Wave Desktop 2.3
User Guide

For use with Agilent Seahorse
XFe96, XFe24, and XFp Analyzers

June 2016

Agilent Technologies

Contents

Chapter 1: Overview & Creating Assay Templates

User Guide Overview

This document provides guidance on how to Wave Desktop 2.3 to create new/customize existing assay template files, analyzing assay results from an assay and best practices for managing Seahorse files. The three steps for any experiment on an Agilent Seahorse XFe or XFp Analyzer are:

1. Create an assay template for the experiment.
2. Transfer and run assay on the Agilent Seahorse XFe or XFp Analyzer.
3. Analyze assay results.

Step 1: Create an Assay Template

Create an assay template using the *Blank* template or customize one of the *Seahorse* templates installed after upgrading to Wave Desktop 2.3. Wave Desktop is the recommended and preferred software for customization of assay template files for the Agilent Seahorse XFe96, XFe24 and XFp Analyzer. See Create a New Assay Template more for information on customizing assay template files.

Note: *Double-click an Assay Design file (*.asyd) created in an earlier version of Wave to open assay design and* ***Save As*** *an assay template file. It is not possible to create assay design files using Wave Desktop 2.3.*

Step 2: Transfer & Run Assay on Agilent Seahorse XFe or XFp Analyzer

After creating an assay template for the experiment, transfer the template to the Agilent Seahorse XFe or XFp Analyzer using a USB flash drive or network drive (active network connection required).

Transfer assay template using a network drive:

Agilent Seahorse XFe Analyzer:

1. Power **ON** the Agilent Seahorse XFe Controller.
2. Start Wave Controller software.
3. Click **New**.
4. Click **Browse.**
5. Locate the template file (.asyt) in network location.
6. Click or tap the template file in the network location to select the template file.
7. Click **Open.**

Agilent Seahorse XFp Analyzer:

1. Power **ON** the XFp Analyzer.
2. Click **Start.**
3. Click the **Network** tab and select the assay template to run.

Transfer assay template using a USB flash drive:

Agilent Seahorse XFe Analyzer:

1. Save template file to a USB flash drive.
2. Plug flash drive into the USB port on the Agilent Seahorse XFe Controller.
3. Click **New.**
4. Click **Browse**.
5. Select the USB flash drive and locate the template file (.asyt).
6. Click or tap the template file on the USB flash drive to select the file.
7. Click **Open.**

Agilent Seahorse XFp Analyzer:

1. Save the template file to a USB flash drive.
2. Plug the flash drive into the front USB port on XFp Analyzer.
3. Press **Start**.
4. Press the **USB** tab and select the assay template to run.

Step 3: Analyze Assay Results

After completion of the assay, transfer the assay result file from the Agilent Seahorse XFe or XFp Analyzer to Wave Desktop for data analysis.

Note: *Wave Controller automatically saves a backup copy of the assay result file locally on the Seahorse XFe Analyzer.*

Transfer assay result file using a network:

Both Agilent Seahorse XFe and XFp Analyzer:

1. Start Wave Desktop on a PC.
2. Click **Results.**
3. Click **Browse** and locate the saved assay result file in the network location specified prior to running the assay.

Transfer assay result file using a USB flash drive:

Agilent Seahorse XFe Analyzer:

1. Locate the assay result file on the Agilent Seahorse XFe Controller.
2. Copy and paste the assay result file to a USB flash drive and transfer to a personal computer with Wave Desktop.

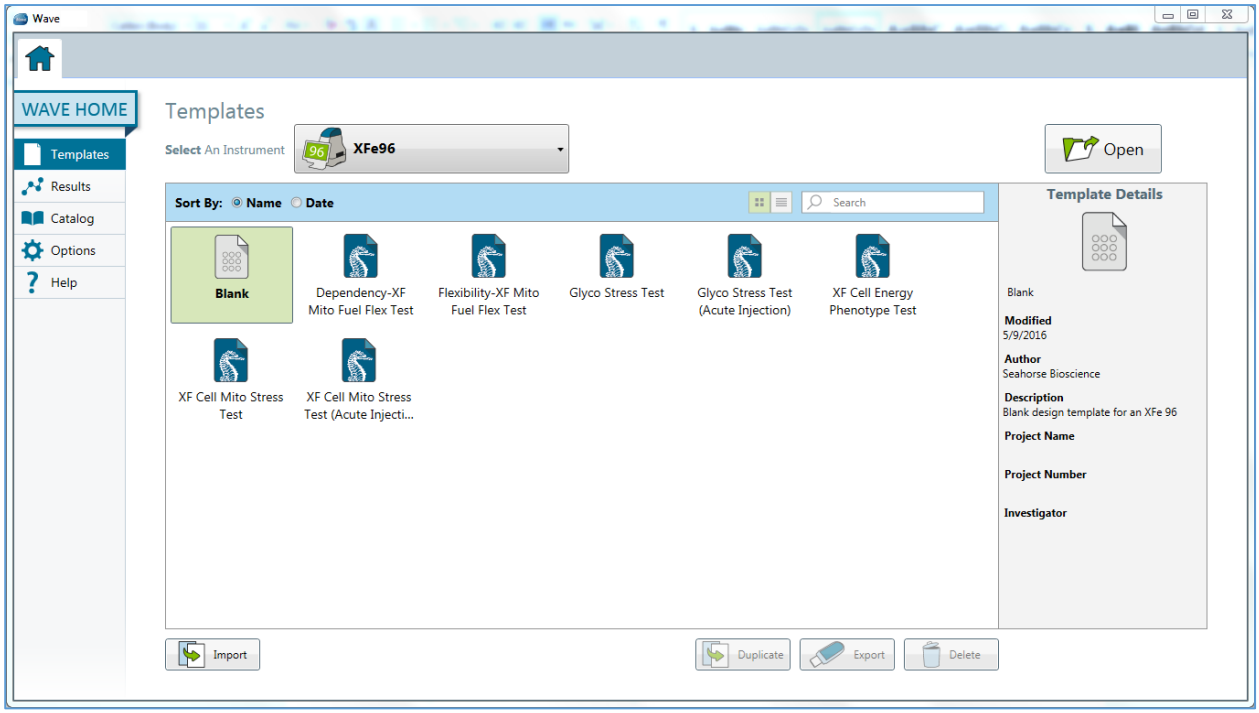
Agilent Seahorse XFp Analyzer:

1. Start the XFp Analyzer.
2. Plug in USB flash drive.
3. Press **Settings**.
4. Press **Assay Results**.
5. Select one or multiple assay result files to export to a USB flash drive.

Note: *Data may also be exported as an Excel file (*.xlsx) or GraphPad Prism file (*.pzfx) from the Agilent Seahorse XFp Analyzer. The assay result file (*.asyr) is always exported.*

The next section outlines how to create assay template files using Wave Desktop 2.3. For more information on the Seahorse XFp Analyzer software and template customization options, please download the Seahorse XFp User Guide from the Agilent website.

Wave Home: Views



Templates

Create, customize, import and export assay template files for a Seahorse assay.

Results

Analyze assay result files and configure *Favorite Places* to quickly access to favorite and frequently used locations (such as a network location or local computer hard drive) where assay result files are stored.

Catalog

Save frequently used Compounds, Pretreatments, Media and Cells. These saved entries can be applied while creating a new Assay Template or customizing a template from an existing template file. The *Catalog* in Wave Desktop 2.3 is prepopulated with Seahorse reagents and compounds that are currently available for each Seahorse assay.

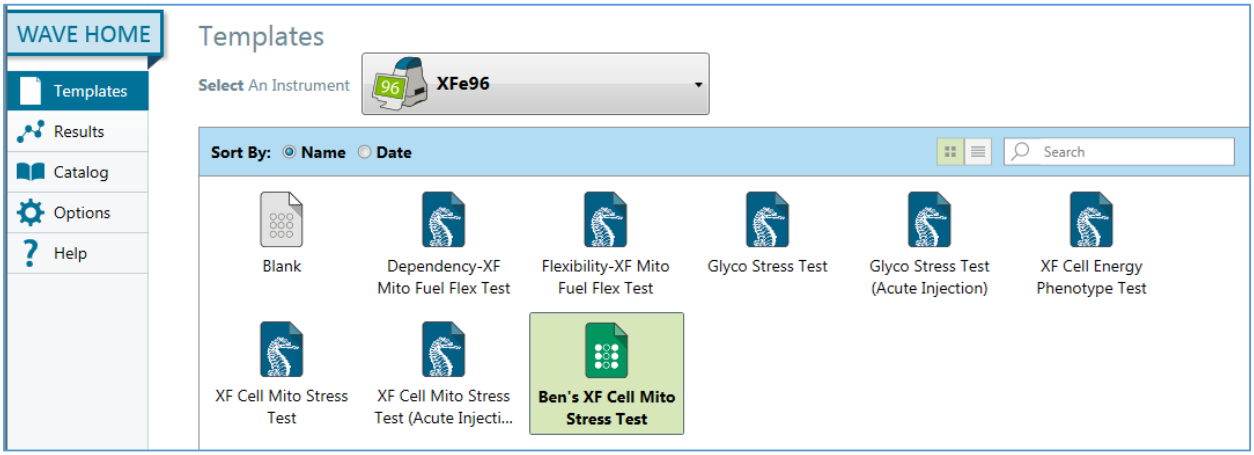
Options

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.*

Wave Home: Templates View



The *Templates* view displays all assay template files saved on the personal computer where Wave Desktop is installed. An a*ssay template* (.asyt) file contains all the necessary information required to run an assay: group definitions, plate map configurations, and a defined instrument protocol. Assay templates can be reused to perform multiple Seahorse assays and customized for other experiments. Assay templates that are automatically installed by Wave are:

- Blank
- Agilent Seahorse XF Cell Energy Phenotype Test
- Agilent Seahorse XF Cell Mito Stress Test (Acute Injection)
- Agilent Seahorse XF Glycolysis Stress Test
- Agilent Seahorse XF Glycolysis Stress Test (Acute Injection)
- Agilent Seahorse XF Mito Fuel Flex Test – Dependency
- Agilent Seahorse XF Mito Fuel Flex Test – Flexibility

All user-customized assay templates will also be displayed in this view. The three icons pictured below provide visual cues to help differentiate the type of template from one another (see below). The Seahorse templates provide a starting point as they contain specific details for each Seahorse XF assay. The Seahorse templates are not editable; Wave will save any changes made to the Seahorse template as a new assay template (green icon).

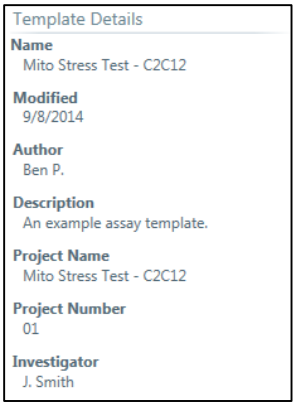
Blank Template

Seahorse Standard Assay Template

User Customized Template

Template Details

Single-click an assay template on the *Templates* view to display the *Template Details*. 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.



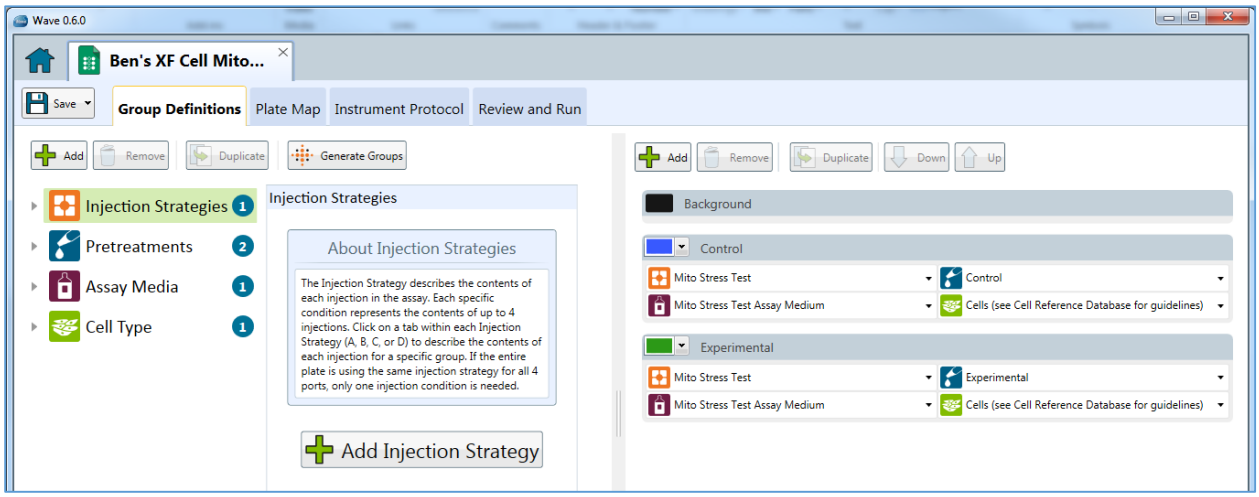
Create a New Assay Template

Steps to create a new assay template:

1. Open Wave Desktop 2.3.
2. Click **Templates** (below *Wave Home*)**.**
3. Select the **Blank** template, a **Seahorse Default Template,** or a custom assay template and click **Open**.
   a. Double-click will also open the template file.
4. Define experimental conditions and create assay groups. See Group Definitions.
5. Assign groups to the plate. See Plate Map.
6. Define the instrument protocol. See Instrument Protocol.
7. Review and run the assay. See Review and Run.

Step 1: Group Definitions

The *Group Definitions* tab is used to define Injection Strategies, Pretreatments, Assay Media, and Cell Types to be used in the assay. The *Seahorse Default Templates* have basic assay information added to the *Injection Strategies*, *Pretreatments*, and *Assay Media* already. The *Blank* template does not contain any default *Group Definition* details. Read each section below for more information on completing *Group Definitions*.

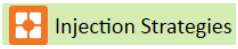


Group Definitions: Injection Strategies

Injection Strategies describe the contents of each set of injections in the assay. Each specific *Injection Strategy* represents the contents of up to 4 injections (one for each of the 4 ports: A, B, C, and/or D). One or multiple *Injection Strategies* can be defined for each assay (example: Seahorse XF Cell Mito and Seahorse XF Glycolysis Stress Test assay on the same cartridge).

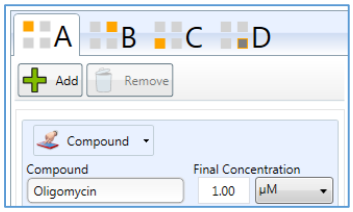
To add one or more *Injection Strategies*:

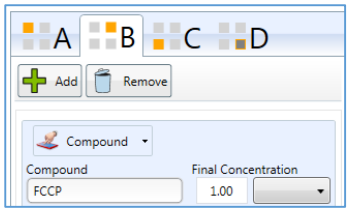
1. Click the **Injection Strategies** bar.

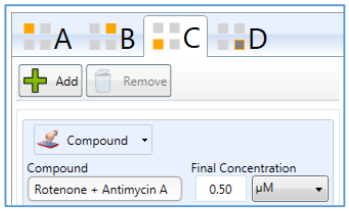


2. Click **Add Injection Strategy**.

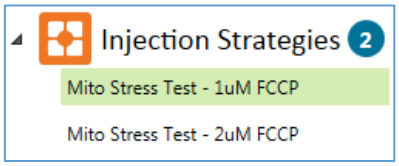
3. Click the name of the new injection condition called **Inj. Strategy 1** (by default) and click **Add** to add a compound for injection Port **A**. After adding details for Port **A**, click the tabs for Ports **B**, **C**, and **D** and repeat the process. Details to add for each Port include: Injection Condition name, contents for up to 4 injection ports (A, B, C, and D), compound name, and compound concentration that is being injected from the port.







The image to the right shows two defined *Injection Strategies* for the Agilent Seahorse XF Cell Mito Stress Test assay for different concentrations of FCCP: **Mito Stress Test – 1uM FCCP** and **Mito Stress Test – 2uM FCCP**

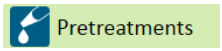


Group Definitions: Pretreatments

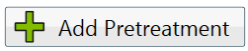
Pretreatments describe a treatment the cells have received, such as a genetic manipulation or exposure to compounds, prior to performing the assay.

To add one or more *Pretreatments*:

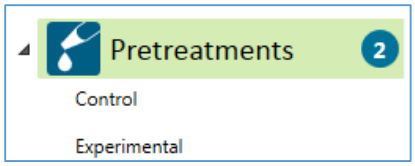
1. Click the **Pretreatments** bar.



2. Click **Add Pretreatment**.



3. Click the name of the new pretreatment condition called **Pretreatment 1** (by default) to display the **Edit Pretreatment** dialog box. Enter the name of the pretreatment in the **Name** field and any additional details in the **Description** field. The image to the right displays two Pretreatment conditions: *Control* and *Experimental*.

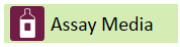


Group Definitions: Assay Media

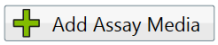
Assay Media describes one or more assay mediums used in the assay. Record the medium and supplements for each medium used in the assay:

To add one or more Assay Medias:

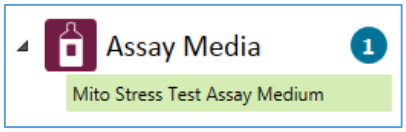
1. Click the **Assay Media** bar.

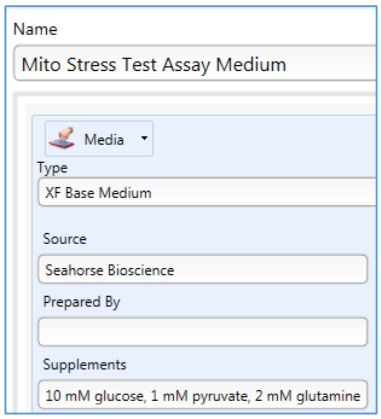


2. Click **Add Assay Media.**



3. Click the name of the new medium called **Media 1** (by default) to display the **Edit Assay Medium** window. Enter the name of the medium in the **Name** field and additional details using the fields below, including: *Source*, *Supplements*, and *Prepared By* info.



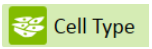


Group Definitions: Cell Type

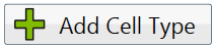
Cell Type describes the biological material/samples used in the assay along with information about the cell line, seeding concentration, and passage number.

To define one or more *Cell Types*:

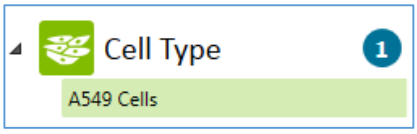
1. Click the **Cell Type** bar.

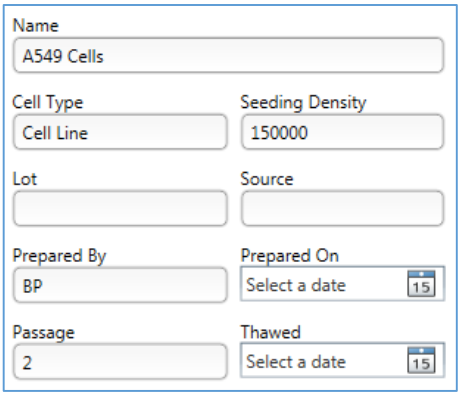


2. Click **Add Cell Type Condition**.



3. Click the name of the new cell type called **Cell Type 1** (by default) to display the **Edit Cell Type** window. Enter the name of the cell type in the **Name** field and additional details using the fields below, including: *Seeding Density*, *Lot*, *Source*, and *Passage* number.





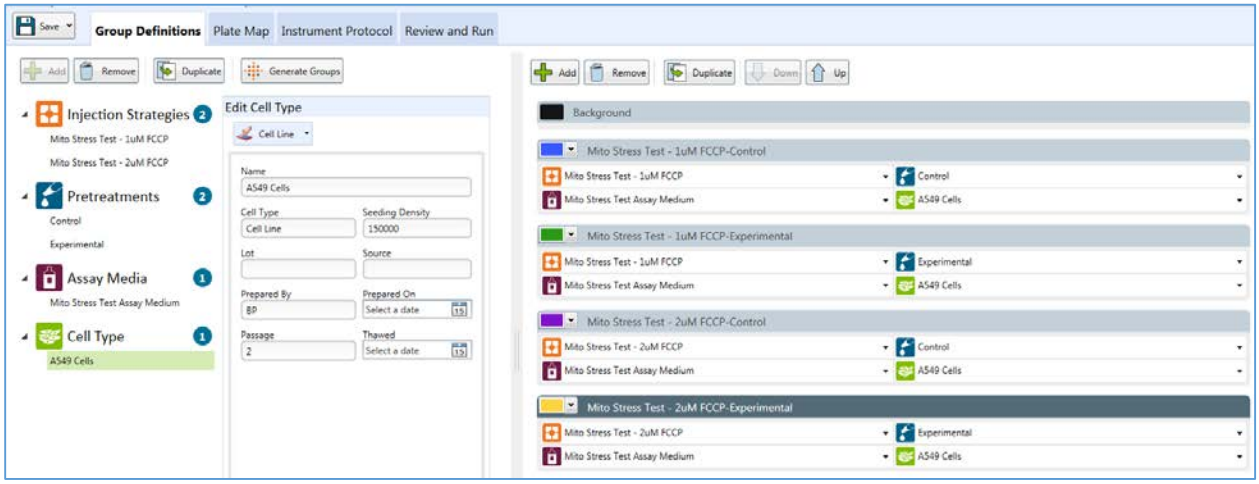
Automatically Generate Groups

Once assay conditions have been defined, click **Generate Groups** button to automatically generate groups for the assay based on the combination of different conditions defined in each **Group Definition** section. The total number of groups generated is based on the number of independent variables defined in the **Group Definitions**. If only one specific condition is defined, Wave will assume that it is a global condition and will not use it to differentiate groups. For more than one condition, Wave will calculate the number of groups, assuming every possible combination of independent conditions.

