

Wave User Guide

Seahorse XF^e96, XF^e24, and XFP Analyzer

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Chapter 1: Designing an XFe Assay

Wave Desktop vs. Wave Controller:

	Hardware Control	Design Assays/Templates	Analyze Data	Export Data to GraphPad/Excel
Wave Desktop	No	Yes	Yes	Yes
Wave Controller	Yes	Yes	Not Recommended	Yes

Overview: Design, Run, and Analyze

There are three major steps to performing an experiment on the Seahorse XF^e and XFp Analyzer: design, run, and analyze. This overview briefly explains each of these steps.

Step 1: Design

Wave Desktop is the recommended software to create an Assay Template file to run on the Seahorse XF^e24/96 or XFp Analyzer. Wave Controller can also be used to create Assay Design and Assay Template files for the XF^e96 and XF^e24 Analyzer. See **Definitions for Templates, Assay Design, and Analysis Files** more for information about Assay Design and Assay Template files.

Step 2: Run

Seahorse recommends designing Assay Template files using Wave Desktop for the XF^e and XFp Analyzer, however templates and design files may also be created using Wave Controller for the Seahorse XF^e24/96. **Transfer the Assay Template to the Seahorse XF^e or XFp Analyzer using a USB flash drive or network drive if the Analyzer is networked.**

Run an Assay Template created on Wave Desktop if the XF^e Controller/XFp Analyzer is networked:

XF^e Controller:

1. Power **ON** XF^e Controller
2. Start Wave Controller
3. Select **Open**
4. Select **Browse**
5. Locate the template file (.asyt) or design file (.asyd) in Network location(s)
6. Click or tap the template/design file in the network location.
7. Click **Open**.

XFp Analyzer:

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

Run an Assay Template created on Wave Desktop if the XF^e Controller/XFp Analyzer is not networked

XF^e Controller:

1. Save the template file to a USB flash drive from Wave Desktop.
2. Plug the flash drive into the USB port on XF^e Controller.
3. Select **Open**
4. Select **Browse**.
5. Locate the template file (.asyt) or design file (.asyd) on the USB
6. Click or tap the template/design file on the USB flash drive
7. Click **Open**

XFp Analyzer:

1. Save the template file to a USB flash drive from Wave Desktop.
2. Plug the flash drive into the front USB port on XFp Analyzer.
3. Press **Start**.
4. Press the **USB** tab.
5. Select the Assay Template to run.

Step 3: Analyze

Seahorse recommends performing data analysis using Wave Desktop. For XFp users, Wave Desktop is required for data analysis.

Transfer analysis files to Wave Desktop

XF^e Controller/XFp Analyzer is networked:

Wave Controller and XFp will automatically save Assay Results file (.asyr) to location specified prior to running assay.

1. Start Wave Desktop.
2. Click **Open**.
3. Click **Browse** and locate the saved assay result file on the network.

Note: XF^e software automatically saves a backup copy of the assay result file locally on the XF^e and XFp.

XF^e Controller is NOT networked:

Wave Controller automatically saves Assay Result files to the location specified prior to running an assay. When the XF^e Controller is not networked, the 2 save locations are USB flash drive or locally on the Analyzer.

1. Locate the Assay Result file on the XF^e Controller.
2. Copy and paste the Assay Result file to a USB flash drive and transfer to a personal computer with Wave Desktop installed.

XFp Analyzer is NOT networked:

The XFp Analyzer automatically saves Assay Result files to the location specified prior to running an assay. When the XFp Analyzer is not networked, the 2 save locations are USB flash drive or locally on the Analyzer. To open files in Wave Desktop, they must first be transferred to a USB flash drive:

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.
 - a. Data may be exported as an Excel file (*.xlsx) or GraphPad Prism file (*.pzfx). The Assay Result file (*.asyr) will always be exported.

The next section outlines how to create Assay Template and Assay Design files using Wave. For more information about the XFp Analyzer please see the XFp Extracellular Flux Analyzer Instrument Manual, available by contacting Seahorse Technical Support.

