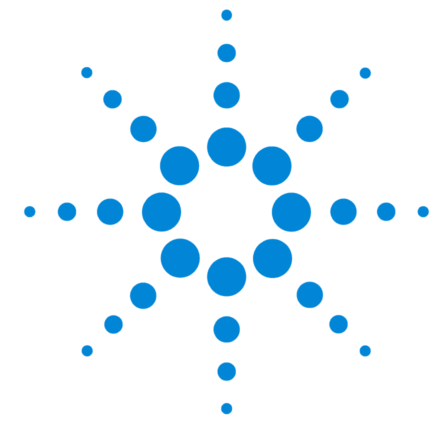

Agilent Seahorse XFe96 and XFe24 Analyzers For use with Wave Desktop

Wave 2.4 User Guide

Notices

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Wave Introduction

Agilent Seahorse Wave software is the experiment design, instrument control, data acquisition, data analysis, and file management software for Agilent Seahorse XF, XFe, and XFp Analyzers. This user guide provides detailed information on each function in Wave software.

Quick Start

The typical Agilent Seahorse XF workflow can be outlined in three steps:

- Design your experiment
- Acquire data
- Analyze results

Design your experiment

First, design your experiment, called an assay template file. Wave software contains several default assay template files for the standardized Agilent Seahorse assay kits (for example, XF Cell Mito Stress Test). Use the Agilent Seahorse default assay templates and customize as necessary. Wave also provides a Blank assay template for those users who prefer to create a new assay template for each experiment.

Wave Desktop is the preferred software for designing or customizing assay templates for Agilent Seahorse XFe and XFp Analyzers, however the same functions can be performed using Wave Controller software (for Agilent Seahorse XFe Analyzers only). See "Create/Edit Assay Templates" on page 14 more for information on creating/customizing assay templates.

Acquire data

After creating an assay template for the experiment, transfer the template to the XFe Analyzer using a USB flash drive or network drive (active network connection required). If the assay template is created using Wave Controller on the XFe Analyzer, the assay can be performed immediately.

Analyze results

After completing the assay, transfer the assay result file from the XFe or XFp Analyzer to Wave Desktop for data analysis. Wave Controller automatically saves a backup copy of the assay result file locally on the XFe Controller (computer).

Wave Home: Views

Templates

View and organize your assay template files from the Templates view. Use the Create, Export, Import, and Duplicate buttons to easily manage your list of template files.

Results

View, open, and manage all your assay result files from the Results view. Click the **Options** button to configure Favorite Places, file locations where assay result files are stored, such as a network directory or local PC folder.

Catalog

Use the Catalog to store Compounds, Pretreatments, Media, and Cells that are frequently used for template design and modification. Wave provides a prepopulated Catalog of the standardized Agilent Seahorse kit components as well as other reagents, media, and cell types. You can add (and delete) entries within the Catalog view only.

Options

Configure the local Template and Catalog file directories as well as the default Instrument Protocol times.

Help

Agilent Seahorse Technical Support contact info, software version and auto-compile feature for System Files.

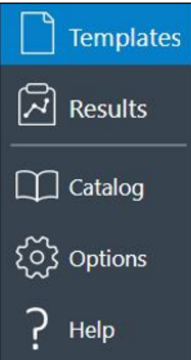


Figure 1 Wave home views

Wave Home: Templates

The Templates view displays all assay template files saved on the XFe Controller (Wave Controller) and on your PC (Wave Desktop). (See Figure 2.) An assay template (.asyt) file contains the required information to run an experiment on the XFe and XFp Analyzers - Group Definitions, Plate Map layout, and Instrument Protocol. Assay templates can be reused for the same assay, or customized and saved as a new template for another assay on the XFe/XFp Analyzer.

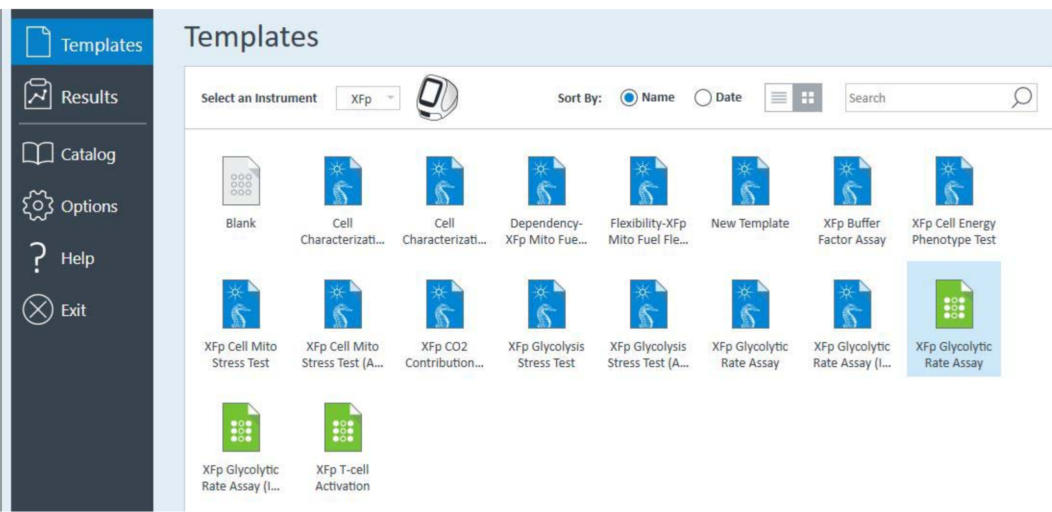


Figure 2 Templates display

Assay templates are color coded based on the type of assay template. (See Table 1.)

Table 1 Assay templates

Template type	Template color
Agilent Seahorse	Blue
Blank	White
User-customized	Green

Blank template | **Agilent Seahorse default template** | **User-customized template**

Blank | XF Cell Energy Phenotype Test | Ben's New Template

Figure 3 Assay template color examples

Wave software automatically installs 10 default Agilent Seahorse assay template files, which contain assay-specific information for standard Agilent Seahorse assays. The blank and Agilent Seahorse templates are not editable; modifications made to a Agilent Seahorse template will be saved as a new assay template (green icon). (See Figure 3.)

- Blank
- XF Cell Energy Phenotype Test
- XF Cell Mito Stress Test (Acute Injection)
- XF Glycolysis Stress Test
- XF Glycolysis Stress Test (Acute Injection)
- Dependency - XF Mito Fuel Flex Test
- Flexibility - XF Mito Fuel Flex Test
- XF Buffer Factor Assay
- XF CO_2 Contribution Factor Assay
- XF Glycolytic Rate Assay
- XF Glycolytic Rate Assay (Induced Assay)

Wave Home: Results

The Results view displays recently opened assay result files, the corresponding file directory where each assay result file is located, and Favorite Places. (See Figure 4.) Opening a new assay result file displays the default analysis view - the Quick View. After modifying and saving an assay result file, Wave automatically displays the last modified analysis view the next time the assay Results file is opened.

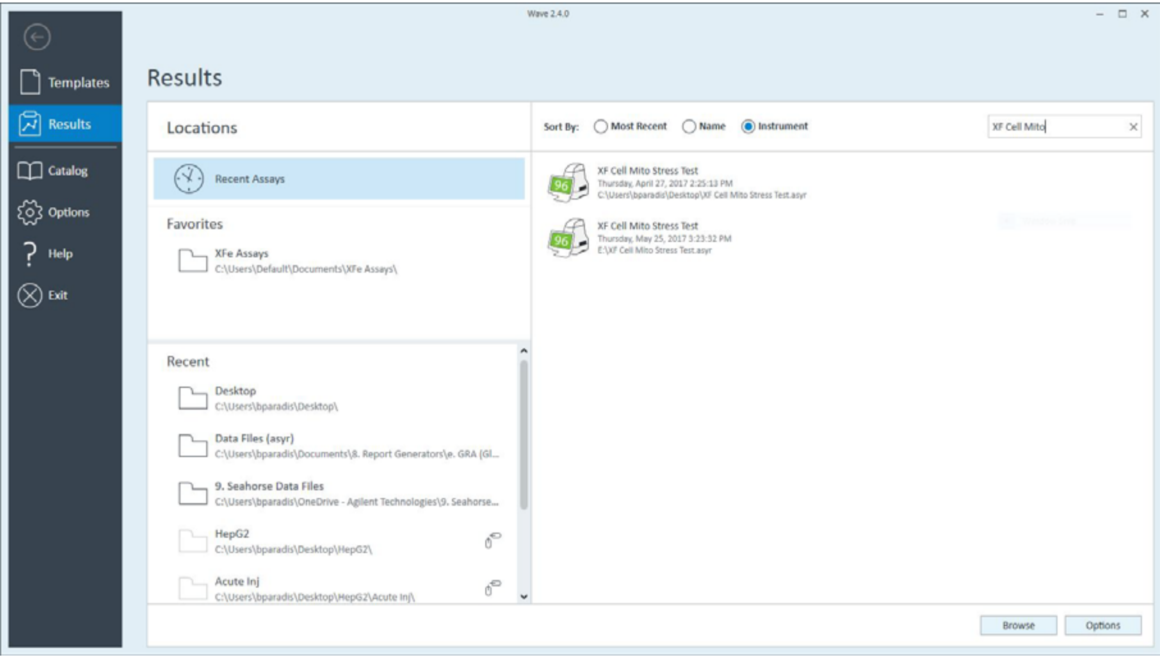


Figure 4 Wave home results

Contents

Wave Introduction

Quick Start 3

Wave Home: Views 4

Wave Home: Templates 5

Wave Home: Results 7

1 Design Agilent Seahorse Experiments with Wave

Create/Edit Assay Templates 14

Step 1: Group Definitions 15
- Injection strategies 15
- Pretreatments 16
- Assay media 17
- Cell type 17
- Duplicate and Delete 18
- Automatically generate groups 18
- Manually generate groups 19

Step 2: Plate Map 20
- Assign groups automatically 20
- Assign groups manually 20
- Background wells 20

Step 3: Instrument Protocol 21
- Default protocol commands 21
- Measurement cycles 21
- Injections 22
- Edit measurement details 23
- Custom cycles 23

Step 4: Run Assay 24
- Assay summary 24
- Errors and Warnings 24

Advanced options 24

Saving Assay Templates 28

2 Perform Assay on the Agilent Seahorse XFe Analyzer

Transfer Assay Template(s) to an Agilent Seahorse XFe Analyzer 30
Transfer assay template using a USB flash drive 30
Transfer assay template using a network drive 30

Start Your Agilent Seahorse XF Assay 31

Add and Remove Measurement Cycles in Runtime 33

Wave Controller Widgets 35
XFe Assays at non-37 °C temperatures 35
Required operational and assay guidelines 35
Set alarm (Temperature tolerance range) 38
Tray control widget 38
Probe control widget 39

XFe Status Indicator 40

Barcode Errors 40
Cartridge barcode read failure 40
Cell plate barcode read failure 41

3 Analyzing Assay Results

Assay Result Files 44

Analysis View #1 - Quick View 45

Analysis View #2 - Overview 46

Analysis View #3 - OCR vs. ECAR 47

Chart Types in Wave 48
Kinetic graph (rate versus time) 48
Scatter plot (rate 1 versus rate 2) 48
Plate Map 49
Bar Graph 50
Group List (legend) 50

Types of Data in Wave 51
Rate data: OCR and ECAR 51

Level data: O2 and pH 51

Introduction to Acidification Data 52

Proton Efflux Rate 53

Display PER data in Wave 53

Data Analysis Using the XF Glycolytic Rate Assay Report Generator 57

Export total PER data 57

Proton Production Rate 58

Report PPR data in Wave 58

Display PPR data in Wave 58

Buffer capacity 58

Baseline to a Rate Measurement (%) 60

Baseline to a Control Group (%) 61

Standard Deviation and Standard Error of the Mean 61

Rate Overlay: OCR, ECAR, PER, PPR, O2, or pH 62

Customizing Data Displays 63

Excluding assay wells 63

Display modes: group and well 64

Graph display options 66

Modifying Assay Result Files 67

Group definitions 67

Plate Map 67

Injection Names 68

General Information 68

Normalization 68

Add normalization values 69

Wave Data Export Options 71

Export to Microsoft Excel 71

Export to GraphPad Prism 74

Export to Agilent Seahorse XF Report Generators 77

Summary View 80

Customize the summary 80

Print and Export summary 82

Data View 83

Column sorting 84

Display values for: Average, Count, Maximum, Minimum, or Sum 84

Group Data by Field 85

4 Managing Agilent Seahorse Files

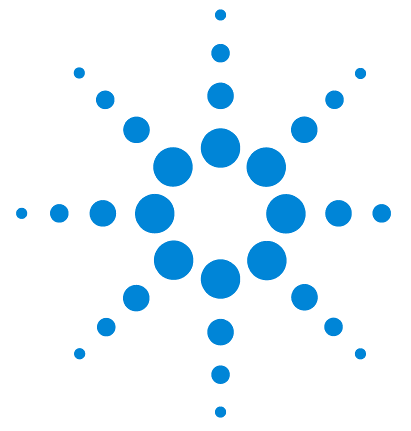
Wave Home: Templates 88
- Import assay templates 88
- Export, duplicate, and remove assay templates 88
- Template Details 89

Wave Home: Results 90
- Recent assays, recent places, and favorite places 91
- Options 91
- Browse 92
- Sort by 92
- Search 92

Wave Home: Catalog 93
- Add a catalog entry 93
- Delete a catalog entry 93
- Share custom catalog 94

Wave Home: Options 95
- General options 95
- Instrument options 96
- Advanced options (Wave controller, Agilent Seahorse XFe analyzers ONLY) 97

Wave Home: Help 99
- Send system files 99



1
Design Agilent Seahorse Experiments with Wave

Create/Edit Assay Templates 14
Step 1: Group Definitions 15
Step 2: Plate Map 20
Step 3: Instrument Protocol 21
Step 4: Run Assay 24
Saving Assay Templates 28

Create/Edit Assay Templates

To create a new assay template or modify an existing template:

1. Open **Wave 2.4**.
2. Click **Templates** (below Wave Home).
3. Select the **Blank** template, a **Agilent Seahorse Default** template, or a **User-customized** template and click **Open** or double-click the template icon.
4. Add, remove, or modify group definitions; and create new assay groups. (See “Step 1: Group Definitions” on page 15.)
5. Assign groups to the Plate Map. (See “Step 2: Plate Map” on page 20.)
6. Review or modify Instrument Protocol. (See “Step 3: Instrument Protocol” on page 21.)
7. Save the template, transfer it to XFe/XFp Analyzer. (See “Saving Assay Templates” on page 28.)
8. Run the assay. (See “Step 4: Run Assay” on page 24.)

Step 1: Group Definitions

Group definitions is comprised of four components:

- Injection Strategies
- Pretreatments
- Assay media
- Cell type

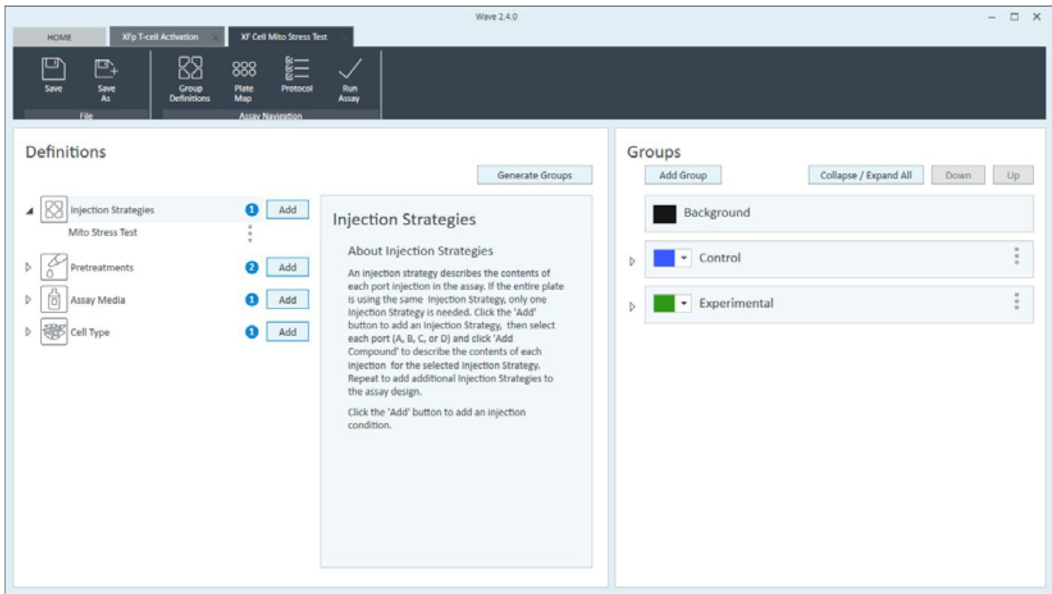


Figure 5 Group definitions

Injection strategies

Injection strategies describe the contents of up to four injections (one for each of the four ports: A, B, C, and D), more than one injection strategy can be performed in an assay. For example, on the same plate, two injection strategies can be defined for the XF Cell Mito and XF Glycolysis Stress Test.

To add one or more injection strategies:

1 Click **Add** next to **Injection Strategies**. (See Figure 5.)

2 Enter a name for the injection strategy if desired. The default name when adding new injection strategies is **Inj. Strategy #** (# corresponds to how many injection strategies have already been added).

3 Next, specify the contents of each injection starting with Port A. Click **Add Compound**, and type in a compound name (for example oligomycin), or use the **Compound Catalog** drop-down menu to select a compound from the catalog. (See Figure 6 on page 16.)

4 If additional injections are called for from ports B, C, and D, repeat step 3.

Figure 6 Additional injection ports

Figure 7 shows two defined injection strategies for the Agilent Seahorse XF Cell Mito Stress Test assay for different concentrations of FCCP:

- Mito Stress Test - 1 µM FCCP
- Mito Stress Test - 2 µM FCCP

Figure 7 Injection strategies for the Agilent Seahorse XF Cell Mito Stress Test assay

Pretreatments

Pretreatments describe a treatment the cells have received prior to performing the assay, such as a genetic manipulation or prolonged exposure to compounds.

To add one or more Pretreatments:

1 Click **Add** next to **Pretreatments**. (See Figure 8.)

2 Enter a name for the pretreatment or use the Pretreatment drop-down menu to select a pretreatment from the catalog. The default name when adding new pretreatments is **Pretreatment #** (# corresponds to how many pretreatments have already been added).

3 If desired, specify the type of pretreatment using the **Description** field.

Figure 8 displays two Pretreatment conditions, **Control** and **Experimental**.

Figure 8 Pretreatment conditions

Assay media

Assay Media describes one or more assay mediums used in the assay. Record the medium and supplements for each medium used in the assay:

To add one or more Assay Medium:

1 Click **Add** next to **Assay Media**. (See Figure 9.)

2 Enter a name for the assay media, or use the Media drop-down menu to select a media from the catalog. The default name when adding a new assay media is **Assay Media #** (# corresponds to how many assay media have already been added). (See Figure 9.)

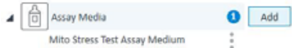


Figure 9 Assay Media

3 Enter additional details if desired for: Source, Supplements, Prepared By, and so forth.

Cell type

Cell Type describes the biological material/samples used, including information about the cell line, seeding density, and passage number.

To define one or more Cell Types:

1 Click **Add** next to **Cell Type**. (See Figure 10.)

2 Enter a name for the cell type, or use the Cell Line drop-down menu to select a cell type from the catalog. The default name when adding a new cell type is **Cell Type #** (# corresponds to how many cell types have already been added). (See Figure 10.)

Figure 10 Cell Type

3 If desired, enter additional details for: Seeding Density, Lot, Source, and Passage number.

Duplicate and Delete

The three dots next to each group definition enable two functions: Delete the group definition entry and Duplicate the group definition entry.

