Agilent

Seahorse XFp Extracellular Flux Analyzer

User Manual

Notices

Manual Part Number

S7802-90001

Edition

First Edition, November 2018

Printed in USA

Agilent Technologies, Inc.
121 Hartwell Ave.
Lexington, MA 02466

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data -Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A CAUTION 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 damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

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.

Contents

1 Introduction

Safety Considerations

WARNING **The protection provided by this instrument may be compromised if it is used in a manner not specified by Agilent.**

Safe operation of the XFp Analyzer requires that all covers are securely attached and plate tray door is closed. This also prevents heat loss and system cooling, which can affect data quality.

The door opens automatically when the tray is extended, allowing the operator to insert or remove the assay consumables. The operator must exercise caution during the loading of the miniplate/cartridge to avoid the possibility of a pinch hazard. After the miniplate/cartridge is placed 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 through the user interface, the tray will move slowly back into the instrument, and the door will close. An optical sensor is used to determine the status of the door.

The XFp Analyzer has heaters around the miniplate that maintain a stable tray temperature. Typically, the tray temperature is maintained at 37 °C, as monitored by temperature sensors and controllers embedded above the tray. A thermal fuse disables the heater when it reaches an abnormally high tray temperature.

The user should not replace the power cord provided with any other power cord that is rated at less than what is specified in **"Technical Specifications"** on page 8, dependent on the power mains of the country in which the instrument will be used.

Overview

The Agilent XFp Analyzer measures the rate of change of dissolved oxygen and pH in the media immediately surrounding living cells cultured in a miniplate. 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 precision and in a completely noninvasive, label-free manner.

A unique feature of XF technology is its ability to make accurate and repeatable measurements in as little as five minutes. This is accomplished by isolating an extremely small volume (approximately 2 ìL) of media above the cell monolayer. Cellular metabolism causes rapid, easily measured changes to the microenvironment in this small volume.

Typically, a measurement cycle is performed for six minutes. The media is gently mixed and the analyte levels are then measured until the oxygen concentration drops approximately 20-30% and media pH declines approximately 0.1-0.2 pH units. The measurement is performed using optical fluorescent biosensors embedded in a disposable cartridge that is placed into the specially designed Seahorse XFp Analyzer Cell Culture miniplate.

Baseline metabolic rates are typically measured 3-4 times, and are reported in pmol/min for Oxygen Consumption Rate (OCR) and in mpH/min for Extracellular Acidification Rate (ECAR). Compound is then 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 XFp Analyzer system includes a bench top analyzer, disposable sensor/compound delivery cartridges, tissue-culture treated miniplates for seeding cells for analysis, calibration solution, and Wave desktop analysis software.

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. XFp Sensor Cartridges are available exclusively from Agilent.

Technical Specifications

Model	Seahorse XFp Analyzer (model number S7802A and 102745-100)
Dimensions	Width x height x depth 12" × 17" × 23" 30 cm × 43 cm × 58 cm
Weight	33 lbs / 15 Kg
Power requirements	100-240V AC 50/60Hz 6A/3A
Power cord rating	3- wire (grounded) AC power cord rated 10A or greater
Power fuse ratings	250V/5A Time Lag (2 fuses) 5 mm × 20 mm
Environmental operating range	+39 °F - 86 °F / +4 °C - 30 °C No direct sunlight Humidity 20 - 70 % RH, non-condensing
Sample temperature and environment	Controlled to user-selected temperature between 16 °C and 40 °C, but at least 10 °C above ambient temperature No gas or humidity control
Data interface	TCP/IP (external) USB Type A (one in front, two in back)
Software OS	Windows 7 embedded

2 Seahorse XFp Analyzer Installation

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

Unpacking and Component Identification

Upon receipt, immediately check each box for damage. Report any shipping damage to the transportation company, and Agilent using **"Technical Support and Ordering Information"** on page 61. The following items are included in a Seahorse XFp Analyzer system:

Instrument/Controller	Quantity	Image
XFp Analyzer	1	
Power cord (region-specific)	1	
Wireless Micro USB adapter (p/n 103203-000)*	1	
Alternate wireless adapter (p/n S7802-80000)*	1	

* Customers will get only one wireless adapter, either S7802-80000 or 103203-000.

Suitable locations for the XFp Analyzer

The XFp Analyzer is designed for laboratory use. The internal environment for the XFp Analyzer Cell Culture miniplate is controlled to a preset tray temperature; therefore, laboratory room temperature must be maintained within the range listed in **"Technical Specifications"** on page 8. The miniplate tray temperature can be monitored using the status display on the upper right portion of the user interface.

The XFp Analyzer uses optical detection technology to measure extremely low levels of fluorescent emission from analyte sensors. Therefore, although the instrument has been designed to shield room light, avoid excessive light (such as direct sunlight).

2 Seahorse XFp Analyzer Installation

Suitable locations for the XFp Analyzer

3 Basic Operation of the XFp Analyzer

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

Power and Warm Up

Power up

To power on the XFp Analyzer, toggle the power switch on the back of the instrument.

Figure 1. XFp Analyzer power switch

Allow at least 20 minutes for the instrument to fully warm and equilibrate to the set temperature.

The status icon in the upper right corner of the screen will display the tray temperature and a green check mark.

Welcome screen on first time power up

On initial power up, a welcome screen displays initial diagnostic tests on the instrument. Follow the on-screen directions to run them.

These tests can be run at any time by using the Diagnostics menu selection on the instrument user interface (For more details, refer to **"Navigating the XFp Analyzer"** on page 17).

XFp Analyzer Communications and Network Connection Setup

The transfer of template and result files between the XFp Analyzer and a computer running Wave can be accomplished through any of the USB connectors on the instrument. Agilent recommends connecting the XFp Analyzer to a local network directory to ease file movement and aid Cell Analysis Technical Support in servicing the instrument. Please contact Cell Analysis Technical Support with any questions.

For instructions on setting up a wired network connection, see **"Wired Network Setup"** on page 49. For instructions on setting up a wireless network connection using the included wireless adapter, see **"Wireless Network Setup"** on page 51.

NOTE **Only the included wireless adapter has been qualified to work with the XFp Analyzer.**

Turning the XFp Analyzer Off

To turn off the instrument, press the power button in the lower-left corner of the Home screen to first shut down the XFp Analyzer screen. After the screen turns black, use the power switch on the back of the instrument to completely shut down power.