Wave Home: New



Wave Home

New – Displays existing template files and option to create a new Assay Template or Assay Design.

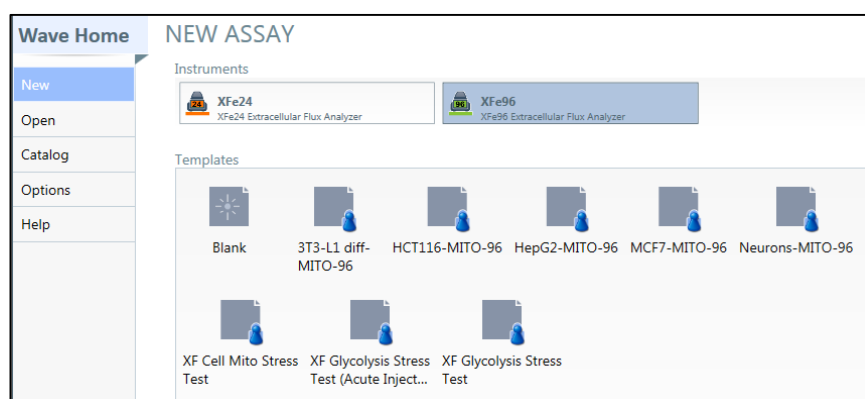
Open – Displays lists of recent analyses and design files opened in Wave (Desktop and Controller).

Catalog - Saves frequently used assay conditions to simplify assay design. The Catalog can contain compounds previously used in Injection Strategies, as well as Pretreatments, Media, and Cell Types.

Options - View, customize, and add default values for any instruments installed on Wave Desktop (XFp, XF²⁴, XF⁹⁶, XF²⁴/XF⁹⁶ Hypoxia Mode). The table below describes these advanced options.

Option	Description
General	View and customize Login Settings, Buffer Capacity, Atmospheric Pressure, Favorite Places, maximum number of Recent Places, Analysis Files, and Design Files for selection when selecting Open .
Instrument	View and customize protocol defaults.
Advanced	Specify the email addresses of recipients to be notified when XF ^e assay has completed.

Definitions for Design, Template and Result Files



Assay Design Files

An *Assay Design file* (.asyd) contains all of the information needed to run an experiment, including group definitions, plate map configuration, and the instrument protocol. Wave Controller on the Seahorse XF^e Analyzer converts the assay design file (.asyd) to an assay result file (.asyr) after the assay has finished. To use an assay design file more than once, it must be saved as an **Assay Template** file.

Assay Design files are NOT supported on the XFp Analyzer and cannot be imported.

Note: Following completion of an assay, the Assay Result file can be converted into an Assay Design file. To do this, open the Assay Result file, click the downward-pointing arrow and select **Save as...** > Select Assay Design as the **Save as type** file.

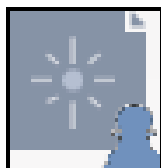
Assay Template Files

An *Assay Template file* (.asyt) contains all of the information needed to run an experiment, including groups and condition definitions, plate map configuration, and protocol definitions. Templates can be reused and customized for other experiments.