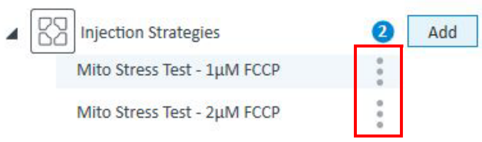


Figure 11 Duplicate and Delete function toggle

Automatically generate groups

After creating the individual Group Definitions, create assay groups, and assign the various conditions to each group based on your experimental design. This can be done automatically by clicking the **Generate Groups** button. This function uses the independent Group Definitions to calculate the number of groups, assuming every possible combination of independent conditions. An example of the combinatorial logic using the Group Definitions below shows how this function results in a total of eight unique groups (2 * 2 * 1 * 2 = 8):

- Injection Strategies = 2 (Mito Stress Test - 1 µM FCCP and Mito Stress Test - 2 µM FCCP)
- Pretreatments = 2 (Control and Experimental)
- Assay Media = 1 (Mito Stress Test Assay Medium)
- Cell Type = 2 (C2C12 and HepG2)

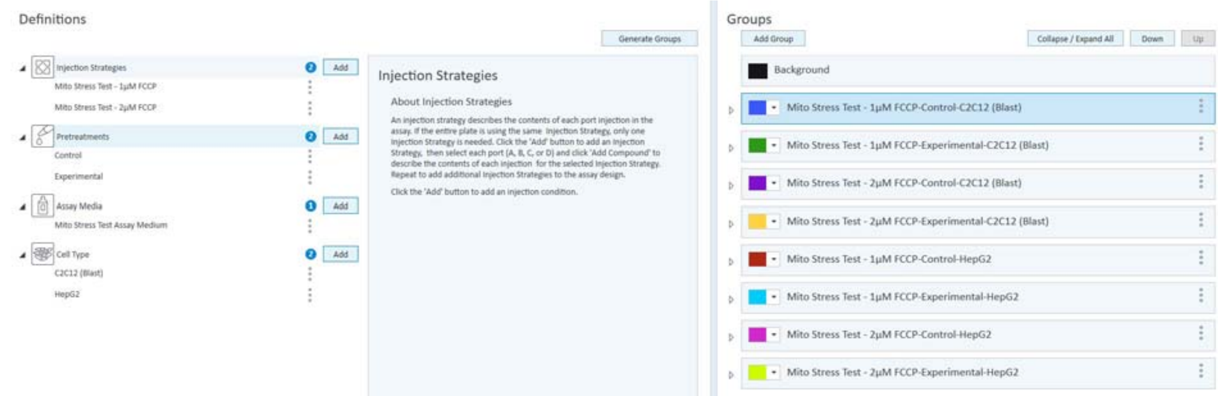


Figure 12 Automatically generate groups

Manually generate groups

If desired, Assay groups can be created manually:

1 Click **Add Group** to create **Group 1**. (See Figure 13.)

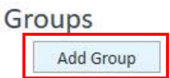


Figure 13 Add groups

2 Use the drop-down menus to assign the appropriate **Group Definitions** to **Group 1**. (See Figure 14.)

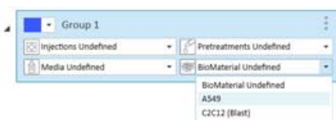


Figure 14 Duplicate and Delete function toggle

3 Repeat for each manually generated group.

To change the name of the group, double-click the **Group 1** text, or right-click and select **Edit**. To change the color of the group, use the drop-down color menu next to the group name. After adding Group Definitions and generating groups, assign groups to the Plate Map. (See “Step 2: Plate Map” on page 20.)

Step 2: Plate Map

The Plate Map displays group assignments on the Cell Plate. Wave uses the group assignments on the Plate Map to calculate group statistics (average rates for each measurement performed and the standard deviation or standard error for each rate measurement) after the assay has completed. There are two ways to assign groups to the Plate Map.

Assign groups automatically

Click **Distribute Groups**. Wave automatically determines the maximum number of replicates per group, and assigns wells to each group starting with column 1. (See Figure 15.)

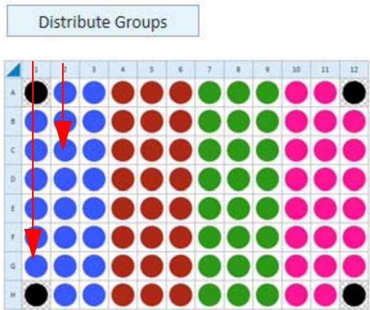
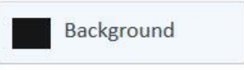


Figure 15 Distribute Groups

Assign groups manually

1. Click the group in the Groups list on the left.
2. Click the well on the Plate Map to assign that individual well to the selected group. You can assign an entire row or column to a group by clicking the row or column label, or drag-and-drop to select an area of the Plate Map to assign to the selected group. (See Figure 15.)

Background wells



Background wells have default Plate Map coordinates based on the type of XFe or XFp Analyzer. You can modify the coordinates of the background wells or add/remove background wells if desired. Use the steps described in "Assign groups manually".

Step 3: Instrument Protocol

The Instrument Protocol is the series of commands performed during an assay, the timing of when these commands occur, and the duration. The default Agilent Seahorse assay templates have a preconfigured Instrument Protocol, and do not require modification.

Default protocol commands

Every Instrument Protocol includes the following steps:

- Calibrate (always ON)
- Equilibrate (highly recommended for every assay)
- Baseline Measurement cycle

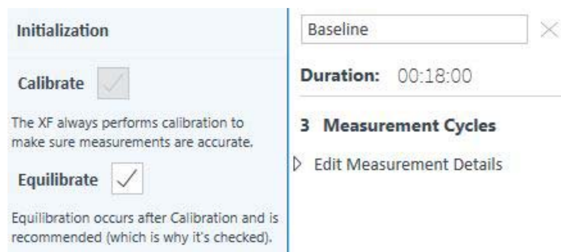


Figure 16 Default protocol commands

Calibration

Calibrate is always the first step in a protocol and cannot be disabled. The Calibrate step reads the coefficients of the Sensor Cartridge and Cell Plate to ensure accurate data acquisition.

Equilibration

Equilibration ensures temperature stability before beginning an assay. The default setting for Equilibration is **ON**. Equilibrate can be disabled although this is strongly discouraged.

Measurement cycles

Measurement cycles indicate the steps in the Instrument Protocol when rate data is collected for oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). The standard Agilent Seahorse Instrument Protocol consists of three measurement cycles before the first injection (called Baseline) and three measurement cycles after each port injection. Each measurement cycle consists of three commands: Mix, Wait, and Measure.

- **Mix**: The amount of time to raise/lower the sensor cartridge to ensure analytes and drug compounds are uniformly mixed in each well before/after an injection, as well as to reintroduce oxygen to the well after forming the microchamber for each measurement.
- **Wait**: The amount of time to delay the Measurement step after the Mix step. This command is required for the XFe24 Analyzers in every assay, but may be added to Instrument Protocol for any analyzer.
- **Measure**: The amount of time to record the flux of analytes in the transient microchamber once the sensor cartridge probes are lowered following a Mix (or Wait) command.

The default measurement cycle times are:

- Agilent Seahorse XFe96 and XFp Analyzer: 3 minutes Mix; 0 minutes Wait; 3 minutes Measure
- Agilent Seahorse XFe24 Analyzer: 3 minutes Mix, 2 minutes Wait, 3 minutes Measure

Baseline measurement cycle

The Baseline measurement cycle is the starting point for every Agilent Seahorse assay and consists of three measurements before the first injection. Data acquired during the Baseline measurement cycle provide valuable information about the bioenergetic status of the cells under starting conditions.

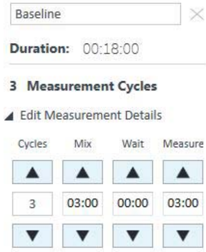


Figure 17 Default protocol commands

Injections

To add an injection command to the Instrument Protocol, click **Injection**. Wave automatically selects the compound injection ports sequentially, therefore, the first time you click **Injection**, port A will be assigned to injection 1. A grayed out port indicates it has already been assigned to an injection. (See Figure 18 on page 23.)

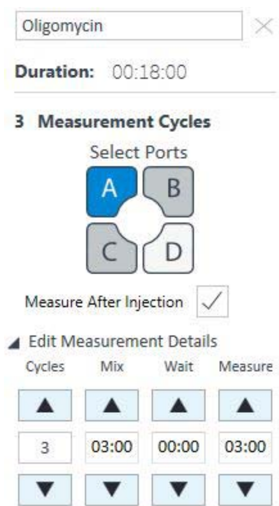


Figure 18 Measurement information

Wave also automatically adds the measurement cycles after adding an injection. Default **Mix**, **Wait**, and **Measure** times are instrument-dependent, and can be modified in **Instrument Options** (**Wave Home** > **Options**).

Edit measurement details

To view and edit the **Mix**, **Wait**, and **Measure** timing, click the drop-down arrow next to **Edit Measurement Details**. (See Figure 18.) Use your keyboard to enter specific **Mix**, **Wait**, and **Measure** times or adjust the number of **Cycles** for each measurement. The up and down arrows can also be used. To achieve optimal results, a minimum of three cycles per measurement is recommended. The default **Mix**, **Wait**, and **Measure** times are instrument-dependent, and can be modified in **Instrument Options** (**Wave Home** > **Options**).

To change the name of each command in the **Instrument Protocol**, click the text field at the top of the column. (See Figure 18.)

Custom cycles

Most Agilent Seahorse assays do not require the use of custom cycles, however depending on the type of experiment or your experiment design, a custom cycle may be appropriate. To add a custom cycle command to the **Instrument Protocol**, click **Custom**. A custom cycle enables multiple Mix and Wait steps without a Measurement. Note that the Agilent Seahorse XF Report Generators are not compatible with Instrument Protocols containing a custom cycle.

Step 4: Run Assay

Assay summary

General Information

The General Information section provides editable fields for project information, plate information, or other important notes related to the assay. Any information entered on this view is saved in the assay result file (*.asyr).

Figure 19 Assay summary

Errors and Warnings

All errors or warnings are displayed on the right side of the Run Assay step. (See Figure 20.) Prior to transferring the template to the XFe or XFp Analyzer, correct any errors that are displayed. A typical error message is the notification that some assay wells have not been assigned to a group on the Plate Map. If a template is designed correctly, Wave displays an **All Set** confirmation message. (See Figure 20.)

Figure 20 Error and Warning messages

Advanced options

Email settings (XFe Analyzers only)

This feature applies to Wave Controller software only, and requires an active internet connection on the XFe Analyzer. Recipients must be added before starting an assay. Wave Desktop does not email assay result files.

To add an email address:

1 Open the template file.
2 On the **Run Assay** navigation step, click **Show Advanced Options**. (See Figure 21.)
3 Type in the email address below **Email Settings**, and click **Add**. (See Figure 21.)
4 Repeat for each email address.

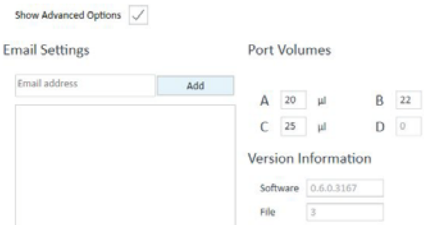


Figure 21 Advanced Options

Port volumes and Version info

Adjust the default **Port Volume** values using the up/down arrows, or by typing in a value using your keyboard. This is for notation-purposes only, and does not affect data calculations. (See Figure 21.)

Version Information displays the Wave software version along with Agilent Seahorse XF **File** version info (internal-use only). (See Figure 21.)

Protocol summary

To display a summary view of the Instrument Protocol, click **Protocol**. (See Figure 22.) To edit the Instrument Protocol, click the **Instrument Protocol** navigation step before running the assay on Wave Controller, or saving/transferring the assay template from Wave Desktop to the XFe or XFp Analyzer.

Figure 22 Protocol

Group summary

To display a summary view of the Group Definitions and Plate Map, click **Group**. (See Figure 23.) To edit the Group Definitions or Plate Map, click the **Group Definitions** or **Plate Map** navigation step before running the assay on Wave Controller, or saving/transferring the assay template from Wave Desktop to the XFe or XFp Analyzer.

Figure 23 Group Summary

Port (A, B, C, or D) summary

Click **Port A** (**B**, **C**, or **D**) for a summary of each injection from the selected port. (See Figure 24 on page 27.) The summary information includes:

- Compound concentration
- Name of the solvent (if specified)
- Percentage of solvent used for each compound
- Port volume

To modify the compound name, compound concentration, solvent name or percentage, click the **Group Definitions** navigation step, then select the appropriate **Injection Strategy** to edit before running the assay on Wave Controller, or saving/transferring the assay template from Wave Desktop to the XFe or XFp Analyzer.

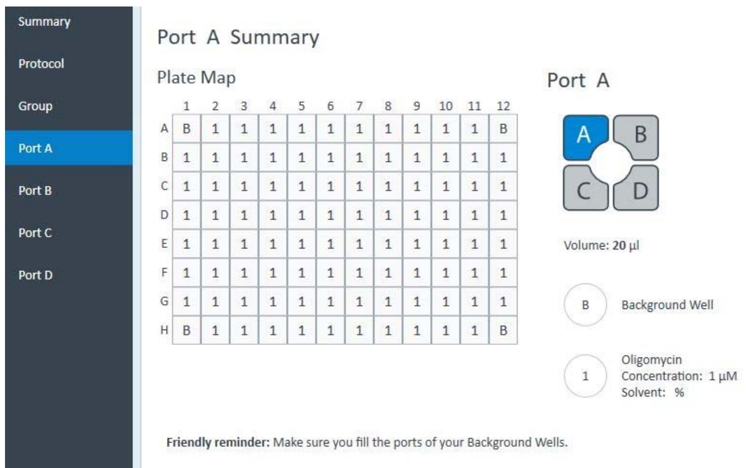


Figure 24 Port A summary

Print summary

Print Summary

To print or save a PDF of the Assay Summary, Protocol, Group, and Port A-D summaries for the assay template, click **Print Summary**.

Saving Assay Templates

After editing an assay template, click the **Save** button to overwrite the template with the newly modified details/content. To create a new template file, and preserve the original template file details, click the **Save As** button.

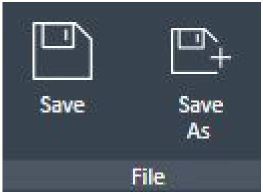


Figure 25 Saving options

Click **Save As** to display the **Save As Template** window (See Figure 26.):

1 Enter the name of the assay template in the **Name** field (required).
2 Enter the **Author** name (optional).
3 Enter a description of the assay in the **Description** field (optional).
4 Click **Save**.

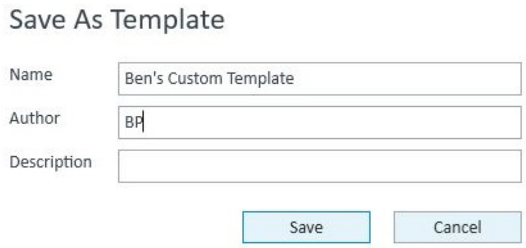
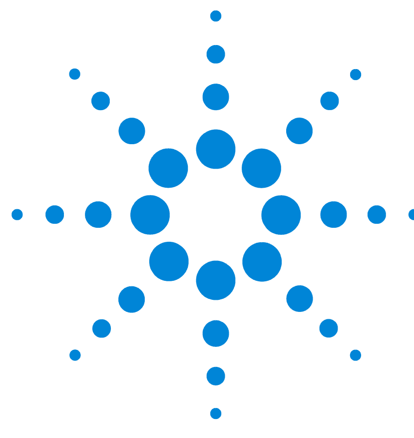


Figure 26 Save As Template window



2
Perform Assay on the Agilent Seahorse XFe Analyzer

Transfer Assay Template(s) to an Agilent Seahorse XFe Analyzer 30
Start Your Agilent Seahorse XF Assay 31
Add and Remove Measurement Cycles in Runtime 33
Wave Controller Widgets 35
XFe Status Indicator 40
Barcode Errors 40

This chapter applies to Wave Controller software for Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Analyzers **ONLY**. The functions described in this chapter do **NOT** apply to Wave Desktop or Agilent Seahorse XFp Analyzer software.

Creating assay template files on Wave Desktop and Controller

Assay template files (.asyt) can be designed using Wave Desktop and Wave Controller. Assay templates created using Wave Controller can be run immediately after designing the template. Assay templates created using Wave Desktop must be transferred to Wave Controller to perform an assay. For nonnetworked XFe Analyzers, assay templates must be exported from Wave Desktop to a USB flash drive, then imported to Wave Controller. If the XFe Controller has an active network connection, assay template files can be transferred/imported directly through a shared network location.

Transfer Assay Template(s) to an Agilent Seahorse XFe Analyzer

Several options to import assay templates to Wave Controller are outlined below.

Transfer assay template using a USB flash drive

Option #1:

1. Save template to a USB flash drive.
2. Plug the flash drive into the USB port on the Agilent Seahorse XFe Controller.
3. Select the USB flash drive, and locate the template file(s) (.asyt).
4. Double-click the assay template to automatically import to Wave Controller software.

Option #2:

1. Save template to a USB flash drive.
2. Plug the flash drive into the USB port on the Agilent Seahorse XFe Controller.
3. Click **Templates**.
4. Click **Import**.
5. Select the USB flash drive, and locate the template file (.asyt).
6. Select one or multiple template files on the USB flash drive, then click **Open**.

Transfer assay template using a network drive

Option #1:

1. Power **ON** the XFe Controller (if OFF).
2. Start **Wave Controller** software.
3. Locate the assay template file (.asyt) in the network directory.
4. Double-click the assay template to automatically import to Wave Controller.

Option #2:

1. Power **ON** the XFe Controller (if OFF).
2. Start **Wave Controller** software.
3. Click **Templates**.
4. Click **Import**.
5. Locate the template file (.asyt) in the network directory.
6. Select one or multiple template files on the network drive, then click **Open**.

Start Your Agilent Seahorse XF Assay

After customizing or transferring an assay template to Wave Controller, click **Start Run** to begin the XFe assay.

CAUTION Once an assay is started, access to Wave Home and other assay template and assay result files is disabled until the assay is completed. Transfer all Agilent Seahorse files from Wave Controller before starting an assay.

1. Click **Start Run**, and select a location to save the assay template file. The assay result file will be saved in the same location following assay completion. Assay template and result files can be saved to a USB Flash Drive, shared network directory, or locally on the XFe Controller. Wave Controller will also automatically save a backup assay result file locally on the XFe Controller in the location: C:\ProgramData\ Seahorse Bioscience, Inc\Seahorse Wave\Assays.
2. After selecting a save location, the tray door on the XFe Analyzer will open. Place the Sensor Cartridge (hydrated and loaded with compounds) along with the Utility Plate onto the tray. Ensure the Cartridge fits properly on the Utility Plate, the lid is removed from the Cartridge, and the direction of the Cartridge is in the proper orientation.
3. After loading the Sensor Cartridge and Utility Plate, press **I'm Ready** to initiate Sensor Cartridge Calibration. The time to complete Calibration for assays at 37 °C is approximately 10-20 minutes. For assays performed at temperatures other than 37 °C, an additional 30 minutes of PreCalibration time will be added to ensure accurate data acquisition.

Figure 27 Load Calibrant Utility Plate prompt

4. After completing Calibration, Wave Controller will display the Load Cell Plate message prompt. Click **Open Tray** to eject the Utility Plate and load the Cell Plate. Ensure the lid is removed from the Cell Plate before loading.

Figure 28 Load Cell Plate prompt

5 Click **Load Cell Plate** to initiate Equilibration. Once Equilibration has finished, the assay will begin acquiring the first Baseline measurement.

Figure 29 Load Cell Plate prompt

6 After completing the final Instrument Protocol step, the assay will finish and display an Unload Sensor Cartridge message prompt. To eject the Sensor Cartridge and Cell Plate from the XFe Analyzer, press **Eject**.

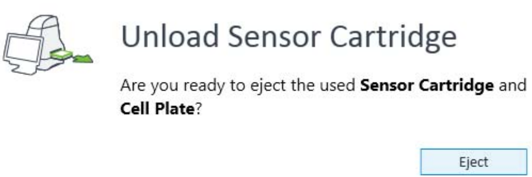


Figure 30 Unload Sensor Cartridge prompt

7 The assay result file will automatically save to the location specified prior to starting the assay, and can be opened immediately following the assay using the on-screen **Assay Complete** prompt.

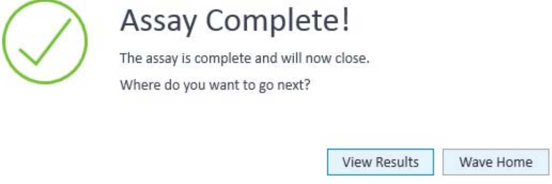


Figure 31 Assay Complete prompt

Add and Remove Measurement Cycles in Runtime

Figure 32 Real-time measurement cycle data

During a Agilent Seahorse XF assay, data is acquired, calculated, and presented in the real-time. For certain assays, it may be necessary to add or remove measurement cycles in real-time.

Wave Controller 2.4 enables real-time Instrument Protocol modification for commands that have not yet been executed. To display the Instrument Protocol for the selected command, click on an **Instrument Protocol** step above the data. (See Figure 33.)

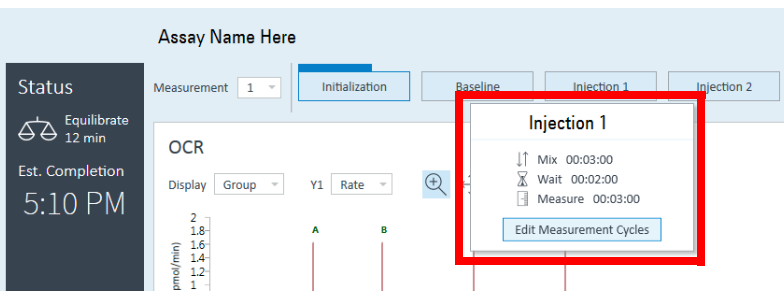


Figure 33 Selected command instrument protocol

Click **Edit Measurement Cycles** to display the **Edit Number of Cycles** window. (See Figure 34.) Use the up/down arrows (or manually type a number) to add/remove measurement cycles from the selected command.