NOTE **Shut down the software before powering off using the rear power switch.**

4 Navigating the XFp Analyzer

This chapter provides navigation information for the Agilent Seahorse XFp Analyzer.

Setting up an XFp Assay

Select a template from Local, USB or Network drive

Agilent provides default templates for most Seahorse XF assay kits. Templates can also be created in Wave Desktop on a desktop or laptop computer and then transferred to the XFp Analyzer through a network connection or USB flash drive.

1 Click **Start** from the Home screen. See **Figure 2**.

Figure 2. Home screen

2 Select a template provided by Agilent to perform a specific assay. These templates can be found in the **Local** tab. See **Figure 3**.

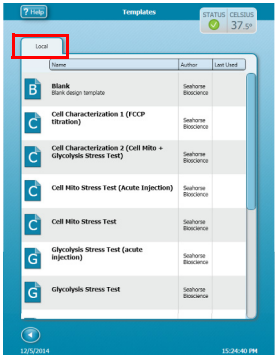


Figure 3. Local tab

3 Templates designed in Wave can be transferred to the XFp Analyzer through a USB or Network location.

- If the instrument is networked, a **Network** tab will appear, and allow selection and running of a template from the networked location.
- If a USB thumb drive containing a valid XFp Analyzer template has been inserted into the instrument, a **USB** tab will appear. Templates can be run directly from this location.

Refer to the Wave User Guide for XFp Analyzer (available from the Agilent website) for more information about creating and transferring templates.

NOTE

Only XFp Analyzer assay template files (*.asyt) are recognized by the XFp Analyzer. Templates must reside on the root of the USB flash drive and NOT in a subfolder.

Verify groups and plate map

After selecting the template file, make any necessary modifications to the Plate Map and Groups to be analyzed.

1 To see the conditions defined for a Group, touch the group name and look at the header information, as shown in **Figure 4**.

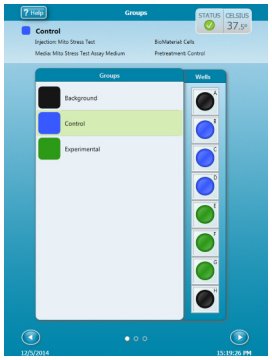


Figure 4. Groups

2 To change the wells assigned to each group, touch the Group name followed by the Well(s) to be included.

3 For other changes to Groups, Wave Desktop must be used.

Please refer to the Wave User Guide for XFp Analyzer for more detailed information about modifying Groups/Conditions.

Review/Edit instrument protocol

Check that all desired steps are selected (check boxes checked). Agilent strongly recommends performing equilibration for all cell-based XF assays. If needed, increase or decrease the number of measurements that will be performed during the assay on the Instrument Protocol page by touching the circled number corresponding to the step to be modified, then adjusting the number of cycles up or down. (Refer to Define the Instrument Protocol section of the Wave User Guide for XFp Analyzer for more information about measurement cycles). See **Figure 5**.

Figure 5. Instrument protocol page

Review summary and start assay

1 Review the **"Safety Considerations"** on page 6, and **"Safety Considerations"** on page 6 to verify that the settings are as desired.

2 Before beginning an assay, the following optional steps may be taken:

   a Click **Edit** next to **Assay Name** to customize the name of the assay result file. See **Figure 6** on page 21.

Figure 6. Assay name

b Click **Edit** next to **Notes** to add any notes related to the assay or protocol being performed.

c Click **Edit** next to **Email Notification** to add email addresses for recipients to be notified when the assay is complete (requires an active network connection).

3 Click **Start Assay** then follow the on-screen instructions.

NOTE

The XFp Analyzer will save assay results to a USB flash drive by default (if available). Agilent recommends inserting a USB flash drive into the instrument at the time of assay setup so that results will automatically be saved. Alternatively, if the XFp Analyzer does not detect a USB flash drive after completing an assay, the system will prompt the user to insert a USB to save their Assay Result file.

Running an XFp Assay

1 Load the Cartridge (hydrated and loaded with compounds) and Utility Plate onto the tray when prompted. Ensure the cartridge fits properly on the Utility Plate, the lid is removed from the cartridge, and the direction of the cartridge matches the image on the screen. See **Figure 7**.

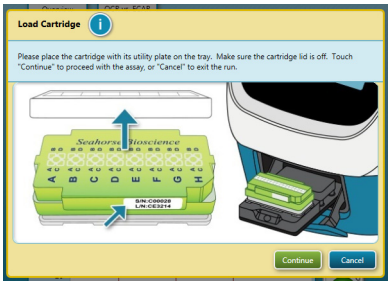


Figure 7. Load cartridge screen

2 The XFp Analyzer will perform Calibration of the sensor cartridge. This takes approximately 20 minutes.

NOTE **Although the sensor calibration may appear to be complete after 15 minutes, the instrument will be busy for a few more minutes. Wait for the user prompt to appear.**

3 Following calibration, the XFp Analyzer tray will open and present the utility plate. Remove the Utility Plate and load the Cell Plate. Ensure the lid is removed from the Cell Plate prior to loading onto the XFp Analyzer tray.

4 Once the Cell Plate is loaded, touch **Continue** to begin the equilibration step. The progress bar in the upper-left area of the screen displays the progress of the equilibration step.

5 **Overview** and **OCR vs. ECAR** are the two run-time views available as data is acquired by the XFp Analyzer. Click either tab to toggle between the views during the assay. See **Figure 8** on page 23.

Overview

The **Overview** tab displays both OCR and ECAR as a function of time. Red vertical lines indicate the injections and are labeled by injection port letter. See **Figure 8**.

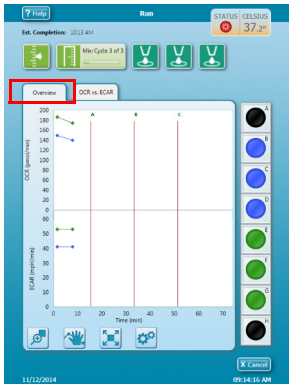


Figure 8. Overview tab

- In this view the charts can be zoomed and scaled by choosing one of the tools below the charts:
  - Zoom
  - Move
  - Reset
- By default, Rate data is displayed in Group mode. Click the **Options** button to change display modes and turn on error bars. See **Figure 9**.