This example shows a total of 4 groups automatically created in Wave Desktop using the preceding **Group Definitions**:

- *Injection Strategies* = 2 (Mito Stress Test – 1 uM FCCP and Mito Stress Test – 2 uM FCCP)
- *Pretreatments* = 2 (Control and Experimental)
- *Assay Media* = 1 (Mito Stress Test Assay Medium)
- *Cell Type* = 1 (A549)

The combinatorial logic using the above **Group Definitions** is 2 x 2 x 1 x 1. This results in a total of 4 unique assay groups.

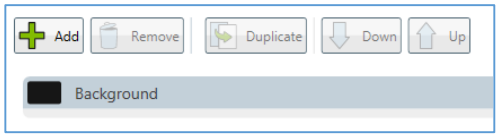


Manually Generate Groups

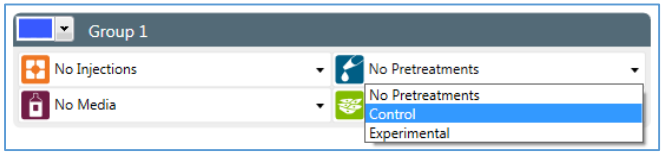
Manually define each group using the drop-down menus for the individual **Group Definition** below the group name.

To manually add groups:

1. Click **Add**.



2. Using the drop-down menus for each **Group Definition,** select the *Injection Strategy*, *Pretreatment*, *Assay Media*, and *Cell Type* to apply to the first group added to the list.



3. Double-click the *Group 1* bar to rename the assay group.

4. Repeat for each additional group.

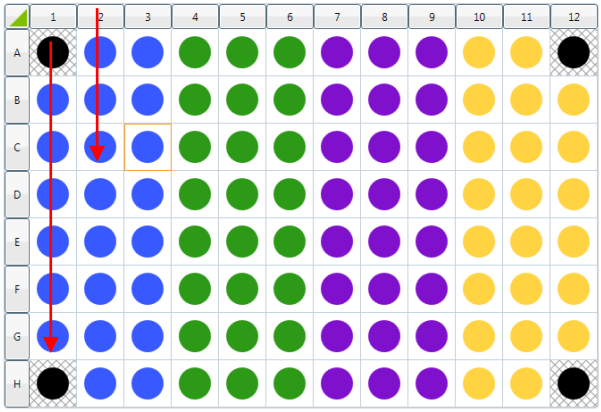
After completing the **Group Definitions** section, the groups must be assigned to the **Plate Map**. See the next section for more information on assigning groups to the **Plate Map**.

Step 2: Plate Map

The **Plate Map** displays the group layout on the Cell Plate. Wave uses **Plate Map** assignments 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.

Automatically Assign Groups – Distribute Groups

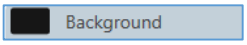
Wave determines the maximum number of replicates per group and distributes wells in vertical sections across the plate map.



Manually Assign Groups

Click the name of the group to assign wells on the left-side group list, then click the well on the Plate Map to assign an individual well. Assign an entire row or column to a group by clicking the row or column label.

Background Wells

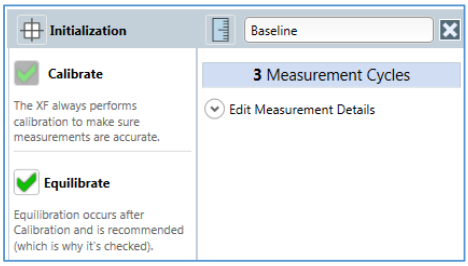


Background wells are automatically assigned to default positions depending on the Agilent Seahorse XFe or XFp Analyzer. To change the Background Well location or add additional Background Wells, first click the **Background** group name on the left-side group list, then click the desired well to assign/unassign as a background well.

Step 3: Instrument Protocol

The **Instrument Protocol** is the series of commands the Agilent Seahorse XFe and XFp Analyzers perform during an assay, such as: what port to inject and when, how many cycles to perform for each measurement, and the time for each cycle (mix, wait, and measure). The Seahorse Default Templates use standardized **Instrument Protocols** and do not require any modifications.

Default Protocol Commands



Every **Instrument Protocol** includes the following steps:

1. Calibrate (always ON).
2. Equilibrate (highly recommended for *every* assay).
3. Baseline Measurement cycle.

Note: *The Agilent Seahorse XFe and XFp Analyzers perform 3 measurements after each injection. The Seahorse Default Templates use standard* ***Instrument Protocol*** *and do not require modification.*

Calibration

Calibrate is always the first step in a protocol and cannot be disabled. The *Calibrate* step reads the coefficients of the Cartridge and Cell Plate to ensure accuracy of the data acquired during the assay.

Equilibration

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

Measurement Cycles

Measurement cycles indicate the steps in the **Instrument Protocol** when data is collected for both rates measured by the Agilent Seahorse XFe/XFp Analyzers – oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

The standard Seahorse **Instrument Protocol** consists of 3 measurement cycles before the first injection (called *Baseline*) and 3 measurement cycles after each port injection.
Each measurement cycle consists of 3 commands: *Mix*, *Wait*, and *Measure*.

Mix – The amount of time raise/lower the sensor cartridge to ensure analytes and drug compounds are uniformly dispersed within the medium in each well before and after an injection and rate measurement.

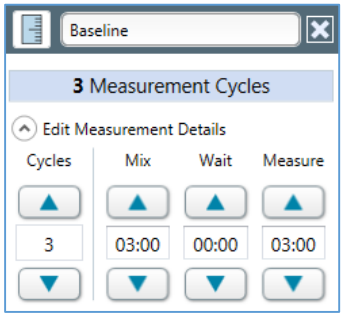
Wait – *Required for Agilent Seahorse XFe24 Analyzer only.* The amount of time to delay the *Measurement* step after the *Mix* step.

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.

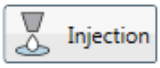
The default measurement cycle times are:
Agilent Seahorse XFe96 and **Agilent XFp Analyzer**: 3 minutes *Mix*; 0 minutes *Wait*; 3 minutes *Measure*.
Agilent Seahorse XFe24 Analyzer: 3 minutes *Mix*, 2 minutes *Wait*, 3 minutes *Measure*.

Baseline Measurement Cycle

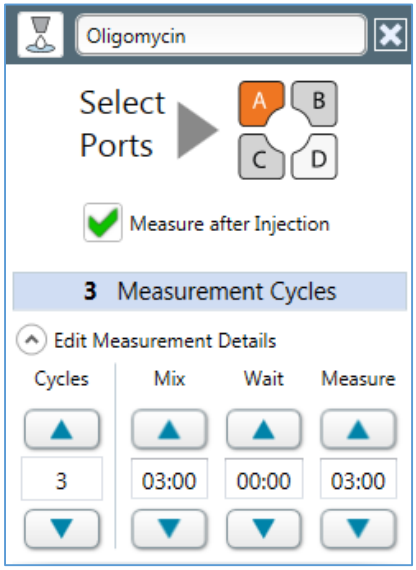
The *Baseline* measurement cycle is the starting point for every Seahorse assay and consists of three measurements *before* the first injection. The *Baseline* measurement displays the OCR and ECAR of cells under starting conditions.



Injections



Click the **Injection** button to add a new injection command in the **Instrument Protocol**. For each new injection, the Wave automatically selects the next available port. Ports that are greyed out indicate the port has been assigned in the **Instrument Protocol**. Wave will also add measurement cycles after the injection step. Default *Mix*, *Wait* and *Measure* times are provided on an instrument-type basis and can be modified.



Edit Measurement Details

View and edit times for the *Mix*, *Wait*, and *Measure* commands by clicking the dropdown arrow next to *Edit Measurement Details*. Use the up/down arrows to adjust the number of *Cycles* for each measurement as well as the *Mix*, *Wait* and *Measure* times for each measurement cycle. A minimum of 3 cycles per measurement is recommended to achieve optimal results. The default *Mix*, *Wait*, and *Measure* times are instrument-dependent and can be customized in the *Instrument Options* (Wave Home > Options). The name of each command in the **Instrument Protocol** can be changed by clicking in the text field at the top of the column.

Custom Cycles

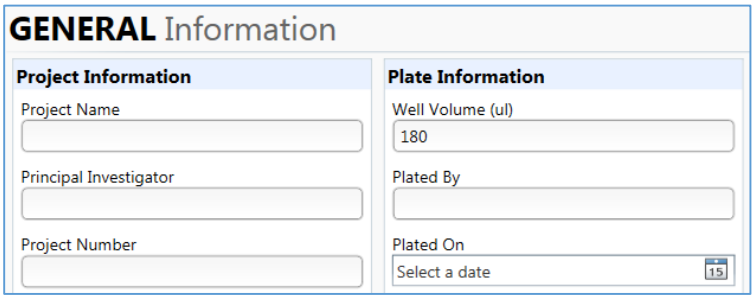
Most Seahorse assays require the default Baseline and Injection cycles only, however depending on the requirements of the assay a custom cycle may be appropriate. Press the **Custom** button to add a custom cycle command to the Instrument Protocol. A custom cycle enables multiple *Mix* and *Wait* steps without a *Measurement*.

Step 4: Review and Run

Summary

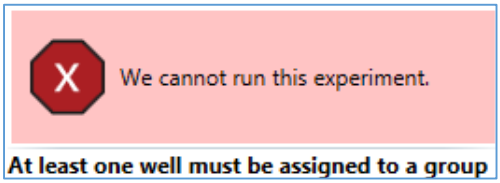
General Information

The *General Information* section provides editable fields for notes or other important information related to the assay. Any information entered on this view will be saved and displayed in the assay result file (*.asyr).



Errors and Warnings

Any errors or warnings will be displayed on the right side of the *Review and Run* tab prior to transferring the template to the Agilent Seahorse XFe or XFp Analyzer to notify the user changes are required before performing the assay. Typical error messages include assay wells on the *Plate Map* that are not assigned to a group defined on the *Group Definitions.*



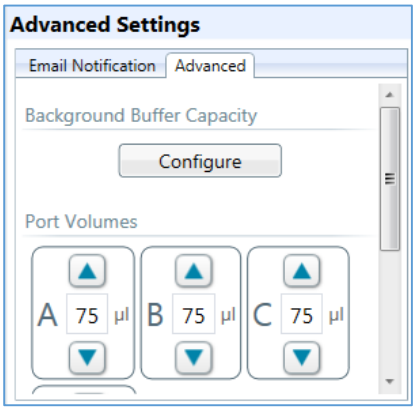
Advanced Settings

Email Notification Tab (Wave Controller for Agilent Seahorse XFe Analyzer only)
This feature requires an active internet connection. Wave Desktop does not email assay result files to recipients configured on this view.

Add email address before starting an assay on the Agilent Seahorse XFe Analyzer:

1. Open the template file.
2. Click the **Review and Run** tab.
3. Go to **Email Notification** tab under *Advanced Settings***.**
4. Type in an email address then click **Add**.
5. Repeat for each email address.

Advanced Tab



1. Assay result files **ONLY** – Configure *Background Buffer Capacity* values for background wells:
   a. Click **Configure**.
   b. Type in the Buffer Capacity (mol/L) for each Background well specified on the Plate Map.

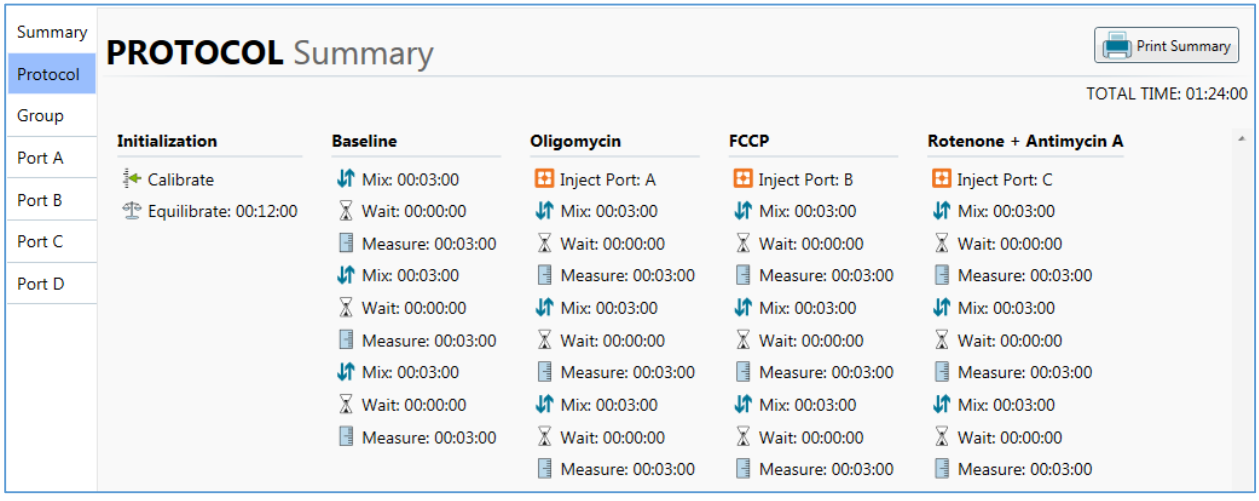
2. Adjust *Port Volume* values:
   a. Type (or use the up/down arrows) to enter a custom value in the *Port Volume* field. This is for notation-purposes only and does not affect calculations.

3. Version Info (below *Port Volumes*):
   a. The software version number for Wave Desktop is displayed along with Seahorse XF File Version info (internal-use only).

***Note**: The displayed PPR kinetic rates do not reflect the custom Buffer Capacity per media until the assay has completed. To correctly display PPR on any analysis view, turn on assay groups and the appropriate background well(s) containing the identical media used. Turn off the assay groups and background wells with a different media/buffer capacity used in the assay.*

Protocol

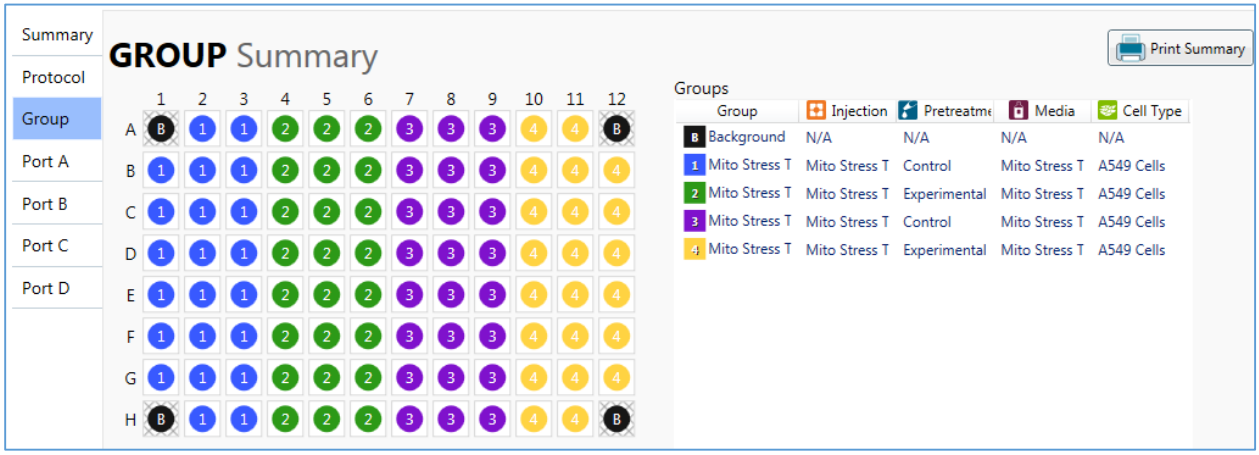
Click **Protocol** to display a preview of the *Instrument Protocol* (below) prior to running the assay. To edit the *Instrument Protocol*, click the **Instrument Protocol** tab before to transferring the assay template to the Agilent Agilent Seahorse XFe or XFp Analyzer.



Agilent Technologies

Group

Click **Group** to display a preview of the *Plate Map* and *Group Definitions* (below) prior to running the assay. To edit the *Group Definitions* or *Plate Map*, click the **Group Definitions** tab or **Plate Map** tab before to transferring the assay template to the Agilent Seahorse XFe or XFp Analyzer.

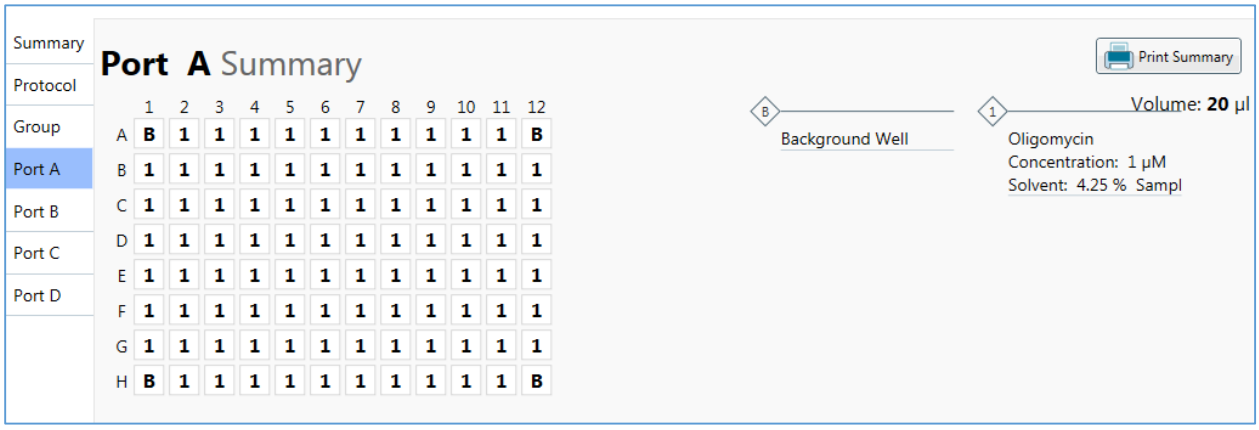


Port A, B, C, and D

Select Port A, B, C, or Port D links to review details of each injection (pictured below):

1. Compound concentration.
2. Name of the solvent (if specified).
3. Percentage of solvent used for each compound.
4. *Port Volume*.

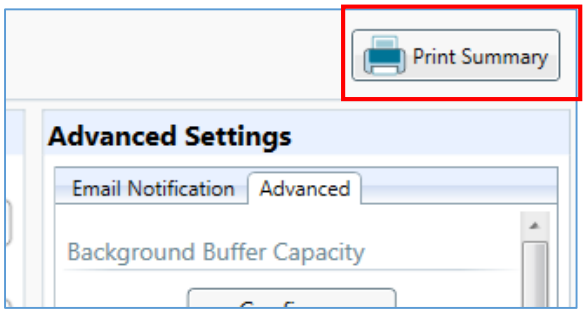
To modify the name and concentration of a compound, or name and percentage of a solvent, click the **Group Definitions** tab then select the appropriate *Injection Strategy* to edit, before to transferring the assay template to the Agilent Seahorse XFe or XFp Analyzer.



Agilent Technologies

Print Summary

Click the **Print Summary** button to print or save a PDF of the Summary (General Information) Protocol, Group, and Port A-D for the assay template.

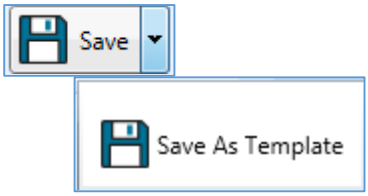


Saving Assay Templates

After making changes to an assay template use the **Save** button to overwrite a custom template with new details/content, or the **Save As Template** button, which will create a new template file and *not* overwrite the original template file contents. Save the assay template to a network or USB flash drive and transfer to the Agilent Seahorse XFe or XFp Analyzer to run the template.

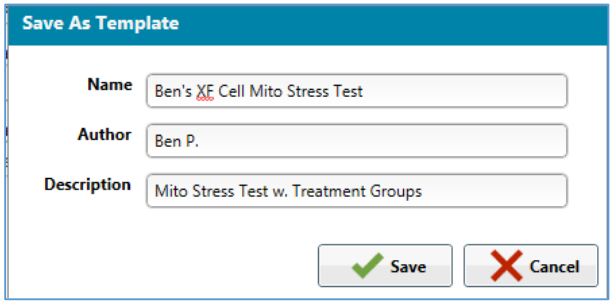
Save As Template

Click the downward-pointing arrow on the **Save** button and choose the **Save as Template** option to save an assay design as an assay template file (.asyt).



