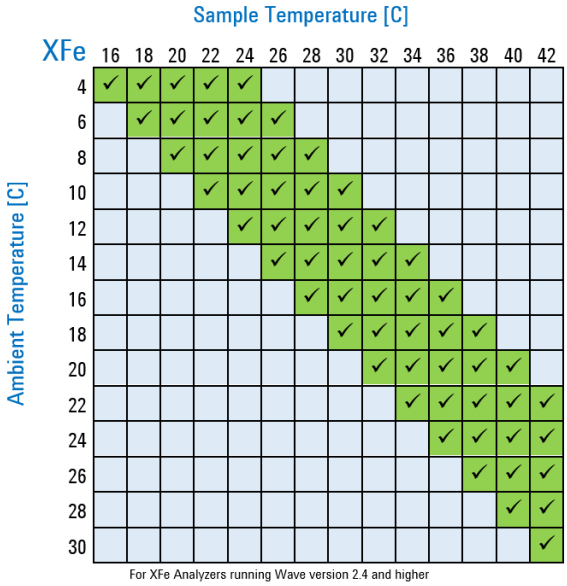


Figure 10. Temperature chart

Operational and assay guidelines for non-37 °C assays

- For all non-37 °C operation, the XFe Analyzer must equilibrate overnight in the required ambient temperature.
- If it is required to set up the XFe 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 11**.)

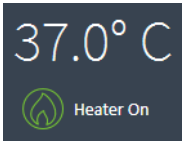


Figure 11. Temperature widget

The Tray Heater dialog box is displayed. (See **Figure 12**.)

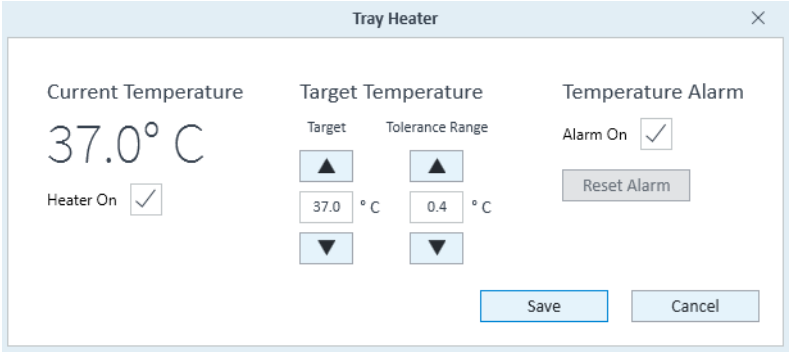


Figure 12. Edit temperature settings (Tray Heater dialog box)

2 Ensure your ambient conditions support the desired target temperature (12-20 °C above ambient). (See the temperature chart shown in **Figure 10** on page 28.)

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 XFe Analyzer) will change from blue to amber. For networked XFe Controllers, Wave 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 Check the **Alarm On** check box in the Tray Temperature window. (See **Figure 11** on page 29.)

2 Click **Save**.

To disable the alarm, uncheck the **Alarm On** 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 XFe Analyzer before beginning an assay. For any suspected temperature issues or unexpected temperature fluctuations, contact Technical Support. (See **"Contact Information"** on page 36.)

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

Cleaning and Routine Maintenance

The XFe 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 your system in good working order.

CAUTION **If you encounter a spill of any reagents or liquids into the sample tray or system, please contact Technical Support. Do not attempt to open the instrument unless specifically instructed to do so by an Agilent technical support representative.**

Troubleshooting

Barcode errors

The XFe 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 Controller displays a message and a choice of three corrective actions. (See **Figure 13**.)

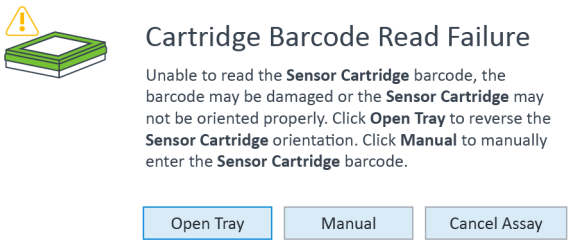

Figure 13. Cartridge Barcode Read Failure dialog box

- **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 36.)
- **Cancel Assay**: Cancel the assay.