Figure 9. Options button

  - Show StdDev turns on display of error bars for the each Group.
  - Level Data shows the concentration data from which the rate data is derived.
  - Well Mode shows the data from the individual wells instead of the average of the wells in each group.

OCR vs. ECAR

The **OCR vs. ECAR** tab displays OCR on the Y-axis and ECAR on the X-axis.

Choose a single time point at which to examine OCR vs. ECAR by using the Measurement selector below the chart. Data are displayed in Group Mode with standard deviations. The scale of this chart is fixed to allow easy comparison of values across measurements. Quadrants are labeled Aerobic, Energetic, Glycolytic, or Quiescent to show the energy preference of the cell during the assay at a given measurement. See **Figure 10**.

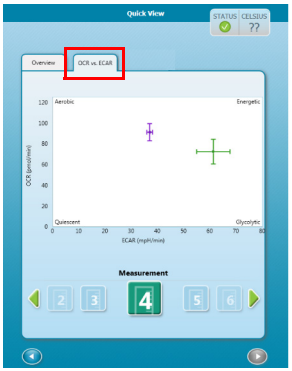


Figure 10. OCR vs. ECAR tab

NOTE **In both the Overview and OCR vs. ECAR views, the display of individual wells can be turned off by touching the well graphic on the right. Background correction is always performed while the experiment is running.**

6 Once the assay has finished, the XFp Analyzer prompts the user to eject the cartridge and remove the cell plate. See **Figure 11**.

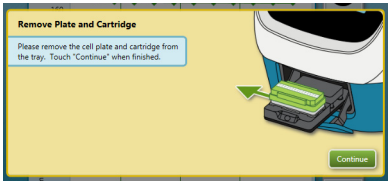
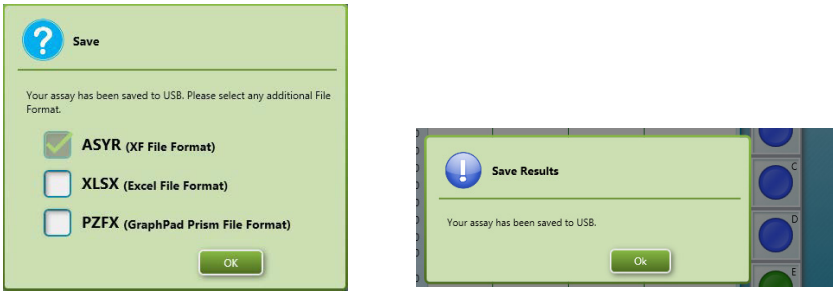


Figure 11. Remove plate prompt

7 After the assay is complete, the data automatically is saved to a USB flash drive (if inserted), the local drive, or the networked drive (if configured). A message appears indicating the location of the save Assay Result File (*.asyr). See **Figure 12**.



File format selection Save results

Figure 12. Save results

NOTE

Canceling midassay may cause the XFp Analyzer to stall. If this occurs, shut down the instrument by pressing the power switch on the back of the instrument. Use the same power switch to turn the instrument ON.

After the assay is complete, data will be automatically saved on the assay result file format to the location specified at the start of your assay (USB flash drive, network location, or locally). It is mandatory to save result data in the assay result file format after completing the assay and will always be checked ON. You can also save result data as an Excel file (.xlsx) and GraphPad Prism file (.pzfx) directly from the XFp Analyzer as well. Check the box next to the desired file formats and click **OK** (**Figure 12**).

Assay result files saved locally on the XFp Analyzer will be stored for 60 days after the assay. It is strongly encouraged you save assay result files on a USB or network drive.

Modifying XFp Analyzer Settings

From the Start page, touch the Settings button to access the XFp Analyzer Settings, see **Figure 13**. XFp Analyzer settings page containing:

- System preferences
  - Environmental settings
  - Assay results
  - Auto assay naming
  - Template management
- Device settings
  - Setup assistant

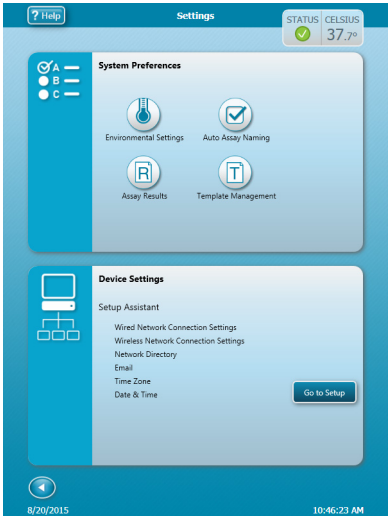


Figure 13. XFp Analyzer settings page

System preferences

Environmental settings

Temperature Target, see Figure 14: Adjust the tray temperature on the XFp Analyzer.

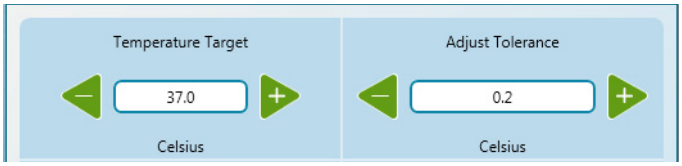


Figure 14. Temperature Target setting

Temperature Control on XFp Analyzer: On instruments containing a heated manifold and firmware version 0.50 or higher, users may adjust the tray temperature of the XFp Analyzer for a wider array of assays and applications. The validated tray temperature range of the XFp Analyzer is 16 °C – 40 °C. See **Figure 15** on page 28 for complete details of the environmental requirements to accurately achieve the desired tray temperature setting.

Temperature Specifications: Users can change the sample (tray) temperature to 16 °C – 40 °C; there is no internal cooling function.

- Lower tray temperatures require that the instrument be placed in a cold room OR refrigerated unit that is 8 °C – 20 °C cooler than the desired sample tray temperature.
- Users can set the tray temperature to any value (in increments of 0.1 °C) within the allowed range. **Figure 13** on page 26 shows the range of supported ambient and sample tray temperatures.