The **Save as Template** browser is displayed:

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 the **Save** button.



Types of Assay Templates

The *Templates* view (below *Wave Home*) displays all assay templates saved on the local drive on the PC in the *Template Directory* folder, which is the default save location for all template files created in Wave Desktop. The three icons pictured below provide visual cues to differentiate the type of template from one another. To view or change the default *Template Directory* save location, see the section on **General Options**.

Blank Template

Seahorse Standard Assay Template

User Customized Template

The default content in the *Blank* and *Seahorse Templates* cannot be modified – only new template files can be generated. The *Blank* template provides an empty canvas whereas the *Seahorse Templates* contain specific content for the Seahorse XF assay they correspond to. Create custom templates for a specific Seahorse XF assay using any of the *Seahorse Templates*. Saving edits to a *Seahorse Template* will be create a new template with a green icon and preserve the original content.

Chapter 2: Run Assay on the Agilent Seahorse XFe Analyzer

(Wave Controller ONLY)

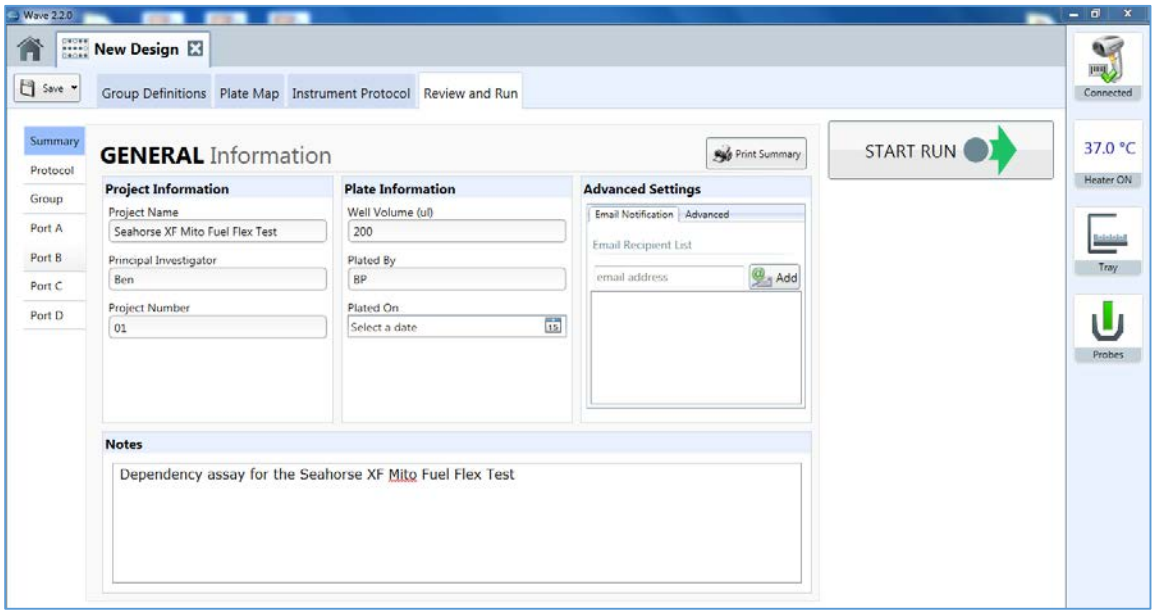
***Note**: This section applies to Wave Controller for the Agilent Seahorse XFe96 and XFe24 Analyzer ONLY. The functions described in this chapter do NOT apply to Wave Desktop or Agilent Seahorse XFp Analyzer software.*

Assay Template files (.asyt) created using Wave Desktop must be imported to Wave Controller on the Agilent Seahorse XFe Analyzer using a USB flash drive or (active) network directory. For non-networked Agilent Seahorse XFe Analyzers, export the assay template from Wave Desktop to a USB flash drive, then import to the Agilent Seahorse XFe Analyzer.

Agilent Seahorse XFe Analyzer

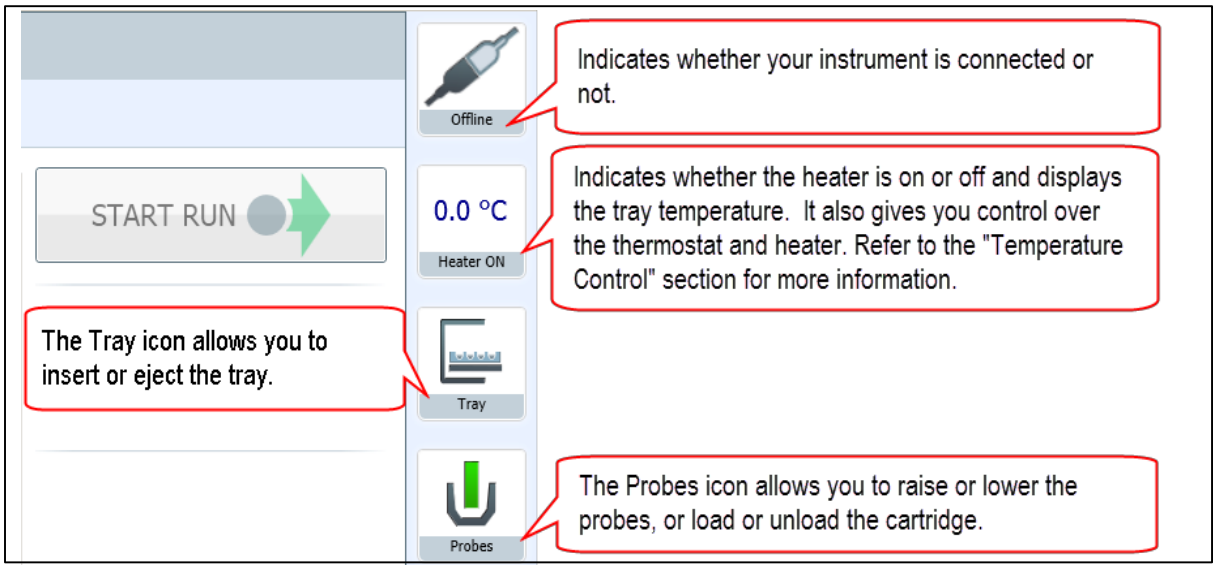
Open Wave Controller to display the *New* view (Template view). To import the assay template:

1. Click **Import**
2. Locate the assay template and click Open.
3. Make any last-minute modifications to the assay template if necessary. When ready to run the assay, navigate to the **Review and Run** tab.



Wave Controller Widgets

The *Widget Icons* located on the right side of the **Review and Run** tab display the Agilent Seahorse XFe Analyzer status, current temperature, and control eject/insert the tray and to raise/lower probes.



Temperature Widget

The Agilent Seahorse XFe Analyzer has been validated to deliver consistent sample temperature when set to 37°C. Performance at temperatures other than 37°C have not been validated.

Click the **Temperature Widget**:

- To turn the heater ON/OFF.
- To set the desired target assay temperature.
- To 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 (see below).

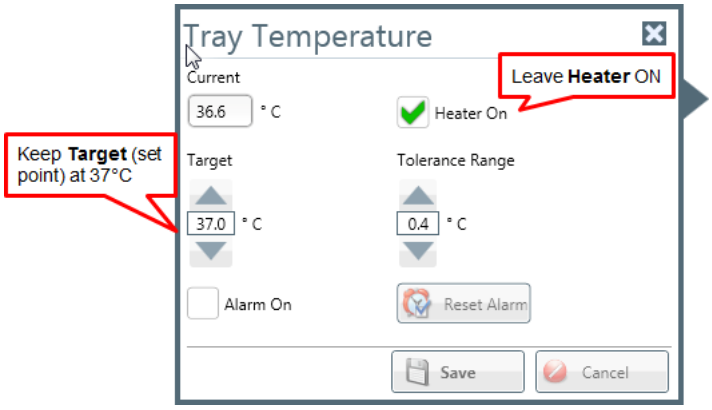
If the temperature exceeds the tolerance range, the Agilent Seahorse XFe Analyzer:

- *Temperature Widget* will change to a magenta color.
- *Status Indicator* light (top of the Agilent Seahorse XFe Analyzer) will change from blue to amber.
- Sends e*mail notifications* to specified recipients sent from the Agilent Seahorse XFe Analyzer (requires active network connection).

Set Target Temperature, Tolerance Range, and Alarm:

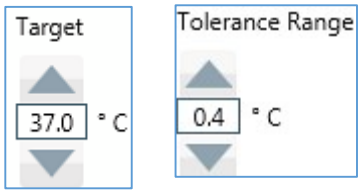
Click on the **Temperature Widget** to display the *Tray Temperature* window (below) to:

- View the current instrument temperature.
- Turn the *Heater* ON/OFF.
- Adjust the *Target Temperature* (set point).
- Adjust the *Tolerance Range.*
- Turn the *Alarm* ON/OFF.



Set Target Temperature and Tolerance Range:

To adjust the **Target Temperature (set point)** or the **Temperature Tolerance Range**, click on the **up**/**down** arrows or type in the desired values using a keyboard. Click **Save** to save any changes on the *Tray Temperature* window.



***Note**: Although the target temperature can be altered, thermal performance of the Agilent Seahorse XFe Analyzer at temperatures other than 37°C has not been validated.*

Set Alarm for Temperature *Tolerance Range*:

1. Check the **Alarm On** box in the Tray Temperature window.
2. Press the **Save** button.

Turn OFF the alarm by unchecking the **Alarm On** box and 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.

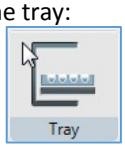
Turn the heater **ON** if the temperature of the Agilent Seahorse XFe Analyzer is lower than the *Set Point Tolerance Range* temperature or **OFF** if the temperature is higher than the *Set Point Tolerance Range* temperature.

Check the current temperature of the Agilent Seahorse XFe Analyzer before beginning an assay to ensure the *Tray Temperature* starts within the *Tolerance Range*. For any suspected temperature issues or unexpected temperature fluctuations, please contact Seahorse Bioscience Technical Support.

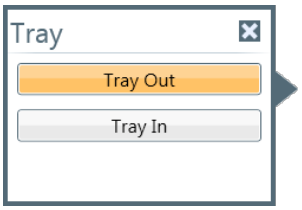
Tray Control Widget

The *Tray Control Widget* allows for manual operation of the tray for the Agilent Seahorse XFe Analyzer. To eject the tray or a Cell Plate already placed on the tray:

1. Press the **Tray Widget**.



2. Click **Tray Out** to eject the tray – remove the Cell Plate if necessary.
3. Click **Tray In** to insert the tray into the Agilent Seahorse XFe Analyzer.

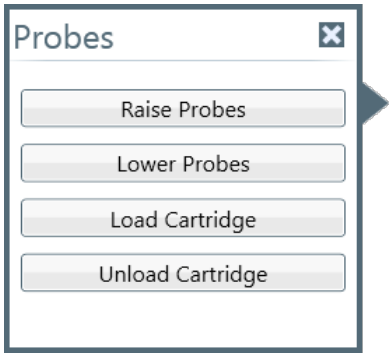


Probe Control

The **Probes Widget** allows for manual operation (raise/lower) probes as well as load/unload a Cartridge:

1. Click the **Probes Widget**

2. Click button for required action to:
   - Raise Probes
   - Lower Probes
   - Load Cartridge
   - Unload Cartridge



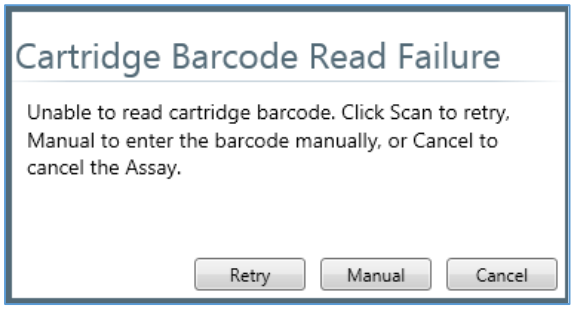
Barcode Errors

The Agilent Seahorse XFe Analyzer reads and records the barcode on the Cell Plate and Cartridge before beginning a Seahorse assay. On the rare occasion the Agilent Seahorse XFe Analyzer is unable to read the barcode, a *Barcode Read* error will appear Wave Controller (see below). Please contact Agilent Seahorse Technical Support to assist with remedying this error in order to start the assay.

Cartridge Barcode Read Failure:

For any Cartridge barcode read errors, Wave Controller will display the following message with 3 corrective actions:

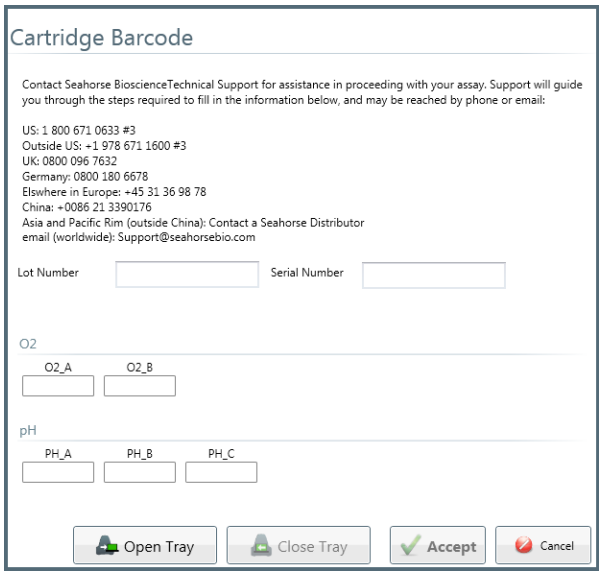
1. **Scan**: Retry scanning the Cartridge barcode.
2. **Manual**: Manually input the Cartridge barcode information. Contact Agilent Seahorse Technical Support for this step.
3. **Cancel**: Cancel the assay.



Manually enter *Cartridge Barcode* information:

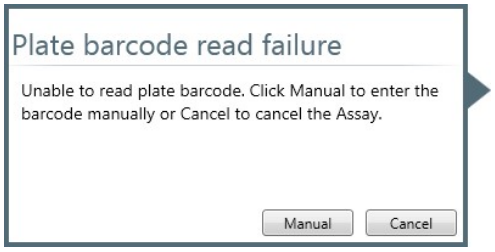
Click **Manual** to display the *Cartridge Barcode* window will appear (pictured right):

Call the appropriate regional Agilent Seahorse Technical Support telephone number. Agilent Seahorse Technical Support will provide the information required to complete the fields on the form in order to proceed with the assay.



Cell Plate Barcode Read Failure

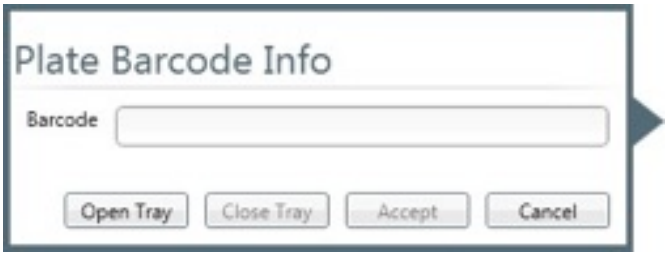
For any Cell Plate barcode read errors, Wave Controller will display the following message with 2 corrective actions:



1. **Manual**: Manually input the Cell Plate barcode information. Contact Agilent Seahorse Support for this step.
2. **Cancel**: Cancel the assay.

Manually enter *Cell Plate Barcode* information:

1. Click the **Tray Widget.**
2. Click **Open Tray** to eject the Cell Plate.
3. The Cell Plate barcode is located on the side of the cell plate – write down the barcode information.
4. Click **Close Tray**.
5. On the *Plate Barcode Info* window, enter the Cell Plate barcode.
6. Click **Accept**.



Status Indicator Color

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

1. To load a Cartridge or Cell Plate.
2. Remove a used Cartridge and/or Cell Plate.
3. Accept or cancel an assay if one or more wells did not calibrate properly after *Calibration*.
4. Any errors that can occur during the run, such as barcode read errors for the Cell Plate or Cartridge, or protocol error.

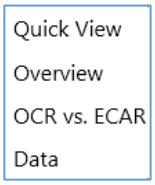
Chapter 3: Analyzing Assay Result Files

Wave Desktop 2.3 is the recommended and preferred software tool for analysis of assay result files. After completing an assay on any Agilent Seahorse Analyzer, save the assay result file to a USB flash drive or network directory to open in Wave Desktop on a personal computer. The default analysis view for new recently performed assay result files is the *Quick View*. After modifying and saving an assay result file, Wave automatically displays the last-modified analysis view upon reopening the assay result.

Assay Result Files

An *Assay Result file* (*.asyr) contains all assay data from a completed run. Examples of the types of data acquired during a Seahorse assay are: rate (OCR, ECAR, PPR), level (mmHg or mpH), calibration results, and consumable information read from the barcodes on the Cell Plate and Cartridge used in the assay. In addition, any details about the experiment, groups, treatments, etc. added to the assay template file is contained in the assay result file. This information is also editable by using the **Modify** function. See Modifying Assay Result Files for more information.

Each analysis view can be added to an assay result file using the **Add View** button.

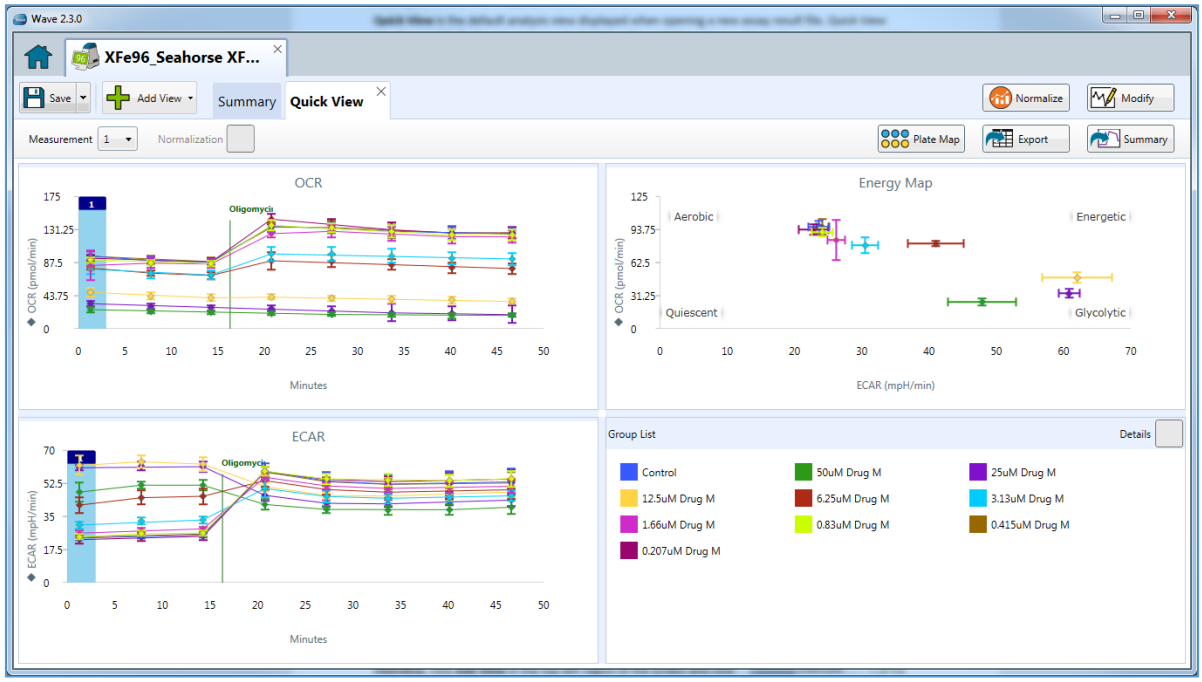


The 4 analysis views in Wave Desktop 2.3 are:

1. Quick View
2. Overview
3. OCR vs. ECAR
4. Data

Analysis View #1 – Quick View:

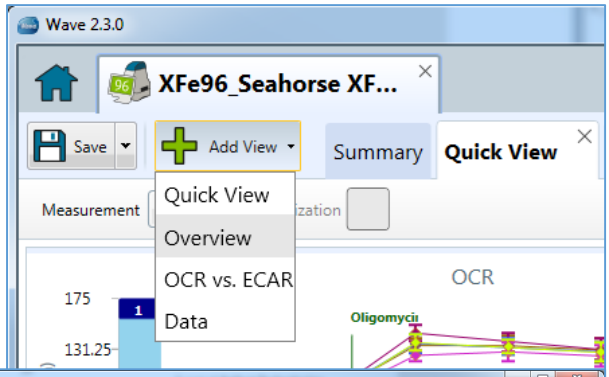
Quick View is the default analysis view displayed when opening a new assay result file. Quick View simultaneously display a kinetic graph of *OCR vs Time*, *ECAR vs Time*, and a scatter plot of *OCR vs. ECAR* (or PPR*).