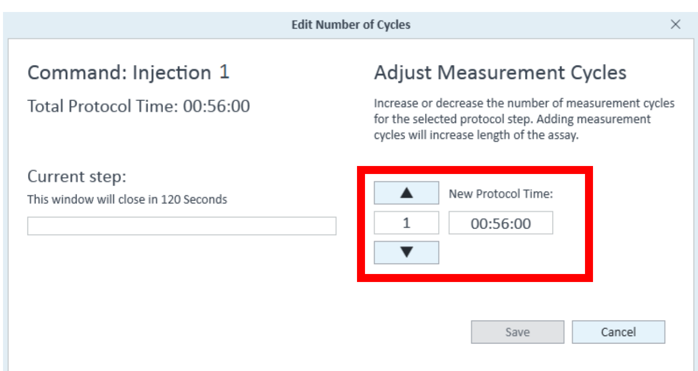
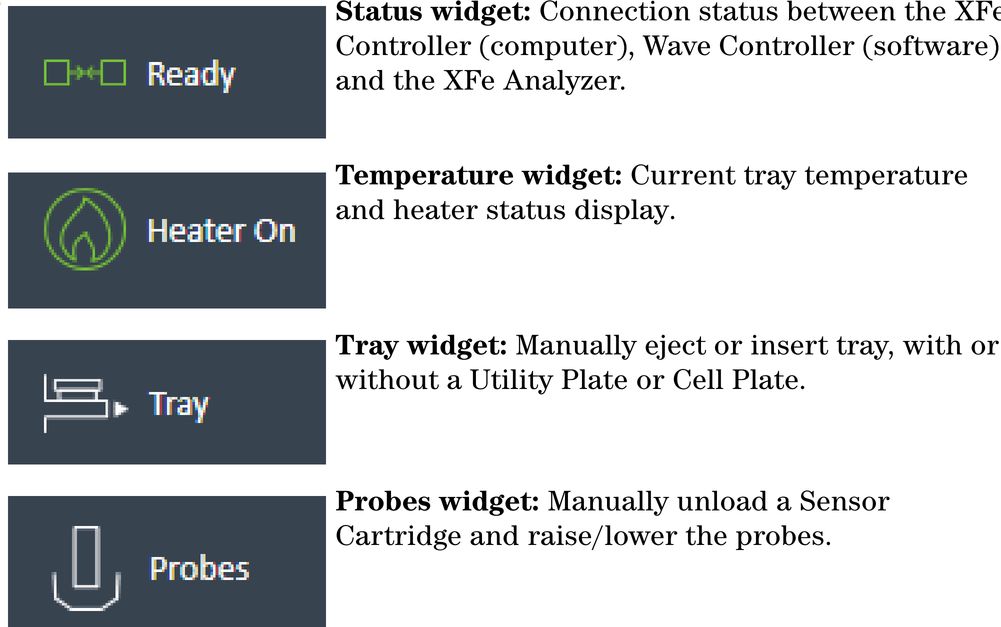


Figure 34 Edit Number of Cycles

Wave Controller Widgets

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



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

Temperature widget: Current tray temperature and heater status display.

Tray widget: Manually eject or insert tray, with or without a Utility Plate or Cell Plate.

Probes widget: Manually unload a Sensor Cartridge and raise/lower the probes.

XFe Assays at non-37 °C temperatures

Agilent Seahorse XFe Analyzers have been validated to deliver desired target temperatures in the range of 16-42 °C provided the ambient room temperature is 12-20 °C below the target temperature, and in the validated operational room temperature range of 4-30 °C. To understand the relationship between the sample temperature and ambient temperature, see Figure 35 on page 36 for the temperature chart.

Required operational and assay guidelines

- For all non-37 °C operation, the XFe Analyzer must equilibrate overnight in required ambient temperature.
- If required to set up the XFe Analyzer in cold room, avoid direct fan sources. (See Figure 35 on page 36.)
- For all non-37 °C operation, the tray heater must remain **ON**, do **NOT** turn the tray heater **OFF**.

- For assay temperatures below 30 °C, hydrate the Sensor Cartridge in the dark at room temperature.
- Prior to starting an assay, an additional 30 minutes of precalibration equilibration time has been added to ensure temperature stability.

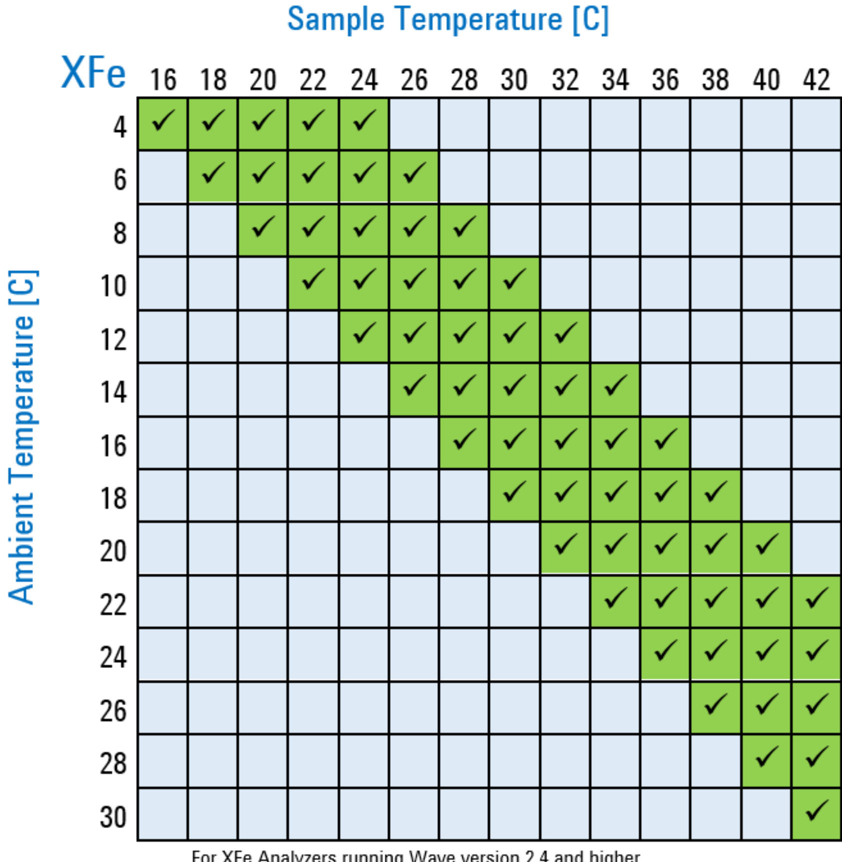


Figure 35 Temperature chart

To adjust the Target Temperature (set point) using the up/down arrows, click on the **Temperature Widget** to display the Tray Temperature window. (Figure 37 on page 37.) Ensure your ambient conditions support the desired target temperature (12-20 °C above ambient). Refer to the temperature chart shown in Figure 35.

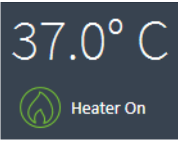


Figure 36 Temperature widget

NOTE

Changing the Target Temperature requires OVERNIGHT equilibration to the new set point.

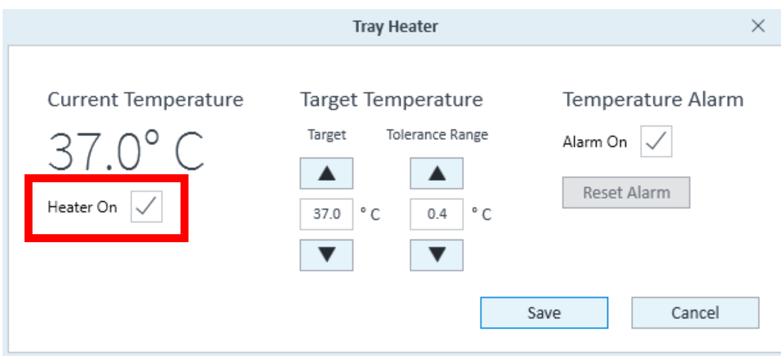


Figure 37 Tray Heater window

Other Temperature Widget functions are:

- Turn the heater **ON/OFF**.
- Set the tolerance range for temperature fluctuation. If the temperature is above or below the acceptable tolerance range from the temperature set point, the Temperature Widget will change color (Figure 38), and the Status Indicator light (top of the XFe Analyzer) will change from blue to amber. For networked XFe Controllers, Wave Controller software will automatically send an email notification to specified recipients.

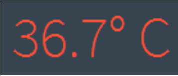


Figure 38 Temperature widget color change

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

Set alarm (Temperature tolerance range)

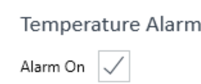


Figure 39 Temperature Alarm

To set the alarm:

1 Check the **Alarm On** box in the Tray Temperature window.

2 Click **Save**.

Disable the alarm by unchecking the **Alarm On** box, then click **Save**. If the Tray Temperature exceeds the Tolerance Range and the alarm is activated, click **Reset Alarm** to acknowledge and reset the Tray Temperature alarm.

To ensure the Tray Temperature starts within the Tolerance Range, check the current temperature of the XFe Analyzer before beginning an assay. For any suspected temperature issues or unexpected temperature fluctuations, contact Agilent Seahorse Products Technical Support.

Tray control widget

Figure 40 Tray control widget

Use the **Tray Control Widget** to manually eject a Utility Plate or a Cell Plate in XFe Analyzer:

1 Click the **Tray Widget**. (See Figure 40.)

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

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

Probe control widget

Use the Probes Widget to load/unload a Sensor Cartridge or raise/lower probes. To display options and select the appropriate action, click the **Probes Widget**. (See Figure 41.)

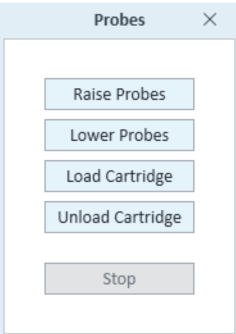


Figure 41 Probes control widget

XFe Status Indicator

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

- To load a Sensor Cartridge or Cell Plate
- To remove a used Sensor Cartridge and/or Cell Plate
- To accept or cancel an assay if one or more wells did not calibrate properly after Calibration
- Any errors that can occur during the run, such as barcode read errors for the Sensor Cartridge, Cell Plate, or a protocol error.

Barcode Errors

The XFe Analyzer reads and records the Cell Plate and Sensor Cartridge barcodes before beginning an assay. A Barcode Read error will be displayed on the rare occasion the barcode cannot be read. (See Figure 42.) Contact Agilent Seahorse Technical Support to assist with resolving this error and to start the assay.

Cartridge barcode read failure

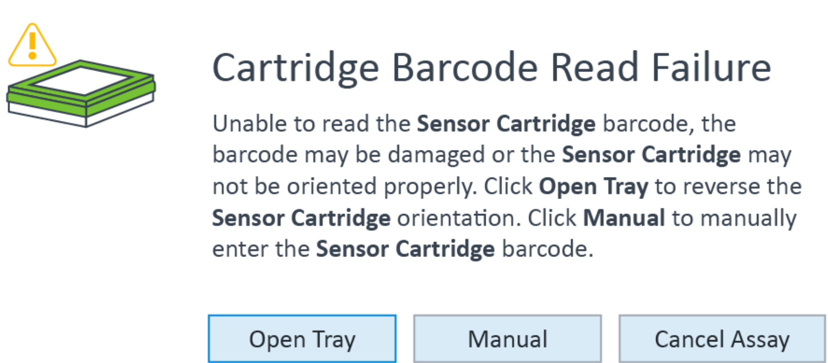


Figure 42 Barcode read failure

For any Sensor Cartridge barcode read errors, Wave Controller will display the message shown in Figure 42 and three corrective actions:

- **Open Tray**: Eject the Sensor Cartridge to inspect barcode quality or to reverse the Sensor Cartridge.
- **Manual**: Manually input the Sensor Cartridge barcode information. Contact Agilent Seahorse Technical Support for this step.
- **Cancel Assay**: Cancel the assay.

Manually enter sensor cartridge barcode

1 To display the Cartridge Barcode window, click **Manual**. (See Figure 43.)

2 Call the appropriate regional Agilent Seahorse Technical Support telephone number to assist with entering the Sensor Cartridge barcode info for each field on the form. (See Figure 43.)

Cartridge Barcode Manual Entry

Contact Agilent Seahorse Technical Support using the telephone numbers below to assist with entering the required **Sensor Cartridge** information below.

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): +65 6420 0900
Email: seahorse.support@agilent.com

Lot Number
Serial Number
O2 A
O2 B
pH A
pH B
pH C

Open Tray | Close Tray | Accept | Cancel

Figure 43 Cartridge barcode manual entry form

Cell plate barcode read failure

Figure 44 Cell Plate Barcode Read Failure

For any Cell Plate barcode read errors, Wave Controller will display the message shown in Figure 44 and two corrective actions:

- **Manual**: Manually input the Cell Plate barcode info.
- **Cancel Assay**: Cancel the assay.

Manually enter cell plate barcode information

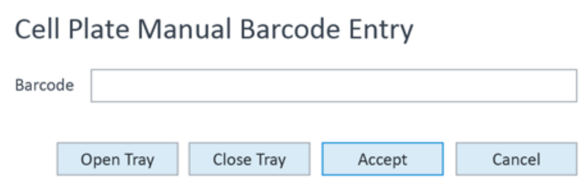
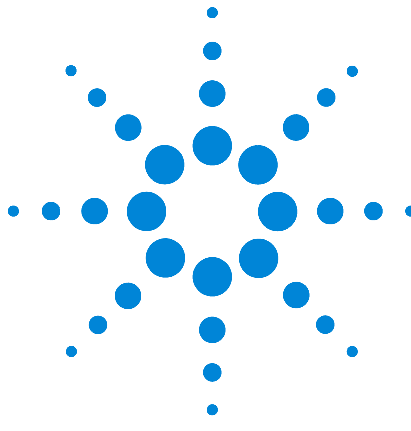


Figure 45 Cell Plate Manual Barcode Entry

1. Click the Tray Widget. (See Figure 40 on page 38.)
2. To eject the Cell Plate, click **Open Tray**.
3. The Cell Plate barcode is located on the side of the plate. Write down the barcode information.
4. Click **Close Tray**.
5. On the Cell Plate Manual Barcode Entry window, enter the Cell Plate barcode, and click **Accept**. (See Figure 45.)



3
Analyzing Assay Results

Assay Result Files 44
Analysis View #1 - Quick View 45
Analysis View #2 - Overview 46
Analysis View #3 - OCR vs. ECAR 47
Chart Types in Wave 48
Types of Data in Wave 51
Introduction to Acidification Data 52
Proton Efflux Rate 53
Data Analysis Using the XF Glycolytic Rate Assay Report Generator 57
Proton Production Rate 58
Baseline to a Rate Measurement (%) 60
Baseline to a Control Group (%) 61
Standard Deviation and Standard Error of the Mean 61
Rate Overlay: OCR, ECAR, PER, PPR, O2, or pH 62
Customizing Data Displays 63
Modifying Assay Result Files 67
Normalization 68
Wave Data Export Options 71
Summary View 80
Data View 83

The primary purpose of Wave Desktop software is for data analysis of result files generated by Agilent Seahorse XF, Agilent Seahorse XFe, and Agilent Seahorse XFp Analyzers. Wave Controller software for XFe Analyzers enables the same analysis features (for XFe data only), but analysis is strongly discouraged on the XFe Controller.

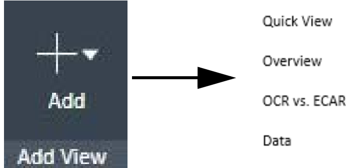
Assay Result Files

Assay result files (*.asyr) are generated by XFe and XFp Analyzers only, and contain all data acquired during an assay. Examples of what type of data is acquired and stored in assay result files include:

- Rate data (OCR, ECAR, PER, and PPR)
- Level data (mmHg or mpH)
- Calibration results
- Consumable information from the barcodes on the cell plate and sensor cartridge used in the assay

In addition, any details added to the assay template file about the experiment, such as groups, compound, pretreatments, is migrated and saved in the assay result file. Most of this information can be edited using the **Modify** function. For more information, see “Modifying Assay Result Files" on page 67 for more information. After completing an XF assay, save the result file to a USB flash drive (or network directory), and open the result file on your personal computer using Wave Desktop. The default analysis view for every new assay result file is called **Quick View**. Reopening a saved result file always displays the last-modified or viewed analysis view. Each analysis view can be added to an assay result file using the **Add View** button. The four analysis views in Wave software are:

- Quick View
- Overview
- OCR vs. ECAR
- Data



Analysis View #1 - Quick View

Quick View is the default analysis view displayed when opening a new assay result file. Quick view simultaneously displays a kinetic graph of OCR vs Time, ECAR vs Time, and a scatter plot of OCR vs. ECAR. (See Figure 46.)

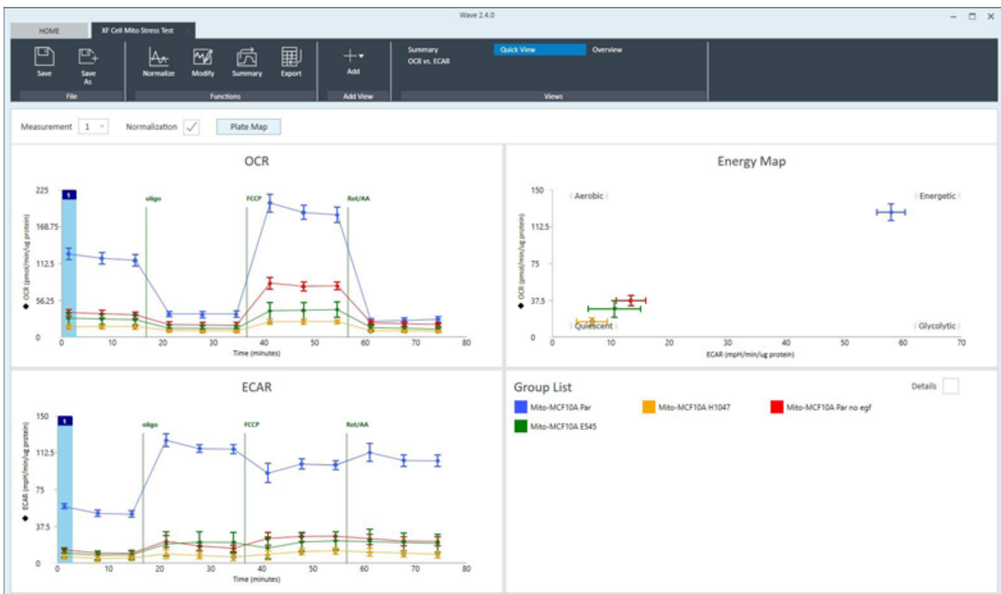


Figure 46 Quick view

Analysis View #2 - Overview

Overview displays a kinetic graph of rate (**OCR**, **ECAR**, **PER**, or **PPR**) versus time. The selected rate is displayed on the y-axis, and **Time** is fixed on the x-axis. Group statistics (average rate and error) for each measurement can be displayed by checking the **Details** box in the **Group List** below the kinetic graph. **Overview** is the most versatile analysis view in Wave software for most routine analysis functions. To display **Overview**, click **Add View** in the center of the ribbon menu, and select **Overview** from the list of views. (See Figure 47.)

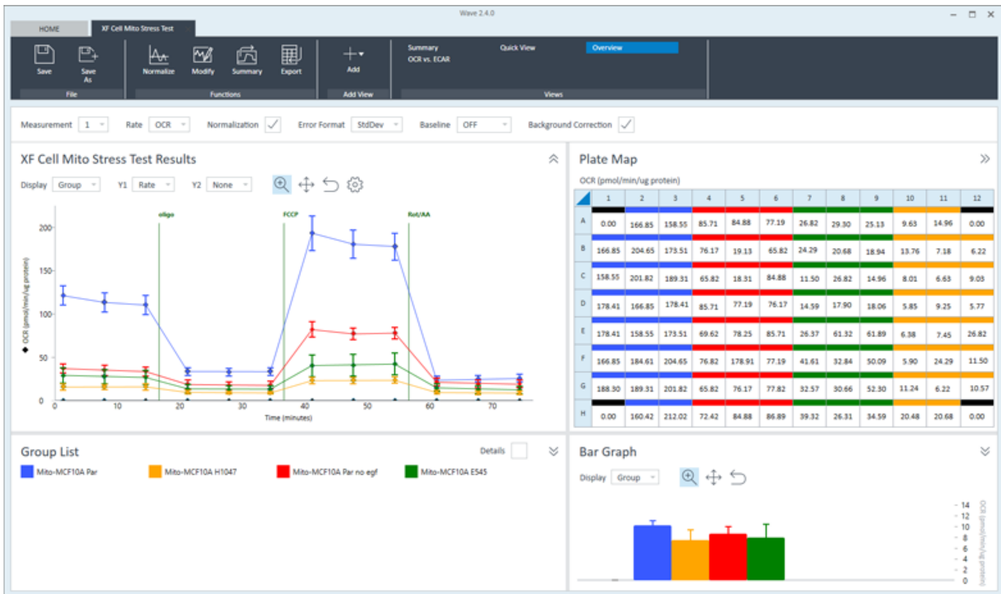


Figure 47 Overview view

NOTE Add multiple Overview analysis views repeating the process of **Add View > Overview** from the list of analysis views. This facilitates generating several customizable overview analysis views to display specific assay groups, or compare a control group versus experimental groups, groups treated with various compound concentrations or other variables measured in the assay.