Manually enter sensor cartridge barcode

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

2 Call the appropriate regional Agilent Cell Analysis Technical Support telephone number on the Cartridge Barcode Manual Entry window for assistance with entering the Sensor Cartridge barcode info. (See **Figure 14**.)

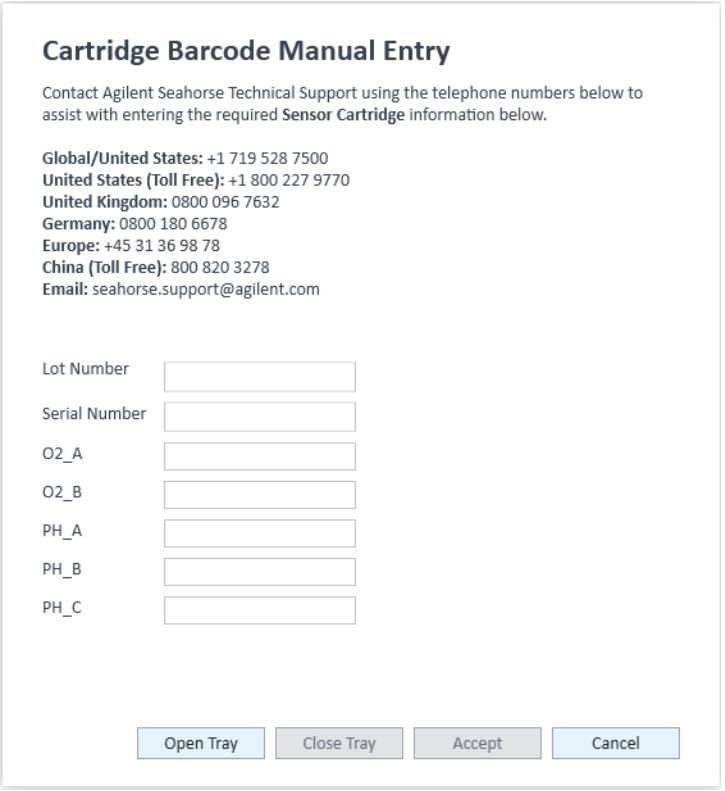

Figure 14. Cartridge Barcode Manual Entry window

Cell plate barcode read failure

For any Cell Plate barcode read errors, Wave Controller displays a message and two corrective actions. (See **Figure 15**.)

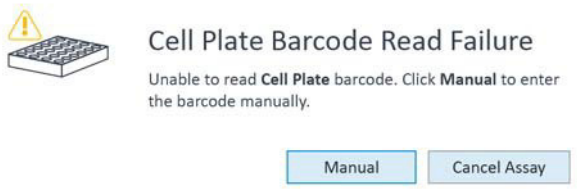

Figure 15. Cell Plate Barcode Read Failure dialog box

- **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 13** on page 33.)
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 window appears. (See **Figure 14** on page 34.)
5. Enter the Cell Plate barcode, and click **Accept**.

Contact Information

Worldwide technical support

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

Email: cellanalysis.support@agilent.com

Phone:

USA and Canada:	1-800-227-9770; select option **3** then **8**
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Germany:	0800 180 66 78
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https://www.agilent.com/en-us/contact-us/page

Additional Resources:

Wave User Guide:	**https://www.agilent.com/cs/library/usermanuals/public/Wave_2_6_User_Guide.pdf**
Software Download Page:	**https://www.agilent.com/en/support/cell-analysis/seahorse-xf-software**
XFe Consumables Web Page:	**https://www.agilent.com/en/products/cell-analysis/seahorse-xfe-consumables**
Links to other useful information:	**https://www.agilent.com/en/promotions/cell-analysis-technology**

Additional Resources:

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