Agilent

Seahorse XF HS Mini Extracellular Flux Analyzer

User Manual

Notices

Manual Part Number

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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 Agilent Seahorse XF HS Mini Analyzer requires all covers to be securely attached and the plate tray door to be 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. Exercise caution when loading the miniplate/cartridge to avoid the possibility of a pinch hazard. After the miniplate/cartridge is placed securely on the tray, the remove your hand from the tray area 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 XF HS Mini Analyzer has heaters around the miniplate that maintain a stable tray temperature. The tray temperature is maintained at 37 °C, and monitored by temperature sensors and controllers embedded above the tray. A thermal fuse disables the heater when it reaches an abnormally high tray temperature.

Do 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 9. This depends on the power mains of the country where the instrument will be used.

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

The XF HS Mini Analyzer measures the rate of change in dissolved oxygen and pH in the media immediately surrounding living cells cultured in a miniplate. Changes in the extracellular media are caused by cell consumption or production of analytes. A sensitive measurement of the media flux can be used to determine rates of cellular metabolism with precision and in a 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 µL) of media above the cell monolayer. Cellular metabolism causes rapid, easily measured changes to the microenvironment in this small volume.

A measurement cycle is performed in six minutes. The media is gently mixed and the analyte levels are then measured until the oxygen concentration drops approximately 20 to 30%, and media pH declines approximately 0.1 to 0.2 pH units. The measurement is performed using optical fluorescent biosensors embedded in a disposable cartridge. The cartridge is placed into an 8-well cell culture miniplate.

Baseline metabolic rates are typically measured three to four times, and are reported in pmol/min for oxygen consumption rate (OCR) and in mpH/min for extracellular acidification rate (ECAR). The 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 XF HS Mini Analyzer system includes:

- Benchtop analyzer
- Disposable sensor/compound
- Delivery cartridges
- Tissue-culture treated miniplates for seeding cells for analysis
- Calibration solution
- Seahorse Analytics for XF data analysis

Consumables are sold separately and include FluxPaks (sensor cartridges, cell plates, and calibrant) as well as various assay kits, reagents, and media. Seahorse XFp Miniplates and XF HS Miniplates are compatible with the XF HS Mini Analyzer. Seahorse XF miniplates and sensor cartridges are available exclusively from Agilent.

Technical Specifications

REF S7852A

Model	Seahorse XF HS Mini Analyzer (model number S7852A)
Dimensions	Width × height × depth 12 in × 17 in × 23 in 30 cm × 43 cm × 58 cm
Weight	33 lb/15 kg
Power requirements	100-240 VAC 50/60 Hz 6 A/3 A
Power cord rating	3-wire (grounded) AC power cord rated 10 A or greater
Power fuse ratings	250 V/5 A time lag (two fuses) 5 mm × 20 mm
Environmental operating range	+39 °F - 86 °F/+4 °C - 30 °C No direct sunlight Humidity 20 to 70% RH, noncondensing
Sample temperature and environment	Controlled to user-selected temperature between 16 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 10 embedded
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 HS Mini Analyzer.

Unpacking and Component Identification

After receiving the package, immediately check each box for damage. Report any shipping damage to the transportation company, and Agilent using **"Technical Support and Ordering Information"** on page 56. **Table 1** shows the Seahorse XF HS Mini Analyzer system components.

Table 1 Seahorse XF HS Mini Analyzer system components

Instrument/Controller	Quantity	Image
XF HS Mini Analyzer	1	
Power cord (region specific)	1	
Wireless Micro USB adapter (p/n S7802-80000)	1	

Suitable Locations for the XF HS Mini Analyzer

The XF HS Mini Analyzer is designed for laboratory use. The internal environment for the XF HS Mini Analyzer cell culture miniplate is controlled to a preset tray temperature. The laboratory room temperature must therefore be maintained within the range listed in **"Technical Specifications"** on page 9. The miniplate tray temperature can be monitored using the status display in the upper right portion of the user interface.

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

3 Basic Operations

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

Power and Warm Up

Power up

To power on the XF HS Mini Analyzer:

1 Press the power switch on the back of the instrument. (**Figure 1**)

Figure 1. XF HS Mini Analyzer power switch.

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

3 Check the status icon in the upper right corner of the screen. When ready the tray temperature and a green check mark is displayed.

Welcome screen on first time power up

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

Run these tests at any time using the Diagnostics menu selection on the instrument user interface. For more details, see **"Navigating the XF HS Mini Analyzer"** on page 15.

XF HS Mini Analyzer Communications and Network Connection Setup

The USB connectors on the instrument transfer template and result files between the XF HS Mini Analyzer and a computer running Seahorse Analytics. Agilent recommends connecting the XF HS Mini 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, **"Worldwide technical support"** on page 56, with any questions.

For instructions on setting up a wired network connection, see **"Wired Network Setup"** on page 47. For instructions on setting up a wireless network connection using the included wireless adapter, see **"Wireless Network Setup"** on page 48.

NOTE

Only the included wireless adapter has been qualified to work with the XF HS Mini Analyzer.

Turning the XF HS Mini 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 XF HS Mini Analyzer screen. This action turns off the HS Mini operation system as well as associated software. After the screen turns black, use the power switch on the back of the instrument to completely shut down power. Do not turn off the power switch on the back of the instrument first.

CAUTION

The shutdown procedure described above must be followed by all users. Improper shutdown of the XF HS Mini Analyzer can result in data or operating system corruption that may require service to correct.

4 Navigating the XF HS Mini Analyzer

This chapter provides navigation information for the Agilent Seahorse XF HS Mini Analyzer.

Setting Up an XF HS Mini 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 XF HS Mini Analyzer through a network connection or USB flash drive.

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

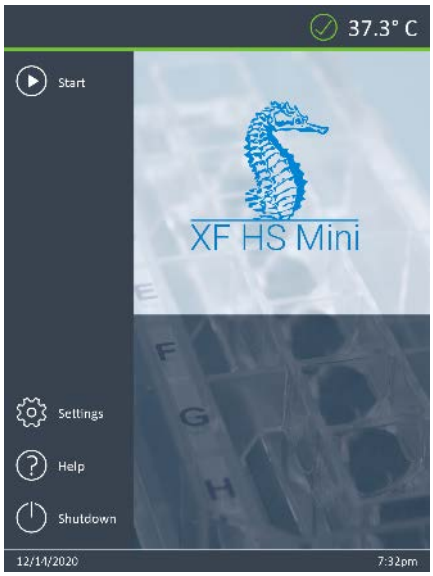


Figure 2. Home screen.

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

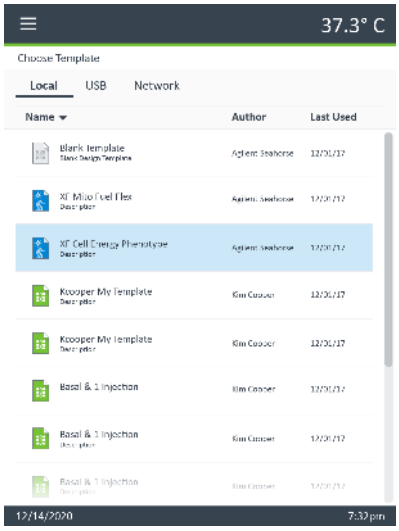


Figure 3. Local tab.

3 Templates designed in Wave can be transferred to the XF HS Mini Analyzer through a USB or network location.
   - If the instrument is networked, click the **Network** tab, select and run the template from the networked location.
   - If a USB drive containing a valid XF HS Mini Analyzer template is inserted into the instrument, click the **USB** tab, select and run the template directly from the USB drive.

NOTE

Only XF HS Mini Analyzer assay template files (*.asyt) are recognized by the XF HS Mini 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, click 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, click the group name followed by the well or wells to be included.