Analysis View #3 - OCR vs. ECAR

The **OCR vs. ECAR** view displays the **OCR** on the y-axis and (by default) **ECAR** on the x-axis. To display **OCR vs. ECAR**, click **Add View** in the upper-left region of the screen, and select **OCR vs. ECAR** from the drop-down menu. Use the rate drop-down menu to change the rate displayed on the x-axis to either **PER** or **PPR**. **OCR** is always displayed on the y-axis, and cannot be changed. (See Figure 48.)

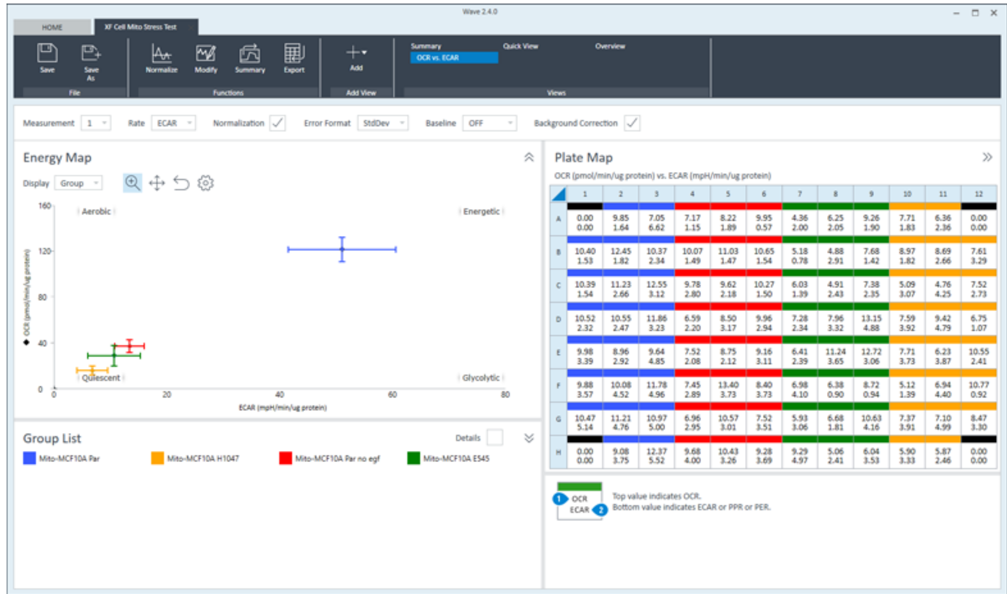


Figure 48 OCR vs. ECAR view

Graph title and Quadrant labels

The default graph title is **Energy Map**. (See Figure 48.) The corners of the Energy Map have labels representing the relative bioenergetic state of the group(s) related to each other for the selected rate measurement. To modify the graph display, including the **Graph Title** and the **Quadrant Labels**, click the **Settings** cog. (See Figure 49.)

Figure 49 Graph display modification options

Dual rate display on Plate Map

The **Plate Map** displays two rate measurements within each well (OCR vs. ECAR, OCR vs. PER, or OCR vs. PPR) as indicated by the legend below the Plate Map. (See Figure 48.)

Chart Types in Wave

Kinetic graph (rate versus time)

A kinetic graph is the most common way to display rate data from Agilent Seahorse XF Analyzers. (See Figure 50.) The kinetic graph displays the rate measurement on the y-axis, and the time on the x-axis. During an assay, data is acquired and plotted in real-time as a kinetic graph. Kinetic graphs are also found in the Quick View and Overview analysis views.

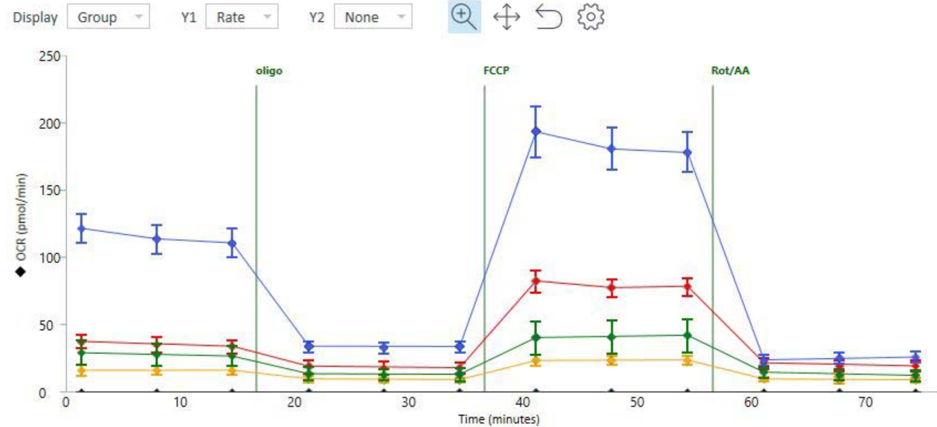


Figure 50 Kinetic graph

Scatter plot (rate 1 versus rate 2)

Another common way to express result data is a scatter plot of OCR vs. ECAR, where OCR is plotted on the y-axis, and ECAR plotted on the x-axis. (See Figure 51.) Data points are displayed on the OCR vs. ECAR scatter plot for a single rate measurement for each group in the assay. The OCR vs. ECAR graph is only available for analysis, not while running an XF assay.

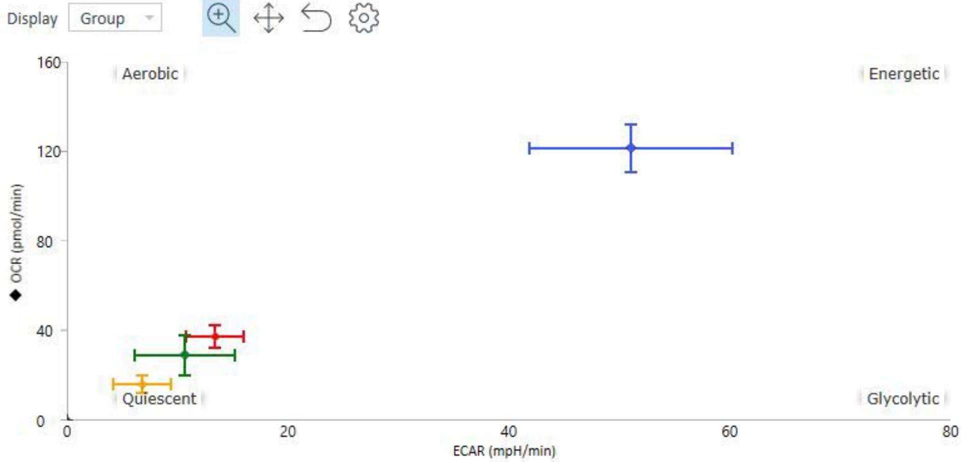


Figure 51 Scatter plot

Plate Map

The Plate Map displays rate data for the selected rate measurement of each assay well. (See Figure 52.) Plate Maps are always displayed in the upper right corner of Wave while running an assay, and on the Quick View, Overview, OCR vs. ECAR analysis views. The Quick View has a button to display the Plate Map, which is hidden by default. The default rate measurement displayed on the Plate Map is rate measurement 1.

Plate Map

OCR (pmol/min/ug protein) vs. ECAR (mpH/min/ug protein)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00 0.00	9.85 1.64	7.05 6.62	7.17 1.15	8.22 1.89	9.95 0.57	4.36 2.00	6.25 2.05	9.26 1.90	7.71 1.83	6.36 2.36	0.00 0.00
B	10.40 1.53	12.45 1.82	10.37 2.34	10.07 1.49	11.03 1.47	10.65 1.54	5.18 0.78	4.88 2.91	7.68 1.42	8.97 1.82	8.69 2.66	7.61 3.29
C	10.39 1.54	11.23 2.66	12.55 3.12	9.78 2.80	9.62 2.18	10.27 1.50	6.03 1.39	4.91 2.43	7.38 2.35	5.09 3.07	4.76 4.25	7.52 2.73
D	10.52 2.32	10.55 2.47	11.86 3.23	6.59 2.20	8.50 3.17	9.96 2.94	7.28 2.34	7.96 3.32	13.15 4.88	7.59 3.92	9.42 4.79	6.75 1.07
E	9.98 3.39	8.96 2.92	9.64 4.85	7.52 2.08	8.75 2.12	9.16 3.11	6.41 2.39	11.24 3.65	12.72 3.06	7.71 3.73	6.23 3.87	10.55 2.41
F	9.88 3.57	10.08 4.52	11.78 4.96	7.45 2.89	13.40 3.73	8.40 3.73	6.98 4.10	6.38 0.90	8.72 0.94	5.12 1.39	6.94 4.40	10.77 0.92
G	10.47 5.14	11.21 4.76	10.97 5.00	6.96 2.95	10.57 3.01	7.52 3.51	5.93 3.06	6.68 1.81	10.63 4.16	7.37 3.91	7.10 4.99	8.47 3.30
H	0.00 0.00	9.08 3.75	12.37 5.52	9.68 4.00	10.43 3.26	9.28 3.69	9.29 4.97	5.06 2.41	6.04 3.53	5.90 3.33	5.87 2.46	0.00 0.00

Figure 52 Plate map

To change the measurement displayed in the Plate Map, use the Measurement drop-down above the kinetic graph or scatter plot. (See Figure 53.) The Rate Highlight tool on the kinetic graph will move to the selected rate. Hide the Rate Highlight tool by clicking **Options** above the kinetic graph/scatter plot, and uncheck **Show Rate Highlight**.

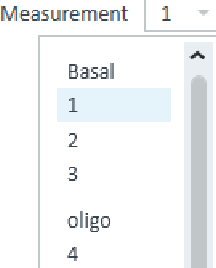


Figure 53 Measurement drop-down

Bar Graph

The Bar Graph below the Plate Map displays the average rate for each group (by default) for a selected measurement. (See Figure 54.) Use the Display drop-down menu to change the rate display from Group (average) to Well (individual well) mode.

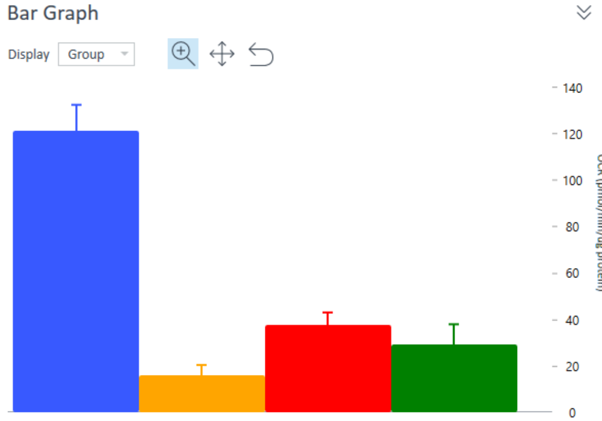


Figure 54 Bar graph

The Bar Chart graph is only available during analysis, not while running an assay. The Bar Chart view provides an alternate data display that can facilitate outlier identification, optimal FCCP concentration, optimal cell seeding density, or other trends that may not be obvious when viewing data as kinetic graph or scatter plot.

Group List (legend)

The Group List is the legend for the data plotted in the kinetic graph/scatter plot. (See Figure 55.) It provides several functions to modify the data plots:

- Hide groups from the data plot by double-clicking the name of the group. To unhide the data from the graph, double-click the hidden group again. When a group is hidden, the mean and standard deviation of the group will be: Mean: 0.00 and Standard Deviation: 0:00.
- Display group statistics for the selected rate measurement by checking the **Details** box in the upper-right corner of the Group List. Statistics are displayed as average and error for the selected rate measurement. Select a different rate measurement to display group statistics for that rate. Assay wells that have been turned **OFF** on the Plate Map are not included in the calculated group statistics.

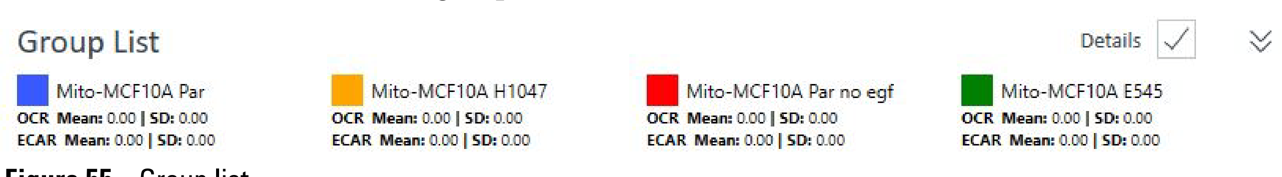


Figure 55 Group list

Types of Data in Wave

Rate data: OCR and ECAR

Use the Rate drop-down box to view the rate data options. (See Figure 56.) The two rates measured by the Agilent Seahorse XF Analyzers are:

- Oxygen Consumption Rate: (OCR) (pmol/min)
- Extracellular Acidification Rate: (ECAR) (mpH/min)

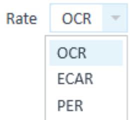


Figure 56 Rate data drop-down

Level data: O_2 and pH

To view **Level** data for O_2 and pH levels for each rate measurement during the assay, use the Y1: drop-down. (See Figure 57.) The O_2 level data is displayed as oxygen tension in units of mmHg. As the biological material in the well consumes oxygen during a measurement, the oxygen tension will decrease. This decrease in oxygen tension during a used to calculate the rate of oxygen consumption (OCR). The pH level data displays the changes in pH for each rate measurement, and is used to calculate ECAR. Level data can be displayed while running an assay, and on the Overview and Data analysis views.

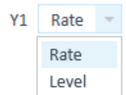


Figure 57 Y1 Drop-down

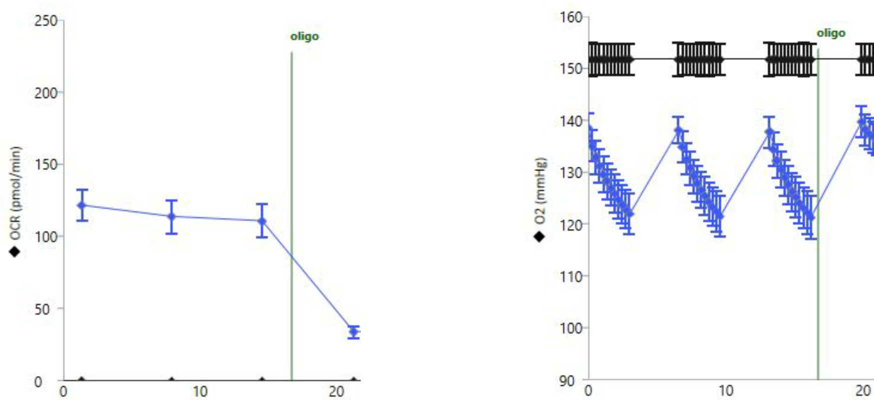


Figure 58 Oxygen Consumption Rate (OCR) data in **Rate** mode (left).
Oxygen tension (O_2) data displayed as mmHg in **Level** mode (right).

Introduction to Acidification Data

The Agilent Seahorse XF Analyzer sensor probes measure changes in the concentration of free protons in the microchamber in real-time; this data is referred to as Extracellular Acidification Rate (ECAR). ECAR can be transformed into a number that reflects the number of protons extruded over time, Proton Production Rate (PPR), and Proton Efflux Rate (PER). Table 2 shows the types of acidification data.

Table 2 Types of acidification data

Type of acidification data:	ECAR	PER	PPR
Detects acidification from all sources?	Yes	Yes	Yes
Changes with media formulation?	Yes	No	No
Accounts for buffer factor of the system?	No	Yes	No
How buffering is accounted for?	None	Buffer factor	Buffer capacity
Displayed while running assay?	No	No	No

Proton Efflux Rate

PER is a new rate measurement calculated in Wave software. PER delivers a quantitative measure of extracellular acidification and is only available in post-run assay results. It is not displayed while running an assay. ECAR is the default reported acidification data in Wave software. The three rate options displayed in the **Rate Measurement** drop-down menu are: OCR, ECAR, and PER (previously PPR was the default). To change the reported acidification data option to PPR, use the **Options** menu under **Wave Home**. PER is calculated using the following equation:

PER (pmol H+/min) = ECAR (mpH/min) × B.F. (mmol/L/pH) × Geometric Volume (µL) × Kvol

Table 3 Wave volume variables

Variable	Unit	Description
Extracellular Acidification Rate (ECAR)	mpH/min	Rate data measured during an assay, reported as the rate of change in mpH in an assay well.
Buffer Factor (BF)	mmol/L/pH	Measure of the *in situ* buffer capacity obtained in XF instruments, and accounts for both medium buffer capacity and XF assay conditions. For standard XF Glycolytic Rate Assay media, this value is prepopulated.
Geometric volume	µL	Physical (geometric) volume of the measurement microchamber.
Volume scaling factor (Kvol)	No units	Volume scaling factor used to account for total proton production in the measurement chamber.

Display PER data in Wave

Wave software calculates and reports total PER on the Overview, OCR vs. ECAR, and Data analysis views for the following XF Analyzers: XFe96, XFe24, XFp, and XF96. Use the **Rate Measurement** drop-down menu to select **PER** as the rate to display on the kinetic graph (Overview analysis view), or scatter plot (OCR vs. ECAR analysis view).

If PER is displayed

Wave automatically displays PER in two scenarios:

- When the Buffer Factor is automatically read from the sensor cartridge
- The assay media selected is the XF Glycolytic Rate Assay

The equation above demonstrates how PER is calculated in Wave software - the only user-changeable variable in the equation is Buffer Factor. A default Buffer Factor is automatically used for the PER calculation. If you are using a custom

assay medium, then Buffer Factor must be determined manually. Refer to the *Agilent Seahorse XF Buffer Factor Protocol* (available in the XF Glycolytic Rate Assay Report Generator download folder) for guidance.

If PER is NOT displayed

Older result files in which the Buffer Factor is not included will show zero for PER. PER will not be displayed:

- For XF24 and XF24-3 sensor cartridges
- For Spheroid and Islet plate types
- For assay template/assay result files that do not have the Glycolytic Rate Assay Medium (DMEM-based) or (RPMI-based) selected, OR if a custom Buffer Factor value is not entered.
- When background well Buffer Factor values have not been configured.

Using Agilent Seahorse XF Glycolytic Rate Assay Medium, display PER data

Figure 59 Assay Media option

1. Open the assay result file in Wave software.
2. Click **Modify**.
3. Click **Add** next to **Assay Media**. (See Figure 59.)
4. To select the assay medium, use the **Media** catalog drop-down menu. (See Figure 60.)
    - **Glycolytic Rate Assay Medium (DMEM-based)**
    - **Glycolytic Rate Assay Medium (RPMI-based)**

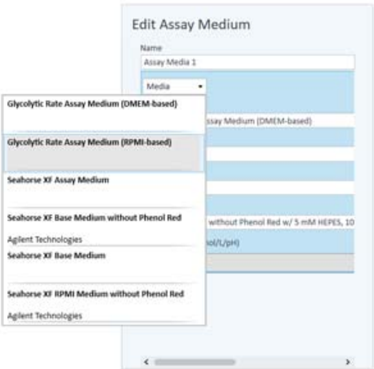


Figure 60 Assay medium media menu

5 To configure Background Well Buffer Factor, click **Assay Media**. (See Figure 61.)

6 Click **Configure**.

7 Under the column header **GRA Media**, check the boxes next to each background well, and click **Save**. (See Figure 61.)

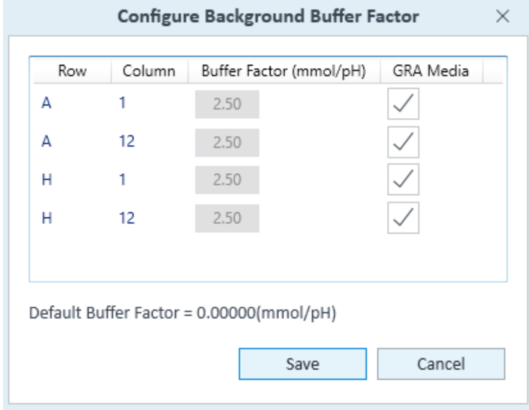


Figure 61 Configure background buffer factor

8 Assign the **Assay Media** to each group in the group list. First click **Collapse/Expand All** to display the Group Definitions for each group.

9 Use the **Media** drop-down menu to select the newly-added Glycolytic Rate Assay Medium (DMEM-based) or (RPMI-based) for each group.

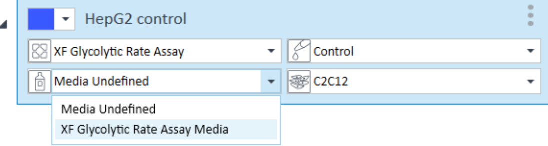


Figure 62 Media drop-down menu

10 When finished, click **Apply**.

11 The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.

12 When finished with analysis, save the changes made to the result file.

Display PER for assays run with custom assay media

Prior to running the assay, calculate the Buffer Factor of the custom assay medium. Follow the steps in the *Agilent Seahorse XF Buffer Factor Protocol* (available in the XF Glycolytic Rate Assay Report Generator download folder) for guidance.

1. Open the assay result file in **Wave** software.
2. Click **Modify**.
3. Click **Add** next to **Assay Media**.
4. In the Edit Assay Medium window, enter the **Buffer Factor** in the field provided.
5. Next, click **Assay Media** again to configure Background Well Buffer Factor.
6. Click **Configure**.
7. Under **Buffer Factor (mmol/pH)**, type in the same Buffer Factor value and click **Save**. (See Figure 63.)