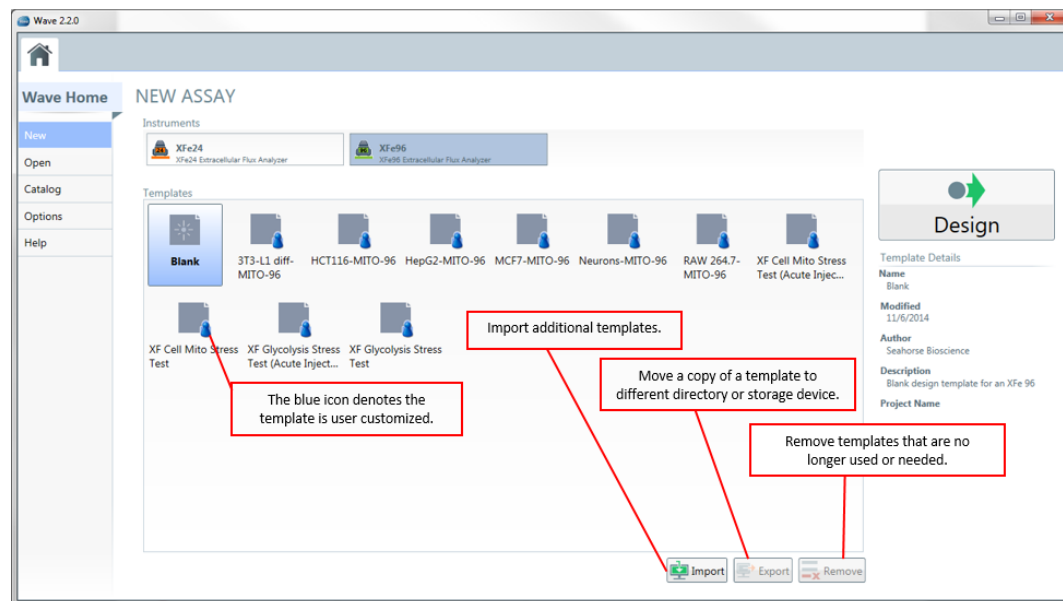
The 5 Seahorse Default Assay Templates available Wave Controller (v2.2) on the XF^e24/XF^e96 Analyzer are:

- Blank Template
- XF Cell Mito Stress Test
- XF Cell Mito Stress Test (Acute Injection)
- XF Glycolysis Stress Test
- XF Glycolysis Stress Test (Acute Injection)

In addition to the 5 Seahorse Default Assay Templates, any user-customized templates will appear in the Templates pane on the **New** tab. The icon below denotes a customized assay template file.



Manage the list of available templates for each XF Analyzer in Wave Desktop by using the **Import**, **Export**, and **Remove** buttons below the Templates pane.



Import, Export, and Remove Buttons

Click the Export button to export a template file to:

- A USB flash drive.
- A shared directory for multiple user access.
- Transfer template file between Wave Desktop and Wave Controller (Seahorse XF^e Analyzer) or the XFp Analyzer.

Note: Seahorse XF^e and XFp Analyzer must be networked for this feature.

To export an assay template file, select the individual Assay Template in Wave Desktop and click/press **Export**. To delete a template file, select the template and click/touch the **Remove** button.

Template Details

The Template Details pane is to the right of the Templates display. The example (right) shows the Assay Template details for a user-defined XF^e Mito Stress Test template for C2C12 cells.

Template Details	
Name	Mito Stress Test - C2C12
Modified	9/8/2014
Author	Ben P.
Description	An example assay template.
Project Name	Mito Stress Test - C2C12
Project Number	01
Investigator	J. Smith

Assay Result Files

An *Assay Result file* (*.asyr) contains all assay data from a completed run, including the level, rate, calibration raw data and consumable information about the Cell Plate and Cartridge that was used in the assay. In addition, the Assay Result file contains the details of the experiment's design, which can be saved as an Assay Design to perform the assay multiple times.