3 For all other changes to **Groups**, use Seahorse Analytics.

Review/edit instrument protocol

1 Check that all desired steps are selected, check boxes checked (**Figure 5**). Agilent strongly recommends performing equilibration for all cell-based XF assays.

2 If needed, increase or decrease the number of measurements that will be performed during the assay on the **Instrument Protocol** page.

a Click the number in the cycle column that corresponds with the step to modify.

b Use the arrows to adjust the number of cycles up or down. See **Figure 5**.

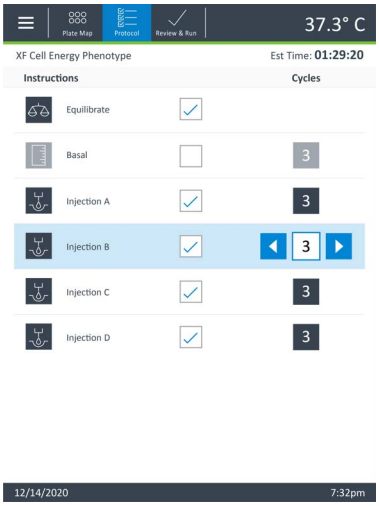


Figure 5. Instrument protocol page.

Review summary and start assay

1 Review the **"Safety Considerations"** on page 5 to verify the desired settings.

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

a Click the **Assay Result File Name** text box to customize the name of the assay result file. See **Figure 6**.

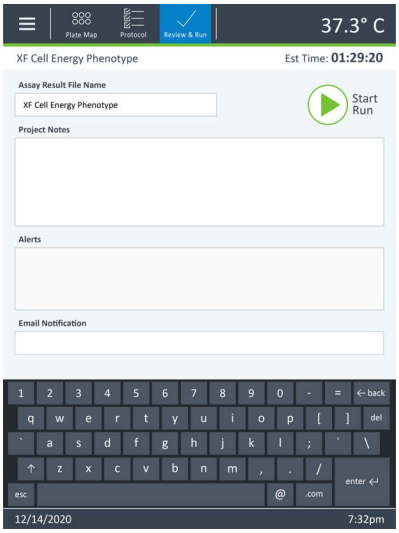


Figure 6. Assay name.

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

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

3 Click **Start Run** then follow the onscreen instructions.

NOTE

The XF HS Mini 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 beginning of assay setup so that results will automatically be saved. If the XF HS Mini 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 Assay with the XF HS Miniplate

The XF HS Mini Analyzer is equipped to analyze samples on the XFp Miniplates and XF HS Miniplates. XFp Miniplates' well bottom area is 11.40 mm^2 while XF HS Miniplates' well bottom area is 3.05 mm^2. The XF HS Miniplates are useful when cell quantity is limited. The plates provide a smaller microchamber, increasing system sensitivity by about 3-fold (The actual improvement in sensitivity may vary by cell type). Agilent recommends using anywhere from 25 to 50% the quantity of cells one might use on an XFp Miniplate.

The XF HS Miniplate consists of an 8-well, tissue culture treated wellplate with a "ring" feature on the bottom surface, a silicone cell-seeding mask, and a mask removal tool. The mask removal tool is reusable.

The XF HS Miniplate was designed to minimize the cell seeding area while remaining compatible with the XF HS Mini Analyzer architecture and Seahorse 8-well sensor cartridges. Plates precoated with Poly-D Lysine are also available, allowing for both adherent and suspension cell models to be used. This protocol will describe a basic overview of how to run an assay with the XF HS Miniplate. Detailed cell seeding protocols may be found online at, **https://www.agilent.com/en/products/cell-analysis/how-to-run-an-assay**.

Running an XF HS Mini Assay

1 Load the cartridge (hydrated and loaded with compounds) and utility plate onto the tray when prompted.

2 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**.

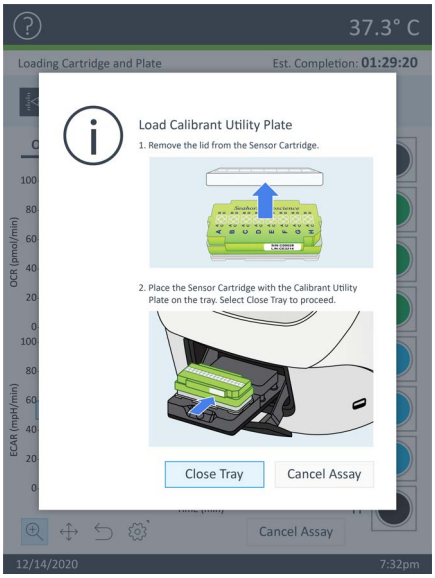


Figure 7. Load cartridge screen.

3 The XF HS Mini Analyzer will perform calibration of the sensor cartridge. This takes approximately 20 minutes.

NOTE

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

4 The XF HS Mini 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 before loading onto the XF HS Mini Analyzer tray.

5 If using an XF HS Miniplate, a dialogue will be presented, providing a reminder to remove the silicone mask from the plate prior to reading. See **Figure 8**. The silicone mask must be removed to prevent any interference with the sensor cartridge, and possible instrument damage. Confirm that the silicone mask has been removed by clicking **Continue** to begin the equilibration step. The progress bar in the upper-left area of the screen displays the progress of the equilibration step.

Figure 8. Silicone insert removal prompt.

6 Click either the **Overview** or **OCR vs. ECAR** tab to toggle between the views during the assay. See **Figure 9** on page 22. Overview and OCR versus ECAR are the two run-time views available as data is acquired by the XF HS Mini Analyzer.

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 9**.

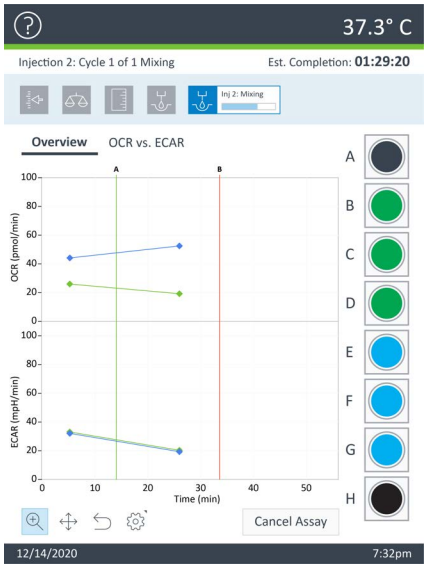


Figure 9. Overview tab.

In this view the charts can be zoomed and scaled by choosing one of the tools below the charts:

- Zoom
- Move
- Reset
- Options. By default, rate data is displayed in **Group** mode. Click the Options icon to change display modes and turn on error bars. See **Figure 10**.

Figure 10. Options icon

- **Show StdDev** turns on display of error bars for 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 versus 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 versus ECAR using the **Measurement** selector below the chart in **Figure 11**. Data is 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 11**.

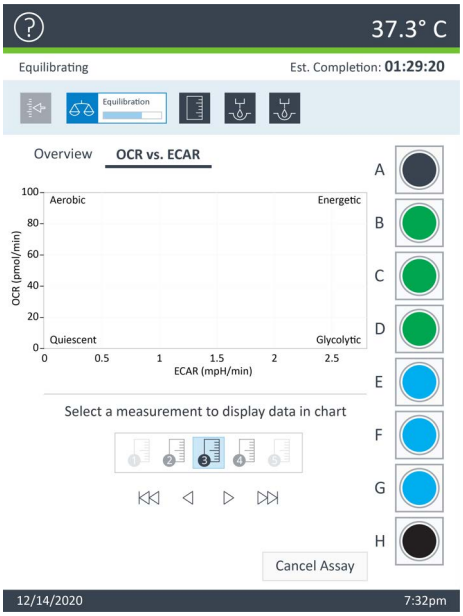


Figure 11. OCR vs. ECAR tab

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.