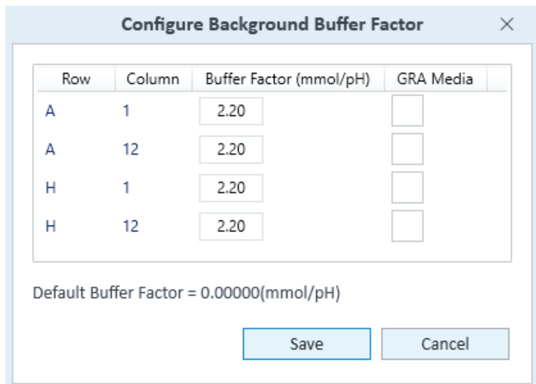


Figure 63

8. Assign the **Assay Media** to each group in the group list. First click **Collapse/Expand All** to display the Group Definitions for each group.
9. To select the appropriate medium for each group, use the **Media** drop-down menu.
10. When finished, click **Apply**.
11. The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.
12. When finished with analysis, save the changes made to the result file.

Data Analysis Using the XF Glycolytic Rate Assay Report Generator

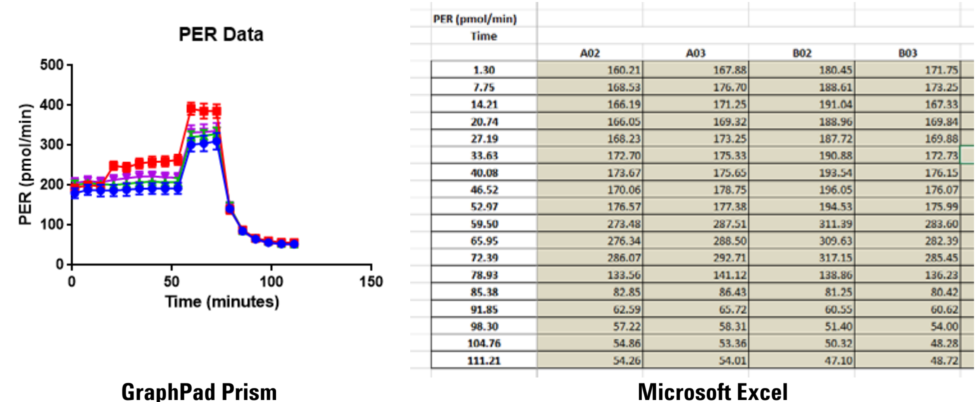
Wave software calculates and reports total PER. If you performed the XF Glycolytic Rate Assay, you must export data to the XF Glycolytic Rate Assay Report Generator to calculate glycoPER, the rate of glycolysis-specific proton efflux. (See Figure 64.)

Figure 64

1. Open the assay result file in Wave software.
2. Click **Export**.
3. Select the **XF Glycolytic Rate Assay Report Generator** from the list.
4. Modify the file name or save location if necessary, then click **Save**.

Export total PER data

PER data is exported to both Microsoft Excel and GraphPad Prism file exports. (See Figure 65.) For manual PER data analysis or graphing purposes, export data from Wave to either file types using the **Export** function located in the top-level ribbon.



PER (pmol/min) Time	A02	A03	B02	B03
1.30	160.21	167.88	180.45	171.75
7.75	168.53	176.70	188.61	173.25
14.21	166.19	171.25	191.04	167.33
20.74	166.05	169.32	188.96	169.84
27.19	168.23	173.25	187.72	169.88
33.63	172.70	175.33	190.88	172.73
40.08	173.67	175.65	193.54	176.15
46.52	170.06	178.75	196.05	176.07
52.97	176.57	177.38	194.53	175.99
59.50	273.48	287.51	311.39	283.60
65.95	276.34	288.50	309.63	282.39
72.39	286.07	292.71	317.15	285.45
78.93	133.56	141.12	138.86	136.23
85.38	82.85	86.43	81.25	80.42
91.85	62.59	65.72	60.55	60.62
98.30	57.22	58.31	51.40	54.00
104.76	54.86	53.36	50.32	48.28
111.21	54.26	54.01	47.10	48.72

Microsoft Excel

Figure 65 Exported data examples

Proton Production Rate

PPR is calculated in Wave software using the following equation:

PPR (pmol H+/min) = ECAR (mpH/min) × Buffer Capacity (mol/L/pH) × Geometric Volume (L)

Report PPR data in Wave

The default Acidification Data displayed in Wave software is PER, PPR is not available for selection in the Rate Measurement Drop-Down Menu until it is enabled in the Wave General Options:

1. Open **Wave** software.
2. Click **Options**.
3. Under **General Settings > Acidification Data** click the radio button next to **Report PPR; Use Buffer Capacity (mol/L/pH)**.
4. Click **Save**.
5. Close/reopen **Wave** software for the changes to take effect.

Display PPR data in Wave

Wave software reports PPR data on the Overview, OCR vs. ECAR, and Data analysis views when enabled, and is calculated using ECAR data and Buffer Capacity of the assay medium used.

Buffer capacity

Buffer Capacity is required for calculating PPR from acquired ECAR data. Buffer Capacity changes based on the constituents of the medium. Individual constituents have different abilities to buffer the medium from pH changes. For accurate PPR data, the Buffer Capacity of each medium used must be measured and entered on **Modify > Group Definitions** tab in an assay result file.

Record Buffer Capacity of an individual assay medium:

1. Open an assay result file.
2. Click **Modify > Group Definitions**.
3. Double-click **Assay Media** (if an assay medium is not specified, use the **Add** button to add an assay medium).

4 Click the assay medium and enter the **Buffer Capacity** value. (See Figure 66.) If no Buffer Capacity is entered, the default value of 0.00078 is used. If more than one assay medium is used, repeat the same steps for each additional media.

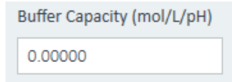


Figure 66 Buffer Capacity value

Record Buffer Capacity for background wells

1 Double-click **Assay Media** below **Group Definitions**.

2 Click **Configure**.

3 In the Configure Background Buffer Capacity window, type in the **Buffer Capacity** value for each background well listed. (See Figure 67.) If only one assay medium is used, enter the same **Buffer Capacity** value. If more than one assay medium is used, enter the **Buffer Capacity** of the assay medium for the appropriate background well.

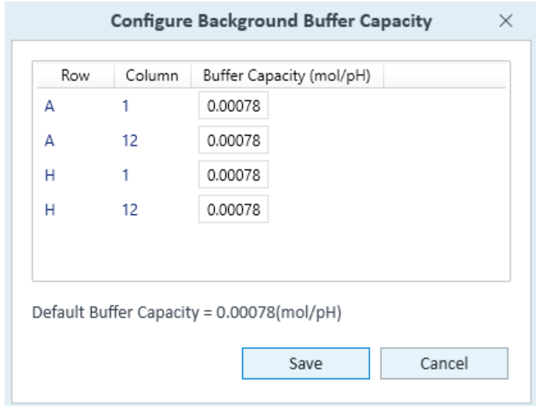
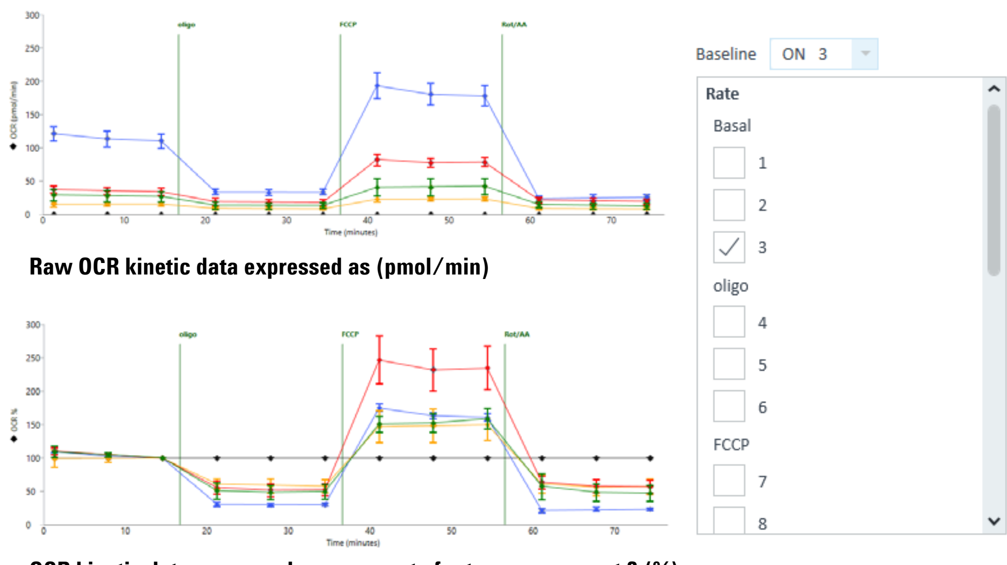


Figure 67 Configure Background Buffer Capacity window

Baseline to a Rate Measurement (%)

The Baseline function in Wave software enables rate data to be expressed as a percent of a selected rate measurement, or as a percent of a control group. Use the **Baseline** drop-down menu located on the Overview and OCR vs. ECAR analysis views (only). Select a **Rate** measurement to express kinetic data as a percent of that rate measurement. (See Figure 68.)



Raw OCR kinetic data expressed as (pmol/min)

OCR kinetic data expressed as a percent of rate measurement 3 (%)

Figure 68 Baseline to Rate measurement examples

Baseline to a Control Group (%)

A new baseline feature allows rate data to be expressed as a percent of a Control Group. The example shown in Figure 69 shows the appropriate use of this feature. A Control group (blue line) with only media injections is selected as the baseline control group to baseline the two assay groups Glucose Dependency (orange line) and Glucose Capacity (green line). Figure 69 shows how this feature can provide a better graphical representation of the effect of glucose inhibition on OCR, and facilitate data interpretation.

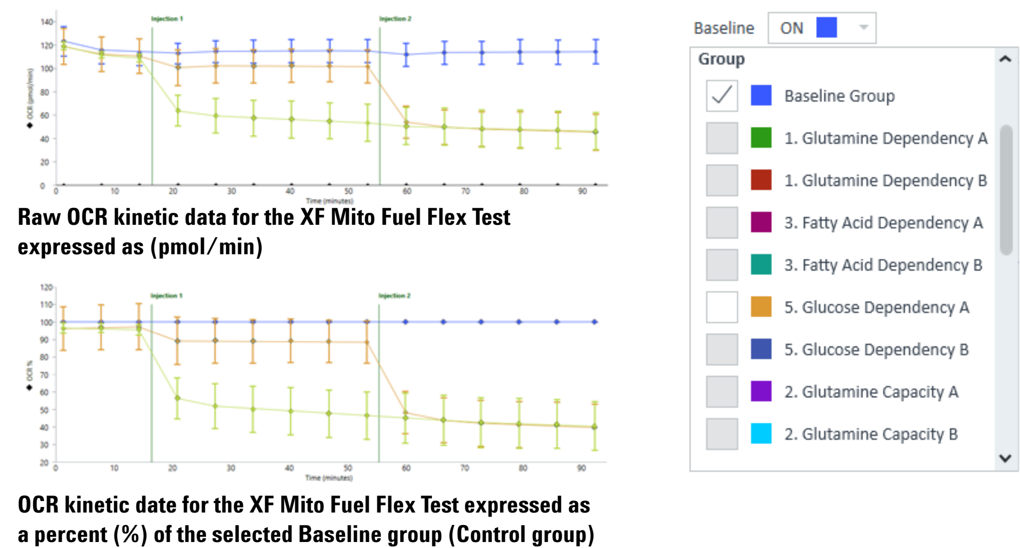


Figure 69 Baseline to Control group examples

Standard Deviation and Standard Error of the Mean

Two types of error bars in Wave are Standard Deviation (**StdDev**) and Standard Error of the Mean (**SEM**). Use the **Error Format** drop-down menu to change the error bar type displayed on the kinetic graph/scatter plot and the Group List. (See Figure 70.) to hide error bars from the data graph, select **None**. In **Well** mode, error bars are not displayed.

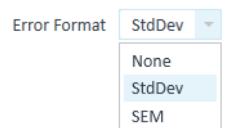


Figure 70 Error Format menu

Rate Overlay: OCR, ECAR, PER, PPR, O_2, or pH

Compare rates or level of data acquired during an assay from on one kinetic graph for one or more groups. Select data to overlay with the Y1 data using the **Y2** drop-down menu above the kinetic graph. (See Figure 71.)

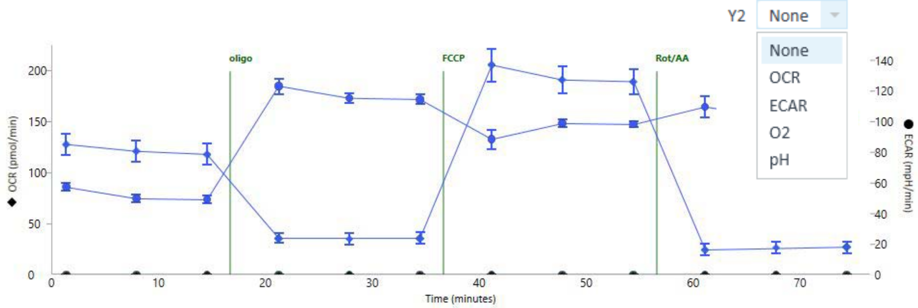


Figure 71 Rate overlay

Customizing Data Displays

Excluding assay wells

By default, all assay wells on the Plate Map are turned **ON** when opening a new assay result file. Click an assay well to change the state of the well, excluded or included in the group statistics and graphed data for the select group. Assay wells that are turned off on the Plate Map have a grey background. (See Figure 72.) Assay wells that are included in the graphs and group statistics have a white background. (See Figure 72.) Assay wells must be manually turned off from each analysis view. Turning off an assay well on one analysis view does not apply to other views.

Two assay wells in this group are turned OFF. These assay wells are excluded from the graph and group calculations.

All assay wells in this group are turned on and included in the graph and group calculations.

Figure 72 Assay well states

Column and row headers can be used to exclude entire vertical or horizontal sections of a Plate Map. Click the numerical column header or letter header for a row to turn the assay wells in that column or row **OFF**.

Click the small triangle in the upper-left corner of the Plate Map to turn off all assay wells on the Plate Map. (See Figure 73.) Charts on this analysis view will not display any groups since all assay wells are turned off.

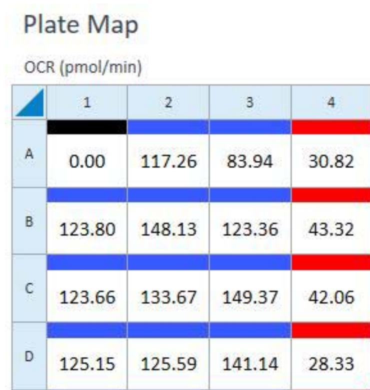


Plate Map

OCR (pmol/min)

	1	2	3	4
A	0.00	117.26	83.94	30.82
B	123.80	148.13	123.36	43.32
C	123.66	133.67	149.37	42.06
D	125.15	125.59	141.14	28.33

Figure 73 Plate Map toggles

Display modes: group and well

Rate data is graphically displayed in Group mode on all analysis views by default. Group mode displays the average rate value based on the assay wells in each group for each measurement. Group mode displays a single kinetic trace for each group on the Overview analysis view, and a single data point per group for each rate measurement on the OCR vs. ECAR view. In Group mode, error within each group is displayed as Standard Deviation or Standard Error of the Mean. Switch to **Well** mode to display individual kinetic traces or data points for each assay well in each group. Error bars are not displayed in Well mode.

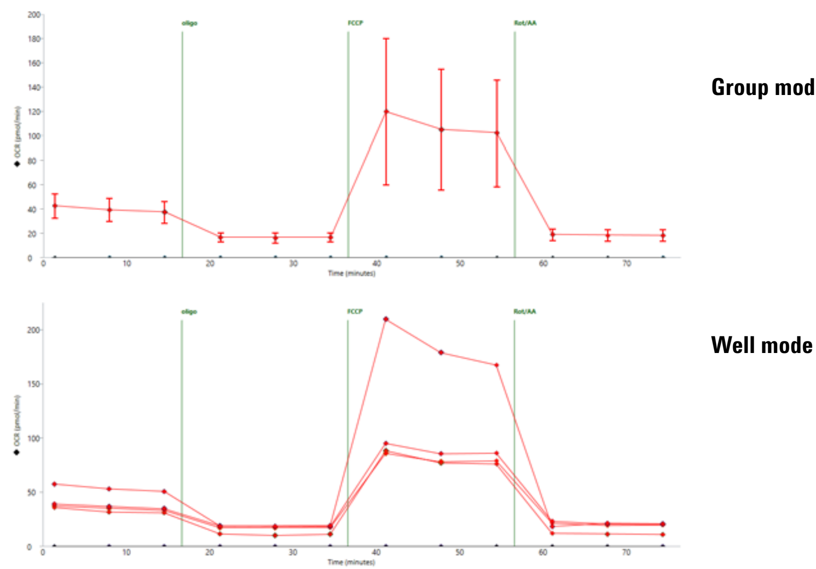


Figure 74 Example of an OCR kinetic graph in Group and Well mode

Resize and Adjust view areas

View areas on the different analysis views can be adjusted in two ways: using the gray borders (red arrows in Figure 75) and the << and >> arrows (outlined in red in Figure 75) to hide entire regions of the view (Overview pictured in Figure 75). Stretch or reduce the size of the kinetic graph using the border between the kinetic graph and Plate Map. Hide the Group List to increase the vertical size of the kinetic graph using the border between the kinetic graph and Group List. Hide the Bar Chart using the border between the Plate Map and Bar Chart.

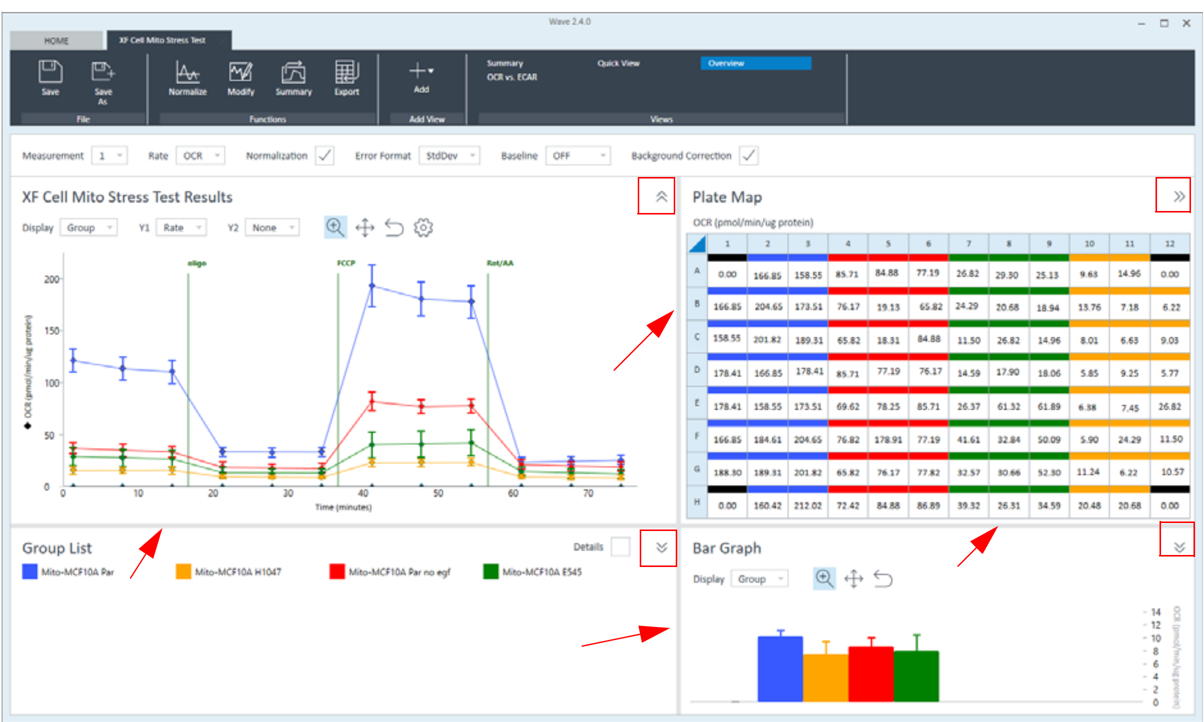
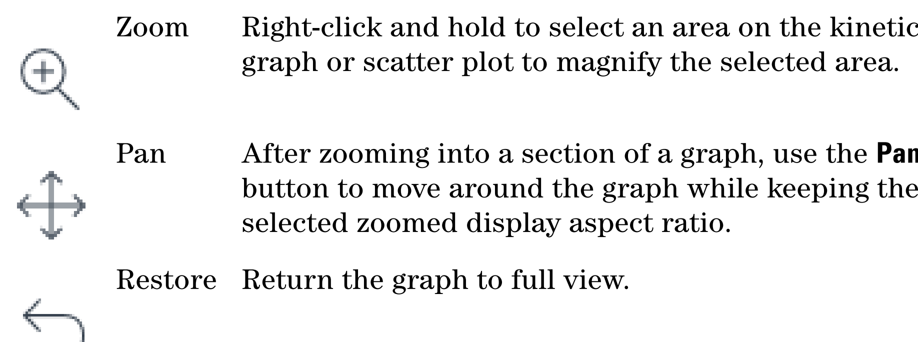


Figure 75 View areas

Zoom, Pan, and Restore

The Zoom, Pan, and Restore buttons are displayed above the kinetic graph on the Overview and the scatter plot on the OCR vs. ECAR view.