XFp 1.1	sample (tray) temperature												
ambient (room) temperature	4	16	18	20	22	24							
	6	16	18	20	22	24	26						
	8	16	18	20	22	24	26	28					
	10		18	20	22	24	26	28	30				
	12			20	22	24	26	28	30	32			
	14				22	24	26	28	30	32	34		
	16					24	26	28	30	32	34	36	37	40
	18						26	28	30	32	34	36	37	40
	20							28	30	32	34	36	37	40
	22								30	32	34	36	37	40
	24									32	34	36	37	40
	26										34	36	37	40
	28											36	37	40
	30												37	40

Figure 15. Ambient room temperature (yellow) required to achieve the desired XFp Analyzer tray temperature setting (green).

Cartridge hydration and equilibration steps for temperatures 28 °C and below

Day prior to Assay:

Hydrate the XFp Cartridge at room temperature in a dark environment (protected from light) overnight.

Day of Assay:

The Utility Plate + Cartridge require 40 minutes of equilibration time inside the XFp when running at temperatures below 20 °C. Approximately 40 minutes before starting the assay:

1. Place the Cell Plate in an incubator at the desired XFp operational temperature.
2. Bring the hydrated Cartridge loaded with compounds and paired with its Utility Plate (with Calibrant solution) to the XFp Analyzer
3. Click **Diagnostics**, then **Click Maintenance**.
4. Press **Tray Out**.

5 Once the tray fully extends, remove the lid from the Cartridge and place the Utility Plate + Cartridge on the tray.

6 Press **Tray In**, then click the lower-left arrow twice to return to the Home screen.

7 Wait 40 minutes.

8 Click **Start** on the home screen, then select the **Assay Template** to run.

9 Click the lower-right arrow twice to navigate to the Summary screen, then click **Start Assay**.

10 The **Load Cartridge** message appears and the tray extends with the Utility Plate + Cartridge. **Do not** remove the Utility Plate + Cartridge (ensure the lid is removed from the Cartridge!).

11 Click **Continue** to start Calibration.

12 After completing Calibration, the XFp will prompt the user for the Cell Plate. Load the Cell Plate and begin assay.

Returning the XFp Analyzer to room temperature after cold room use

To use the XFp Analyzer at room temperature after use in the cold room, complete the following steps:

1 Unplug the analyzer and move it to the desired location.

2 Leave the instrument UNPLUGGED in the destination OVERNIGHT.

3 Resume normal usage.

   a **Adjust tolerance:** The temperature tolerance can be set from 0.2 °C – 9.9 °C. The tolerance defines the acceptable range the tray temperature may deviate from the target tray temperature.

   b **Temperature alarm:** When enabled, the XFp Analyzer will automatically notify users (**Figure 16** on page 30) when the tray temperature of the XFp Analyzer is out of the acceptable range as specified by the temperature tolerance above (only while running an assay on the XFp Analyzer).

      Example: At a target tray temperature of 37 °C and a tolerance of 0.3 °C, the acceptable tray temperature range is: 36.7 – 37.3 °C.

   c **Atmospheric pressure:** Customize the atmospheric pressure set on the XFp Analyzer to match that of the lab in which the instrument is located. This value must be entered manually, the local atmospheric pressure is not automatically detected by the XFp Analyzer.

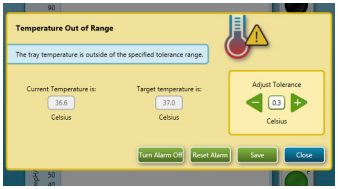


Figure 16. Temperature alarm window

Assay results

Assay Results contains a copy of each assay performed within the previous 60 days on the local storage within the XFp Analyzer. After 60 days, Assay Result files are deleted from the XFp Analyzer.

1 **Assay Results Menu:** Individual Assay Results can be viewed by clicking the small checkbox next to the result file to view and clicking the **View** button. See **Figure 17**.

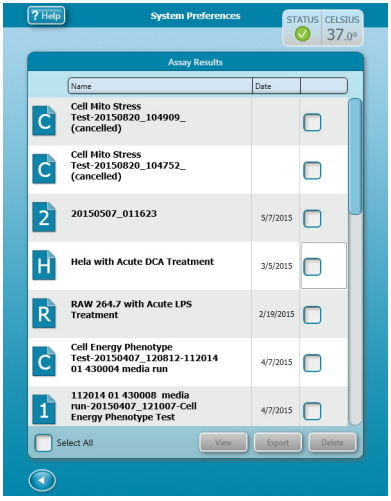


Figure 17. Assay results view

2 **Export Options:** Assay Result files may be exported to Microsoft Excel or GraphPad Prism file formats (requires a USB flash drive or shared network directory). See **Figure 18** on page 31.

- To export these files either individually or collectively to a network location or USB, click the individual check box or click **Select All,** and then click **Export**.

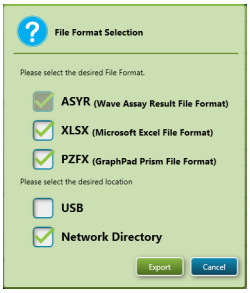


Figure 18. Export options dialog box

3 **Delete:** To remove Assay Result files from the XFp Analyzer, click the checkboxes next to all result files to remove, and click **Delete**.

Auto assay naming

Auto Assay Naming allows one to specify the default name for each Assay Result File (*.asyr) that is created on the XFp Analyzer. See **Figure 19** on page 32.

1 Adjust the order of each variable by dragging and dropping the selection in the order desired. **Figure 19** on page 32 shows the configuration (Template Name) – (Timestamp)

2 The **Custom** field can be used to add a keyword, instrument name, and so forth to all assay results files.

3 Click **Apply** to complete the process of setting up a custom name.

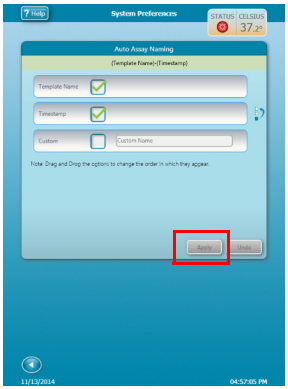


Figure 19. Auto assay naming window

Template management

Template Management provides a simple way to import or export Assay Template files to/from the XFp Analyzer.

Import assay template file(s):

1. First, transfer Assay Template file(s) to a USB flash drive or shared network directory location.
2. On the XFp Analyzer, select the location where the Assay Template file(s) will be imported from (insert the USB flash drive, then select the tab when it appears).
3. Check the box next to an individual assay template, or click **Select All** to select all the assay template files.
4. To import Assay Template files to the **Local** tab, click **Import**.
5. A successful Assay Template import results in an **Import Complete** message, click **OK**.