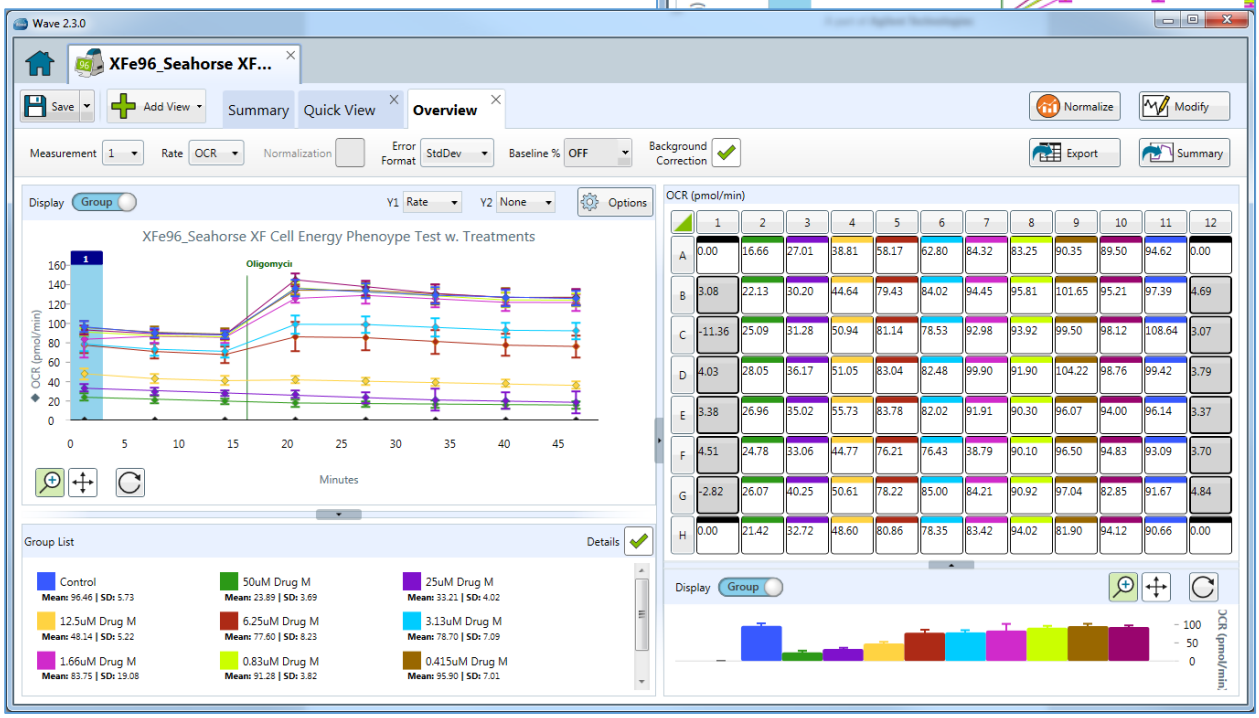


** PPR is displayed when the Assay Media buffer capacity is specified within Groups/Conditions.*

Analysis View #2 – Overview:

Overview displays kinetic graphs for all rates (OCR, ECAR, PPR) versus time. The selected kinetic rate is displayed on the y-axis and Time is always displayed 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 for data analysis. To display **Overview**, click **Add View** in the upper-left region of the screen and select **Overview** from the dropdown menu.

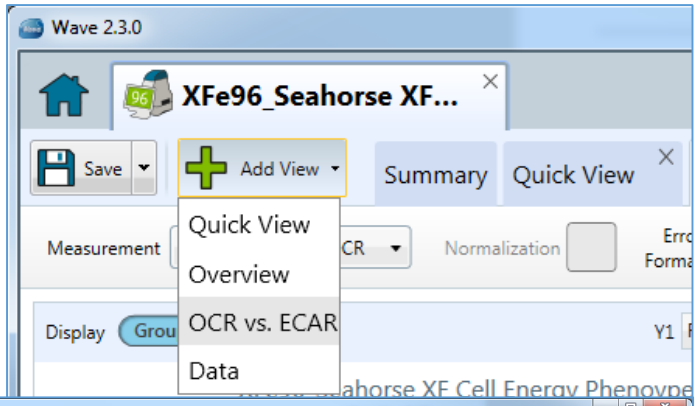




***Note**: Add any number of **Overview** tabs by clicking **Add View** and selecting **Overview** from the dropdown menu. This allows for generating several customizable **Overview** tabs to display specific assay groups, compare a control group vs. experimental groups, groups treated with various compound 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 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 dropdown menu.



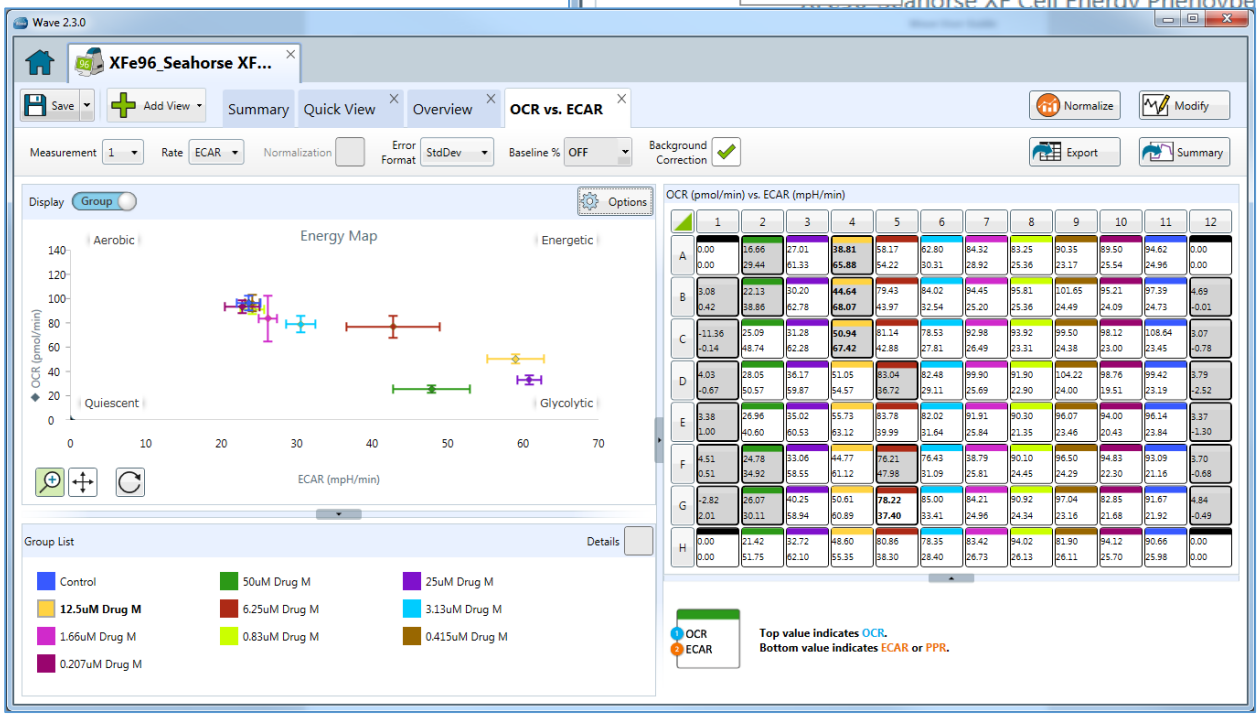
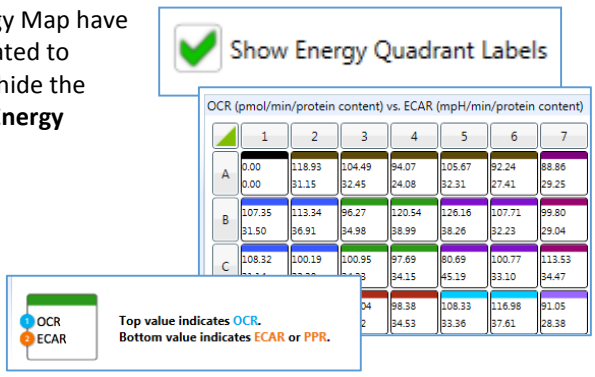


Chart Title and Quadrant Labels

The default graph title is: Energy Map. The corners of the Energy Map have labels representing the bioenergetics status of the group(s) related to other groups in the assay for a selected rate measurement. To hide the quadrant labels, click **Options** then uncheck the box for **Show Energy Quadrant Labels**.



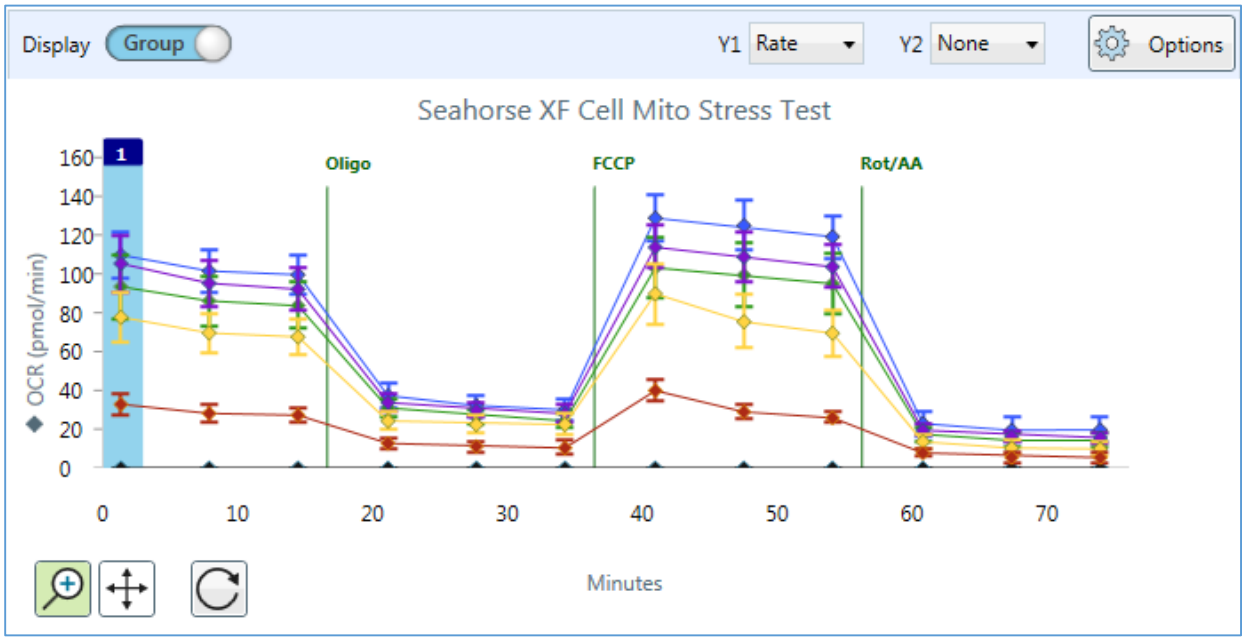
Dual Rate Display on Plate Map

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

Chart Types in Wave Desktop:

Kinetic Graph (Rate vs. Time)

A *kinetic graph* is the most common way to display data from the Agilent Seahorse XFe Analyzer. The kinetic graph displays the rate measurement on the y-axis and the time on the x-axis and is visible on the **Overview** tab.



Scatter Plot (OCR vs. ECAR)

The other most commonly used data display is an OCR vs. ECAR scatter plot – OCR plotted on the y-axis and ECAR plotted on the x-axis. Data points are displayed on the OCR vs. ECAR scatter plot for a single rate measurement from each group in the assay.

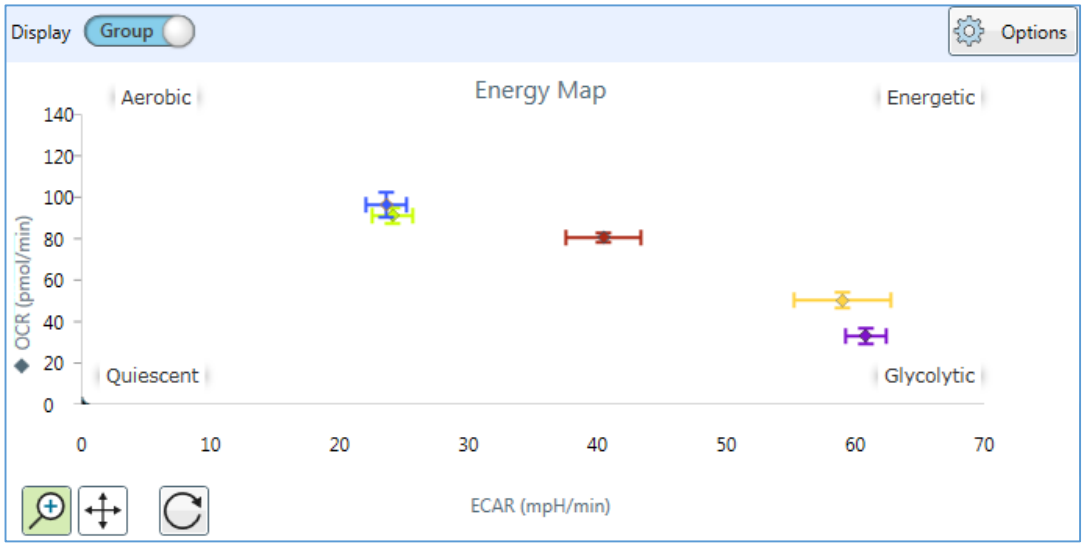
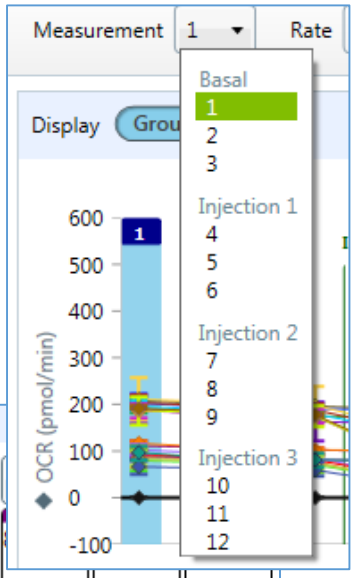


Plate Map

The *Plate Map* displays rate data for each assay well per group for the selected rate measurement. *Plate Maps* are displayed in the upper right-hand corner of the **Overview** and **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 always rate measurement 1.



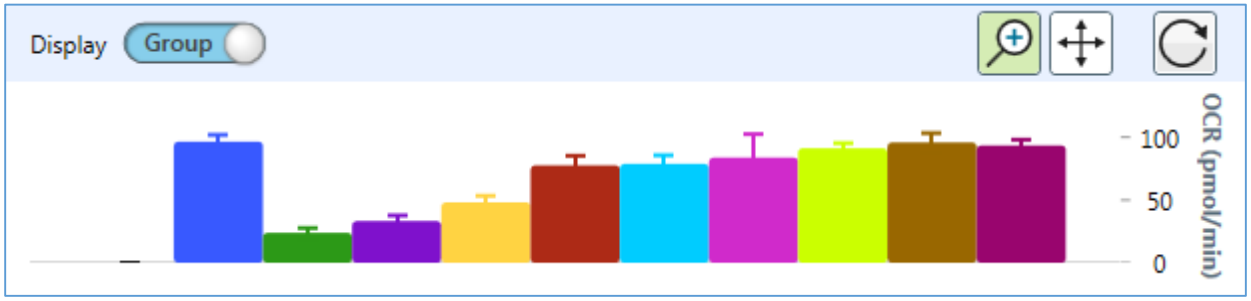
To change the measurement displayed in the *Plate Map*, use the *Measurement* dropdown above the kinetic graph or scatter plot. The *Rate Highlight* tool on the kinetic graph will move to the selected rate. Hide the *Rate Highlight* tool by clicking **Options** above the kinetic graph/scatter plot and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

OCR (pmol/min)

	1	2	3	4	5	6	7	8	9			
A	0.00	212.43	200.43	209.39	209.92	203.42	88.86	94.90	87.28			
B	228.98	192.36	199.15	196.89	208.65	209.93	110.75	96.20	117.63	119.58	114.03	86.40
C	194.11	194.85	178.83	187.16	143.38	194.01	120.36	119.28	114.96	114.91	101.86	84.45
D	205.87	273.94	186.93	205.06	187.60	198.36	95.30	109.34	97.77	105.54	99.27	71.83
E	176.57	189.40	221.33	200.64	185.01	212.27	115.86	88.88	100.08	73.34	87.98	51.46
F	199.78	185.29	194.34	132.39	183.64	191.46	112.48	102.42	95.72	96.92	82.33	52.57
G	198.29	165.18	190.94	193.61	191.86	193.85	95.23	81.70	90.12	79.43	84.26	54.20
H	0.00	182.60	220.16	190.60	197.16	189.26	62.09	63.14	61.17	70.76	59.39	0.00

Bar Graph

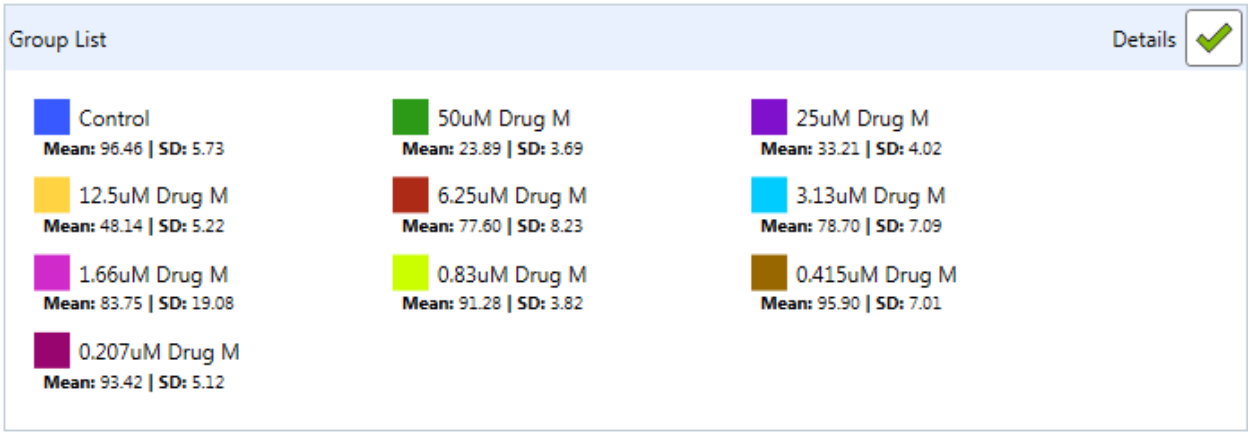
The **Bar Graph** below the *Plate Map* displays the average rate for each group for the selected measurement. Use the **Display** toggle switch to change the rate display from *Group* (average) to *Well* (individual well) mode.



The **Bar Chart** is useful for viewing rate data from each assay group at a specific rate measurement. This view helps facilitate outlier identification or other trends in the results that may not be obvious when viewing a kinetic graph or scatter plot.

Group List (Legend)

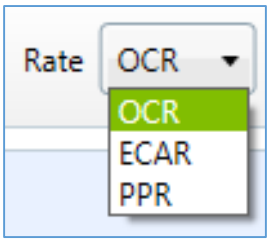
The *Group List* below the kinetic graph or scatter plot, functions as a legend for the chart data above. To hide a group from the graphed data, simply double-click the name of the group in the *Group Details*. Double-click the hidden group name to unhide the data from the graph. The mean and standard deviation of hidden groups will change to **Mean: 0.00 and Standard Deviation: 0:00**.



The *Group List* also displays group statistics for each assay group for the selected rate measurement. To display the group statistics, check the **Details** box in the upper-right corner of *Group List*. Statistics that are displayed (per group) are average and error of the selected rate measurement. Select a different rate measurement to recalculate and display the average and error value for the newly selected rate. Assay wells that have been turned OFF on the *Plate Map* are not included in the calculated group statistics.

Types of Data in Wave Desktop:

Rate Data: OCR, ECAR, and PPR



The two rates measured by the Agilent Seahorse XF Analyzers are:

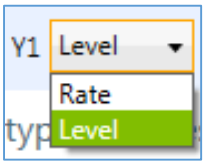
- OCR: Oxygen Consumption Rate (pmol/min)
- ECAR: Extracellular Acidification Rate (mpH/min)

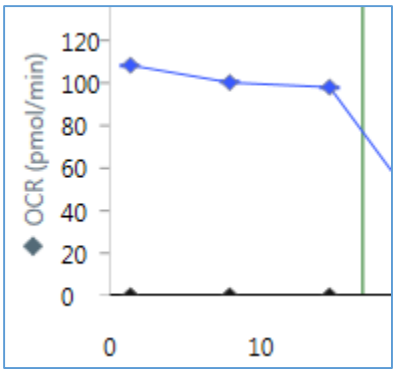
Note: *Proton Production Rate (PPR; pmol/min) is calculated based on the ECAR data and the Buffer Capacity of the media used for each group.*

The **Overview** and **OCR vs. ECAR** analysis views have a *Rate* drop-down menu to switch the data displayed on the chart a different rate.