	Zoom	Right-click and hold to select an area on the kinetic graph or scatter plot to magnify the selected area.
	Pan	After zooming into a section of a graph, use the **Pan** button to move around the graph while keeping the selected zoomed display aspect ratio.
	Restore	Return the graph to full view.

Graph display options

To show the graph display formatting options menu, click the cog next to the restore button.

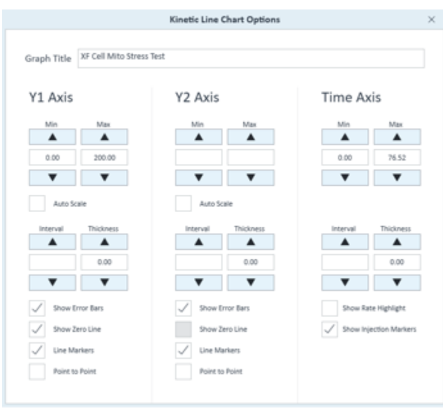
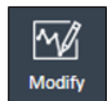


Figure 76 Graph display options

- **Minimum** and **Maximum**: Set the minimum and maximum bounds for the Y1, Y2, and X-axes.
- **Interval**: Set the major units for the Y1, Y2, and X-axes.
- **Thickness**: Add and adjust thickness of Y1, Y2, and X-axes gridlines. Adjust values in 0.50 intervals. Default value is '0.00' (no gridlines displayed).
- **Show Error Bars**: Toggle error bars **ON/OFF**.
- **Show Zero Line**: Display a horizontal reference line at the zero when the y-axis is negative.
- **Point-to-Point**: Display point-to-point rates for the selected axis.
- **Show Rate Highlight**: Toggle **ON/OFF** display of the vertical blue rate highlight bar on the kinetic graph (indicates the selected rate measurement that corresponds to values displayed on Plate Map).
- **Show Injections Marker**: Toggle the vertical injection lines on the kinetic graph **ON/OFF**.

Modifying Assay Result Files

Modify enables you to add or edit details of an assay result file. Modify is most-frequently used to add important experiment notes or add additional Group Definition details (group names, compound injections, pretreatment details, and reassign assay wells to an outliers group or unassign assay wells from the Plate Map entirely). To display the Group Definitions, Plate Map, Injection Names, General Information as tabs, click **Modify** (in the **Functions** ribbon). The Group Definitions is always displayed by default.



The views in Modify mode look almost identical to the template designer views. When actively modifying an assay result file, Wave will indicate the program is in Modify mode in the upper-left corner of Wave (upper-left corner of Wave). To return to assay result data, click **Apply** (upper-right corner of Wave) to apply modifications to the assay result file, or click **Cancel** to cancel out of Modify mode and prevent any changes from taking effect.

Modify Assay Mode

NOTE The Modify function enables textual changes only, and is for adding notes or updating details of an assay. Wave does not allow or enable access to edit rate data acquired during the assay.

Group definitions

To display the Injection Strategies, Pretreatments, Assay Media and Cell Type details entered in the assay template prior to running the assay, select **Group Definitions**. Modifications to the Group Definitions tab include:

- Renaming assay groups
- Reconfigure details for one or more groups
- Add additional group information that was left out of the initial assay template design, or
- Add Buffer Factor to calculate PER

Plate Map

To display and edit conditions of each assay group using the drop-down menus below the group name, select **Plate Map**. This tab also enables reassigning group locations on the Plate Map. select a group, then select the appropriate assay wells on the Plate Map.

Injection Names

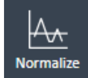
To edit the name of each compound injection during the assay, select **Injection Names**. The specified injection names from the Instrument Protocol tab are displayed above each injection line on a kinetic graph. The default name of each injection is: 'Injection 1', 'Injection 2', Injection 3', and 'Injection 4'. Type an Injection Name for each compound injection, and click **Apply** to display the new name above the corresponding injection line.

General Information

To edit details of an assay result file, such as: Project Name, PI, Well Volume, or Port Volumes, select **General Information**.

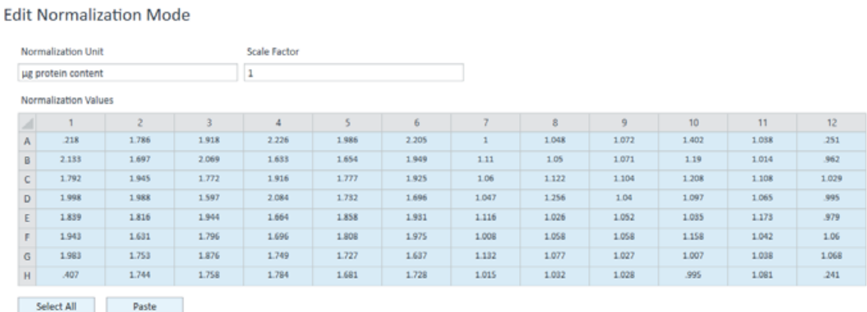
Normalization

In the context of a Agilent Seahorse XF assay, normalization is the process of recalculating rate data to account for variations in a relevant cell parameter (such as cell count or protein concentration) that can cause well-to-well variability. The Normalization function in Wave provides a simple method to apply normalization data to the measured rate data. To use the normalization function, an independent assessment of the assay wells for cell number, DNA content, protein concentration or similar parameter is required. (See Figure 77 on page 69.)



To normalize data in Wave, three components are used:

- **Normalization Values (required):** The numeric data generated from the independent assessment of the well (cell count, protein concentration, DNA content, and so forth).
- **Normalization Unit (required):** This alphanumeric field describes the units to which the data are to be normalized. It comprises the desired value to which the data will be normalized (see Normalization Scale Factor) plus the unit of measure of your normalization values (such as “cells”, “mg”, “ng”, and so forth).
- **Normalization Scale Factor:** This number determines what value the rate data will be normalized to. Default is 1 and adjustment is optional.


Edit Normalization Mode

Normalization Unit: µg protein content

Scale Factor: 1

Normalization Values

	1	2	3	4	5	6	7	8	9	10	11	12
A	.218	1.786	1.918	2.226	1.986	2.205	1	1.048	1.072	1.402	1.038	.251
B	2.133	1.697	2.069	1.633	1.654	1.949	1.11	1.05	1.071	1.19	1.014	.962
C	1.792	1.945	1.772	1.916	1.777	1.925	1.06	1.122	1.104	1.208	1.108	1.029
D	1.998	1.988	1.597	2.084	1.732	1.696	1.047	1.256	1.04	1.097	1.065	.995
E	1.839	1.816	1.944	1.664	1.858	1.931	1.116	1.026	1.052	1.035	1.173	.979
F	1.943	1.631	1.796	1.696	1.808	1.975	1.008	1.058	1.058	1.158	1.042	1.06
G	1.983	1.753	1.876	1.749	1.727	1.637	1.132	1.077	1.027	1.007	1.038	1.068
H	.407	1.744	1.758	1.784	1.681	1.728	1.015	1.032	1.028	.995	1.081	.241

Select All | Paste

Figure 77 Normalization mode

Add normalization values

To display the Edit Normalization Mode, click **Normalize** (in the **Functions** ribbon). The Normalization Values table is where to copy/paste normalization data in Wave, two ways to add normalization data are:

Option #1:

1 Open the independent normalization data file, the data must be in the same grid format as the Normalization Values table.
2 Select the normalization data and copy to the clipboard [**Ctrl+C**].
3 Open a Agilent Seahorse assay result file in Wave Desktop.
4 Click **Normalize**.
5 To insert the copied data the Normalization Table, click **Select All > Paste**.
6 Click **Apply**.

Option #2:

1 Open a Agilent Seahorse assay result file in Wave Desktop.
2 Click **Normalize**.
3 Type each value into the Normalization grid manually.
4 Click **Apply**.

NOTE Background wells should be left blank on the Normalization Table.

Normalization unit

Before applying normalization values, Wave requires a Normalization Unit. The Normalization Unit is displayed on the Y1 axis title on the kinetic graph when normalization is applied and checked **ON**.

Normalization Unit

µg protein

For example, type µg Protein into the **Normalization Unit** field, and the Y1 axis label for OCR will be displayed on the kinetic graph as: (pmol/min/µg Protein). When using the Normalization Scale Factor, the scale factor value must also be entered to the Normalization Unit field.

Normalization scale factor

The Normalization Scale Factor allows you to scale normalized data to a specific factor, such as cell number. It is often preferable to normalize to an average or rounded number.

Enter a scale factor value to recalculate and multiply normalized rate data based on the equation shown below:

Normalized Rate (well)=[Rate (well)/(Normalization Value)]*Normalization Scale Factor

For example, if assay wells contain values of 12052, 12503, and 12757 cells per well, a user might enter `10000` as the normalization scale factor.

NOTE Be sure to enter the same value in the Normalization Unit field to display the factor correctly on the y-axis. For example, if using cell count and the scale factor is 10000, enter 10000 cells as the Normalization Unit.

Viewing normalized data

After applying changes made to the Normalize function, the Normalization checkbox will become active on all analysis views. Toggle the checkbox **ON/OFF** to change the data display between normalized and nonnormalized rates on the kinetic graph, scatter plot, and Plate Map. All data graphs will display the Normalization Unit entered. (See Figure 78.)

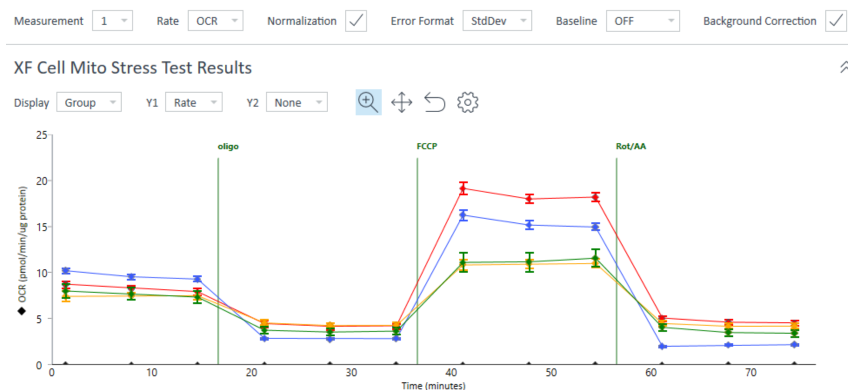
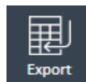


Figure 78 Normalized data

Wave Data Export Options

Assay result data can be exported from Wave for additional analysis, graphing, or other purposes. Use the **Export** button to display the available export options from any analysis view, or right-click a graph/Plate Map to display the menu options for exporting data. To export raw data, select **Microsoft Excel** or **GraphPad Prism** options from the list. Create a customizable summary report by exporting data to an XF Report Generator. Select the XF Report Generator that matches the Agilent Seahorse assay type.



Export to Microsoft Excel

Export assay result data to Microsoft Excel from Wave for additional data analysis or graphing purposes.

1 Click **Export** (in the Functions ribbon).

2 Click **Microsoft Excel**.

3 Choose a save location, and click **Save**.

Using the export function described above, there will be between 7–13 tabs in the exported Excel file depending on the data configured in Wave:

- **Raw data**: Exporting raw result data (not normalized, baseline OFF) will have seven tabs of data. (See Figure 79.)

Measurement 1	
	1
A	0.00
B	46.24
C	36.60
D	44.44
E	64.52
F	56.29
G	47.87
H	0.00

Rate (Plates) Tab: Raw OCR rates for rate measurement 1 (from an Agilent Seahorse XFp Analyzer).

Figure 79 Raw data

a **Assay configuration**: Information from the assay result file including the Group Layout (Plate Map), Cartridge/Cell Plate Barcode and other information, Instrument Protocol, and normalization data and normalization unit.

b **Rate**: Raw OCR, ECAR, and PER or PPR data in a column format organized by measurement.

c **Rate (Columns)**: Raw OCR, ECAR, and PER or PPR data in a column format organized by group. Similar output format to the GraphPad Prism export.

d **Rate (Plates)**: Raw OCR, ECAR, and PER or PPR data organized by measurement displayed as a Plate Map.

e **Raw**: Raw assay well data for temperature, emission values and reference values. Often used for troubleshooting by Agilent Seahorse Technical Support.

f **Calibration**: Raw calibration data for both the O2 and pH channels.

g **Operation log**: List of each protocol command executed by the analyzer, timing of the command, and outcome (success/fail).

- **Normalized data**: Exporting result data that has been normalized (baseline OFF) will have three additional data tabs in the Excel export. (See Figure 80.)

Measurement 1	
	1
A	0.00
B	0.00385
C	0.00305
D	0.00370
E	0.00538
F	0.00469
G	0.00399
H	0.00

Normalized Rate (Plates) Tab: Normalized OCR rates for rate measurement 1 (from an Agilent Seahorse XFp Analyzer).

Figure 80 Normalized data

a **Normalized rate**: Normalized OCR, ECAR, and PER or PPR data in a column format organized by measurement.

b **Normalized rate (Columns)**: Normalized OCR, ECAR, and PER or PPR data in a column format organized by group. Similar output format to the GraphPad Prism export.

c **Normalized rate (Plates)**: Normalized OCR, ECAR, and PER or PPR data organized by rate, displayed as a Plate Map.

- **Baselined data**: Exporting result data with baseline ON (recalculated as a percent of a rate measurement or of a control group) will have three additional data tabs in the Excel export. (See Figure 81.)

Measurement 1	
	1
A	100.00
B	128.34
C	139.25
D	124.75
E	126.51
F	124.18
G	128.18
H	100.00

Baselined Rate (Plates) Tab: Baseline OCR rates for rate measurement 1 displayed as a percentage (from an Agilent Seahorse XFp Analyzer).

Figure 81 Baseline data

a. **Baselined rate**: Baselined (%) OCR, ECAR, and PER or PPR data in a column format organized by measurement.

b. **Baselined rate (Columns)**: Baselined (%) OCR, ECAR, and PER or PPR data in a column format organized by group. Similar output format to the GraphPad Prism export.

c. **Baselined rate (Plates)**: Baselined (%) OCR, ECAR, and PER or PPR data organized by rate, displayed as a Plate Map.

Excel export legend

The legend on each tab indicates the assay wells that have been excluded from calculations in Wave (turned **OFF** or unassigned). (See Figure 82.) Assay well B in the images below was turned **OFF** in Wave before exporting to Excel.

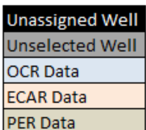


Figure 82 Excel export legend

Export select graph data to Microsoft Excel (from any analysis view)

If you wish to only export select data that is displayed on a kinetic graph, scatter plot, or Plate Map to Microsoft Excel.

1 Right-click anywhere on a chart or **Plate Map** to display an option menu and select **Export Graph Data to Excel**. (See Figure 83.)

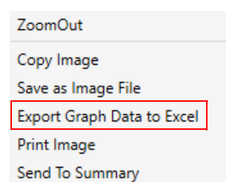


Figure 83 Option menu

2 Browse to the desired save location, type in the file name for the export.

3 Click **Save**.

The data values exported to Microsoft Excel will be in the exact same format as they are displayed on the chart or Plate Map.

NOTE

This Microsoft Excel file export is not compatible with the XF Report Generators. Use the **Export** button in Wave software to export data directly to the XF Report Generators from any analysis view.

Export to GraphPad Prism

Export assay result data to GraphPad Prism for additional data or statistical analysis, or graphing purposes.

1 Click **Export** (on any analysis view).

2 Select **GraphPad Prism**.

3 Choose a save location, and click **Save**.

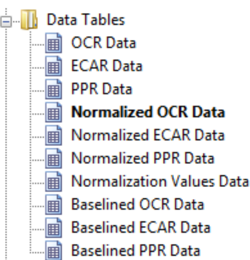
NOTE

Prism export compatibility has been validated using GraphPad Prism versions 6 and 7.

GraphPad Data tables

Wave exports rate data (OCR, ECAR, PER or PPR) to GraphPad as:

- **Raw rate data:** always exported
- **Normalized rate data:** Normalization data must be applied to the assay result file in Wave before export
- **Baseline rate data:** Rate data as a percent of the selected baseline measurement or control group



Sample data output in GraphPad Prism from an Agilent Seahorse XFp Analyzer with two assay groups, **Disease Group** and **Healthy Group**.
(See Figure 84.)

X	Group A			Group B		
Time (minutes)	Disease Group			Healthy Group		
X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1.5591	36.5971	44.4419		64.5248	56.2883	47.8664
8.0789	30.7173	39.6487		56.5285	49.8633	41.6654
14.6040	27.7426	36.9345		53.6014	46.7723	38.8526
21.1255	26.2812	35.6242		51.0053	45.3292	37.3431
27.7413	14.0176	17.1571		25.6648	22.5966	17.3317
34.2693	13.7931	17.2244		26.3632	23.8603	18.1830
40.7907	13.7985	16.4647		25.5408	23.3045	17.3062
47.3159	13.1798	15.6891		24.9620	22.5543	17.4538
53.9360	37.7011	29.9699		74.9459	69.0456	53.5484
60.4695	32.8831	24.3168		69.2413	61.1567	50.3059
66.9959	30.8536	20.7413		63.5366	56.0195	46.1478
73.5296	28.8017	17.9396		59.6601	52.3409	43.6067
80.1601	7.5714	8.5284		14.9611	13.7690	8.6728
86.6962	7.5500	7.7343		15.7009	14.1531	9.1453
93.2338	7.0782	8.9587		14.9156	14.4237	8.4967
99.7749	5.9633	6.9644		14.0657	12.4098	7.0267

Figure 84 Sample data output

- **Column-format Data Display:** Time is a fixed column next to the assay groups, and will scroll horizontally when there are more assay groups.
- **Assay Wells and Columns:** The number of columns with rate data (below each group header) reflects the number of assay wells in each group.

- **Excluded Assay Wells:** Assay wells that have been excluded in Wave will appear as an empty column in Prism. Column A:Y3 below Group A (called Disease Group) in Figure 84 on page 75 shows rate data that has been exported for two wells in the Disease Group. Refer to Project Info or the assay result file in Wave for excluded assay wells in the exported Prism file.
- **Rows and Rate Measurements:** The number of rows reflects the number of rate measurements captured during the assay. In Figure 84 on page 75, there are a total of 16 measurements performed during the assay (16 rows).

GraphPad Kinetic graphs

Every Data Table in the GraphPad Prism file export also contains a corresponding kinetic graph. The graph in Figure 85 represents the data set on the previous page from the Agilent Seahorse XFp Analyzer. The Graphs folder displays the available kinetic graphs based on the type of data exported from Wave, that is, raw data, normalized data, or data as a percent of the baseline. Assay wells or groups that are turned OFF in Wave prior to export will be excluded from the file export.

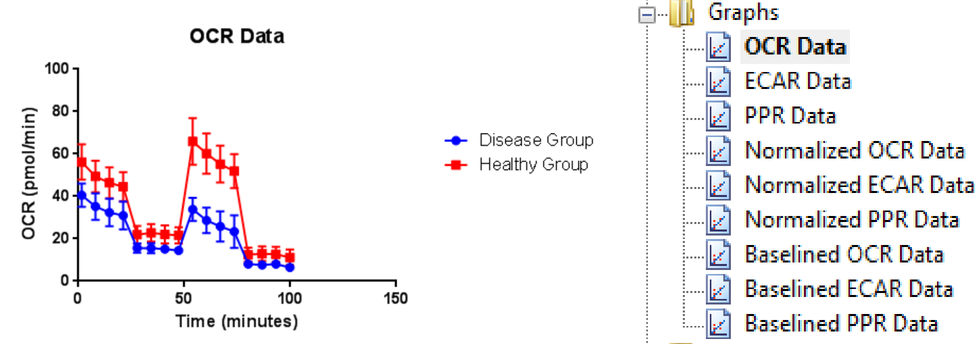


Figure 85 Kinetic graph

GraphPad project info

Information about the experiment and assay properties is displayed in Project info 1 (in the Info folder) including the date the assay was performed, the assay result file name, and normalization unit. (See Figure 86.) The Notes display on the Project Info view shows the group layout, assay wells in each group, and indicates excluded assay wells using brackets (example: [A2]).

Experiment Date	2/5/2015
Experiment ID	XFp_Seahorse XFp Cell Mito Stress Test_Control vs. Disease
Notebook ID	
Project	
Experimenter	
Protocol	
Background Correction	True
Normalization Scale Factor	5000
Normalization Unit	cells

GROUP LAYOUT
Wells between [ ] are unselected wells and they are not exported in the Data Tables.
Group Names between [ ] are unselected groups and they are not exported in the Data Tables.
Background wells and wells not assigned to a group are not exported in the Data Tables.