Creating a new Assay Template or Assay Design

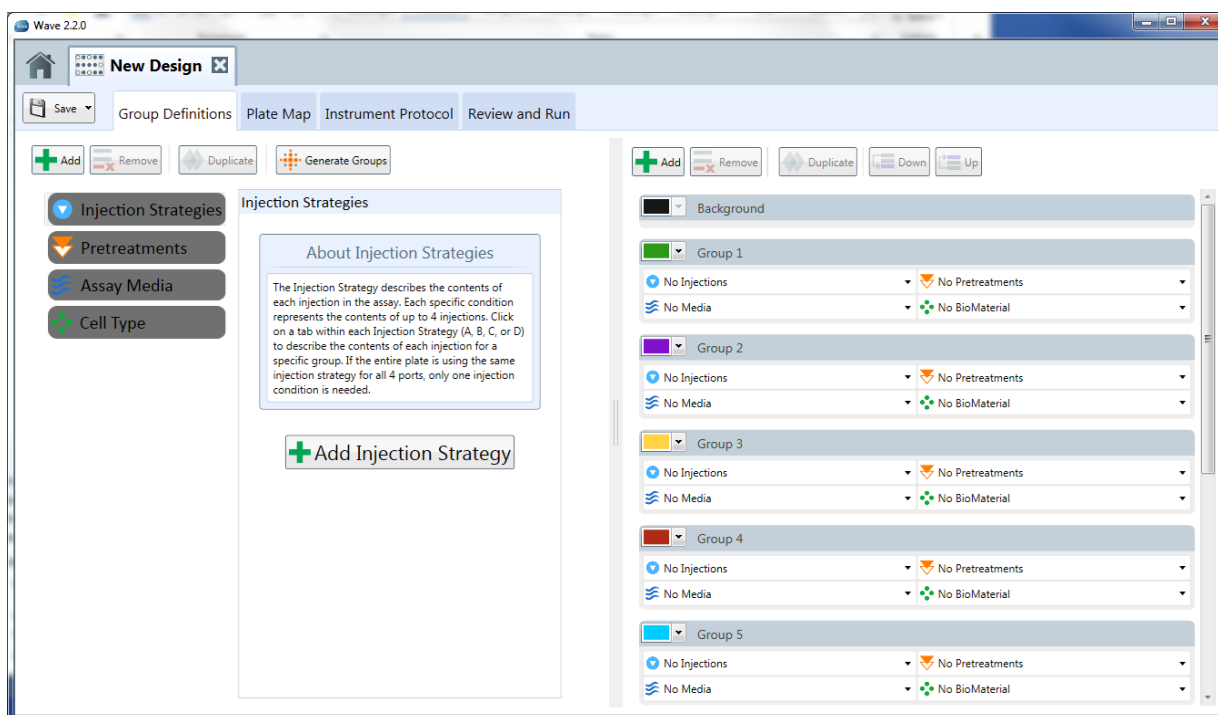
1. Open Wave.
2. Click/Press **New**.
3. Select **Blank** (or a Seahorse Default Assay Template or user customized Assay Template) from the list of available Templates.
4. Click the **Design** button to create a **New Design** tab.
5. Define groups and conditions. See **Define Groups and Conditions**.
6. Map groups to the plate. See **Map Groups to the Plate Map**.
7. Define the assay protocol. See **Define a Protocol**.
8. Review and run your assay. See **Review and Run**.

Group Definitions

The Group Definitions tab is used to define the Injection Strategy, Pretreatments, Assay Media, and Cell Type to be used in the assay. Groups can be created manually or Wave can generate groups automatically based on the combination of different variables defined in each Group Definition.

The 4 different types of Group Definitions are:


- Injection Strategies
- Pretreatments
- Assay Media
- Cell Type



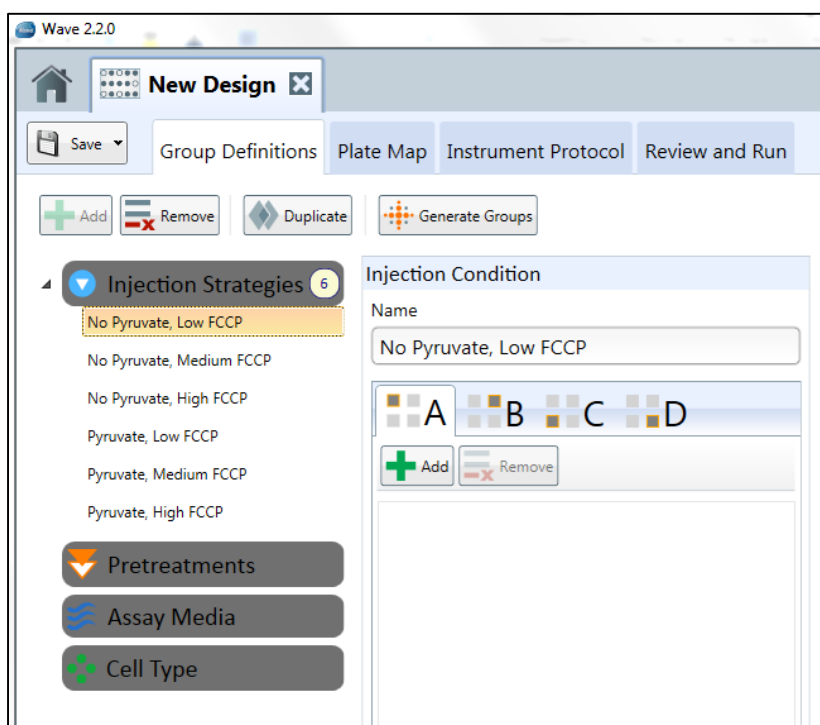
Assay Conditions: Injection Strategies

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 Glycolysis Stress Test assay on the same Cartridge).

To define one or more *Injection Strategies*:

1. Click/press the **Injection Strategies** bar.
2. Click/press the **Add Injection Strategy** button 
3. Click/press the name of the new injection strategy on the left (Default name is **Inj. Strategy 1**) to display a dialog box. Complete the fields to name the Injection Strategy and define the contents of each injection port (A, B, C, and D).

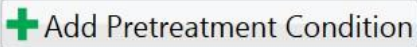
In the example below, 6 injection strategies were defined: **No Pyruvate, Low FCCP; No Pyruvate, Medium FCCP; No Pyruvate, High FCCP; Pyruvate, Low FCCP; Pyruvate, Medium FCCP; Pyruvate, High FCCP**

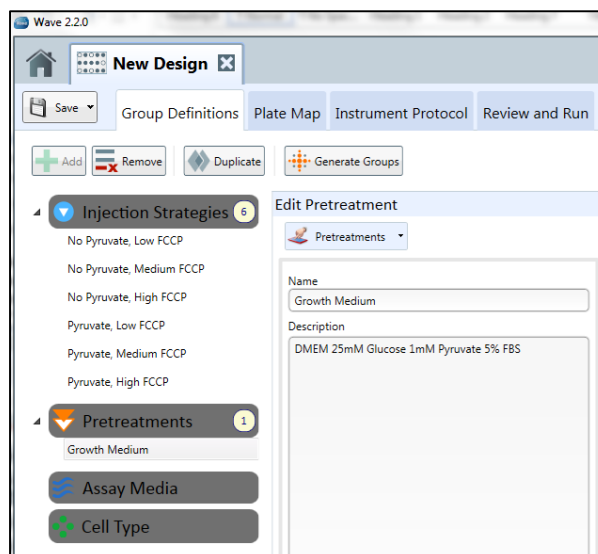


Assay Conditions: Pretreatments

Pretreatments describe any treatment the cells have received prior to the assay, such as a genetic manipulation or a drug treatment.

To define one or more *Pretreatments*:

1. Click/Press the **Pretreatments** bar
2. Click/Press the **Add Pretreatment** button

3. Click/Press **Pretreatment 1** to display the **Edit Pretreatment** dialog box. In the following example, *Pretreatment 1* has been renamed *Growth Medium*
4. Enter the name of the pretreatment in the **Name** field and provide record-keeping details in the **Description** field