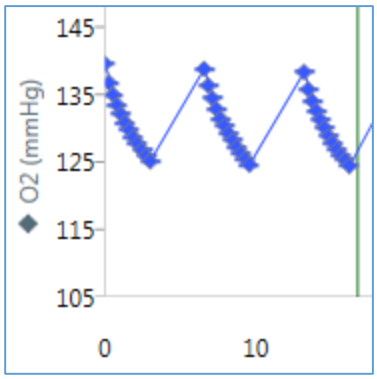
Level Data: O2 and pH

Use the **Y1:** drop-down to select *Level* to view the O2 levels and pH levels for each rate measurement during the assay. The O2 level data is displayed as oxygen tension in units of mmHg. The drop in oxygen tension during a measurement is used to calculate the OCR. The pH level data displays the changes in pH for each rate measurement and is used to calculate ECAR. View level data on the **Overview** and the **Data** analysis views only.





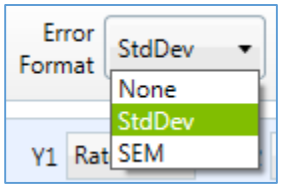
Oxygen Consumption Rate (OCR) data in **Rate** mode.



Oxygen tension (O2) data displayed as mmHg in **Level** mode.

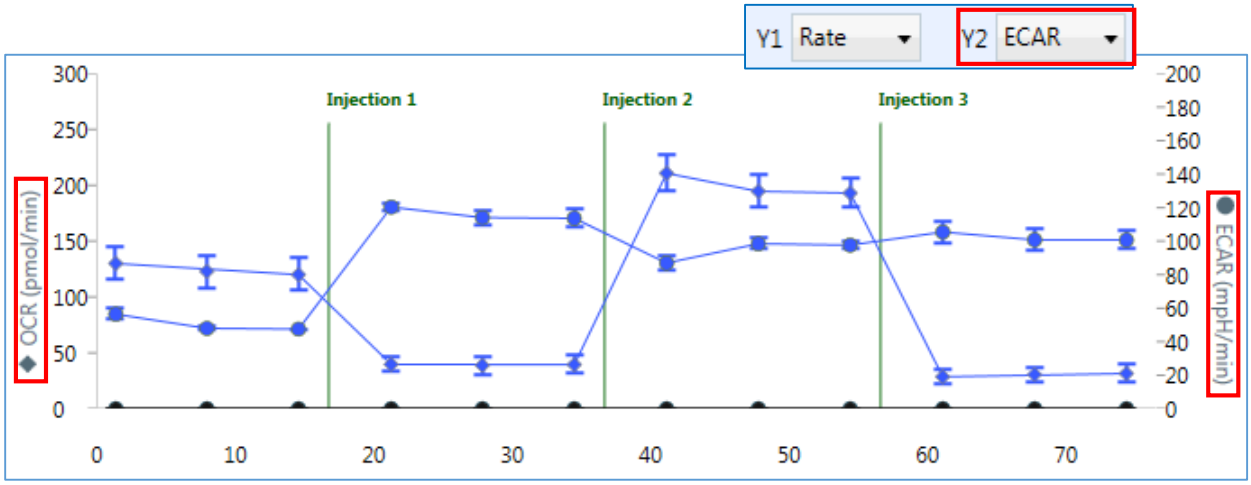
Standard Deviation and Standard Error of the Mean

The two error bar types in Wave are **Standard Deviation** and **Standard Error of the Mean (SEM)**. Use the *Error Format* dropdown menu to change the error bar type displayed on the kinetic graph/scatter plot and the **Group List**. Select **None** to hide error bars from the data graph. In *Well* mode, error bars are not displayed.



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

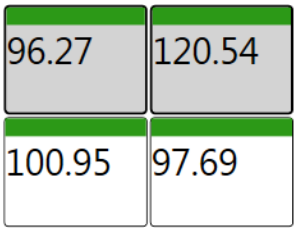
Compare rates or level of data acquired during an assay from on one kinetic graph for one or more groups. Select data to overlay with the Y1 data using the **Y2:** drop-down menu above the kinetic graph.



Customizing Data Displays

Excluding Assay Wells:

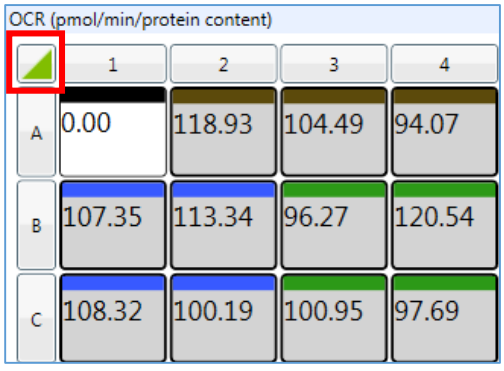
By default, all assay wells on the *Plate Map* are turned ON when opening a new assay result file. Click an assay well to exclude (or include) the assay well from the group statistics and graphed data for that group. Assay wells that are turned off on the *Plate Map* have a grey background (pictured right). Assay wells that are included in the graphs and group statistics have a white background (also pictured right). 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.

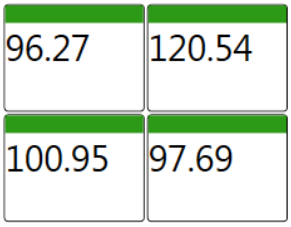


Two assay wells in this group are turned *OFF*. These assay wells are excluded from the graph and group calculations.

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 (pictured below) in the upper-right corner of the *Plate Map* to turn off *all* assay wells on the *Plate Map*. Charts on this analysis view will not display any groups since all assay wells are turned off.

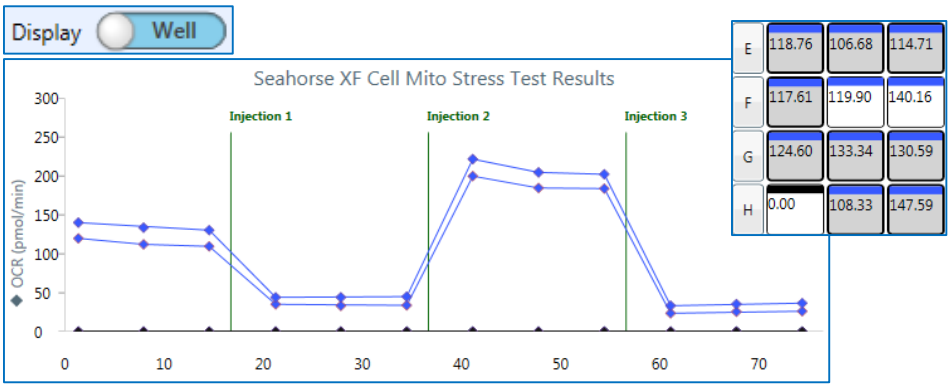




All assay wells in this group are turned on and included in the graph and group calculations.

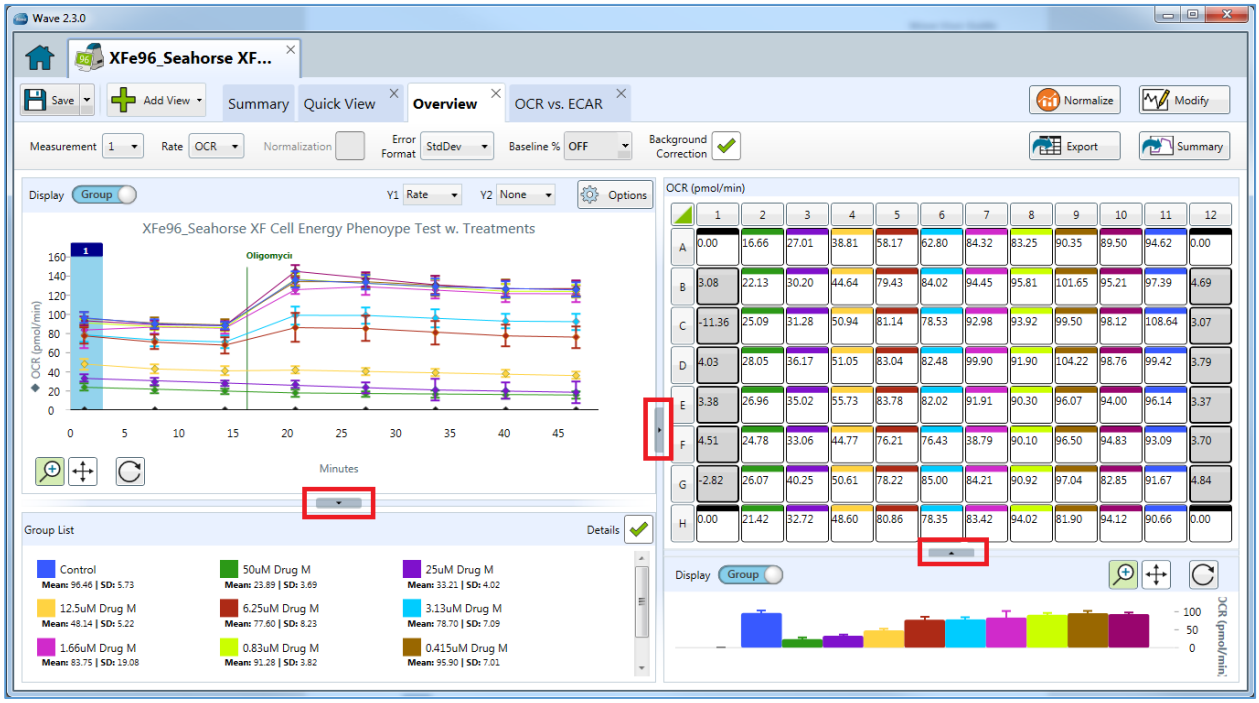
Display Modes: Group and Well

Rate data is graphically displayed in *Group* mode by default 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.



Resize a Graph or Plate Map

Click resize arrow or drag the border to resize the kinetic graphs, *Plate Map* or *Bar Chart* (**Overview** pictured below – resize arrows outlined in red). Stretch the size of the kinetic graph using the outlined arrow or border between the kinetic graph and *Plate Map*. Hide the *Group List* to increase the vertical size of the kinetic graph using the outlined arrow between the kinetic graph and *Group List*. Hide the *Bar Chart* using the outlined arrow between the *Plate Map* and *Bar Chart.*



Agilent Technologies

Zoom, Pan, and Restore

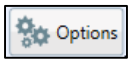
The **Zoom, Pan,** and **Restore** buttons are displayed below the kinetic graph on the **Overview** and the scatter plot on the **OCR vs. ECAR** view:

Zoom – Right-click and hold to select an area on the kinetic graph or scatter plot to magnify the selected area.

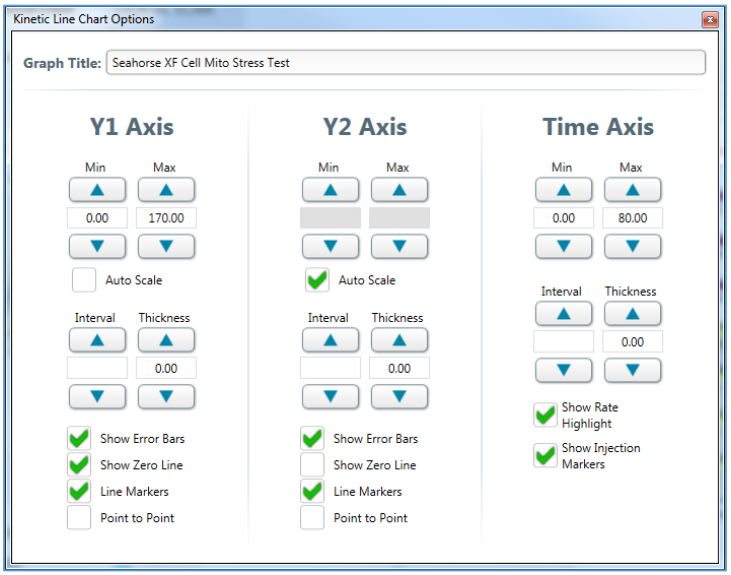
Pan – After zooming into a section of a graph, use the **Pan** button to move around the graph while keeping the selected zoomed display aspect ratio.

Restore – Return the graph to full view.

Graph Formatting Options



Click **Options** (upper-right corner of a kinetic graph or scatter plot) to format *Kinetic Line Chart Options* and *OCR vs. ECAR Chart 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 values 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 ON/OFF the vertical injection lines on the kinetic graph.

Modifying Assay Result Files

Modify enables users to add or edit details of an assay result file. **Modify** is most frequently used to add notes about the experiment, further define groups, compounds or pretreatment details, reassign assay wells to an 'outlier' group or un-assign assay wells from the *Plate Map* entirely. Click **Modify** (upper-right corner of an assay result file) to display a tabular view of:

- Groups Definitions
- Plate Map
- Injection Names
- General Information (assay properties)

Modify mode looks nearly identical to the tabular view when crating or customizing an assay template file. When actively modifying an assay result file Wave Desktop will indicate the program is in **Modify** mode (pictured below). To return to viewing data in the assay result file, click **Apply** (upper-right corner of Wave) to apply modifications to the assay result file or click **Cancel** to cancel out of **Modify** mode to prevent any changes from taking effect.

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

Group Definitions Tab

Select the **Group Definitions** tab to display the *Injection Strategies*, *Pretreatments*, *Assay Media* and *Cell Type* details entered in the assay template prior to running the assay. Modifications to the **Group Definitions** tab include: renaming assay groups, reconfigure details for one or more groups or add additional group information that was left out of the initial assay template design.

Plate Map Tab

Select the **Plate Map** tab to display and edit conditions of each assay group using the drop-down menus below the group name. 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 Tab

Select the **Injection Names** tab to edit the name of each compound injection during the assay. 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 Tab

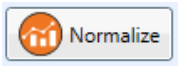
Select the **General Information** tab to edit details of an assay result file, such as: Project Name, PI, Well Volume, or Advanced Settings (email notification or background well buffer capacity.

Normalization

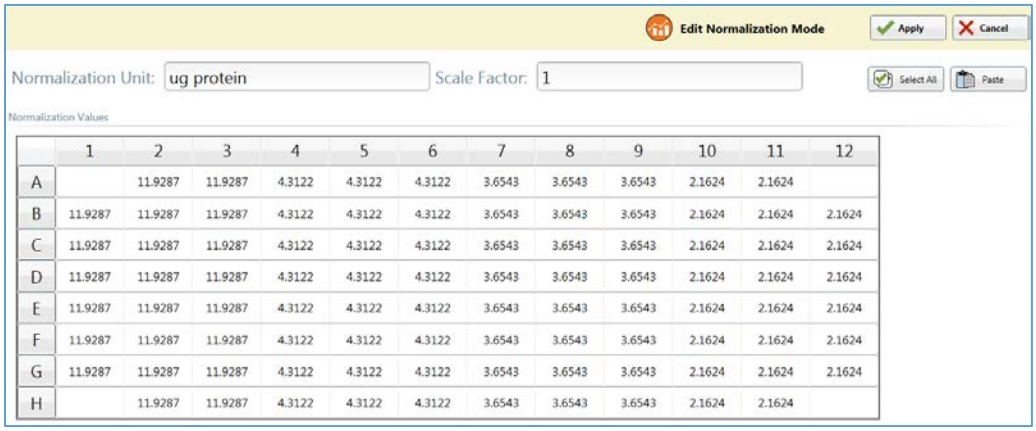
In the context of a Seahorse XF assay, normalization is the process of adjusting rate data for variations in a relevant cell parameter (such as cell count or total cell mass) that can occur among wells causing well-to-well variability. The *Normalization* function in Wave can help correct for these variations by entering the values for the cellular parameter and adjusting the data to 'normalize' to a single value. To use the normalization function, an independent assessment of the assay wells for cell number, protein mass, DNA content, or similar parameter is required. In order to normalize data in Wave, three components are used:

1. *Normalization Values* (required) – The numeric data generated from the independent assessment of the well (cell count, protein content, DNA content, etc.).
2. *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", etc.).
3. Normalization Scale Factor – This number determines what value the rate data will be normalized to. Default is 1 and adjustment is optional.

Add *Normalization Values*



Click **Normalize** to apply normalization data to rate calculations in a Seahorse assay result file. The **Edit Normalization Mode** will appear (completed *Edit Normalization Mode* below).



	1	2	3	4	5	6	7	8	9	10	11	12
A		11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	
B	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
C	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
D	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
E	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
F	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
G	11.9287	11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	2.1624
H		11.9287	11.9287	4.3122	4.3122	4.3122	3.6543	3.6543	3.6543	2.1624	2.1624	

The two ways to add normalization data to an assay result file are:

Copy/paste normalization values from a secondary source (ex. Excel File)

1. Open the independent normalization data file – the data must be in a grid format, similar to the plate map in Wave Desktop.
2. Select the normalization data and copy to the clipboard (Ctrl+C).
3. Open a Seahorse assay result file in Wave Desktop.
4. Click the **Normalize** button.
5. Click **Select All** to select all wells on the plate map.
6. Click **Paste** to insert the copied data from the clipboard into the normalization grid in Wave.
7. Click **Apply.**

Enter normalization values manually:

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

***Note**: Enter '0' as the normalization value for each background well.*

Normalization Unit

A *Normalization Unit* **must** be entered to apply normalization values to the rate measurements. This unit will be displayed on the Y1 axis title on the kinetic graph when *Normalization* is checked ON. 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*, be sure to add the scale factor into the unit field as well.

Normalization Scale Factor

The new *Normalization Scale Factor* allows users to normalize to a specific cell number of other normalization value. It is often desirable to normalize to an average or rounded number. For example, if wells contain 12052, 12503, and 12757 cells per well, "10000" would be a suitable *normalization scale factor*. Enter a scale factor value to recalculate and multiply normalized rate data based on the equation shown below:

$$Normalized\ Rate\ (well) = \left[\frac{Rate\ (well)}{Normalization\ Value}\right] * Normalization\ Scale\ Factor$$

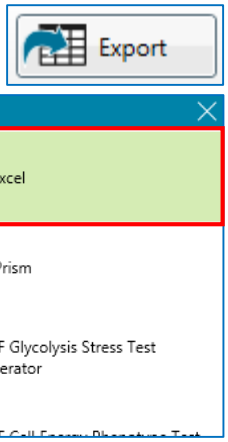
***Note**: Be sure to enter the same value in the **Normalization Unit** field to display the factor correctly on the y-axis. For example, if using cell count and the scale factor is 10000, enter "10000 cells" as the **Normalization Unit**.*

Viewing Normalized Data

After normalization data has been entered, the **Normalization** checkbox will become active on all analysis tabs. Toggle the checkbox ON/OFF to view the normalized/non-normalized rates on the kinetic graph, scatter plot, and plate map. All data graphs will display the *Normalization Unit* entered.

Export Options

Export to Microsoft Excel



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

1. Click **Export** (on any analysis view).
2. Select **Microsoft Excel** (pictured right).
3. Choose a save location and modify the file name (if necessary).
4. Click **Save**.

The two default tabs in an Excel file export are: **Assay Configuration** and **Rate (Plates).**

The **Assay Configuration** tab provides 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. The **Rate (Plates)** tab displays raw OCR, ECAR, and PPR data in a *Plate Map* format for each rate measurement acquired during an assay.

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

Unassigned Well	Unselected Well	OCR Data	ECAR Data	PPR Data

Normalized and Baselined data are also exported to Excel when applied to an assay result file in Wave. Normalization data that has been applied to an assay result file will be contained in the third tab called: **Normalized Rate (Plates)**

Data that has been baselined to a rate measurement in Wave will be contained in the fourth tab called: **Baselined Rate (Plates)**

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 a Seahorse XFp Analyzer).

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 a Seahorse XFp Analyzer).

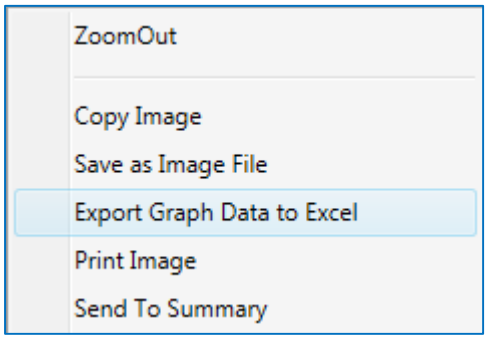
Measurement 1	1
A	100.00
B	128.34
C	139.25
D	124.75
E	126.51
F	124.18
G	128.18
H	100.00

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

Export Selected Chart Data to Microsoft Excel from any Analysis View

Export data 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**.
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.

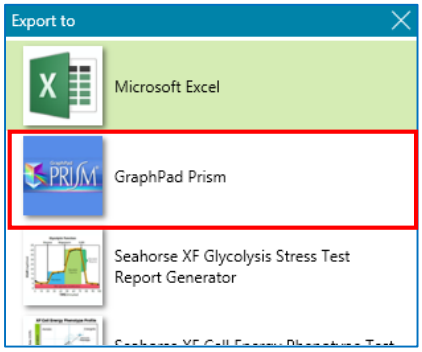
Note: This Microsoft Excel file export is **not** compatible with the Seahorse XF Report Generators. Export data from Wave Desktop 2.3 directly to the Seahorse XF Report Generators using the **Export** button on any analysis view.