7 Once the assay has finished, the XF HS Mini Analyzer prompts the user to eject the cartridge and remove the cartridge and cell plate. See **Figure 12**.

Figure 12. Remove cartridge and cell plate prompt.

8 After the assay is complete, the data is automatically 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 (*.xflr). See **Figure 13**.

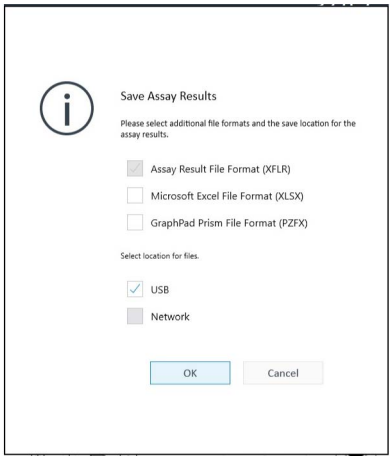


Figure 13. Save results.

NOTE

Canceling during an assay may cause the XF HS Mini Analyzer to stall. If this occurs, shut down the instrument by pressing the power switch on the back. Use the same power switch to turn the instrument ON.

9 After the assay is complete, data is 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 XF HS Mini Analyzer as well. Check the box next to the desired file formats and click **OK** (**Figure 13**).

Assay result files saved locally on the XF HS Mini Analyzer will be stored for 60 days after the assay. Saving assay result files to a USB or network drive is encouraged.

Modifying XF HS Mini Analyzer Settings

From the Start page, click **Settings** to access the XF HS Mini Analyzer settings, see **Figure 14**. The XF HS Mini Analyzer settings page contains:

- System Files
- Diagnostic Tests
- System Settings
- Assay Results
- Template Management

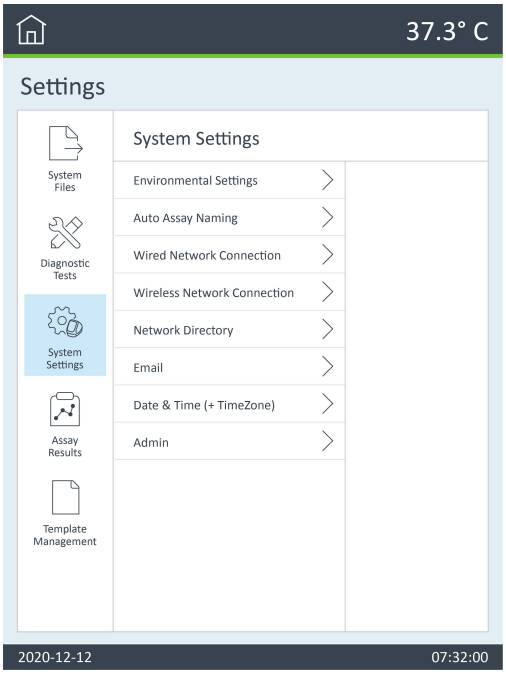


Figure 14. XF HS Mini Analyzer settings page.

XF HS Mini Analyzer system files

System files

To access the **System Files** page, go to the Home page and select **Settings > System Files**. (**Figure 15**) Cell Analysis Technical Support routinely requests that System Files be sent if an XF HS Mini Analyzer encounters an error.

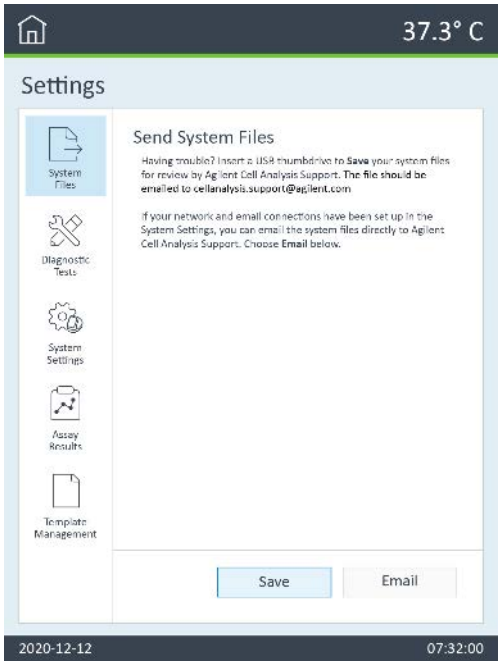


Figure 15. Send system files.

Option 1 - Send system files to a USB flash drive

Save System Files to a USB flash drive as a compressed (zipped) folder. This can then be attached to an email to Cell Analysis Technical Support. **Save** will become active after a USB is connected to one of the three USB ports.(**Figure 15**)

NOTE

The file will be named: Diagnostic Report_Date_Time (Example: Diagnostic Report_2020_05_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 XF HS Mini Analyzer. The XF HS Mini Analyzer must have an active wired or wireless network connection to directly send system files to Cell Analysis Technical Support.

Diagnostics tests

The XF HS Mini Analyzer **Diagnostic Tests** page contains various functions that assist Cell Analysis Technical Support in debugging any issues or errors encountered during normal operation. Perform the **System Check** diagnostic function at the initial start of the XF HS Mini Analyzer and when Cell Analysis Technical Support request.

To access the **Diagnostic Tests** page, go to the Home page and select **Settings > Diagnostic Tests**. (**Figure 16**)

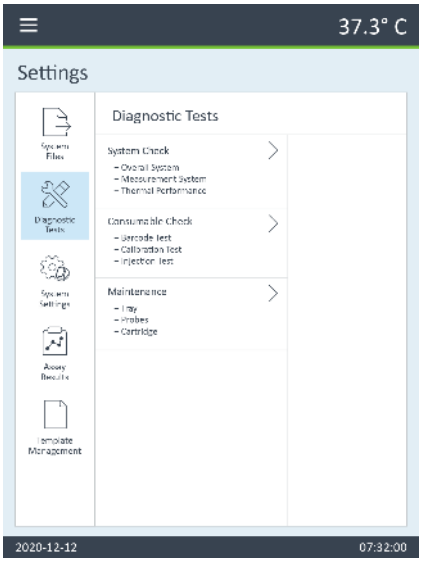


Figure 16. Diagnostic tests.

The Diagnostic Tests page has the following sections:

- System Check
- Consumable Check
- Maintenance

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 that the system records LED intensity signal when ON.
- **Autozero 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** - Conduct an amplifier rise time test.

Thermal performance

- **Thermal test** - Verifies that the temperature remains within tolerance after a “Wait” period.

Consumable check

- **Barcode test** - Verifies XF HS Mini Analyzer can read barcodes (requires user interaction to complete).
- **Calibration test** - Performs calibration procedure and will verify that all wells are calibrated correctly.
- **Injection test** - Verifies proper performance of injection step.

Maintenance

The XF HS Mini 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 17**)

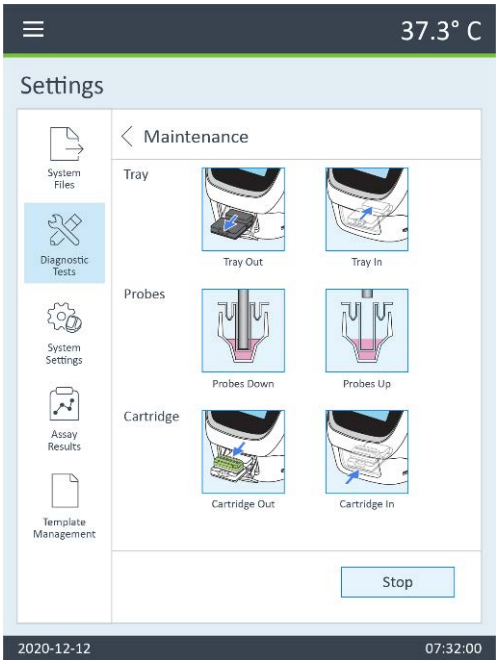


Figure 17. Maintenance window.