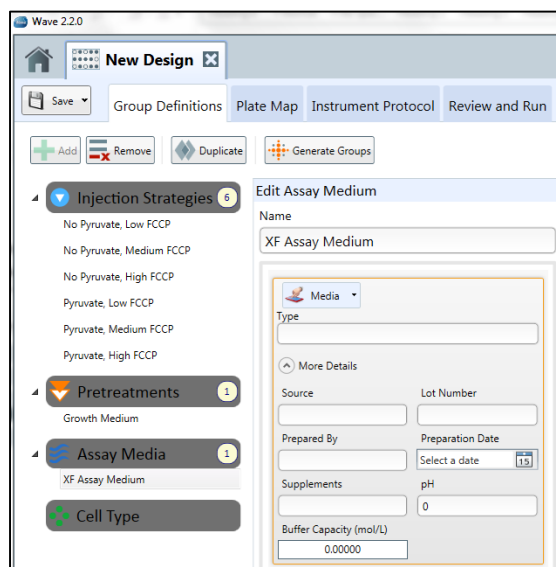


Assay Conditions: Assay Media

Assay Media describes the assay medium used in the assay. Record the medium and supplements for each medium used in the assay.

To define one or more *Assay Medias*:

1. Click/Press the **Assay Media** bar
2. Click/Press the **Add Media Condition** button
3. Click/Press **Media 1** to display the **Edit Assay Medium** window
4. Enter the name of the Assay Medium Condition in the Name field
5. Click/Press **More Details** to display fields that provide record-keeping details. In the following example, the assay medium is named XF Assay Medium



Assay Conditions: Cell Type

Cell Type describes the source(s) of cellular material used for the assay with information about the cell line, seeding concentration, and passage number.

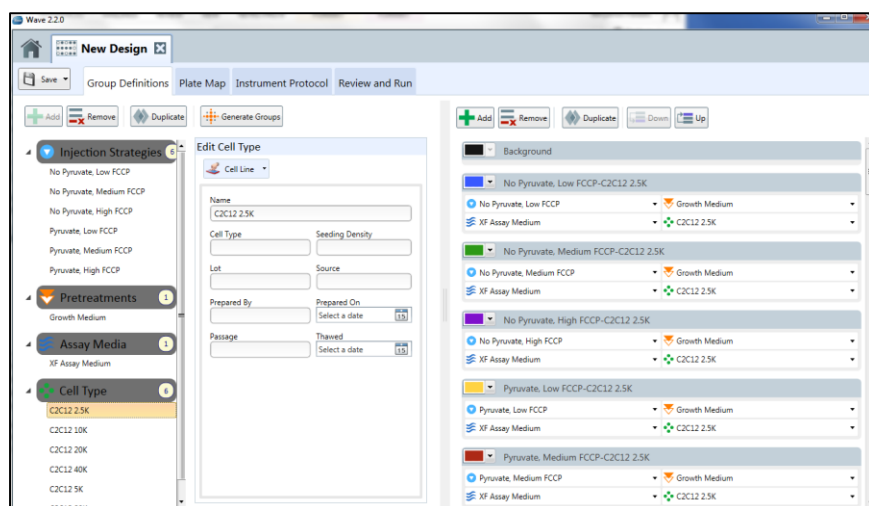
To define one or more *Cell Types*:

1. Click/Press the **Cell Type** bar.
2. Click/Press **Add Cell Type Condition** button
3. Select **Cell Type 1** to display the Cell Type window
4. Enter additional details in the appropriate fields

Automatically Generate Groups

Wave automatically generates groups 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. Once finished defining all assay conditions, click/press the **Generate Groups** button.