Export to GraphPad Prism 6*

***Note:** Export compatibility has been validated with GraphPad Prism 6.*

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

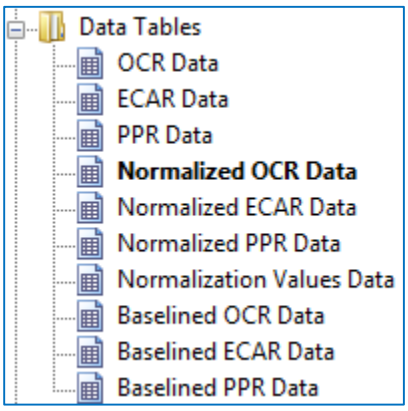
1. Click **Export** (on any analysis view).
2. Select **GraphPad Prism** (pictured right).
3. Choose a save location and modify the file name (if necessary).
4. Click **Save**.



GraphPad Data Tables

Wave Desktop exports OCR, ECAR and PPR data to GraphPad in 3 different formats:

- 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.



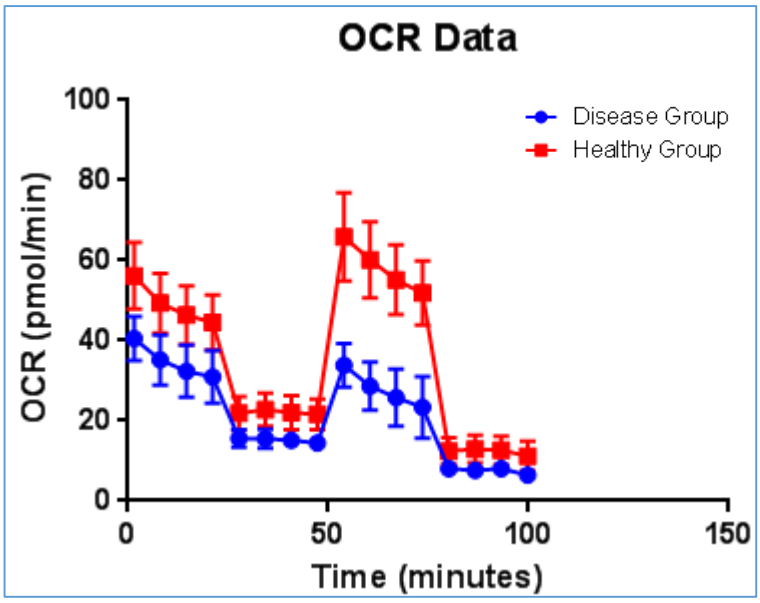
Sample data output in GraphPad Prism from a Seahorse XFp Analyzer with two assay groups called: *Disease Group* and *Healthy Group*.

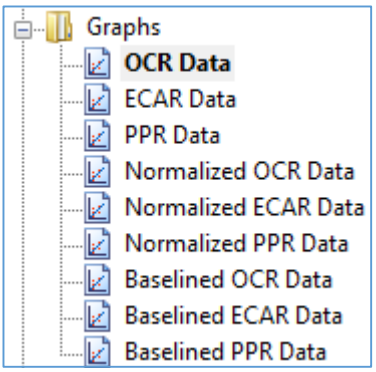
X	Group A			Group B		
Time (minutes)	Disease Group			Healthy Group		
X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1.5591	36.5971	44.4419		64.5248	56.2883	47.8664
8.0789	30.7173	39.6487		56.5285	49.8633	41.6654
14.6040	27.7426	36.9345		53.6014	46.7723	38.8526
21.1255	26.2812	35.6242		51.0053	45.3292	37.3431
27.7413	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

- *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 the above image shows rate data that has been exported for two wells in the *Disease Group.* Refer to **Project Info** or Wave Desktop 2.3 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 the above example 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 below 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 – 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.





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. 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

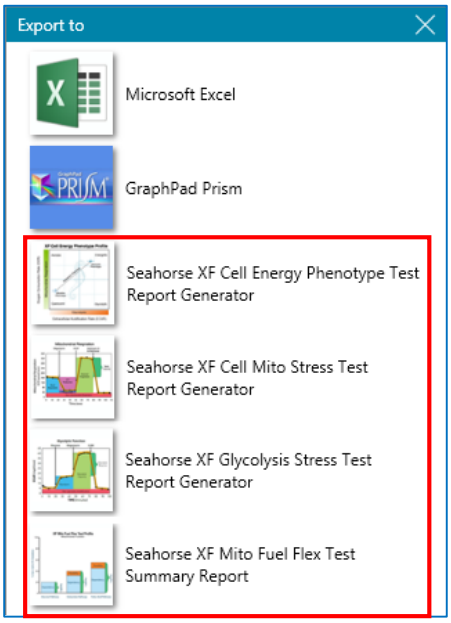
GROUP LAYOUT
Wells between [ ] are unselected wells and they are not exported in the Data Tables.
Group Names between [ ] are unselected groups and they are not exported in the Data Tables.
Background wells and wells not assigned to a group are not exported in the Data Tables.

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

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

Group Name: Background
Group Wells: A1, H1

Export to the Seahorse XF Report Generators



Export data to the Seahorse XF Report Generators directly from Wave Desktop 2.3 to generate a Summary Report of an assay. Seahorse XF Report Generators are Microsoft Excel macro files that automatically calculate the parameters of the selected Seahorse XF assay and present the data in a one-page, customizable Summary Report. Modifications performed in Wave Desktop, such as turning off outlier assay wells or normalizing data, will persist to the exported Seahorse Report Generator file.

The four Seahorse Report Generators available to export to are:

- Seahorse XF Cell Energy Phenotype Report Generator
- Seahorse XF Cell Mito Stress Test Report Generator
- Seahorse XF Glycolysis Stress Test Report Generator
- Seahorse XF Mito Fuel Flex Test Report Generator

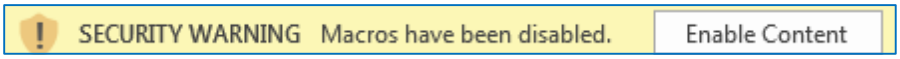
Export data from any analysis view in Wave Desktop to a Seahorse XF Report Generator:

1. Open assay result file and modify data as necessary.
2. Click **Export.**
3. Select the Seahorse XF Report Generator that applies to the result data.
   ***Hint**: When exporting an assay result file that has data from two different assays on the same Cell Plate (such as a Seahorse XF Cell Mito Stress Test and Glycolysis Stress Test), exclude assay groups for one assay and export to the appropriate Report Generator, then repeat the same process for the other assay groups and Report Generator.*
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 a new Seahorse XF Report Generator file is created by Wave Desktop.
6. Click the **Save** button to save the Summary Report.

For more information on how to use a specific Seahorse XF Report Generator, please refer to the appropriate Seahorse XF Report Generator User Guide on the Agilent website.

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

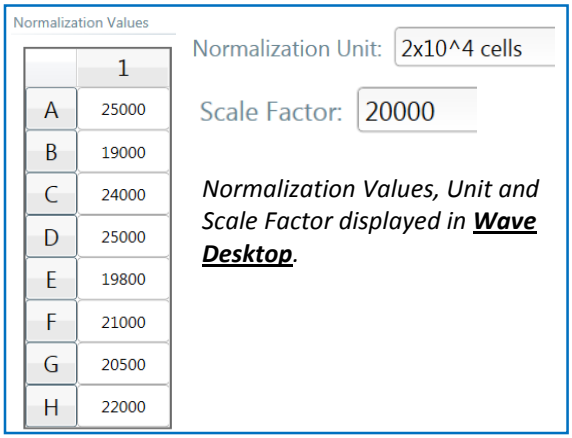
Enable Macros in the Trust Center: Before using a Seahorse XF Report Generator, macros must be enabled. Macros are *disabled with notification* in the *Microsoft Excel Trust Center* by default. Opening a newly-exported Seahorse XF report Generator from Wave Desktop will display a security warning along with a button to enable the macro (pictured below). Click **Enable Content** to begin using the Seahorse XF Report Generator. This will have to be enabled each time a Seahorse XF Report Generator file is created.

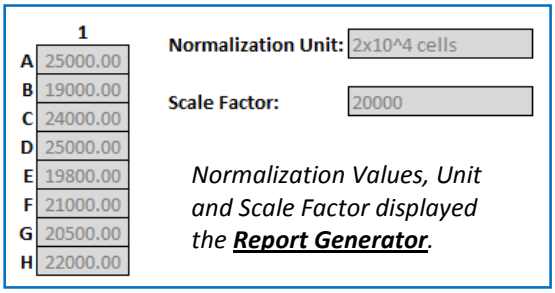


To always enable macros and not be prompted to **Enable Content** each time a Report Generator file is created, enable all macros in the Microsoft Trust Center:

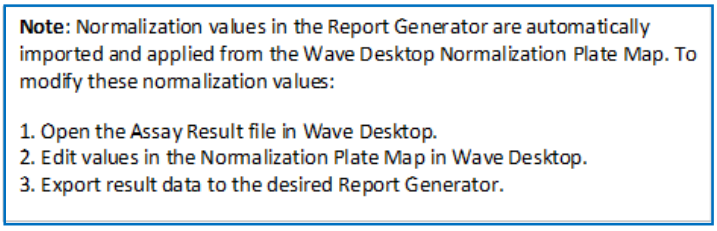
1. Open Microsoft Excel.
2. Click **File**, then click **Options**.
3. Click **Trust Center**, then click **Trust Center Settings**.
4. Click **Macro Settings**.
5. Select *Enable all macros* and click **OK.**

Exporting normalized data to a Report Generator: Rate data that has been normalized in Wave Desktop 2.3 will be exported to the Report Generator and used for parameter calculations. Normalized data is displayed by default after selecting groups to display in the Report Generator. Use the **Normalize** button on the Summary Printout page to toggle the data display between normalized and raw rate data. The **Normalize** tab in the Report Generator will display the normalization values, unit, and scale factor from Wave (pictured below). 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.

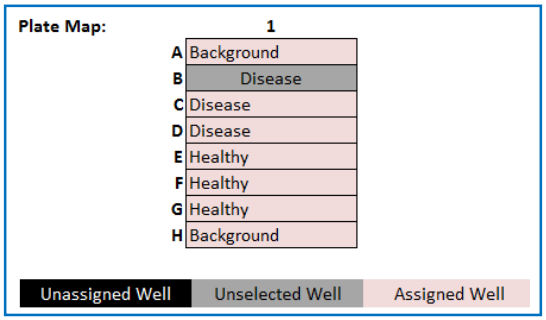




The **Normalize** tab in the Report Generators provides a step-by-step explanation on how to edit the normalization values imported to the Report Generator (pictured right).

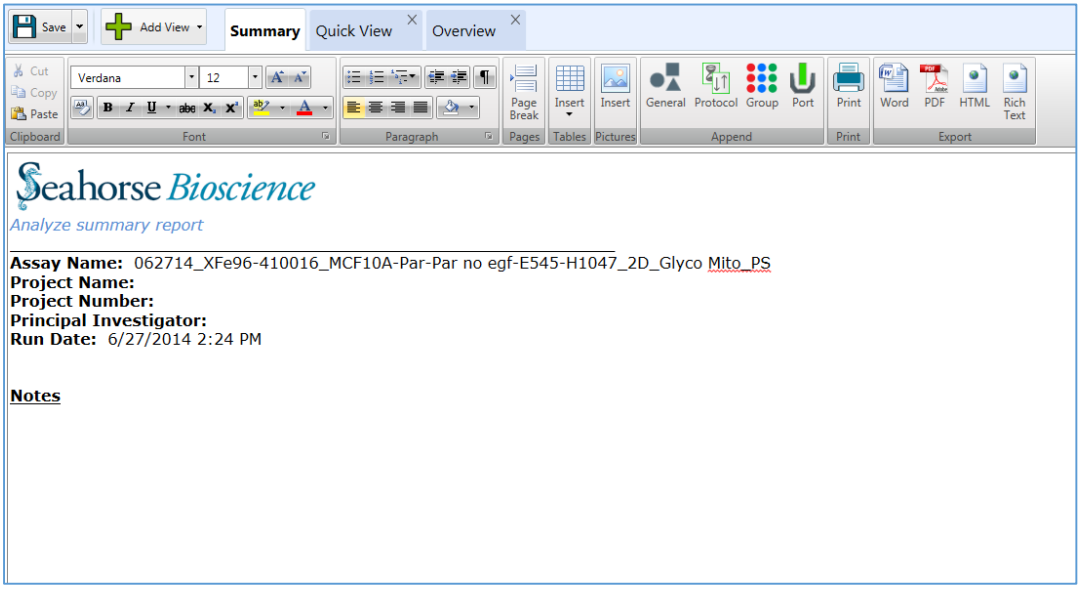


Excluded assay wells in Report Generator: Assay wells that are turned off in Wave Desktop will not be exported or included in parameter calculations for each group in the Report Generators. This also applies to entire groups in an assay result file – Groups turned off in Wave will not be exported to 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.



Summary Tab:

The **Summary** tab is always the first tab in an assay result file cannot be closed or deleted. The **Summary** tab provides a place for adding custom notes or valuable information about the assay performed, appending graphs, charts, and/or plate maps from other views, or adding details of the **Instrument Protocol** or **Groups/Conditions** used in the assay. The **Summary** tab is the only location in Wave that supports a *print* function as well as a file export to Microsoft Word, Adobe PDF, HTML, or Rich Text Format (.rtf).



Customize the Summary:

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

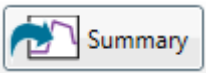
- Cut, copy, and paste to the clipboard.
- Change the font type, size, text and highlight color, and style.
- Subscript or superscript a character or characters.
- Add bullet points or numbers.
- Insert a table, picture or page break.

Append to Summary

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

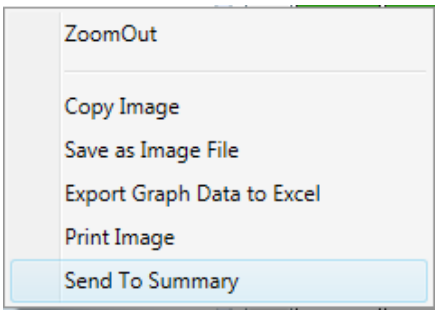
Summary button:

Click **Summary** in the top left-hand corner of the **Quick View, Overview, OCR vs. ECAR** view. This button sends all content on the analysis view to the **Summary** tab.



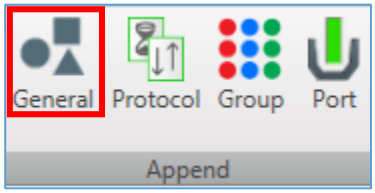
Right-click Options:

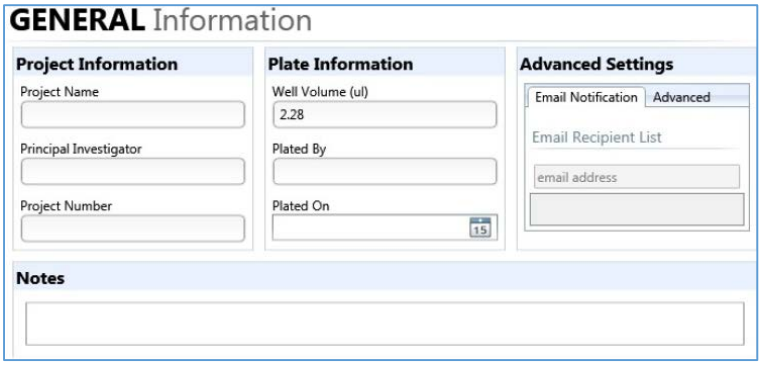
Right-click a kinetic graph, scatter plot, or *Plate Map* and click **Append to Summary** from the dropdown list. To view appended content, click the **Summary** tab.



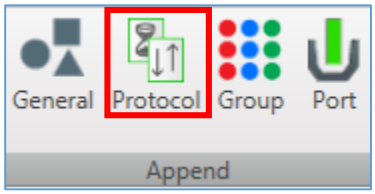
Append Tool Bar (*Summary* tab):

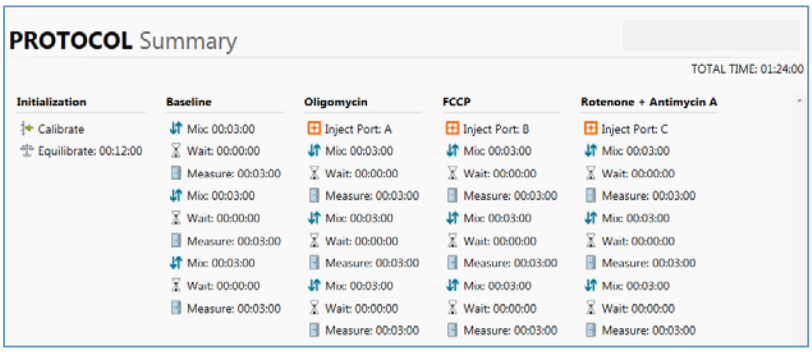
General Information: General information about the assay, including project and plate information.



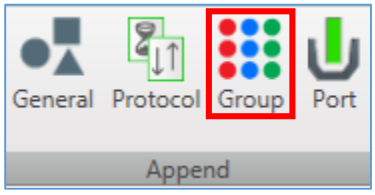


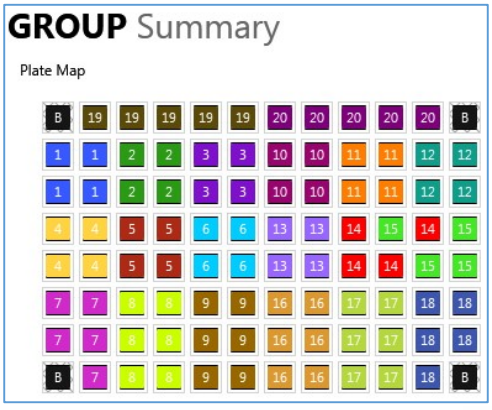
Protocol Summary: The Protocol Summary displays the Instrument Protocol (Mix, Wait, and Measure steps) as well as the time and number of cycles for each measurement.



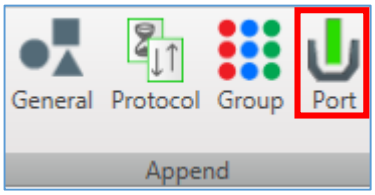


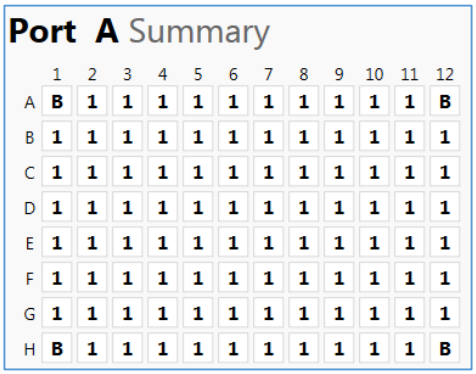
Group Summary: The Group Summary button appends a *Plate Map* that displays the location of each group in addition to a table of the Group Definitions for each group. The *Plate Map* is shown below.





Port Summary: The Port Summary displays information about the contents of each compound port in the assay.

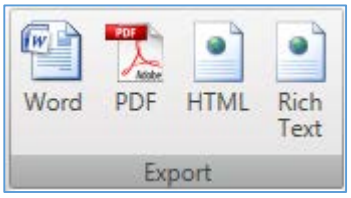




Print and Export Summary:

1. To print the **Summary** tab contents, click the **Print** button on the **Summary** tab toolbar, then select the printer to use and press **Print.**

2. Export the contents of the **Summary** tab as a:
   - Microsoft Word Document
   - PDF file
   - HTML file
   - Rich Text Format



Click the appropriate icon from the *Export* section of the **Summary** toolbar, browse to the desired file location to save to press **Save**.