- **Tray** - Press **Tray Out** to open the door and eject the tray. Press **Tray In** to return the tray inside the XF HS Mini Analyzer.
- **Probes** - Probe movement will adjust the probes within the XF HS Mini Analyzer up or down.
- **Cartridge** - Use this function to take a **Cartridge Out** or put a **Cartridge In** the XF HS Mini Analyzer.

NOTE

If a cartridge is suspected to be inside the XF HS Mini Analyzer, use this function to remove the cartridge before beginning another assay.

System settings

The XF HS Mini Analyzer System Settings page contains various functions that permit assay customizations and IT integration options.

To access the **System Settings** page, go to the Home page and select **Settings > System Settings**. (**Figure 19**)

The **System Settings** page has the following sections:

- Environmental Settings
- Auto Assay Naming
- Wired Network Connection
- Wireless Network Connection
- Network Directory
- Email
- Date and Time (plus TimeZone)
- Admin

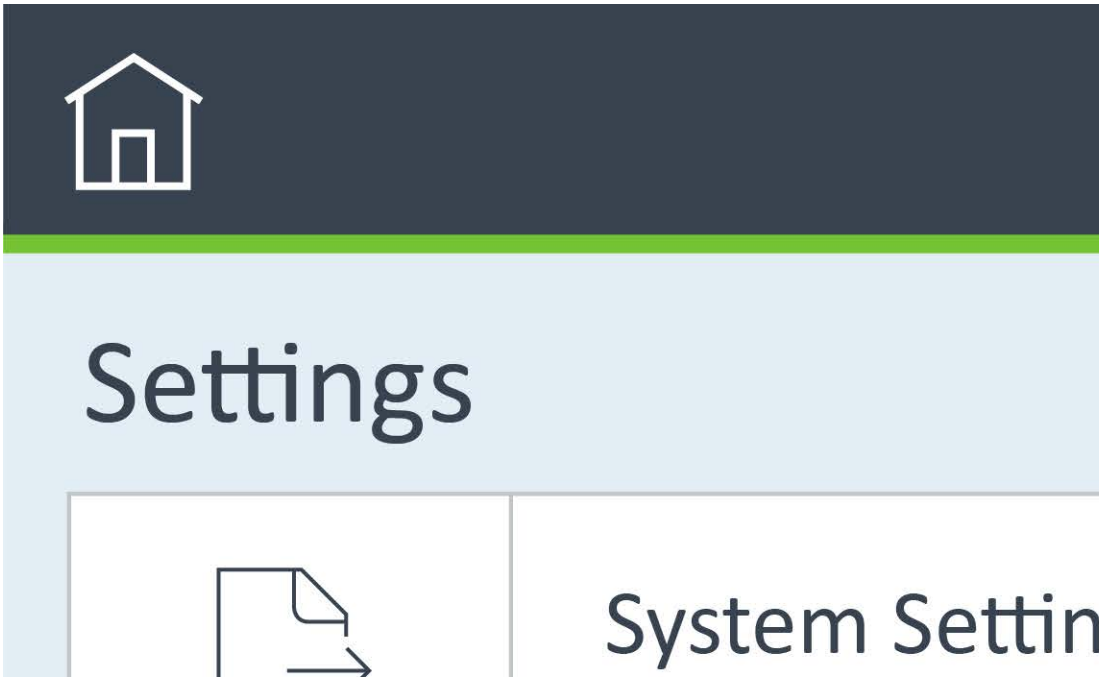


Figure 18. System Setting page.

Environmental settings

Temperature target: Adjust the tray temperature on the XF HS Mini Analyzer. See **Figure 19**.

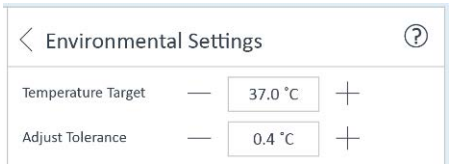


Figure 19. Temperature target setting.

Temperature Control on XF HS Mini Analyzer: Users may adjust the tray temperature of the XF HS Mini Analyzer for a wider array of assays and applications. The validated tray temperature range of the XF HS Mini Analyzer is 16 to 40 °C. See **Figure 20** for complete details of the environmental settings required to accurately achieve the desired tray temperature setting.

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

- Lower tray temperatures require the instrument to be placed in a cold room or refrigerated unit that is 8 to 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 20** 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 20. Ambient room temperature (yellow) required to achieve the desired XF HS Mini Analyzer tray temperature setting (green).

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

Day before assay:

Hydrate the 8-well sensor cartridge In a non-CO_2 37 °C incubator overnight.

Day of Assay:

The utility plate + cartridge require 40 minutes of equilibration time inside the XF HS Mini 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 XF HS Mini operational temperature.
2. Bring the hydrated cartridge loaded with compounds and paired with the utility plate (with calibrant solution) to the XF HS Mini 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 and 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 and cartridge. *Do not* remove the utility plate and cartridge (but ensure the lid is removed from the cartridge).
11. Click **Continue** to start calibration.
12. After completing calibration, the XF HS Mini will prompt the user for the cell plate. Load the cell plate and begin assay.

Returning the XF HS Mini Analyzer to room temperature after cold room use

To use the XF HS Mini 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 use.
   a. **Adjust tolerance**: The temperature tolerance can be set from 0.2 to 9.9 °C. The tolerance defines the acceptable range in which the tray temperature may deviate from the target tray temperature.
   b. **Temperature alarm**: When enabled, the XF HS Mini Analyzer will automatically notify users (**Figure 21** on page 32) when the tray temperature of the XF HS Mini Analyzer is out of the acceptable range, as specified by the temperature tolerance above (only while running an assay on the XF HS Mini 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 to 37.3 °C.
   c. **Atmospheric pressure**: Customize the atmospheric pressure set on the XF HS Mini Analyzer to match that of the lab where the instrument is located. This value must be entered manually, the local atmospheric pressure is not automatically detected by the XF HS Mini Analyzer.

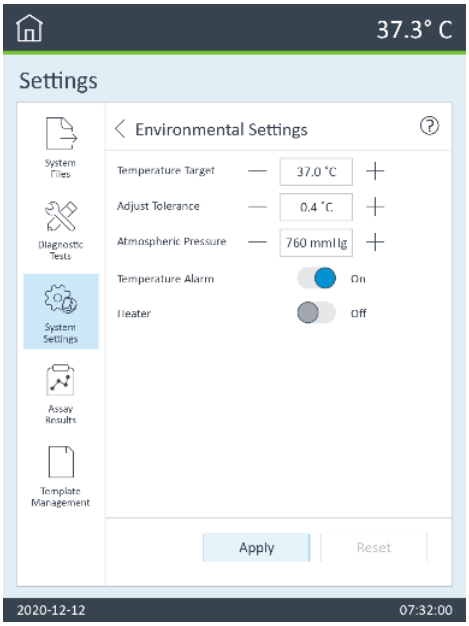


Figure 21. Temperature alarm window.

Auto assay naming

Auto assay naming allows users to specify the default name for each Assay Result File (*.xflr) that is created on the XF HS Mini Analyzer. See **Figure 22**.

1. Adjust the order of each variable by dragging and dropping the selection in the order desired. **Figure 22** shows the configuration (template name) - (Timestamp).
2. The **Custom** field can be used to add a keyword, instrument name, or other items to all assay results files.
3. Click **Apply** to complete the process of setting up a custom name.