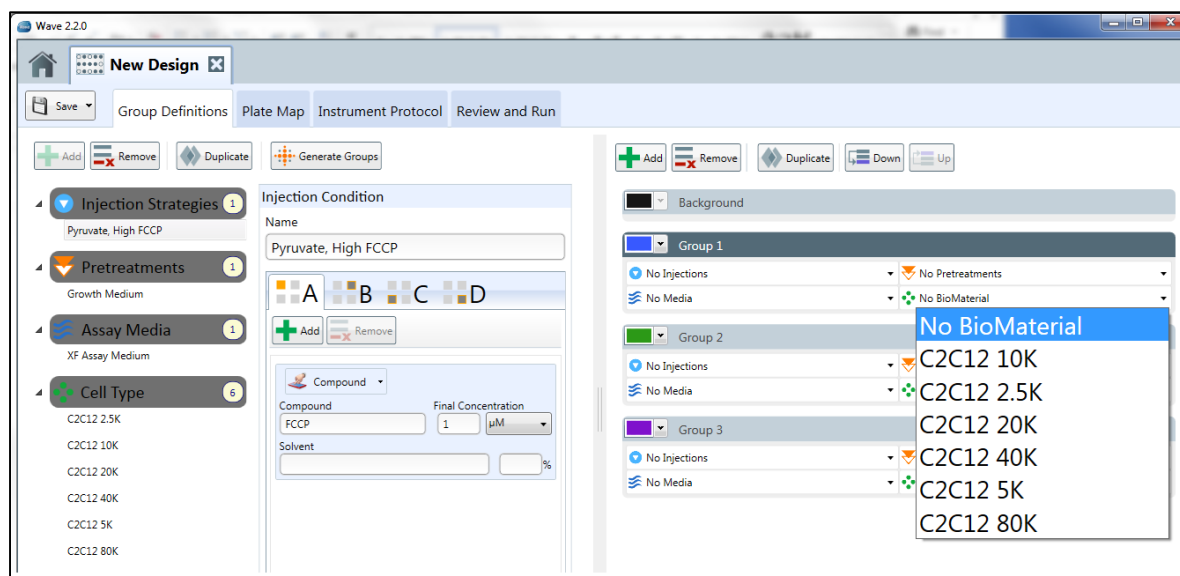
This example shows 6 *Injection Strategies*, 1 *Pretreatment*, 1 *Assay Media*, and 6 *Cell Types* defined. Clicking the **Generate Groups** button automatically creates 36 unique assay groups for each possible combination of Injection Strategies, Assay Medium, and Cell Type.



The combinatorial logic is $6 \times 1 \times 1 \times 6$, resulting in the generation of 36 groups. (Example: 3 *Injection Strategies*, 1 *Pretreatment*, 1 *Assay Media*, and 6 *Cell Types*, the combinatorial logic would be $3 \times 1 \times 1 \times 6$, thus a total of 18 unique assay groups.)

Manually Generate Groups

Wave also allows assay groups to be defined manually by selecting each individual Group Definition that distinguish one group from another.



To add a group:

1. Click the **Add** button.
2. Define the assay group by selecting each Group Definition from the dropdown list of conditions that have been entered in the **Group Definitions** (Injection Strategy, Pretreatments, Assay Media, and Cell Type).
3. Repeat the steps above to add another group.

Once assay groups have been defined, assign the groups to the **Plate Map** for each group.

Plate Map

The **Plate Map** displays the layout of assay groups defined in the previous section on the Cell Plate for the assay. Wave uses group assignments to calculate group statistics in the Assay Result file.

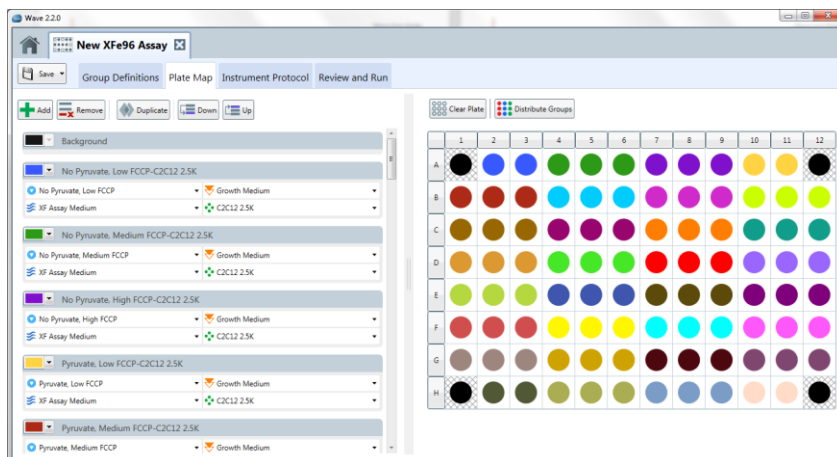
There are 2 ways to assign assay groups to the Plate Map:

1. **Distribute Groups** button – Automatically distributes assay groups to the Plate Map (quickest method).
2. Assign groups manually to the Plate Map.