Export Assay Template File(s):

1. On the **Local** tab, click the checkbox next to the Assay Template file(s) to be exported.
2. Select the desired location to export the Assay Template file (USB flash drive or shared network directory).

3 A successful Assay Template export results in an **Export Complete** message, click **OK**.

Device settings

The Setup Assistant is a series of screens that guide configuration of network settings.

NOTE

Agilent recommends that the Device Settings be configured by the institution IT department using the "XFp Analyzer Network Setup" on page 43 as a reference.

- **Wired Network** – An ethernet cable is required to set up wired network access. The Ethernet cable port is located on the lower backside of the XFp Analyzer.

Figure 20. Wired network

- **Wireless Network** – A wireless USB adapter can be plugged into one of the available USB ports (recommended on lower back side of XFp Analyzer) to gain wireless access. Once finished, select an available SSID from the list and click **Connect**. If there are no visible networks available, click **Refresh**.

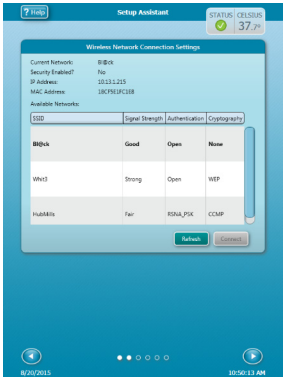


Figure 21. Wireless network

NOTE **The XFp Analyzer must be restarted after the Wireless USB adapter is plugged in.**

NOTE **Saving Assay Template or Result files to a shared network directory requires an active wired or wireless network connection.**

- **Email Configuration** – Configure an email account to automatically send 'Assay Complete' email notification as well as the Assay Result file to specified email addresses entered before starting an assay (see **"Review summary and start assay"** on page 20 for more information).
- **Time Zone** – Set the time zone for the XFp Analyzer. Once set, the correct time will be visible on the screen as well as when using the time stamp function for auto assay naming.
- **Date & Time** – Adjust the date or time. This can be done automatically by connecting to a time server (requires an active network connection) or set manually.

XFp Analyzer Diagnostics

The XFp Analyzer Diagnostics page contains a variety of functions that assist Cell Analysis Technical Support in debugging any issues or errors encountered during normal operation. The System Check diagnostic function should be performed upon initial startup of the XFp Analyzer and upon Cell Analysis Technical Support request.

To access the Diagnostics page, go to the Home page, then click **Diagnostics**.

Figure 22. Diagnostics page

The Diagnostics page has the following sections:

- System files
- Diagnostic tests
- Remote assistance
- Maintenance

System files

Cell Analysis Technical Support routinely requests that System Files be sent if an XFp Analyzer encounters an error. Click **Send** to collect the system files.

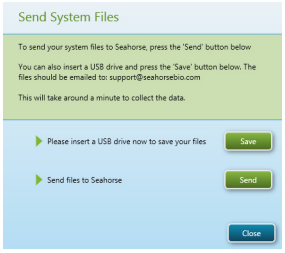


Figure 23. Send system files

Option 1 – Send System Files to a USB Flash Drive

Save System Files to a USB flash drive as a compressed (zipped) folder, which then can be attached to an email to Cell Analysis Technical Support.

NOTE **The file will be named: Diagnostic Report_Date_Time (Example: Diagnostic Report_2015_08_19_15_34_14.zip.**

Option 2 – Send System Files directly to Cell Analysis Technical Support

The Diagnostic Report compressed folder may also be emailed directly to Cell Analysis Technical Support from the XFp Analyzer. The XFp Analyzer must have an active wired or wireless network connection to directly send system files to Cell Analysis Technical Support.

Diagnostic tests

System check

Overall System

- **Communication Test** – Verifies all system modules are communicating appropriately.
- **Voltage Test** – Ensures voltage levels are within specified ranges for operation.
- **Motor Test** – Verifies the movements and positions of the tray and probes

- **Holding Pressure Test** – Ensures the injection system does not have any leaks.
- **Injection Zero Test** – Ensures function of the zero pressure sensor.
- **Injection Solenoid Test** – Verifies proper open/close operation of internal valves.

Measurement System

- **LED Reference Test** – Verifies system records LED intensity signal when ON.
- **Auto-zero Test** – Analyze and record any offset values found within the channels on each board.
- **Noise Test** – Analyze and record the amplitude of the noise level on each channel.
- **High-Gain Rise Time Test** – Conducts an amplifier rise time test.

Thermal Performance

- **Thermal Test** – Verifies that the temperature remains within tolerance after a "Wait" period.

Advanced Measurement

- **Drift Test** – Tests stability of each channel.
- **LED Drift Test** – Tests stability of each LED.
- **LED Noise Test** – Analyze the amplitude of the noise level of each LED when ON.

Consumable check

- **Barcode Test** – Verifies XFp Analyzer can read barcodes (requires user interaction to complete).
- **Calibration Test** – Performs Calibration procedure and will verify all wells are calibrated correctly.
- **Injection Test** – Verifies proper performance of Injection step.

Remote assistance

Cell Analysis Technical Support has the ability to remotely access each XFp Analyzer that has an active network connection.

Figure 24. Remote assistance window

1 To perform a remote session with Cell Analysis Technical Support (formerly Seahorse Bioscience), call the appropriate geographically located support line then click **Start**.

2 An ID and Password will be presented; Cell Analysis Technical Support will request this information to connect to the XFp Analyzer.

NOTE **To find a list of Cell Analysis Technical Support telephone numbers go to the Help page on the Home screen, see "Technical Support and Ordering Information" on page 61.**

Maintenance

The XFp Analyzer is designed to require minimal cleaning and maintenance. All consumables are disposable, and none of the instrument components are exposed to the cell plate at any time, preventing cross-contamination of biological or chemical materials. However, it may be necessary to perform some tasks.

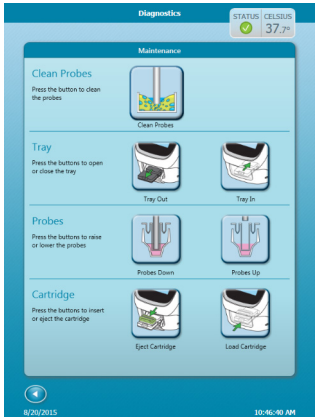


Figure 25. Maintenance window

- **Probe Cleaner** – Performed only by trained Agilent personnel.
- **Tray** – Press **Tray Out** to open the door and eject the tray. Press **Tray In** to return the tray inside the XFp Analyzer.
- **Probes** – Probe Movement will adjust the probes within the XFp Analyzer up or down.
- **Cartridge** – Use this function to **Eject** or **Load** a cartridge into the XFp Analyzer*.