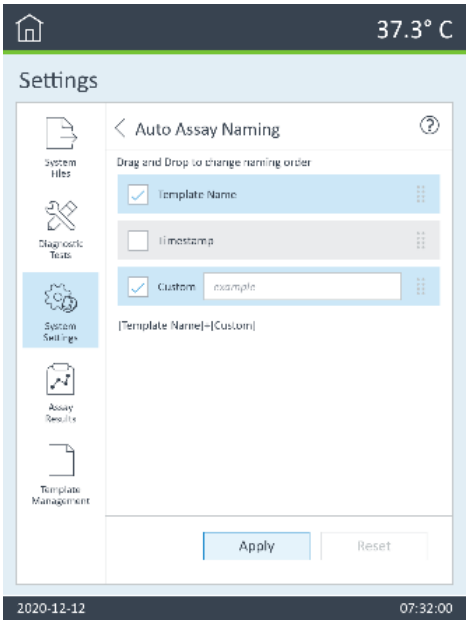


Figure 22. Auto assay naming window.

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 XF HS Mini Analyzer.

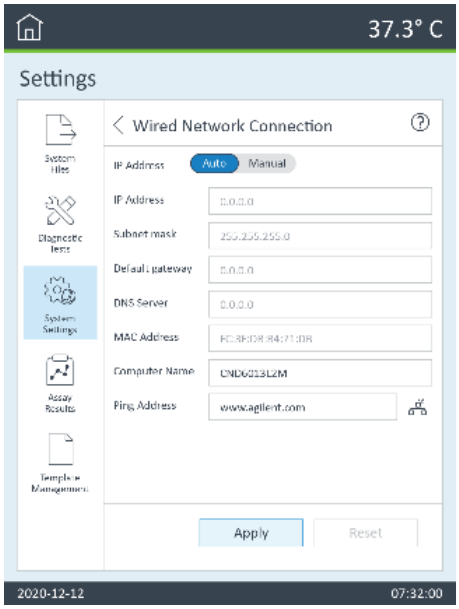


Figure 23. Wired network.

NOTE

Agilent recommends that the device settings be configured by the institution IT department using the "XF HS Mini Analyzer Network Setup" on page 43 as a reference.

Wireless network

A wireless USB adapter can be plugged into one of the available USB ports (recommended on lower back side of XF HS Mini 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**.

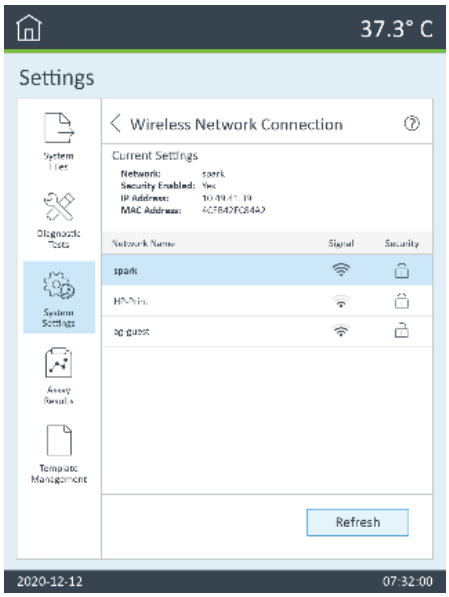


Figure 24. Wireless network.

NOTE

The XF HS Mini 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.

Network directory

Network configuration is covered in **"XF HS Mini Analyzer Network Setup"** on page 43.

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 18 for more information).

Date and Time (plus Time Zone)

Adjust the date or time. This can be done automatically by connecting to a time server (requires an active network connection) or set manually.

Set the time zone for the XF HS Mini 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.

Admin

Exit and re-enter Kiosk mode to perform Windows Updates or install security software such as Trend Micro's Safe Lock application. The Using Safe Lock section provides the steps to reach the Windows desktop and return to XF HS Mini application.

Assay results

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

To access the **System Settings** page, go to the Home page, then select **Settings > Assay Results**.

1 **Assay results**: Individual assay results can be viewed by clicking the small check box next to the result file to view and click **View**. See **Figure 25**.

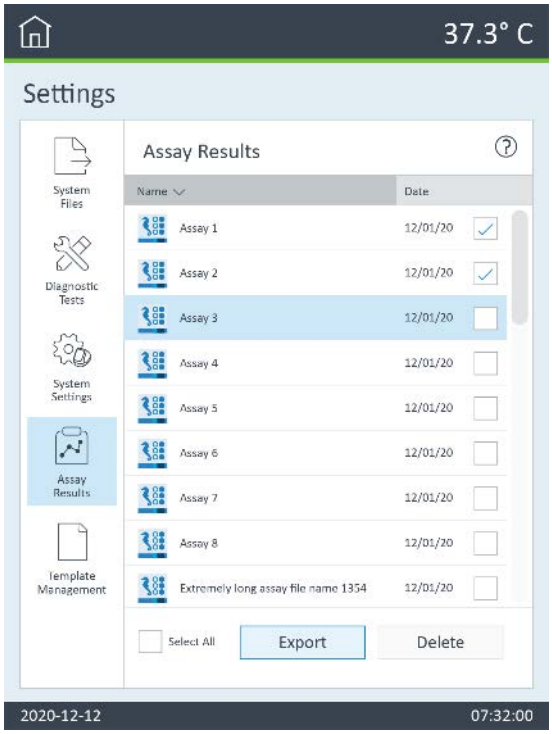


Figure 25. Assay results view.

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 26**. 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**.

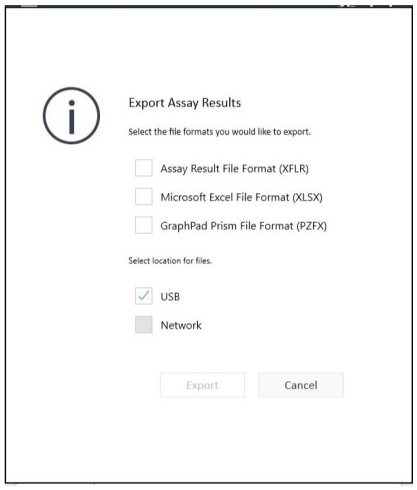


Figure 26. Export options dialog box.

3 **Delete**: To remove assay result files from the XF HS Mini Analyzer, click the check boxes next to all result files to remove, and click **Delete**.

Template management

Template management provides a simple way to import or export assay template files to/from the XF HS Mini Analyzer.

Import assay template file (or files):

1 First, transfer assay template file to a USB flash drive or shared network directory location.

2 On the XF HS Mini Analyzer, select the location where the assay template file 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 files:

1 On the **Local** tab, click the check box next to the assay template file to be exported.

2 Select the desired location to export the assay template file (USB flash drive or shared network directory).

3 If the assay template has exported successfully, an **Export Complete** message will appear. Click **OK**.

Help

From the Home page Click **Help**. The Help page has the following sections:

- Support (see **"Support"** on page 55)
- Software version (see **"Support"** on page 55)
- Getting started
- License agreement (see **"Support"** on page 55)
- Remote assistance

Getting started

Provides useful tips and information regarding the setup and operation of the XF HS Mini.

Remote assistance

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

To access the Remote Assistance page, go to the Home page, then select **Help > Remote Assistance**. See **Figure 27** on page 37.

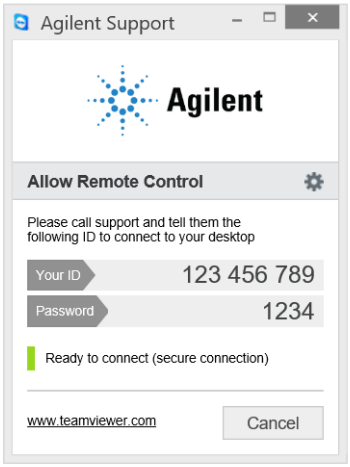


Figure 27. Remote assistance window.

1 To begin a remote session with Cell Analysis Technical Support, 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 XF HS Mini Analyzer.