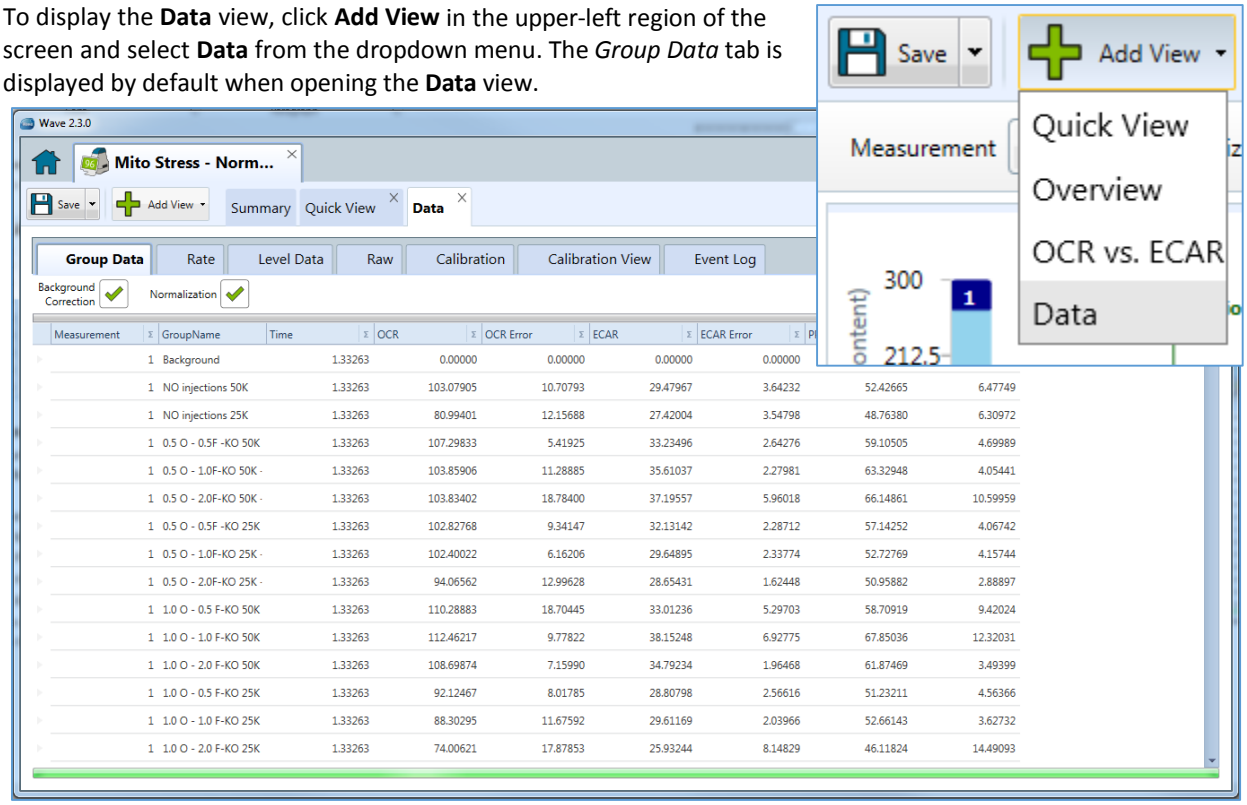
Data Tab:

The **Data** analysis view is a comprehensive data log containing every detail about the assay result file. Each tab in the **Data** view can be exported to Microsoft Excel. The 7 data tabs in the **Data** view are outlined below:

- *Group Data*: OCR, ECAR, PPR rate data and error values by group for measurement performed.
- *Rate*: OCR, ECAR, PPR individual well data. No error values displayed.
- *Level Data:* Individual well data displayed as Level data – O2 level (in mmHg) and pH raw values.
- *Calibration View*: Plate map of the outcome of calibration for each well.
- *Calibration*: Calibration results in table format or all raw data values.
- *Raw*: Raw data values obtained during the assay including LED values, temperature at each measurement and internal LED reference values for the Agilent Seahorse XFe / XFp Analyzer.
- *Event Log*: Agilent Seahorse XF Analyzer serial number, consumable lot numbers used during assay and a command log for processes performed during assay and whether they were successful or failed.

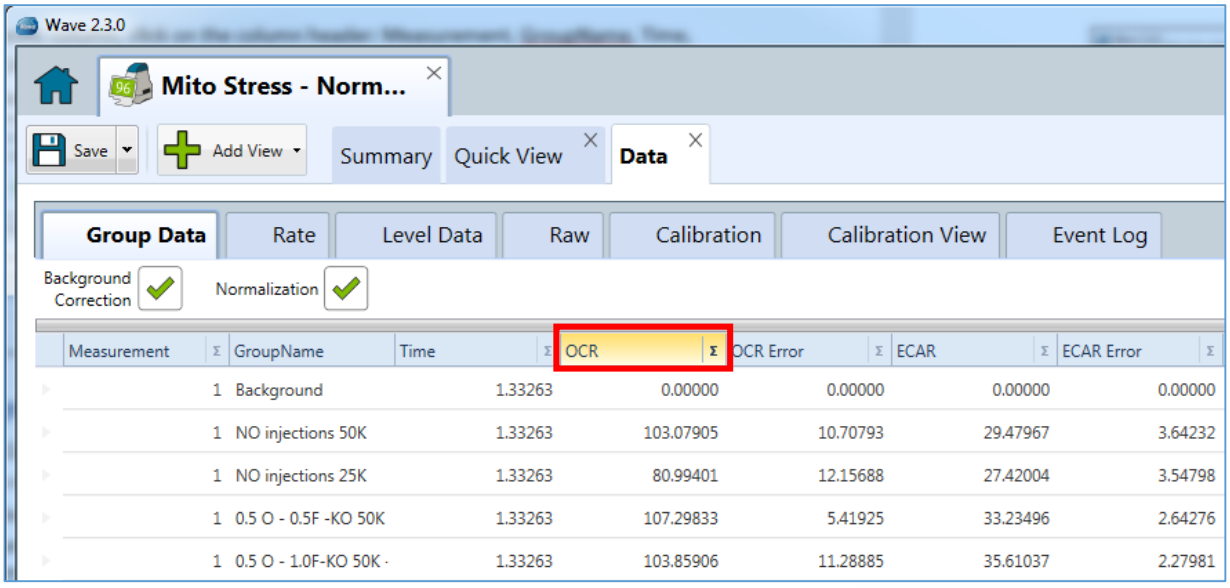
The *Calibration, Raw,* and *Event Log* tabs are used primarily for internal purposes, including QC, validation processes, and Agilent Seahorse Technical Support:

To display the **Data** view, click **Add View** in the upper-left region of the screen and select **Data** from the dropdown menu. The *Group Data* tab is displayed by default when opening the **Data** view.



Column Sorting

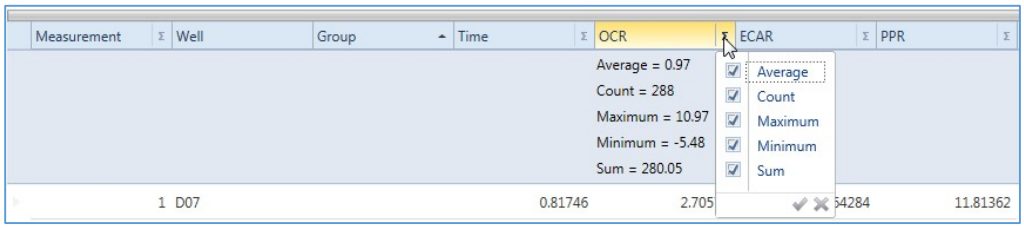
To sort the data by a specific column, click on the column header: Measurement, GroupName, Time, Rate or error for OCR, ECAR, or PPR. Wave will automatically sort the data in ascending or descending order in the selected column.



Agilent Technologies

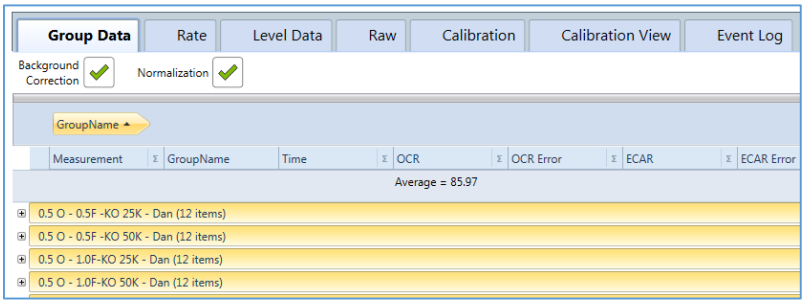
Display values for: Average, Count, Maximum, Minimum, or Sum

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 following example all values are checked for OCR data – Wave will display the average, count, maximum value, minimum value, and sum for the OCR data.

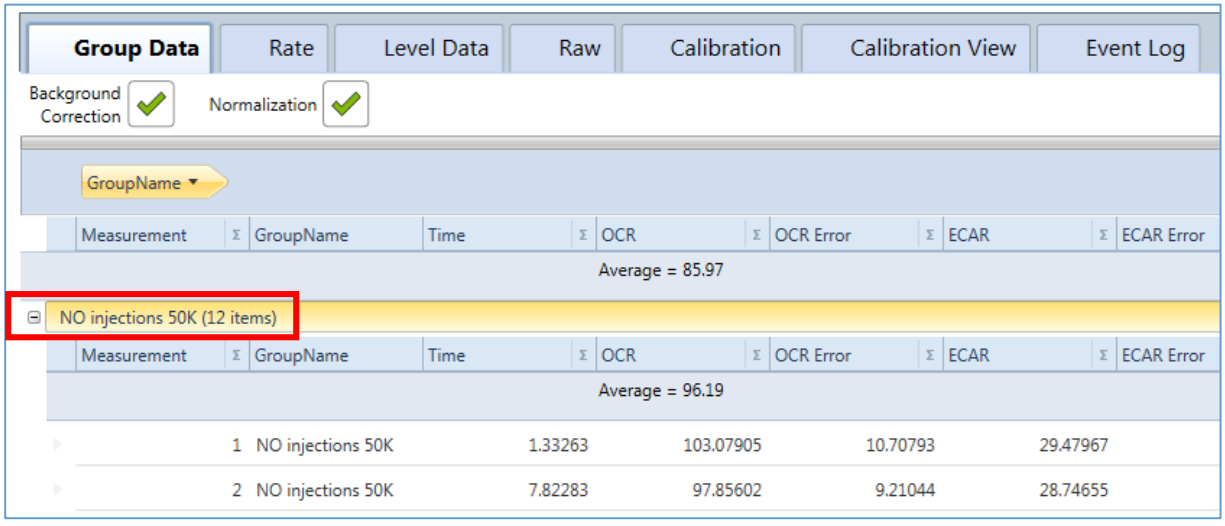


Group Data by *Field*

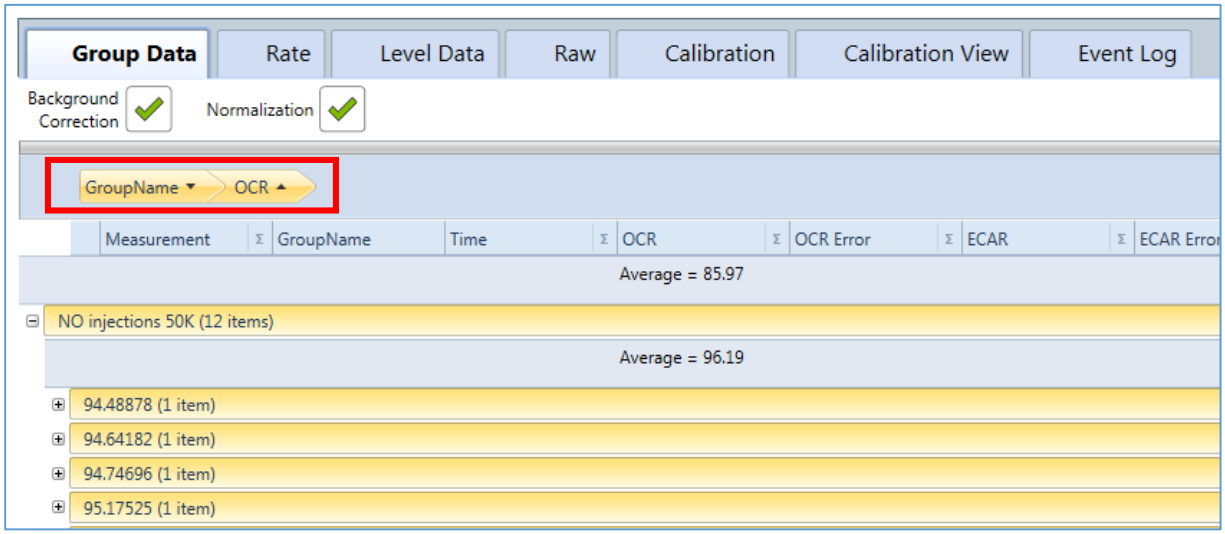
To group the data by field click on a column heading and drag it up to the gray bar. In the example to the right, the **GroupName** heading was dragged to the gray bar.



After sorting, *expand* or *collapse* data by clicking on the *plus* or *minus* sign to the left of a group.



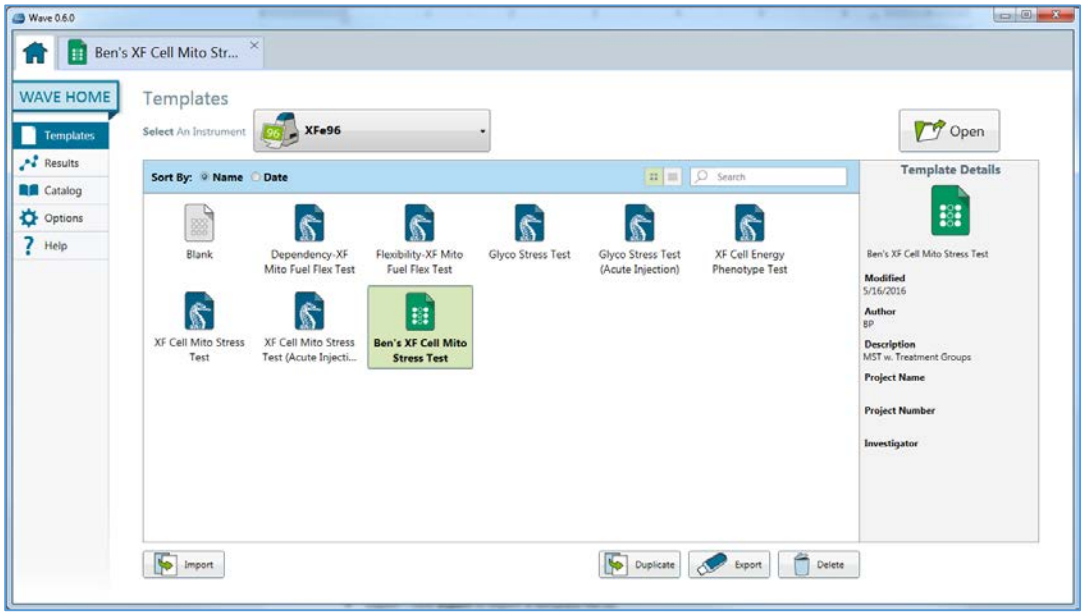
After expanding a group, select another column heading to define the groupings further. In the example below, the heading **OCR** is dragged to the grey bar after *GroupName*.



Chapter 4: Managing Seahorse Files

Wave Home: Templates

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



Import Assay Templates

Import an assay template to Wave Desktop 2.3 in two ways:

Double-click the assay template in Windows Explorer. Wave Desktop will automatically import the assay template to the *Templates* view.

Within Wave Desktop – Open the *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:

Export to export a template file to:

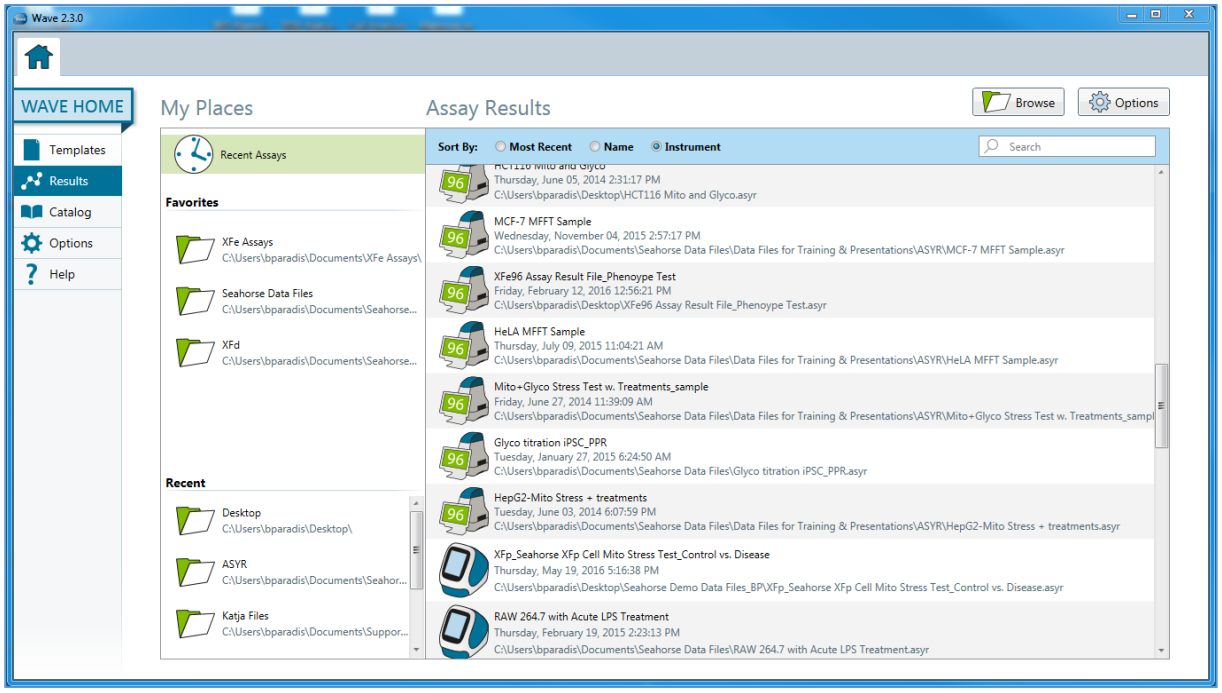
- A USB flash drive.
- A shared network directory for multiple user access.
- Wave Controller (Agilent Seahorse XFe Analyzer) or the XFp Analyzer.

***Note**: The Agilent Seahorse XFe and XFp Analyzer must have an active network connection for this feature.*

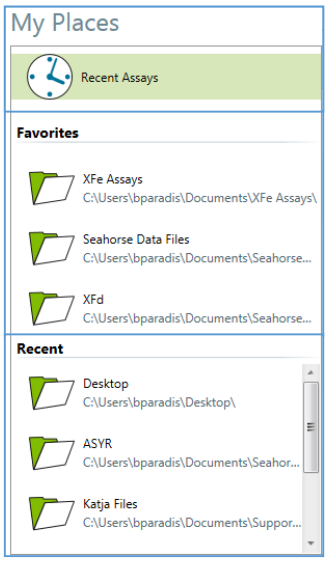
Duplicate to create an identical copy of a template.
Delete a template – this will permanently delete the template file.

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*. Opened a **new** assay result file will display 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.

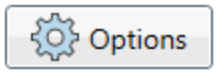


Recent Assays, Favorite Places and Recent Places

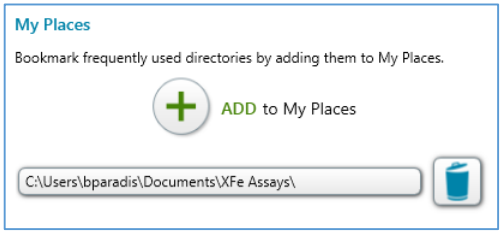


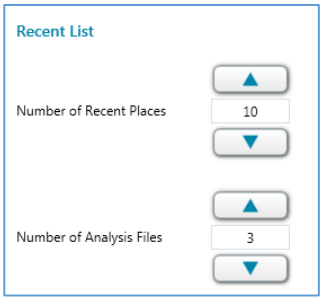
- *Recent Assays* displays a sortable and searchable list of recently viewed and analyzed assay result files.
- *Favorites* – configure local or network directories where assay result files are frequently added to, or accessed from. Once configured, each local directory added as a *Favorite Place* will appear below *Recent Assays* as a folder icon (pictured right).
- *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.

Options



Click **Options** to add *Favorite Places* (from local or network directory locations) or configure the number of *Recent Places* and assay result files to display on the *Results* view.