Group Name: Disease
Group Wells: [B1], C1, D1

Group Name: Healthy
Group Wells: E1, F1, G1

Group Name: Background
Group Wells: A1, H1

Figure 86 Project info view

Export to Agilent Seahorse XF Report Generators

Agilent Seahorse XF Report Generators are Microsoft Excel macro files that automatically calculate the parameters of the selected Agilent Seahorse XF assay, and present the data in a one-page, customizable Summary Report. Wave enables a simple, one-click direct export of result data to the Report Generators. Any modifications in Wave will be incorporated in the exported Report Generator file, including excluded outlier assay wells and normalizing data.

The five Agilent Seahorse Report Generators embedded in Wave are:

- XF Glycolysis Stress Test Report Generator
- XF Glycolytic Rate Assay Report Generator
- XF Cell Energy Phenotype Report Generator
- XF Cell Mito Stress Test Report Generator
- XF Mito Fuel Flex Test Report Generator

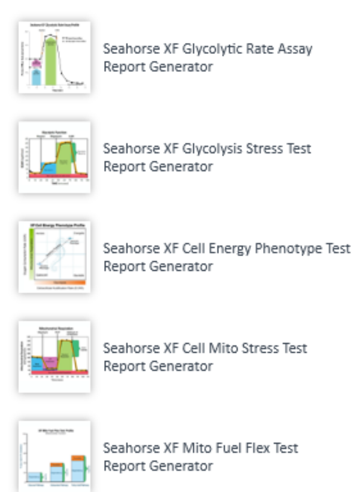


Figure 87 Embedded report generators

NOTE The Agilent Seahorse XF Report Generators are validated for compatible with MS Excel 2010, 2013, and 2016 on Windows PCs and MS Excel for Mac 2011 and 2016 for Apple PCs. For more information on a specific XF Report Generator, refer to the appropriate Report Generator User Guide on the Agilent website.

Export data to an XF Report Generator from any analysis view

1. Open assay result file and modify data as necessary.
2. Click **Export**.

3 Select the Agilent Seahorse XF Report Generator that applies to the result data.

4 The Report Generator file name matches the assay result file name by default. Enter a new file name if desired, select a save location, and click **Save** to save the Report Generator file.

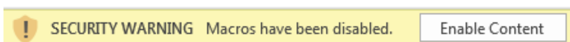
5 Open the **Report Generator** file, and select groups to display. The Select Groups dialogue box is displayed automatically the first time data is exported to the XF Report Generator.

6 To save the Summary Report, click **Save**.

NOTE When exporting an assay result file that has data from two different assays on the same Cell Plate (such as a Agilent Seahorse XF Cell Mito Stress Test and Glycolysis Stress Test), turn **OFF** groups that do not apply to the Report Generator you are exporting to first. Repeat the same process for the other assay groups and Report Generator.

Best practices for exporting data to the Agilent Seahorse XF Report Generators

Enable Macros in the Trust Center Before using a XF Report Generator, macros must be enabled. Macros are disabled with notification in the Microsoft Excel Trust Center by default. Opening a newly-exported Report Generator will display a security warning to enable the macro. To use the XF Report Generator, click **Enable Content**. This must be enabled for subsequent XF Report Generator file exports.



To always enable macros and not be prompted to **Enable Content** each time a Report Generator file is created, enable all macros in the Microsoft Trust Center.

1 Open **Microsoft Excel**.

2 Click **File>Options**.

3 Click **Trust Center>Trust Center Settings**.

4 Click **Macro Settings**.

5 Select **Enable all macros**, and click **OK**.

Exporting normalized data to a Report Generator Normalized rate data in Wave will be exported and used for parameter calculations in the Report Generator. Normalized data is displayed by default after selecting groups to display. To toggle the rate data display between normalized and raw data, use the **Normalize** button on the Summary Printout page. The **Normalize** tab in the Report Generator will display the normalization values, unit, and scale factor as exported from Wave. (See Figure 88 on page 79.) For data integrity purposes, when normalized data is exported to a Report Generator from Wave, the **Normalize** tab in the Report Generator is locked for editing.

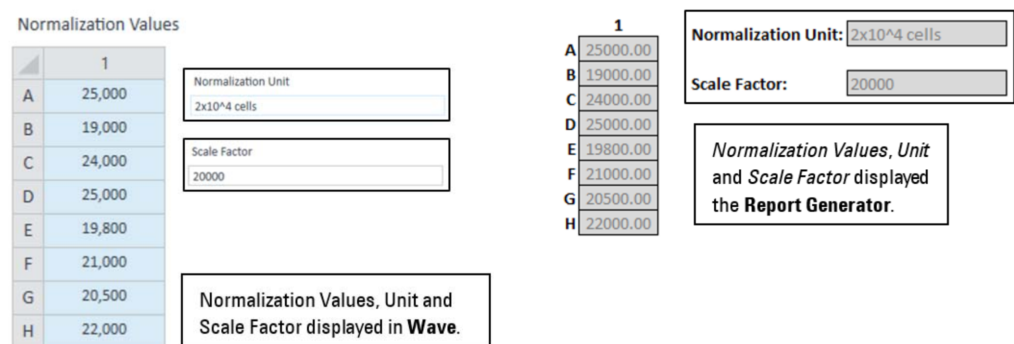


Figure 88 Normalize tab view

The **Normalize** tab in the Report Generators provides a step-by-step explanation on how to edit the normalization values imported to the Report Generator. (See Figure 89.)

Note: Normalization values in the Report Generator are automatically imported and applied from the Wave Desktop Normalization Plate Map. To modify these normalization values:

1. Open the Assay Result file in Wave Desktop.
2. Edit values in the Normalization Plate Map in Wave Desktop.
3. Export result data to the desired Report Generator.

Figure 89 Instructions to edit the normalization values

Excluded assay wells in Report Generator Assay wells that are turned off in Wave will not be exported or included in parameter calculations for each group in the Report Generators. This also applies to entire groups in an assay result file. Groups turned off in Wave will not be exported to selected Report Generators. The Measures Sheet displays the group names, Plate Map layout, and any assay wells that have been excluded in the group calculations in the Report Generator. (See Figure 90.)

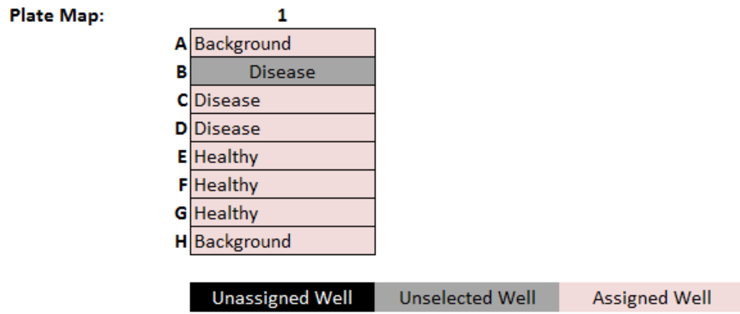


Figure 90 Measures sheet

Summary View

The Summary view is added to every assay result file by default, and cannot be closed or deleted. The Summary is a place to add notes or other valuable information about the assay. You may also append graphs, charts, Plate Maps from other views, or add details of the Instrument Protocol or Groups/Conditions used in the assay. The Summary is the only location in Wave that enables export to Microsoft Word, Adobe PDF, HTML, or Rich Text Format (.rtf). (See Figure 91.)

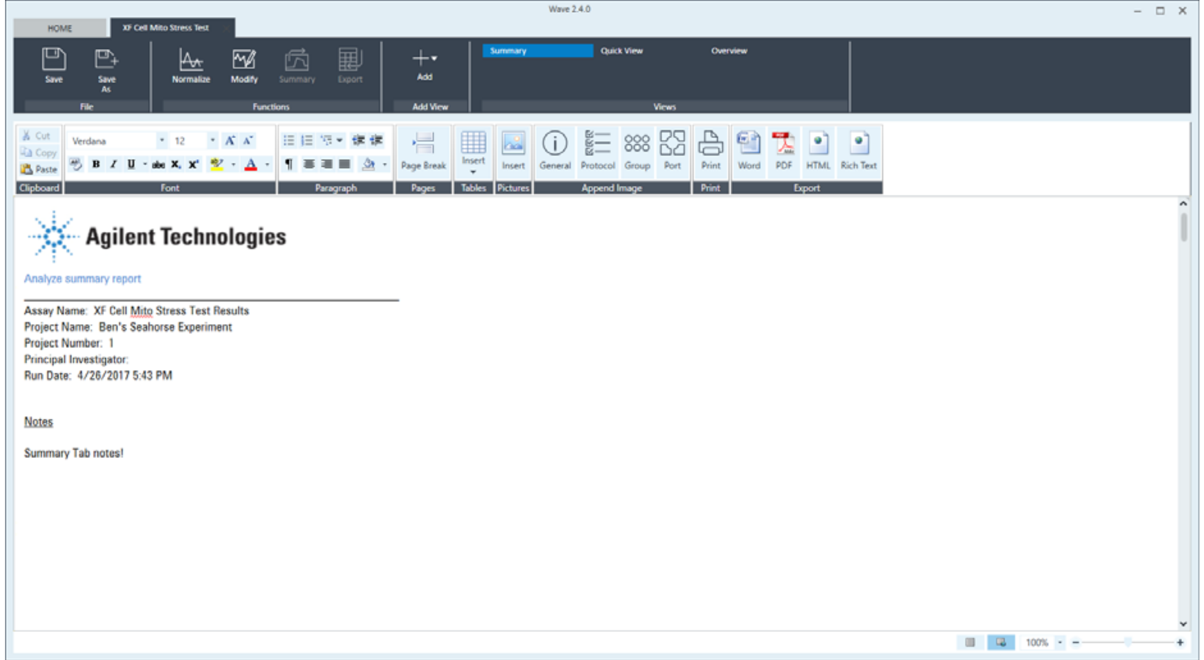


Figure 91 Summary view

Customize the summary

Use the ribbon toolbar to add, edit, and format edit the content added to the Summary, such as:

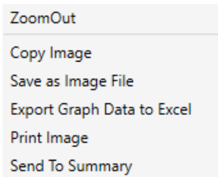
- Cut, copy, and paste to the clipboard.
- Change the font type, size, text, highlight color, and style.
- Subscript or superscript a character or characters.
- Add bullet points or numbers.
- Insert a table, picture or page break.

Append to summary

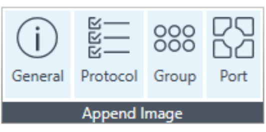
Append content from any analysis view directly to the Summary in three ways:

- **Append to Summary:** Click **Summary** (in the **Functions** ribbon) on the Quick View, Overview, OCR vs. ECAR views to send all data on the current analysis view to the Summary.

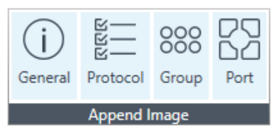
- **Right-click > Send to Summary:** Right-click a kinetic graph, scatter plot, or Plate Map and click **Append to Summary** from the drop-down list. To view appended content, click the **Summary** view.



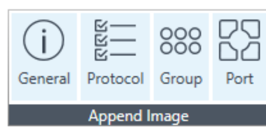
- **Append Image (Summary view):** General Information: The General Information button appends an image to the Summary view containing information about the assay, including project and plate information.



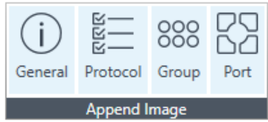
  - **Protocol Summary:** The Protocol Summary appends the Instrument Protocol (Mix, Wait, and Measure steps) as well as the time and number of cycles for each measurement to the Summary view.

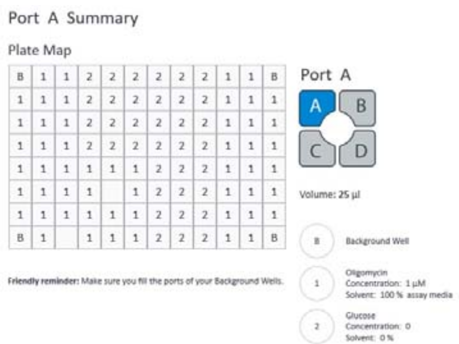


- **Group Summary:** The Group Summary button appends an image of the Plate Map and a table of the Group Definitions to the Summary view. The Plate Map is shown below.



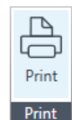
- **Port Summary:** The Port Summary button appends an image to the Summary view of the contents injected from each compound port in the assay.



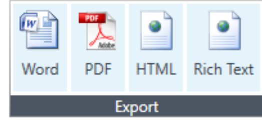


Print and Export summary

To print the Summary view, click the **Print** button on the **Summary** toolbar, select the printer to use, and click **Print**.



Export the contents of the Summary view to a Microsoft Word Document, PDF file, HTML file, or Rich Text Format file. Click the appropriate icon from the Export section of the Summary toolbar, browse to the desired file location to save and click **Save**.



Data View

The Data view contains every piece of data associated with assay result file, such as:

- **Group Data**: OCR, ECAR, PER, or PPR rate data and error values organized by group, and ordered by measurement.
- **Rate**: OCR, ECAR, PER or PPR individual assay well data. Error values are not displayed.
- **Level Data**: Individual assay well data displayed as Level data - O2 level (in mmHg) and pH raw values.
- **Raw**: Raw data values obtained during the assay including LED values, temperature at each measurement, and internal LED reference values.
- **Calibration**: Calibration results for each assay well are displayed in a table format.
- **Calibration View**: Calibration results for each assay well are displayed in a Plate Map format.
- **Event Log**: XF Analyzer serial number, consumable lot numbers used during assay, a command log for processes performed during assay, and whether they were successful or failed.

Export the data in a selected tab to Microsoft Excel using the right-click **Export** function. The Calibration, Raw, and Event Log tabs are used primarily for internal purposes, including QC, validation processes, and Agilent Seahorse Technical Support.

To display the Data view, click **Add View** on the top-level ribbon and select **Data** from the drop-down menu. The **Group Data** tab is displayed by default when opening the Data view. (See Figure 92.)

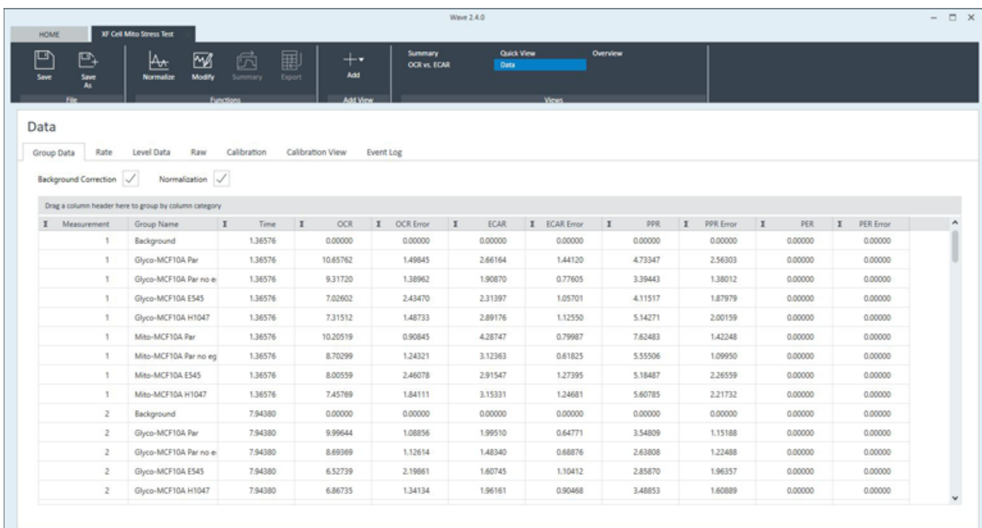
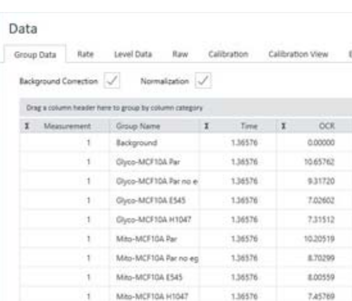


Figure 92 Data view

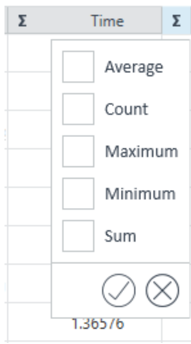
Column sorting

To sort the data by a specific column, click on the column header: Measurement, Group Name, Time, Rate or error for OCR, ECAR, or PER/PPR. Wave will automatically sort the data in ascending or descending order in the selected column.



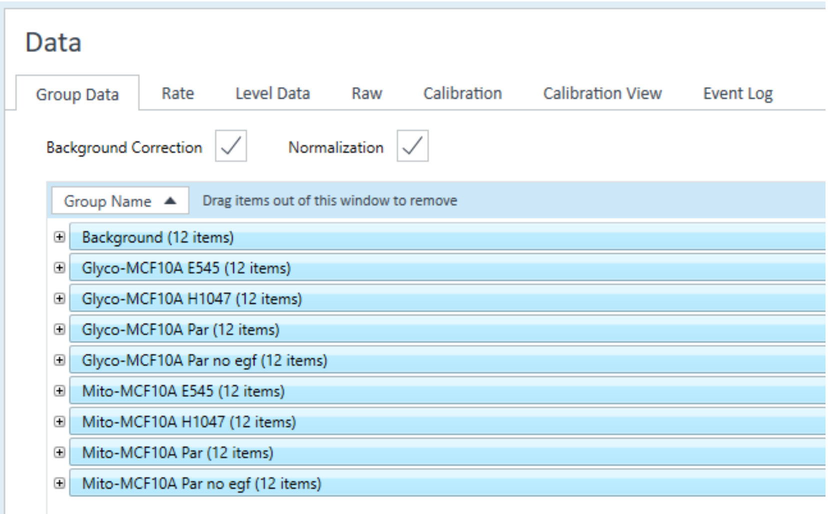
Display values for: Average, Count, Maximum, Minimum, or Sum

To display the Average, Count, Maximum, Minimum, or Sum, or any combination of these values, click the **Sigma** sign to the right of the arrow, and check the applicable boxes. In the example shown below, all values are checked for OCR data - Wave will display the average, count, maximum value, minimum value, and sum for the OCR data.

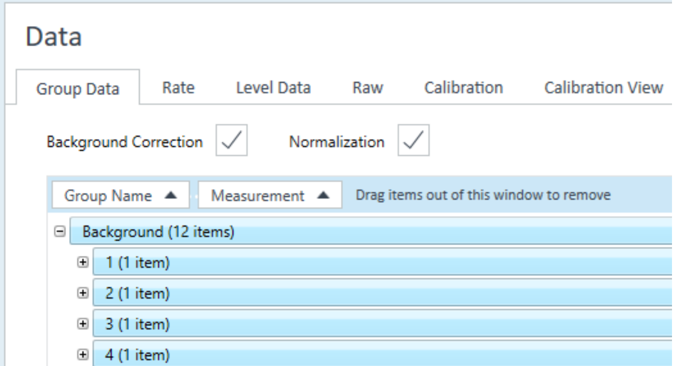


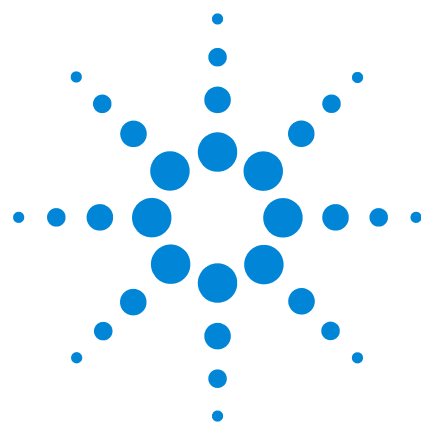
Group Data by Field

To group the data by field, drag and drop a column header to the gray bar above the table. In the example shown below, the Group Name heading was dragged to the gray bar. After grouping the data, expand or collapse the grouped by clicking on the **plus/minus** sign to the left of the group.



After expanding a group, select another column heading to define the groupings further. In the example below, the heading OCR was dragged to the grey bar after Group Name.





4
Managing Agilent Seahorse Files

Wave Home: Templates 88
Wave Home: Results 90
Wave Home: Catalog 93
Wave Home: Options 95
Wave Home: Help 99

Wave Home: Templates

Manage the list of available templates for the Agilent Seahorse XFe and XFp Analyzer on the Templates view, Figure 93, by using the **Import**, **Export**, **Duplicate**, and **Delete** buttons.