Using Safe Lock (Trend Micro)

The computer security application Safe Lock by Trend Micro has been validated on the XF HS Mini Analyzer. Safe Lock provides several levels of security while allowing the XF HS Mini Analyzer to perform its functions. More information can be found at www.trendmicro.com. To reach the Windows 10 desktop to install application, follow the steps below:

1. From the Home page tap **Settings > System Settings > Admin**.
2. Click **Exit Kiosk Mode**.
3. The Windows 10 desktop is now available
4. Follow the installation instructions for the Safe Lock application
5. Once all the steps and configurations are complete, double-click the **Exit Maintenance Mode** icon on the XF HS Mini Desktop
6. The system will reboot and start the XF HS Mini controller application

Cleaning and Routine Maintenance

The XF HS Mini 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.)

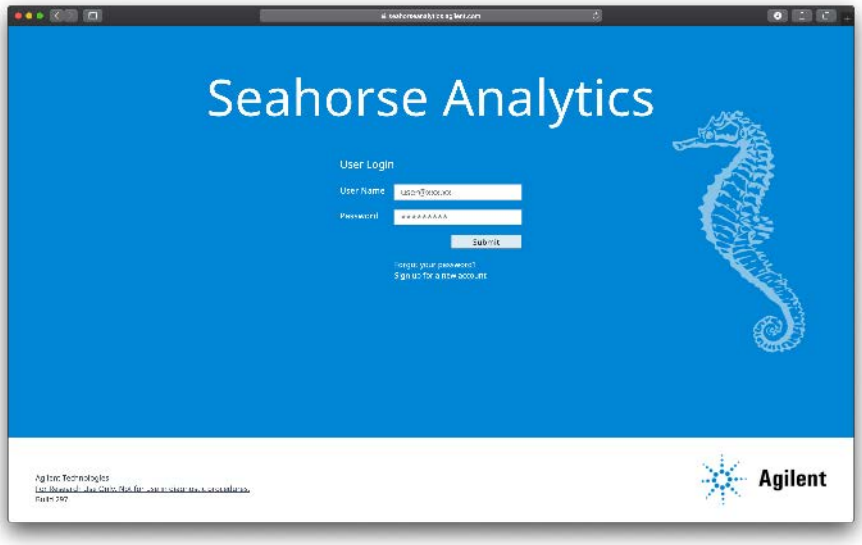
Analyzing XF HS Mini Analyzer Data Using Seahorse Analytics

Agilent Seahorse Analytics is a web-based analysis and file management software application for XF HS Mini assay data. Use the integrated assay kit companion analysis views to quickly report and share result data, or create a custom analysis view using the 30+ graphing options available.

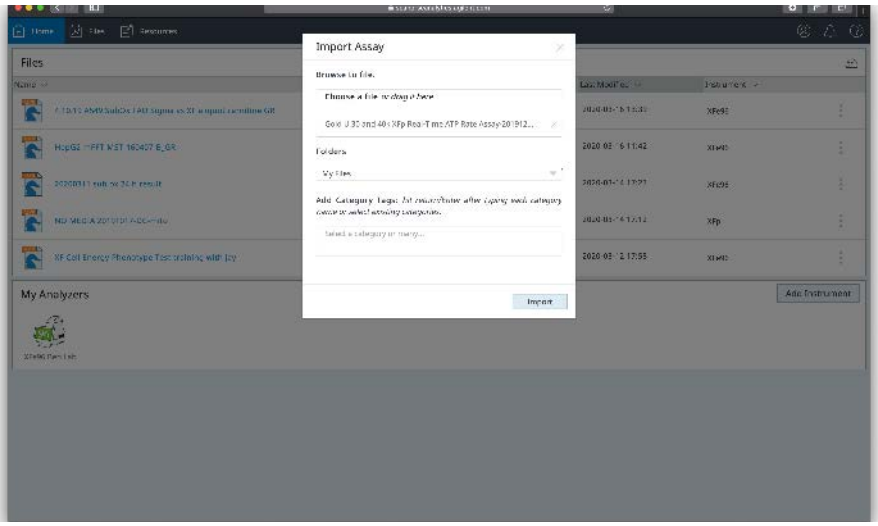
Create a Seahorse Analytics account: **https://seahorseanalytics.agilent.com**

Basic data analysis:

1. After the assay is completed, transfer your assay result file to your personal computer using a USB drive or network drive.
2. Go to **https://seahorseanalytics.agilent.com** to register or log in to your Seahorse Analytics account.

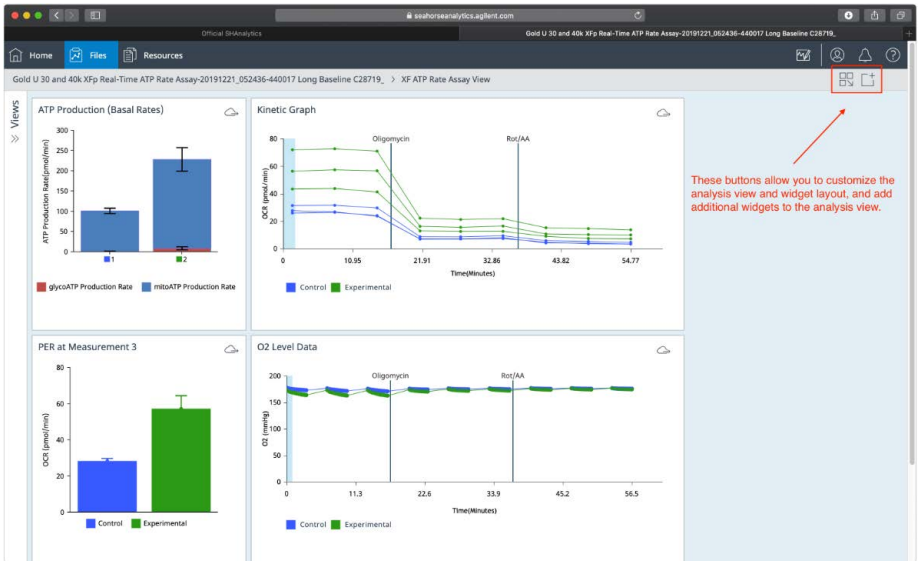


3. From the home view, import your XF HS Mini assay result file to your account.

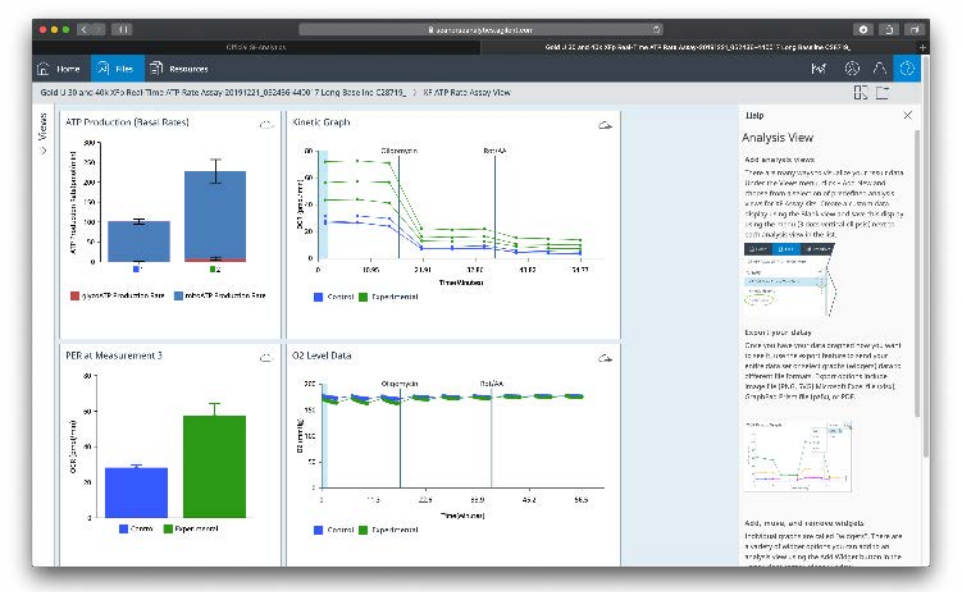


4 Click the file to open it. You will be presented with various analysis view options that you can apply to your data file, including kinetic graphs or bar charts as well as analysis specific for an assay kit.