NOTE **If a cartridge is suspected to be inside the XFp Analyzer, use this function to remove the cartridge before beginning another assay.**

Cleaning and Routine Maintenance

The XFp Analyzer is designed to require minimal cleaning and maintenance. All consumables are disposable and none of the instrument components are exposed to the cell plate at any time, preventing cross-contamination of biological or chemical materials.

Air filter replacement

At approximately one-year intervals, the air filter at the upper rear of the analyzer should be replaced. The filter retainer is held in place by magnets, and can be pulled away from the housing by hand. [Replacement filters can be ordered using part number: 102799-000]

Analyzing XFp Analyzer Data Using Wave Desktop

Wave Desktop is the assay design, data analysis and file management software for the Seahorse XFp Analyzer. Use Wave Desktop software to create new or customize existing assay template files. Wave Desktop also provides a standard set of graphing options and analysis views for data analysis and interpretation, and a one-click direct export of result data to the XF Report Generators.

Download Wave Desktop software:

https://www.agilent.com/en/products/cell-analysis/software-download-for-wave-desktop

Wave Desktop User Guide:

https://www.agilent.com/cs/library/usermanuals/public/Wave_2_6_User_Guide.pdf

Wave Desktop installation instructions (PC with Windows 7 OS or later):

https://www.agilent.com/cs/pubimages/misc/ReadMe_Wave_Desktop_2-6.pdf

5 XFp Analyzer Network Setup

This chapter provides network setup information for the Agilent Seahorse XFp Analyzer.

Introduction

Why should I network my XFp Analyzer?

Connecting your XFp Analyzer to a network provides unique workflow advantages to users, such as:

- Immediate acquisition of Assay Results.
- Simple File Transfer for Assay Templates and Results, no need for USB drives.
- Remote access for Cell Analysis Technical Support.

The XFp Analyzer Supports Two Types of Network Connections

- **Wide Area Network (WAN):** A computer network that covers a broad area, such as the internet.
- **Local Area Network (LAN):** Smaller computer network, typically found in offices or schools, which connects multiple devices (computers, printers, lab devices, etc) to facilitate file sharing between those networked devices in addition to internet access. LANs also have some level of security to access the file sharing directory.

Both a WAN and LAN can be configured through a wired (Ethernet cable) or wireless (USB WiFi adapter) connection on the XFp Analyzer. Depending on your company's IT infrastructure, configuring WAN or LAN access may require additional support from your local IT department to complete set up.

The **"Network Checklist"** on page 58 of this guide should be passed to your IT department to facilitate network setup on the XFp Analyzer.

Figure 26. Networked XFp Analyzer

Network access features on the XFp Analyzer

Immediate data delivery

Before starting an assay, users may enter in one or multiple email addresses to receive a copy of the Assay Result file (*.asyr) for data analysis. The email service also informs users when they can begin another assay.

File transfer made simple

Use a shared network directory to simplify data transfer between Wave Desktop and the XFp Analyzer. A shared network directory allows users to easily transfer Assay Template files to the XFp Analyzer to perform an assay, then retrieve the Assay Results for analysis on a personal computer.

Rapid remote assistance

An active network connection on the XFp Analyzer allows a Cell Analysis Technical Support representative to diagnose and troubleshoot potential issues quickly by allowing remote access to view and control the XFp Analyzer.

Send 'System Files' directly to cell analysis technical support

When an issue is encountered by a user, Cell Analysis Technical Support routinely requests System Files from the XFp Analyzer, which assist Cell Analysis Technical Support in identifying the root cause of an issue. With network access, users are able to send System Files directly to Cell Analysis Technical Support by clicking a single button.

XFp Analyzer System Information

Required Materials

- Approved Wireless USB Adapter or Wired Connection (ethernet cable)
- Network connection settings from IT Department

NOTE

XFp Analyzer instruments are shipped with Microsoft Security Essentials configured for Real Time protection and weekly scheduled virus scans using default actions for its four alert levels. Microsoft Security Essentials (MSE) is the default antivirus software on all XFp Analyzers. MSE is configured for real-time protection and weekly scheduled virus scans using default actions for its four alert levels. MSE will not scan media automatically upon connection/insertion. Modifications to MSE or installation of other antivirus software is not supported at this time.

- The XFp Analyzer can be connected to any Microsoft Windows compatible network and the Local Area connections can be configured as required by the network.
- Complete the **"Network Checklist"** on page 58 to ensure you have everything needed to successfully connect to a new network.
- The XFp Analyzer ships with an integrated 100Mbps Ethernet network adapter.
- The XFp Analyzer ships with a Netgear USB Wi-Fi Adapter (only the provided adapter is qualified for use). See **Figure 27** on page 48.

NOTE

The software required to use the USB WiFi Adapter is installed on the XFp Analyzer by default.

- There is no internal WiFi adapter in the XFp Analyzer instrument.
- The Ethernet (RJ-45) jack is located at the base of the instrument in the back. See **Figure 28** on page 48.

Part number 103203-000

Part number S7802-80000

Figure 27. USB WiFi Adapters for the XFp Analyzer

Figure 28. Ethernet (RJ-45) jack location outlined in red.

Wired Network Setup

Wired connection

1 Plug the wired network connection (Ethernet cable) into the Ethernet port (**Figure 29**), located on the back of the XFp Analyzer instrument. Ensure that the connection is firmly seated.

Figure 29. Wired network connection with Ethernet cable.

2 Power XFp Analyzer **OFF**.

3 Power XFp Analyzer **ON**.

4 Wait for the temperature to display in the upper right-hand corner of the home screen before proceeding with the Wired Setup instructions, see **"Option 2: Manual IP address assignment"** on page 50.