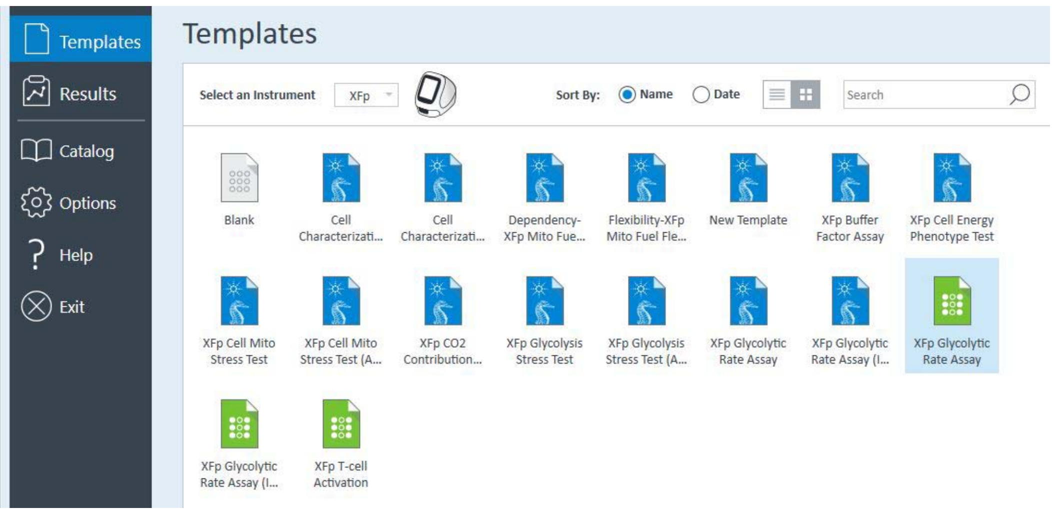


Figure 93 Templates view

Import assay templates

Import assay template files to Wave two ways:

- Double-click the assay template on a USB flash drive, network drive, or shared in an email, and Wave will automatically import the template to the Templates view.
- Manual Import - Open **Wave** > **Templates** view and click **Import**. Locate the template file, and click **Open**.

Export, duplicate, and remove assay templates

Single-click an assay template on the Templates view to:

- **Duplicate:** Create an identical copy of a template.
- **Delete:** Permanently delete an assay template file from Wave.
- **Export:** Export an assay template to:
  - A USB flash drive
  - A shared network directory for multiple user access

Template Details

To display details about an assay template without opening the template file, single-click the template to display **Template Details**. (See Figure 94.) The **Modified** date reflects the most recent date the template was modified and changes were saved. **Description**, **Project Name**, **Project Number**, and **Investigator** are optional details and not required to save a template.

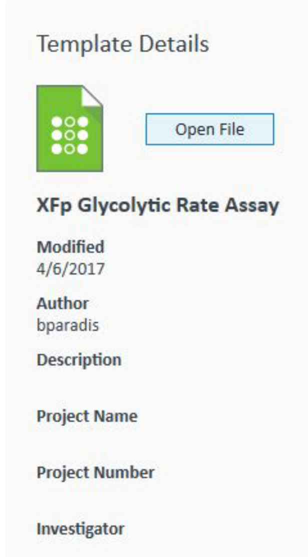


Figure 94 Template Details

Wave Home: Results

The Results view, Figure 95, displays recently opened assay result files, the corresponding file directory where each assay result file is located, and Favorite Places. Opening a new assay result file displays the default analysis view, the Quick View. After modifying and saving an assay result file, Wave automatically displays the last modified analysis view the next time the assay result file is opened.

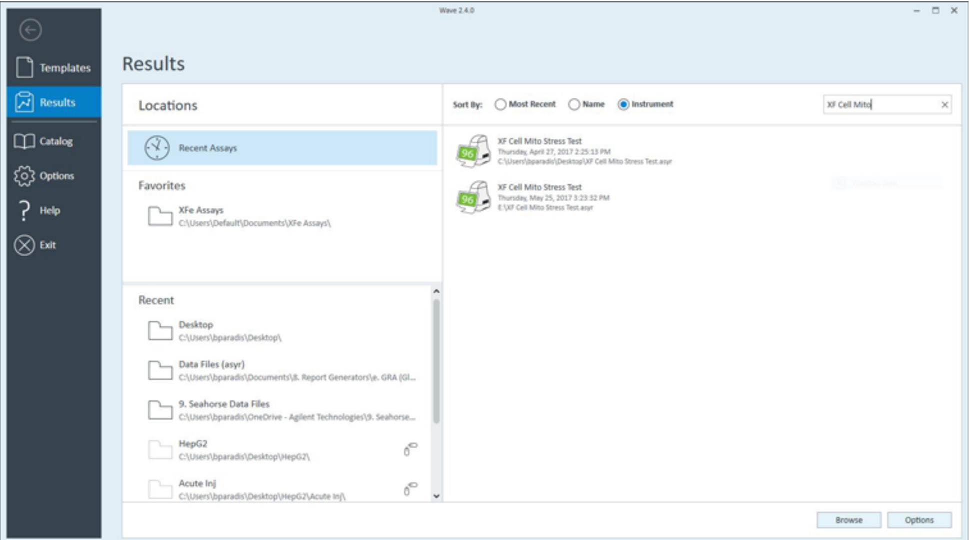


Figure 95 Results view

Recent assays, recent places, and favorite places

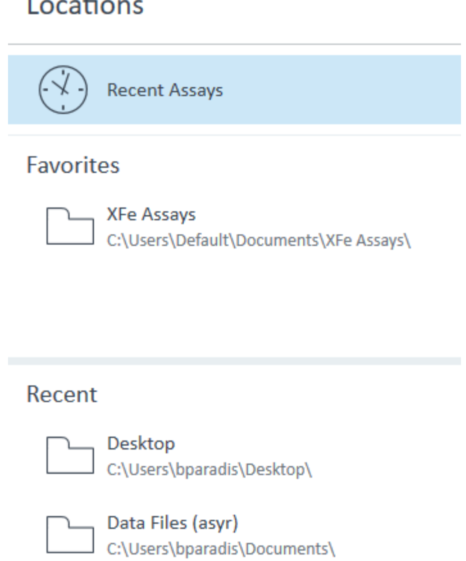


Figure 96 Recent Assays, recent places, and favorite places

- **Recent Assays** displays a sortable and searchable list of recently viewed and analyzed assay result files. (See Figure 95 on page 90.)
- **Favorites** displays user-defined locations (local or network directories) where assay result files are frequently added to, or accessed from. Once configured, each local directory added as a Favorite Place will appear below **Recent Assays** as a folder icon. (See Figure 96.)
- **Recent** displays the file location (for both local and network directories) of recently opened assay result files in the order of when each result file was opened. (See Figure 96.)

Options

Click **Options**, next to **Browse** in Figure 95 on page 90, to add Favorite Places (from local or network directory locations) or configure the number of **Recent Folders** and **Recent Assay Files** to display on the Results view. (See Figure 97.)

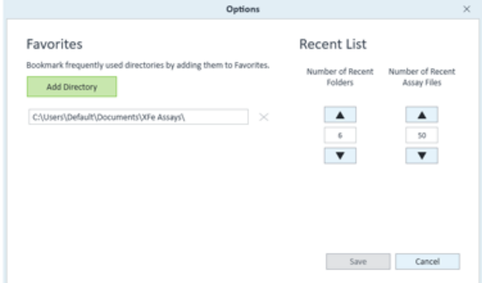


Figure 97 Number options for results view

Browse

To manually open an assay template or assay result file, click **Browse**. (See Figure 95 on page 90.)

The file types compatible with Wave 2.4 are:

- Assay result files (*.asyr)
- Assay templates (*.asyt)
- Agilent Seahorse XF data files (*.xfd)

NOTE Double-click to open an assay design file (*.asyd) created by earlier versions of Wave and **Save As** an assay template file. It is not possible to create assay design files using Wave 2.4. If you have *.asy files, these must be converted to *.asyr before opening in Wave. Contact Agilent Seahorse Technical Support for help with this.

Sort by

Assay result files are default-sorted by **Most Recent**, which displays a list of the most-recently opened or modified assay result file first. **Name** sorts assay result files alphabetically based on the file name and **Instrument** sorts by the type of Agilent Seahorse XF Analyzer used to generate the assay result file.

Figure 98 Sort by from the results view

Search

Type specific keywords in the **Search** bar to filter and display assay result files whose file name contains matching keywords. Searching using the file directory or date is not supported.

Figure 99 Search from the results view

Wave Home: Catalog

The **Catalog** provides a location to save frequently used **Compounds**, **Pretreatments**, **Media**, and **Cells**. (See Figure 100.) Save time when creating a new assay template or customizing a template file by inserting Catalog entries. The Catalog is prepopulated with Agilent Seahorse reagents and compounds that are used in commercially available Agilent Seahorse assay kits.

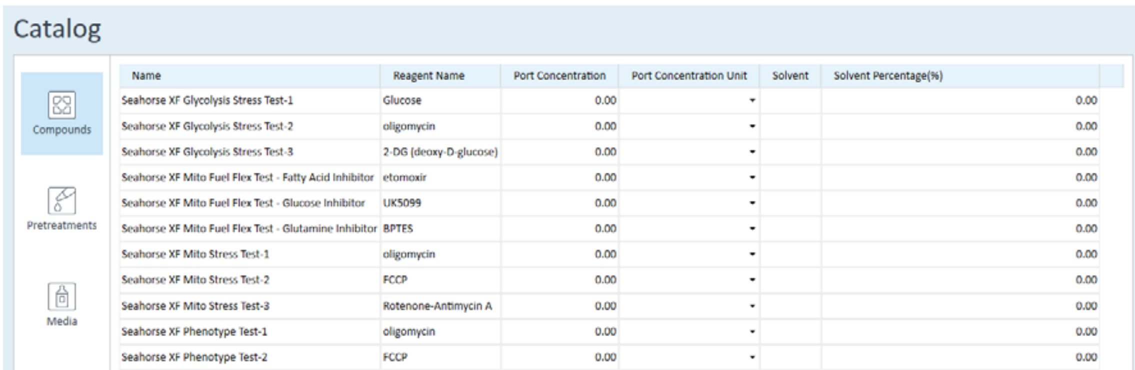


Figure 100 Catalog

Add a catalog entry

1 Open the **Catalog** view.

2 Select a condition (**Compounds**, **Pretreatments**, **Media**, and **Cells**). This example used **Pretreatments**.

3 At the bottom of the **Catalog > Pretreatments** view, type in a **Name** (required), and any additional fields to describe the **Pretreatment** entry (optional).

4 After adding details, click **Add Pretreatment** to save the entry.

5 When finished, click **Save** in the lower-right corner of the **Catalog** view.

Delete a catalog entry

1 Open the **Catalog** view.

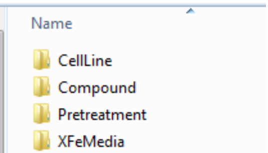
2 Click the red **X** at the end of the row to delete.

Share custom catalog

User-customized Catalogs can be shared and uploaded to Wave on XFe Analyzer or another user's Wave Desktop.

Computer #1:

1 Plug in the USB flash drive (if applicable).
2 Start **Wave**, and open the **Options** view.
3 The **Catalog** directory is found under the **Directories** header on the **General Options** view.
4 Highlight the path and copy/paste into Windows Explorer to display the **Catalog** folders: **CellLine**, **Compound**, **Pretreatment**, and **XFeMedia**.



5 Select each Catalog condition to share and copy/paste to the USB flash drive (or a network directory). Do *NOT* cut the Catalog entries.
6 Eject the USB flash drive (if applicable).

Computer #2:

1 Insert the USB flash drive (or open the network directory) containing the saved Catalog entries.
2 Start **Wave**, and open the **Options** view.
3 In **General Options**, copy/paste the **Catalog** directory location in Windows Explorer.
4 Close **Wave**.
5 In Windows Explorer, open each **Catalog** folder (from Computer #1), and copy/paste the contents into the appropriate **Catalog** folder on Computer #2.
6 Eject the USB flash drive (if applicable) and open **Wave**.
7 Open the **Catalog** view, and verify the transferred entries are displayed in the list for each condition.

Wave Home: Options

The three types of modifiable settings in the **Options** view are: **General**, **Instrument**, and **Advanced**. (See Figure 101.)

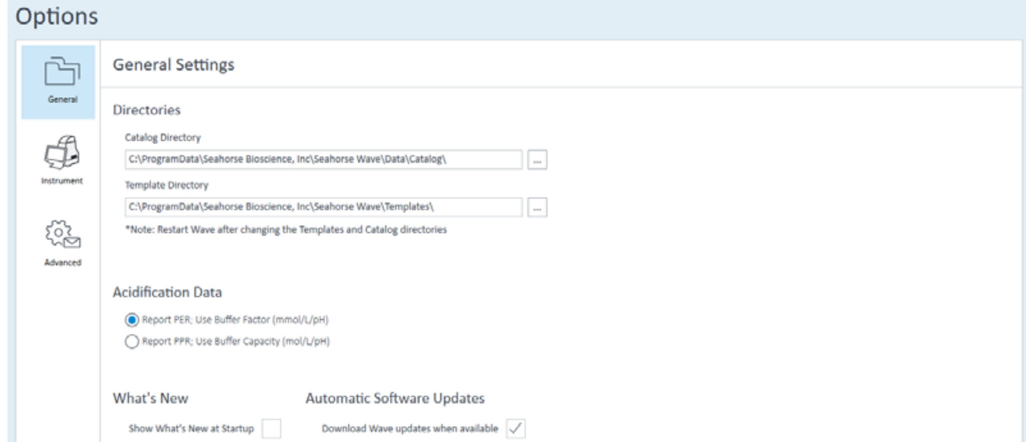


Figure 101 Options view

General options

General settings displays options to:

- View/change Agilent Seahorse file directories (save locations) for Catalog and Assay Templates files in Windows Explorer.
- Modify the reported acidification rates - PER (default) and PPR.
- Enable/disable the **What's New** pop-up (list of new features) upon startup of Wave 2.4 software.
- Enable/disable automatic Wave software updates.

After making changes to **General** options, click **Save** (lower-right corner) to save changes. To restore changes to the factory default setting, click the **Default** button.

Directories

Directories displays the default save location for **Catalog** entries and **Template** files. (See Figure 101.) The prepopulated Catalog conditions and assay template files installed with Wave are stored in this directory, as well as any new Catalog entries and assay template files. To change the location of either directory, click the [**...**] button to browse to the preferred location. Wave software must be restarted for changes to take effect.

What's new message box

The **What's New** message box appears upon Wave startup, and displays the new features and functions available in version 2.4. This message box will always appear unless either the **Don't show this again** box is checked, or it is disabled in the **Options** view.

Instrument options

Instrument options displays default values (by analyzer type) for the Instrument Protocol. (See Figure 102.) To change the XF Analyzers displayed in Wave Desktop, see the *Wave Desktop 2.4 ReadMe* PDF.

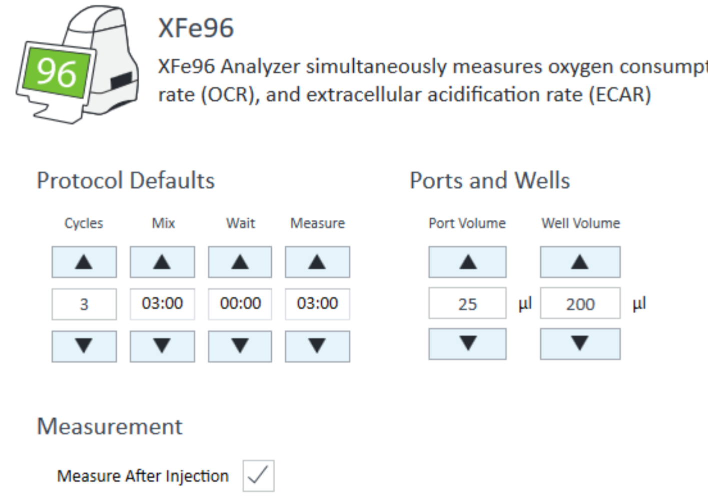


Figure 102 Instrument options

Other settings that can be modified include:

- **Cycles**: Number of repeat **Mix**, **Wait**, and **Measure** cycles in the default protocol
- **Mix**, **Wait**, and **Measure** times: Time to complete each command in the Instrument Protocol
- Default **Port Volume** (notation purposes only)
- Default **Well Volume** (notation purposes only)

Protocol defaults

Leave the default **Protocol Defaults** untouched, and modify the Instrument Protocol in the individual assay template file. To change the **Protocol Defaults**:

1 Use the up/down arrows or type in the new values for **Mix**, **Wait**, **Measure**, and **Cycles**.

2 Click **Save** at the bottom of the screen to save changes.

Ports and Wells

The port and well volumes are unique to each Agilent Seahorse analyzer. Modifying these values do not change the function of the analyzer or calculations, and are for record-keeping only. During an assay, the contents of the port will be injected. To change the **Port Volume** or **Well Volume**:

1. Use the up/down arrows, or type the new value in the **Port Volume** field or the **Well Volume** fields.
2. To save changes, click **Save**.

Advanced options (Wave controller, Agilent Seahorse XFe analyzers ONLY)

The Agilent Seahorse XFe Analyzer can automatically notify users when certain functions are complete or need user interaction, such as:

- **Calibration**: The Calibration Plate must be replaced with the Cell Plate, and the assay must be started.
- **Assay Complete**: Following completion of an assay, Wave Controller will automatically email the assay result file to the specified email addresses.

An active internet connection configured on Wave Controller for the Agilent Seahorse XFe Analyzer is required for use of automatic email notification features. (See Figure 103.)

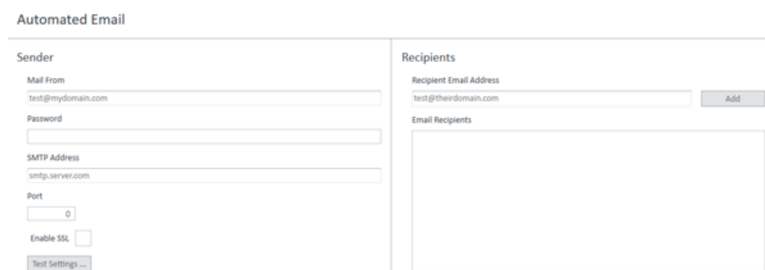


Figure 103 Automated Email features

Add email recipients

1. Type an email address in the **Mail From** field, and enter a password for this address in the **Password** field. This email address will be used to send all notifications and will be displayed in the **Mail From** field after receiving a notification from the Agilent Seahorse XFe Analyzer.
2. Specify the **SMTP Address** and the access **Port** field.
3. Check **Enable SSL** if required by the local IT group.
4. Type the email address for a single recipient in the **Recipient Email Address** field.
5. Click **Add**.

6. Repeat Steps 4 and 5 for each recipient.
7. When finished adding recipients, click **Save**.
8. To send a test notification email to all email recipients, click **Test Settings**. If the test email is not received, verify the correct network settings have been configured in Wave Controller.

Remove email addresses

Select the email address under **Email Recipients**, and click **Delete**.

Wave Home: Help

To quickly and easily send system files for support and diagnostic purposes, the Help view displays software version information, Agilent Seahorse technical support contact information, and **Send System Files**. (See Figure 104.)

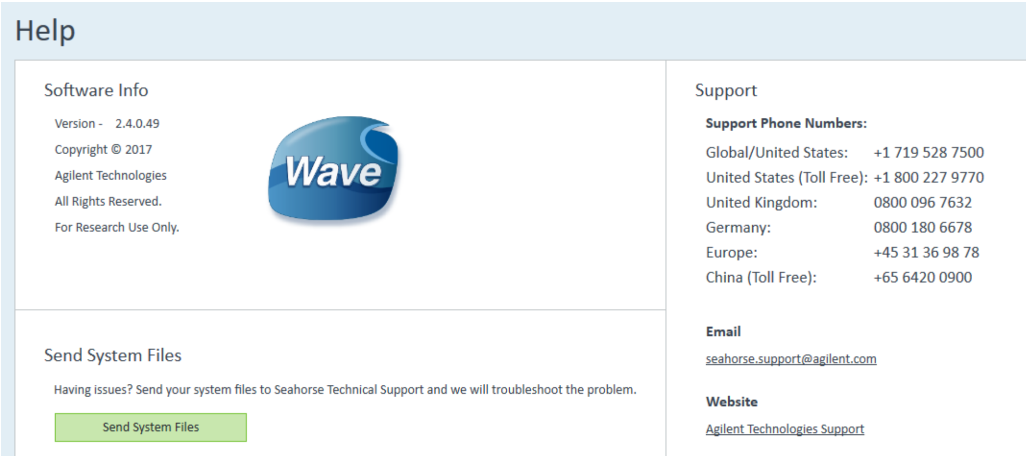


Figure 104 Help view

Send system files

Use the **Send System Files** function to automatically compile Wave System Files into a compressed folder, create a blank email to attach the System Files, and send to Agilent Seahorse Technical Support. (See Figure 105.)

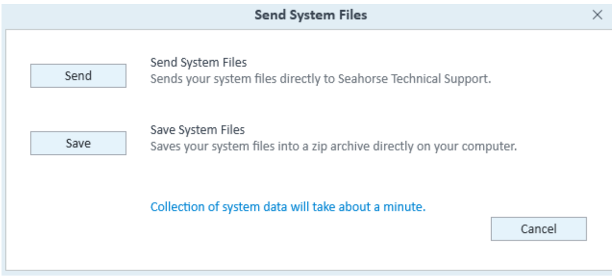


Figure 105 Send system files function

Save system files in a compressed folder:

1 Open the **Help** view.

2 Click **Send System Files**.

3 Click **Save** to select a file location to save the compressed folder.

Send system files to Agilent Seahorse technical support:

1. Open the **Help** view.
2. Click **Send**. An email message will appear populated with the following information:
    a. Email Recipient - Agilent Seahorse Technical Support email address.
    b. Subject - Email subject line.
    c. Email Body Text - displays the default save location of the System Files on the local drive.
3. Locate the saved System Files compressed folder, and attach it to the email.
4. Click **Send**.

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Not for use in diagnostic procedures.

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