5 Once you add an analysis view, you can add extra graphs - called widgets - to your analysis view and customize how those widgets appear on the analysis view by moving and resizing them.



6 Use the embedded help seen on each software screen to learn about other features available.



5 XF HS Mini Analyzer Network Setup

This guide provides network setup information for the Agilent Seahorse XF HS Mini Analyzer.

Introduction

Why should I network my XF HS Mini Analyzer?

Connecting your XF HS Mini 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 XF HS Mini 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)**: Typically found in offices or schools, a computer network the connects multiple devices (computers, printers, lab devices, etc.) to enable file sharing between those networked devices in addition to internet access. LANs also have some level of security to access the shared file directories.

Both a WAN and LAN can be configured through a wired (Ethernet cable) or wireless (USB WiFi adapter) connection on the XF HS Mini 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.

Give the **"Network Checklist"** on page 54 to your IT department to use during the network setup of the XF HS Mini Analyzer.

Figure 28. Networked XF HS Mini Analyzer

Network access features on the XF HS Mini Analyzer

Immediate data delivery
Before starting an assay, users enter in one or multiple email addresses to receive a copy of the Assay Result file (*.xflr) 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 to and from the XF HS Mini Analyzer. A shared network directory allows users to easily transfer Assay Template files to the XF HS Mini Analyzer to perform an assay, then retrieve the Assay Results for analysis using **Agilent Seahorse Analytics**.

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

Send 'System Files' directly to cell analysis technical support
When an issue is encountered, Cell Analysis Technical Support routinely requests System Files from the XF HS Mini Analyzer. System Files 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.

XF HS Mini Analyzer System Information

Required materials

- Approved wireless USB adapter or wired connection (Ethernet cable)
- IT department network connection settings

NOTE

XF HS Mini Analyzer instruments use Windows Defender with default settings. These include Quick Scan, real-time protection and cloud-protection turned on.

- The XF HS Mini 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 54 to ensure you have everything needed to successfully connect to a new network.
- The XF HS Mini Analyzer ships with an integrated 100Mbps Ethernet network adapter. The XF HS Mini Analyzer ships with a Netgear Wireless AC Adapter AC600 Dual Band (only the provided adapter is qualified for use). See **Figure 29**.

NOTE

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

  - There is no internal WiFi adapter in the XF HS Mini Analyzer.
- An Ethernet cable is using a wired network setup. The Ethernet (RJ-45) jack is located at the base of the instrument in the back. See **Figure 30**.

Figure 29. USB WiFi adapter for the XF HS Mini Analyzer. Part number S7802-80000

Figure 30. 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 31**), located on the back of the XF HS Mini Analyzer. Ensure that the connection is firmly seated.

Figure 31. Ethernet port with wired network connection cable

2 Power XF HS Mini Analyzer **OFF**.

3 Power XF HS Mini Analyzer **ON**.

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

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

6 Click **System Settings** to access 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 XF HS Mini Analyzer and automatically uses a DHCP server on the network to retrieve IP address values. The XF HS Mini 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"** or contact your local IT administrator.

Option 2: Manual IP address assignment

1 In the Wired Network Connection screen, switch the toggle from **Auto** to **Manual**.

2 Manually enter the information provided by the local IT department in each field.

3 After setting the IP options, click **Save**.

4 Power XF HS Mini Analyzer **OFF**.

5 Power XF HS Mini Analyzer **ON**.

6 Once the temperature is displayed in the upper-right corner, click **Settings**, and then click **Settings**.

7 To ensure you have a working connection, click **Ping**. 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 Use the left right arrow to continue editing instrument settings or exit the settings options.

Wireless Network Setup

Wireless connection

1. Plug the USB WiFi Adapter into one of the available USB ports on the back of the XF HS Mini Analyzer. See **Figure 32**.
2. Power XF HS Mini Analyzer **OFF**.
3. Power XF HS Mini Analyzer **ON**.
4. Wait for the temperature to display in the upper-right corner of the home screen before proceeding with the Wireless Setup instructions.

Figure 32. USB WiFi adapter inserted into an available USB port on the back of the XF HS Mini Analyzer

Joining a wireless network

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

2 Click **System Settings**.

3 Click the right arrow (bottom right) once to access the Wireless Network Connection Settings screen shown in **Figure 33**.

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 Refresh.

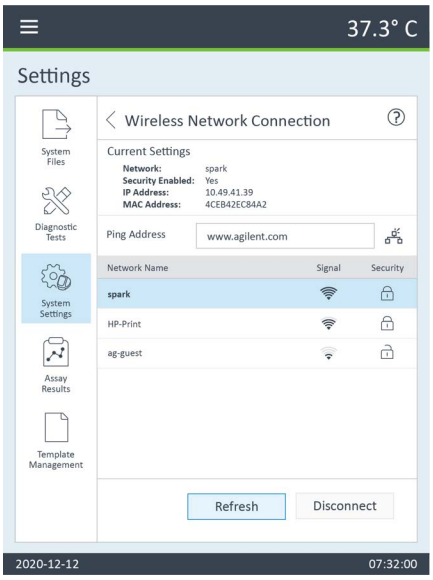


Figure 33. Example wireless network selection table

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 XF HS Mini Analyzer **OFF**.

8 Power XF HS Mini Analyzer **ON**.

9 After the XF HS Mini Analyzer starts up and the temperature value in the upper-right corner is displayed, click **System Settings**.

10 Click **Ping** 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 right arrow to continue editing instrument settings or exit the settings options.

NOTE

An active Internet connection is required for this feature.

Shared folder setup

This procedure requires an active wired or wireless network connection.

1 From the Home screen, click **Settings**, then **System Settings**.

2 Select 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 54 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 for access.
- **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.

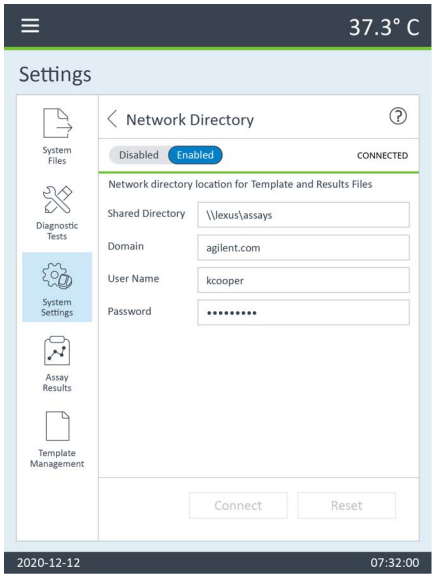


Figure 34. Example of a complete Network Directory configured on the XF HS Mini Analyzer

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 **CONNECTED** message on the XF HS Mini Analyzer.