5 From the **Home** screen click the **Settings** button.

6 Click the **Go To Setup** button. This brings up the Wired Network Connection Settings screen.

There are two options depending on the network settings required by your facility's IT Department:

Option 1: Automatic IP address assignment (DHCP)

Dynamic Host Configuration Protocol (DHCP) is enabled by default on the XFp Analyzer and automatically uses a DHCP server on the network to retrieve IP address values. The XFp Analyzer is set to obtain the IP address and subsequent information (Subnet mask, Default gateway, etc.). If these fields do not automatically populate, follow the steps in **"Option 2: Manual IP address assignment"** on page 50 or contact your local IT administrator.

Option 2: Manual IP address assignment

1. Remove the green check from the **Obtain IP Address Automatically** checkbox.
2. Manually enter the information provided by the local IT department in each field.
3. After setting the IP options click the **Save** button
4. Power XFp Analyzer **OFF**.
5. Power XFp Analyzer **ON**.
6. Once the temperature in the upper right corner of the user interface shows 37 °C, click the **Settings** button, and then click **Go To Setup**.
7. To ensure you have a working connection, click the **Ping** button . A **Pass** message appears if the setting are working and the connection is active.
8. If the connection fails, reconfirm the IP address setting with the IT department, and confirm the information in the hardware setup steps.
9. To continue editing instrument settings or exit the settings options, use the left and right arrows.

Wireless Network Setup

Wireless connection

1 Plug the USB WiFi Adapter into one of the available USB ports on the back of the XFp Analyzer. See **Figure 30**.

2 Wait 1-2 minutes after installing the adapter, and ensure that the lights on the adapter are on or blinking.

3 Power XFp Analyzer **OFF**.

4 Power XFp Analyzer **ON**.

5 Wait for the temperature to display in the upper right hand corner of the home screen before proceeding with the Wireless Setup instructions.

Figure 30. USB WiFi Adapter inserted into an available USB port on the back of the XFp Analyzer.

Joining a wireless network

1 From the Home screen click the **Settings** button

2 Click the **Go To Setup** button.

3 Click the right arrow (bottom right) once. This will bring up the Wireless Network Connection Settings screen shown in **Figure 31** on page 52.

4 Select the network you want to connect to, and click **Connect**.

NOTE **If you do not see your wireless network on the list of available networks, click the Refresh button.**

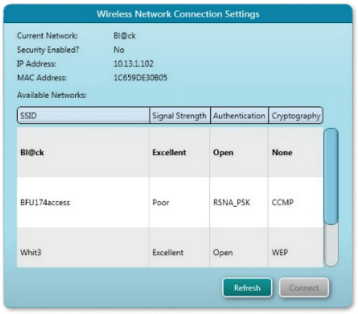


Figure 31. Example wireless network selection table.

5 Enter the password when prompted by the system.

6 If no errors are received, select the desired network again and verify that the connect button now displays disconnect.

7 Power XFp Analyzer **OFF**.

8 Power XFp Analyzer **ON**.

9 After the XFp Analyzer starts up and the temperature value in the upper right corner is displayed, click **Settings** then **Go To Setup**.

10 Click the **Ping** button in the **Wired Network Connection Settings** screen.

11 If you received a **Pass** message and icon, your wireless setup is complete. If not, confirm the information in the wireless networking setup steps and reattempt connecting to a wireless network.

12 Use the left and right arrows to continue editing instrument settings or exit the settings options.

NOTE **An active Internet connection is required for this feature.**

Shared folder setup

(Requires active wired or wireless network connection)

1 From the Home screen click **Settings**, then **Go to Setup**.

2 Click the right arrow (bottom right) two times. This brings up the **Network Directory** tab.

3 Enter the information for the shared network drive into the dialogue boxes. Refer to the **"Shared directory settings"** on page 58 in the Network Checklist for the correct information. (IT should provide this information.)

- **Shared Directory:** The desired location on the LAN where all Assay Template and Assay Result files will be saved and retrieved from.
- **Domain:** Name of the LAN.
- **User Name:** User name of the Windows account that has read/write permission to access the shared directory location.
- **Password:** Password for the Windows account above.

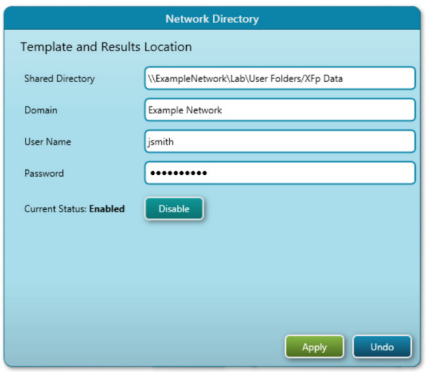


Figure 32. Example of a complete Network Directory configured on the XFp Analyzer.

NOTE **Accuracy is essential; double check your entry before checking access.**

4 Once all information has been entered, click **Enable**. Successfully configuring a LAN results in a **Success** message on the XFp Analyzer. (**Figure 33**)

Figure 33. Successful network connection status.

5 To continue editing instrument settings or exit the settings options, use the left and right arrows.

Email configuration

1 From the Home screen click **Settings**, then **Go to Setup**.

2 Click the right arrow (bottom right) three times. This brings up the **Email Configuration** tab (**Figure 34**).

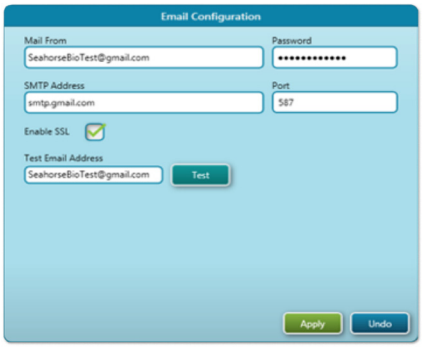


Figure 34. Example of a completed Email Configuration setup.

3 Enter the information below:

- **Mail From:** The email address that will send Assay Result files to email recipients.
- **Password:** Password for the email address account in the **Mail From** field.
- **SMTP Address:** Simple Mail Transfer Protocol for the email account in the Mail From field. See **Table 1** for example SMTP Addresses.
- **Port:** Contact your local IT Administrator for the correct port.

- **Enable SSL:** Typically required by email providers, SSL protects data transmission between devices.

4 Verify the email configuration is completed by entering an email address to send a test email message from the XFp Analyzer. Click **Test** once an email address has been entered into the field. If an email is not received, ensure the information provided is correct.