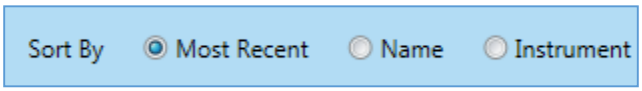
Browse

Click **Browse** to manually open an assay template or Seahorse data file in Wave. Seahorse file types compatible with Wave Desktop 2.3 are:

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

Note: *Double-click an Assay Design file (*.asyd) created in an earlier version of Wave to open assay design and* ***Save As*** *an assay template file. It is not possible to create assay design files using Wave Desktop 2.3.*

Sort By and Search



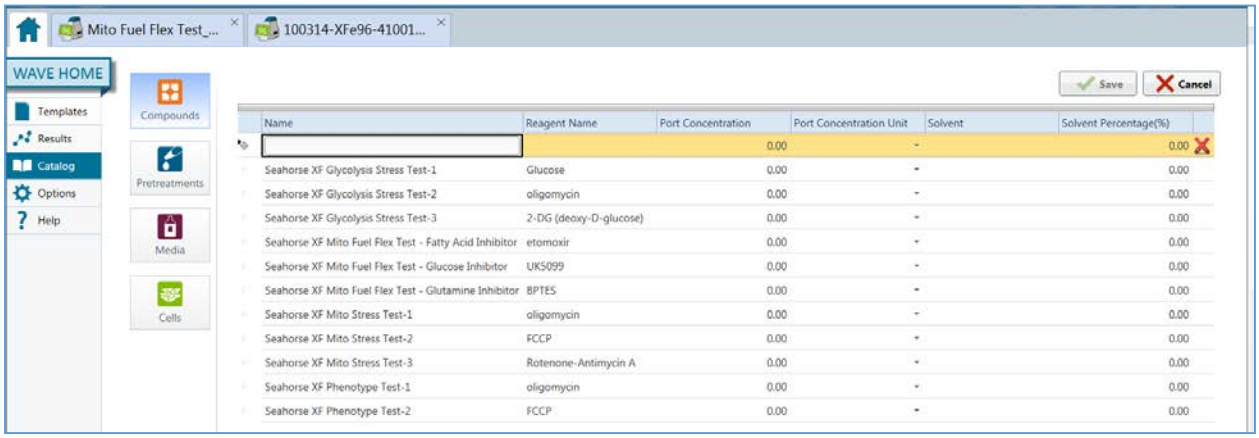
Assay result files are default-sorted by *Most Recent*, which displays the most-recently opened or modified assay result file first in the list. *Name* sorts assay result files alphabetically based on the file name. *Instrument* sorts by the type of Agilent Seahorse XF Analyzer used to produce the assay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

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.

Wave Home: Catalog

The **Catalog** provides a location to save frequently used *Compounds, Pretreatments, Media* and *Cells*. Save time when creating a new assay template or customizing an template file by inserting **Catalog** entries. The Catalog in Wave Desktop 2.3 is prepopulated with Seahorse reagents and compounds that are currently available for each Seahorse assay.



Add a Catalog entry:

1. Open the **Catalog** view.
2. Select a condition (*Compounds, Pretreatments, Media, or Cells*).
3. Single-click below the **Name** column to add a *Name*. The *Name* field is required – additional fields to describe the **Catalog** entry are optional.
4. After adding desired details, press **Enter** (on keyboard) to save the **Catalog** entry.

When finished entering all Catalog entries, press **Save** (upper-right corner of the **Catalog** view).

Delete a Catalog entry:

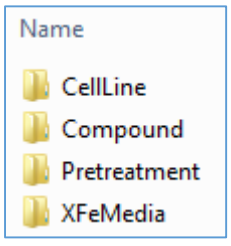
1. Open the **Catalog** view.
2. Click the red 'X' at the end of the Catalog entry row to delete.

Share Custom Catalog:

User-customized **Catalogs** can be shared and uploaded to Wave Controller for the Seahorse XFe Analyzer or another user's Wave Desktop software.

Computer #1:

1. Plug in USB flash drive (if applicable).
2. Start Wave Desktop and open the **Options** view.
3. The **Catalog** directory can be 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 USB flash drive (if applicable).

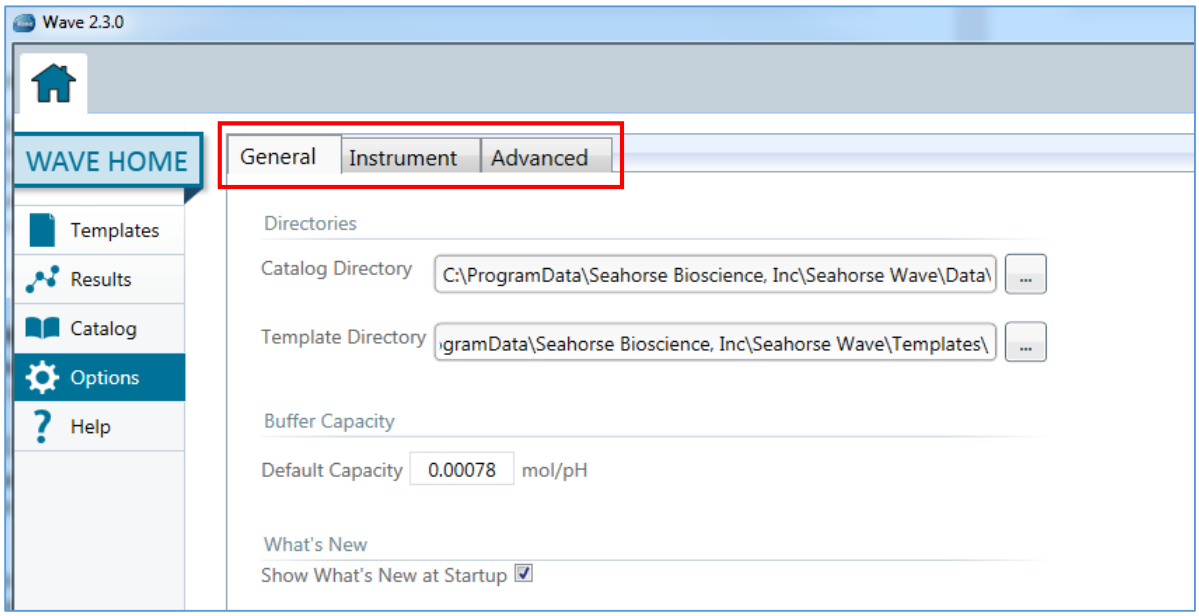


Computer #2:

1. Insert USB flash drive (or open the network directory) containing the saved **Catalog** entries.
2. Start Wave Desktop (or Wave Controller for Agilent Seahorse XFe Analyzers) and open the **Options** view.
3. In *General Options*, copy/paste the **Catalog** directory location in Windows Explorer.
4. Close Wave Desktop (or Wave Controller).
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 Desktop (or Wave Controller).
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*.



General Options

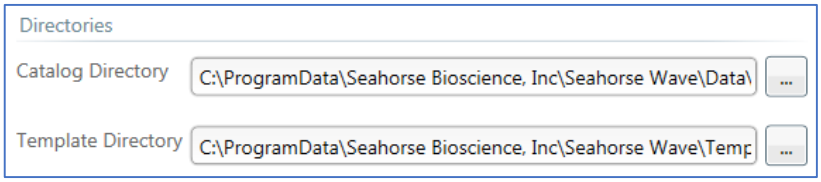
The *General* tab provides options to for:

- View or change the *Catalog* and *Assay Templates* file directory in Windows Explorer.
- View or edit the default *Buffer Capacity* value.
- Disable the *What's New* pop-up (new features in Wave Desktop 2.3) upon startup of Wave Desktop 2.3.

After making changes to the *General Options* tab, click **Save** (bottom of the window) to save all changes made to the *General Options* tab.

Directories

Directories displays the default location of the *Catalog* and *Template* directories. Files for the *Catalog* conditions as well as each assay template file created or customized in Wave Desktop are stored in the default directory. To change the location of either directory, click the **[…]** button to browse to the preferred file location.

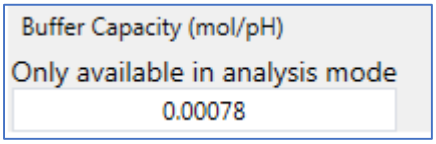


Buffer Capacity

Seahorse determined the default buffer capacity setting for Agilent Seahorse XF Assay Medium and XF Base Medium. Enter a custom default buffer capacity in the *Default Capacity* field. Buffer capacity is used to calculate the PPR (Proton Production Rate) from the measured ECAR (Extracellular Acidification Rate) values. Buffer capacity **must** be adjusted in the assay result file on a per media basis.

Note: *Buffer capacity changes based on the constituents of the medium; different constituents have different capacity to buffer the medium from pH changes. For accurate PPR data, the buffer capacity of each medium used **must** be measured and entered in the Groups/Condition within the assay result file.*

Buffer Capacity per Media



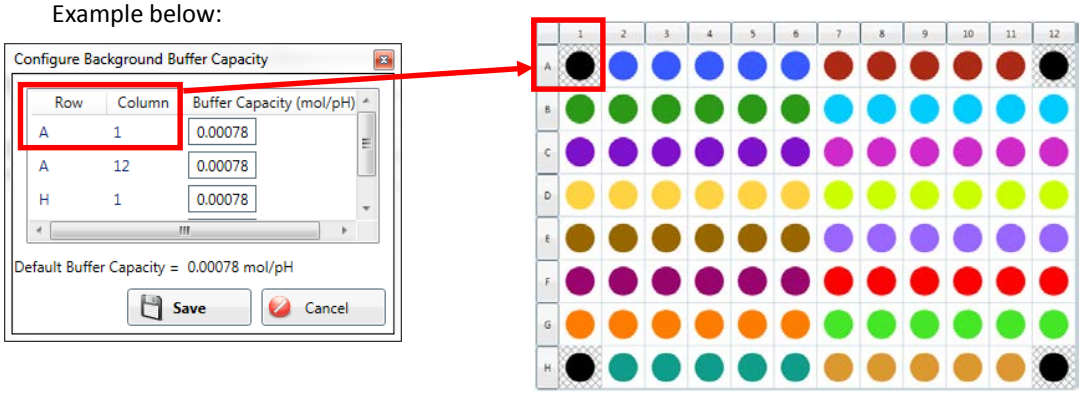
To record the buffer capacity of individual media:

1. Open an assay result file.
2. Click **Modify** and select the **Groups/Conditions** tab.
3. Double-click **Assay Media** to display media used in the assay.
4. Select the individual media and enter a custom buffer capacity value (the *Default Buffer Capacity* value is always the default value unless specified). Repeat for each media.

Background Well Buffer Capacity

To record the buffer capacity for background wells for the same assay result file:

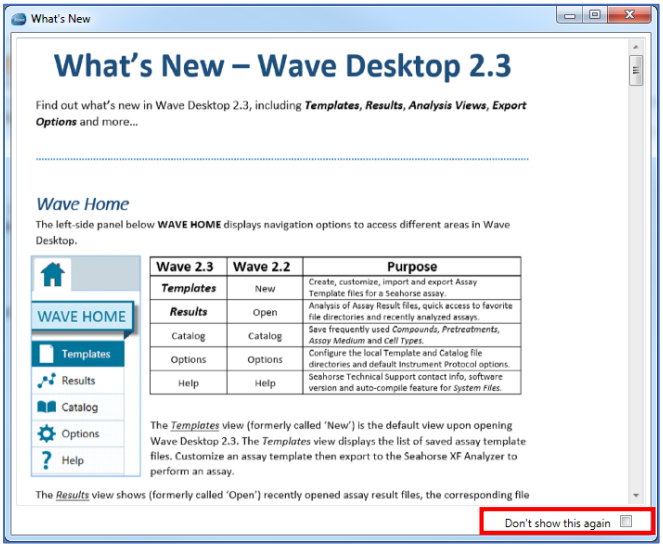
1. After modifying the **Assay Media** buffer capacity values, click the **General Information** tab (within **Modify**).
2. Under **Advanced Settings**, click the **Advanced** tab to display *Background Buffer Capacity*.
3. Click **Configure** to enter the buffer capacity for each background well.
   a. Wells that have been assigned as background wells will automatically populate the list. Example below:



Please see the corresponding *Data Sheet* PDF files for more information on the default buffer capacity values for both the **Agilent Seahorse XF Base Medium** and **Agilent Seahorse XF Assay Medium.**

What's New Message Box

The **What's New** message box appears on Wave Desktop 2.3 startup and displays the new features and functions available. This message box will always appear unless it's toggled off from the message box itself, or on the **Options** view.



Instrument Options

The **Instrument** options tab displays the *Protocol Default* times for the Agilent Seahorse XFe or XFp Analyzer(s) configured for use with Wave Desktop 2.3 (during installation). To modify the Agilent Seahorse Analyzers that are compatible with Wave Desktop, please see the **Wave Desktop 2.3 ReadMe** PDF.

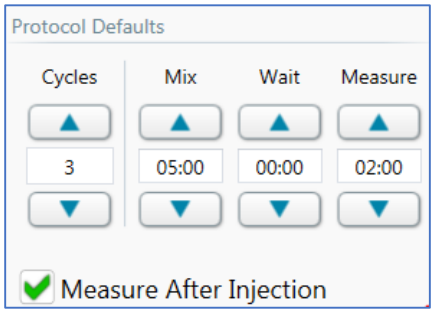
To modify the *Protocol Defaults*, first select the Agilent Seahorse XFe or XFp Analyzer instrument tab, then modify the default settings for:

- **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

It is recommended to use the original *Protocol Defaults* set in Wave Desktop and to modify the *Instrument Protocol* for each individual assay template file. To change the *Protocol Defaults*:

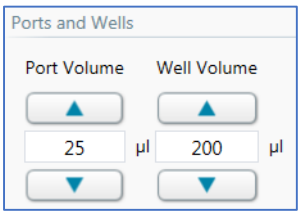
1. Use the **up/down** arrows or type in the new values for **Mix, Wait, Measure** and **Cycles**.
2. Click **Save** at the bottom of the screen to save changes.



Ports and Wells

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

1. Use the **up/down** arrows or type in the new value in the **Port Volume** field or the **Well Volume** fields.
2. Click **Save** at the bottom of the screen to save changes.

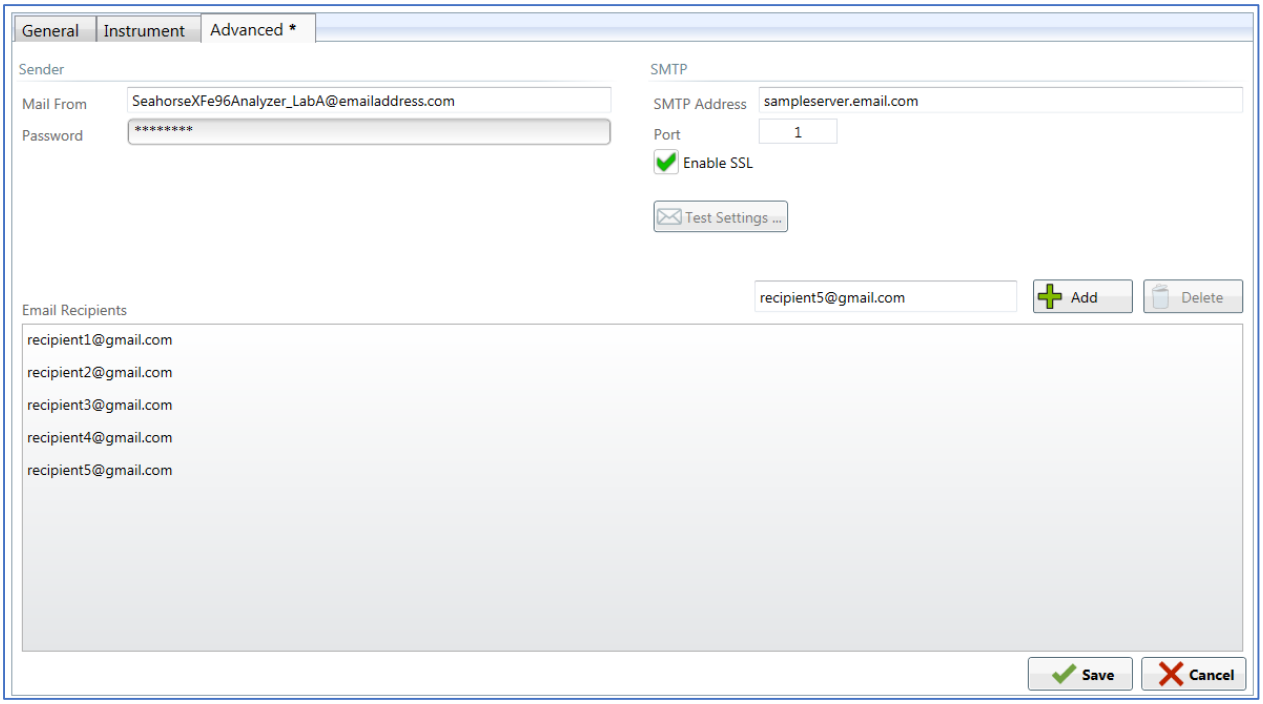


Advanced Options (Wave Controller for Agilent Seahorse XFe Analyzer ONLY)

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.



Add Email Recipients:

1. Type an email address in the *Mail From* field and corresponding a password for this address in the *Password* field below. This email address will be used to send all notifications and displayed in the '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 *Email Address* field.
5. Click **Add**.
6. Repeat Steps 4 and 5 for each recipient.
7. When finished adding recipients, click **Save**.

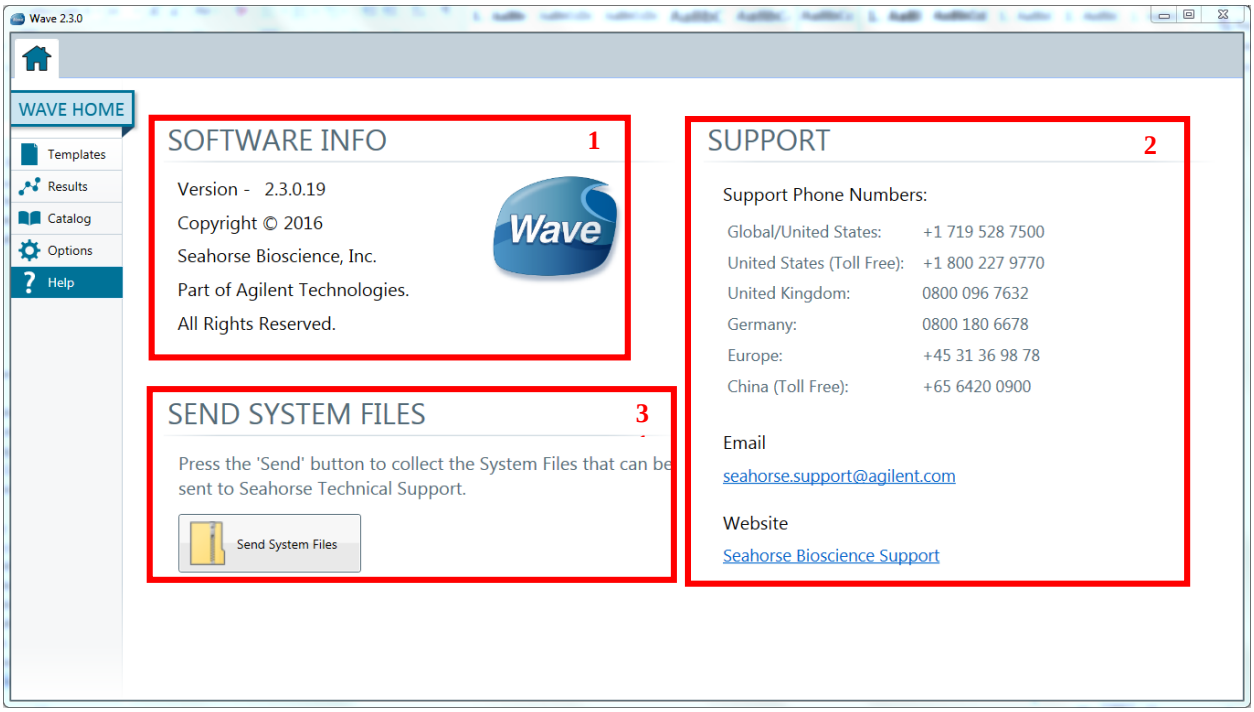
To remove email addresses from the recipient list, first select the email address under *Email Recipients*, then click **Delete**.

Test Notification Settings:

Click **Test Settings** to send a test notification email to all email recipients. If the test email is not received, verify the correct network settings have been configured in Wave Controller.

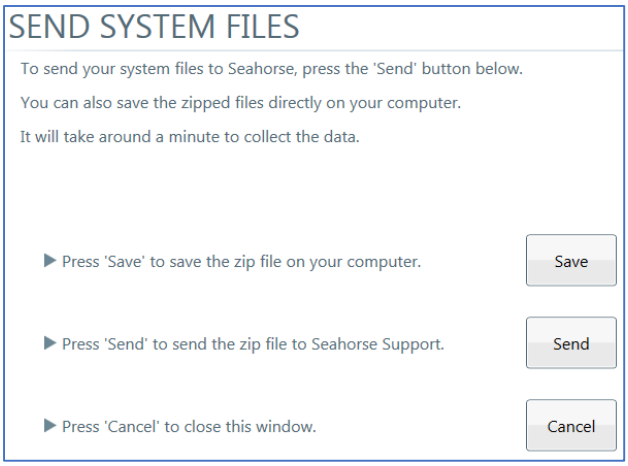
Wave Home: Help

The **Help** view displays software version information for Wave Desktop (1), Agilent Seahorse Technical Support contact information (2), and **Send System Files** to quickly and easily send *System Files* for support and diagnostic purposes (3).



Send System Files

Use the **Send System Files** function to automatically compile *System Files* for Wave Desktop into a compressed folder, create an email to attach the *System Files* to, and send directly to Agilent Seahorse Technical Support.



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. After saving *System Files*, click **Send System Files** a second time.
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* for Wave Desktop.
3. Locate the saved *System Files* compressed folder and attached to the email.
4. Click **Send**.

Agilent Technologies

102617-407 Rev. C