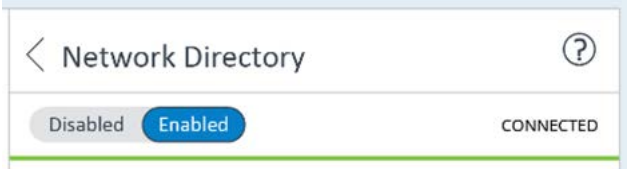


Figure 35. Successful network connection status

5 Use the left right arrow to continue editing instrument settings or exit the settings options.

Email configuration

1 From the Home screen, click **Settings**, then **System Settings**.

2 Select the **Email Configuration** tab.

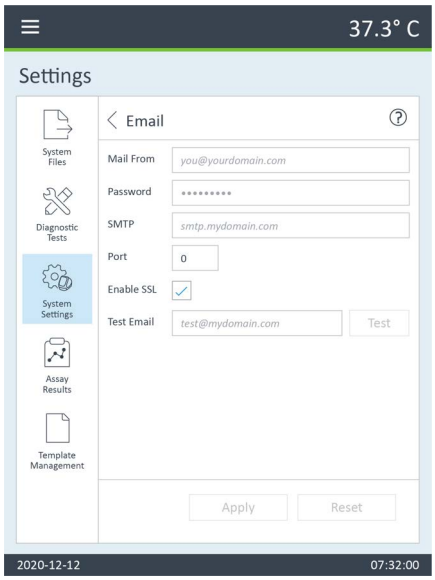


Figure 36. Example of a completed Email Configuration setup

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.
- **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 XF HS Mini 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 Use the left right arrow to continue editing instrument settings or exit the settings options.

Time zone setup

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

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

2 Select the **Date & Time** tab. (**Figure 37**)

3 Select the **Time Zone** from the list provided.

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

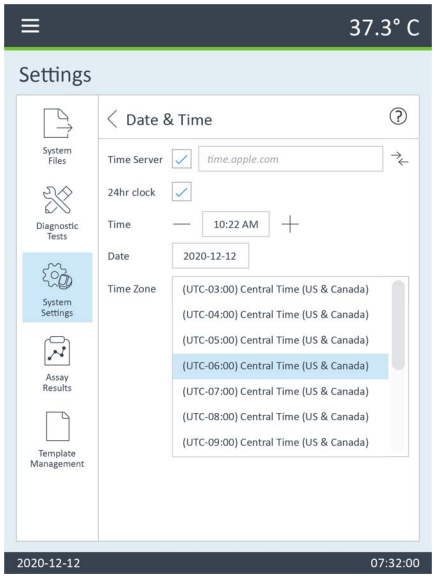


Figure 37. Select the local time zone for your XF HS Mini Analyzer

Setting the time

1 From the Home screen click **Settings > System Settings > Data & Time (+ TimeZone)**.

2 Select the **Date and Time** settings tab. (**Figure 38**)

3 Manually enter the appropriate date and time.

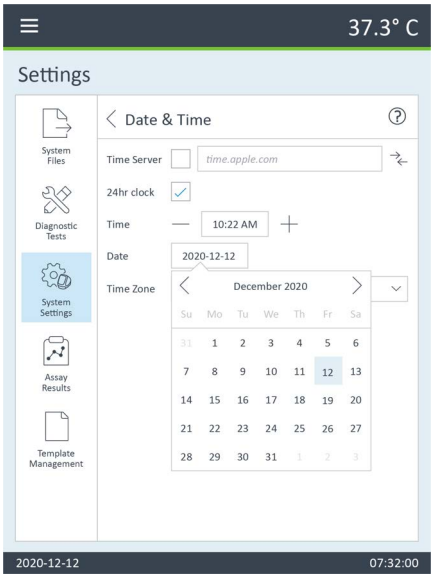


Figure 38. Configure date and time for the XF HS Mini Analyzer

Time server (optional)

For XF HS Mini Analyzers with an active network connection, the XF HS Mini Analyzer can sync to a network's time server, if provided. (**Figure 39**)

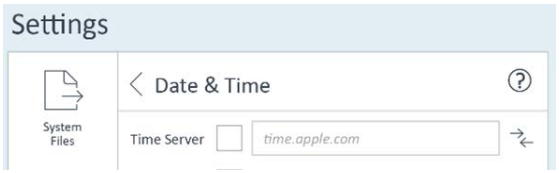


Figure 39. Configuring a time server on the XF HS Mini Analyzer

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 top left menu button to exit the settings options and return to Home screen.

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 XF HS Mini Analyzer.

Troubleshooting Guide

Problem	Possible cause	Solution
Instrument gives a "load error message" and it is not clear whether a cartridge has already been loaded		• Click **Settings** from Home Screen. • Click **Diagnostic tests**. • Click **Maintenance**. • Click **Cartridge out**. • The XF HS Mini 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 Wi-Fi adapter from the XF HS Mini Analyzer and store in a safe location
Low rates	Not enough cells	• Consult the **Cell Reference Database** to identify a working range or perform a dilution series of your cell type. The XF HS Mini is compatible with XFp Miniplates and XF HS Miniplates. The XFp Miniplates are the same dimensions as those of the XFe96 standard plates. The XF HS Miniplate seeding area is approximately one-third of the area of the XFp or XFe96 plate.
"An error has occurred" window pops up and displays the message: "A Task's Exception(s) were not observed."	Instrument was not shut down properly. See **"Turning the XF HS Mini Analyzer Off"** on page 14.	• Contact technical support. See **"Technical Support and Ordering Information"** on page 56.

Technical Support and Ordering Information

Worldwide technical support

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

Email: cellanalysis.support@agilent.com

Phone:

- Global/United States +1 719 528 7500
- United States (toll free) +1 800 227 9770
- United Kingdom 0800 096 7632
- Germany 0800 180 6678
- Europe +45 31 36 98 78
- China (toll free) 800 820 3278

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**.

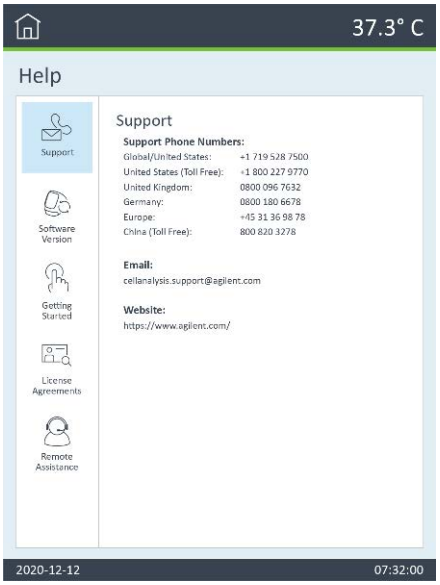


Figure 40. Help window.

Support

Worldwide Agilent Support contact information.

Version of software/hardware

Currently installed software and firmware versions on XF HS Mini Analyzer are displayed on this screen. If the XF HS Mini Analyzer is networked, software updates (Product Update Available) or firmware updates (Firmware Update Available) will be selectable. See **Figure 41**.

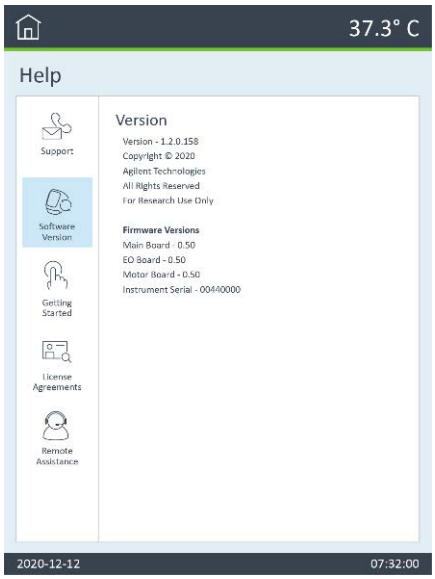


Figure 41. Software version

“Help” text info

Most screens on the XF HS Mini Analyzer have a small **Help** button that will display help text related to the current screen. See **Figure 42**.

Figure 42. Help button.

Additional Resources

XF HS Mini data analysis software registration	**https://www.agilent.com/en/products/cell-analysis/cell-analysis-software/data-analysis/seahorse-analytics**
XF HS Mini Consumables webpage	**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	**www.agilent.com/chem/discoverxf**

www.agilent.com

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