5 To exit the settings options, use the left and right arrows.

Table 1 Example SMTP addresses for common providers

Provider	URL	SMTP Settings
Comcast	Comcast.net	Smtp.comcast.net
Gmail	Gmail.com	Smtp.gmail.com
Outlook.com	Outlook.com	Smtp.live.com
Verizon	Verizon.net	Outgoing.verizon.net
Yahoo	Yahoo.com	Mail.yahoo.com

Time can be set manually, or if you are connected to a network, it can be auto set. First, you must choose a Time Zone.

Time zone setup

1 From the Home screen click **Settings**, then click **Go to Setup**.

2 To navigate to the Time Zone settings (**Figure 35** on page 56), click the lower-right arrow four times.

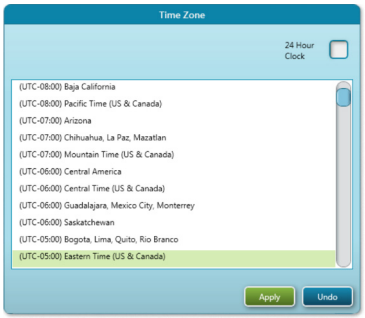


Figure 35. Select the local time zone for your XFp Analyzer.

3 Select the time zone from the list provided.

Optional: Check the box 24 Hour Clock to activate the 24 hour clock setting.

Setting the time

1 From the Home screen click **Settings**, then click **Go to Setup**.

2 To navigate to the Date &Time settings (**Figure 36**), click the lower-right arrow five times.

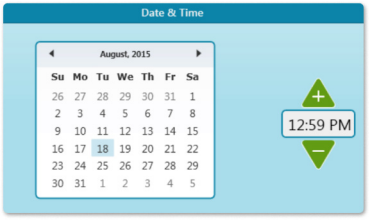


Figure 36. Configure date and time for the XFp Analyzer.

3 Manually enter the appropriate date and time (**Figure 36**).

Time server (optional)

For XFp Analyzers with an active network connection, the XFp Analyzer can sync to a network's time server, if provided (**Figure 37** on page 57).

Figure 37. Configuring a time server on the XFp Analyzer.

Optional: A national time server (example: time.nist.gov) can be used to automatically set the time and account for daylight savings adjustments, or any time server provided by your local IT department. Once entered, click **Sync**, then click **Apply** for the changes to take place. Use the left and right arrows to exit the settings options.

Network Checklist

Only complete the section that pertains to the desired network connection type.

Wired network settings

- IP Address
- Subnet mask
- DNS Server
- Computer Name

Wireless network settings

- Wireless Network Name
- Wireless Network Password

Email settings

- Email Address
- Password
- SMTP Address
- Email Port
- SSL Required?

Shared directory settings

- Shared Directory Address
- Shared Directory Domain
- Shared Directory User Name
- Shared Directory Password
- Time Server Address

6 Support

This chapter provides support and troubleshooting information for the Agilent Seahorse XFp Analyzer.

Troubleshooting Guide

Problem	Possible Cause	Solution
Slow response, particularly when selecting an Assay Template or Result file from a shared network directory.	An incomplete or broken network connection	• Click **Settings**. • Click **Go to Setup** on the Settings page. • Browse to the Network Directory page. • Click **Disable**. When the button changes to Enable, click **Enable**. • If (after a brief delay) a green "OK" icon appears next to the Enable/Disable button then the network connection is functional. • If a "Fail" icon appears, contact your local IT administrator as there may be a network connectivity issue.
Instrument gives a "load error message" and it is not clear whether a cartridge has already been loaded		• Click **Diagnostics** from Home Screen. • Click **Maintenance**. • Click **Eject Cartridge.** • The XFp Analyzer will eject a cartridge if one is left inside the instrument.
Intermittent Barcode Read Errors	USB Wi-Fi Adapter plugged into back USB port not actively being used	• Unplug the USB WiFi Adapter from the XFp Analyzer and store in a safe location.
Low Rates	Not enough cells	• Consult the **Cell Reference Database** to identify a working range and/or perform a dilution series of your cell type. The wells of the XFp miniplate are the same dimensions as those of the XFe96 standard plate.
"An error has occurred" window pops up and displays a message saying "A Tasks Exception(s) were not observed."	Instrument was not shut down properly. See **"Turning the XFp Analyzer Off"** on page 16.	• Contact Technical Support. See **"Technical Support and Ordering Information"** on page 61.

Technical Support and Ordering Information

Worldwide technical support

For questions about XF technology, the XFp 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**
UK:	0800 096 7632
Germany:	0800 180 66 78
Netherlands:	0800 022 7243
Other EU countries:	+45 3136 9878
Other countries:	Visit **https://www.agilent.com/en-us/contact-us/page** for country specific contact information.

Ordering

Link to online store: **https://www.chem.agilent.com/store/**

US Direct Ordering:

- Email: css_afo_fax@agilent.com
- Phone: 1.800.227.9770 option #1 #1
- Fax Purchase Orders to: 302.633.8901

Europe:

Contact your local Customer Care Center
https://www.agilent.com/en-us/contact-us/page

Online help and support

To access the Help page, go to the Home screen then click **Help**.

Figure 38. Help window

Support

Worldwide Agilent Support contact information.

Version of Software/Hardware

Currently installed software and firmware versions on XFp Analyzer are displayed on this screen. If the XFp Analyzer is networked, Software Updates (Product Update Available) or Firmware Updates (Firmware Update Available) will be selectable.

"Help" Text Info

Most screens on the XFp Analyzer have a small **Help** button that will display Help text related to the current screen. See **Figure 39**.

Figure 39. Help button

Additional Resources

Wave User Guide:	**https://www.agilent.com/cs/library/usermanuals/public/Wave_2_6_User_Guide.pdf**
Wave Desktop Download Page:	**https://www.agilent.com/en/products/cell-analysis/software-download-for-wave-desktop**
XFp Analyzer Release Notes and latest Software Downloads:	**https://www.agilent.com/en/products/cell-analysis/software-download-for-seahorse-xfp-analyzer**
XFp Consumables Web Page:	**https://www.agilent.com/en/products/cell-analysis/seahorse-xfp-consumables**
Cell Reference Database:	**https://www.agilent.com/cell-reference-database/**
Links to other useful information:	**https://www.agilent.com/en/promotions/cell-analysis-technology**

www.agilent.com

First Edition, November 2018

S7802-90001_REV A