Automatically Assign Groups



This command determines the maximum number of replicates and distributes the groups in vertical sections across the plate.



Manually Assign Groups

To manually assign assay groups to the Plate Map, simply click the group name on the left-side group list, then click the well on the Plate Map. Click the row or column label to assign all wells in an entire row or column to a particular group.

Background Wells



Wave will automatically default to Seahorse recommended Background Wells for each XF Analyzer. To change the location of 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.

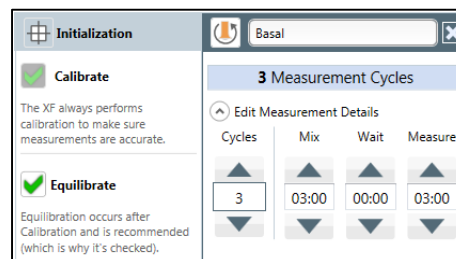
Instrument Protocol

Click the **Instrument Protocol** tab to modify the assay protocol (including measurement and injection cycles) to add additional measurement cycles or adjust the mix, wait, measure times during the assay.

Default Protocol Commands

The predefined assay protocol includes the following steps:

1. Calibrate (Always ON).
2. Equilibrate (User-editable however Seahorse strongly recommends an Equilibration step for every assay).
3. Measurement Cycles.



Note: Modifying the Instrument Protocol is not required. By default the XF^e and XFp Analyzer perform a measurement step after each injection. Predefined measurement cycles are defined by default for all Seahorse XF Default Assay Templates.

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 accurate assay data from the XF assay.

Equilibration

Equilibration ensures temperature stability before beginning an XF assay and is ON by default. Equilibrate can be turned OFF however it is strongly discouraged.

Measurement Cycles

Measurements of the Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) are taken at various points during an assay:

1. During the baseline measurement prior to the first injection.
2. After each injection (for custom Assay Templates, injections are user-defined for each port).

Seahorse recommends three measurement cycles for each rate measurement (baseline and after each injection). The default measurement cycle consists of 3 commands: *Mix*, *Wait*, and *Measure*, repeated 3 times.

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 Seahorse XF^e24 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.

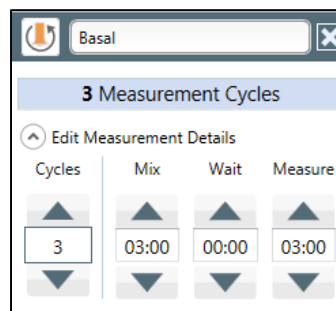
The time for each *Mix*, *Wait*, and *Measure* command are user-adjustable. The Seahorse recommend default times for are:



Seahorse XF^e96 and XFp Analyzer: 3 minutes *Mix*; 0 minutes *Wait*; 3 minutes *Measure*.

Seahorse XF^e24 Analyzer: 3 minutes *Mix*, 2 minutes *Wait*, 3 minutes *Measure*.

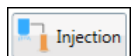
Baseline Measurement Cycle

The Baseline Measurement Cycle consists of measurements *before* the first injection. This command is the default starting point for all XF assays. Touch or click on the dropdown arrow on the **Edit Measurement Details** bar to change the default **Mix**, **Wait**, and **Measure** times. The recommended **Mix**, **Wait** and **Measure** times are instrument dependent. Defaults can be defined in the **Instrument Options** tab.



If necessary, use the up arrow  or the down arrow  to increase or decrease the number of **Cycles** for each measurement. Seahorse Bioscience recommends a minimum of 3 cycles per measurement for optimal results. Change the name of the Baseline Measurement Cycle by clicking/touching the text field at the top of the column and typing in the desired information.

Injections

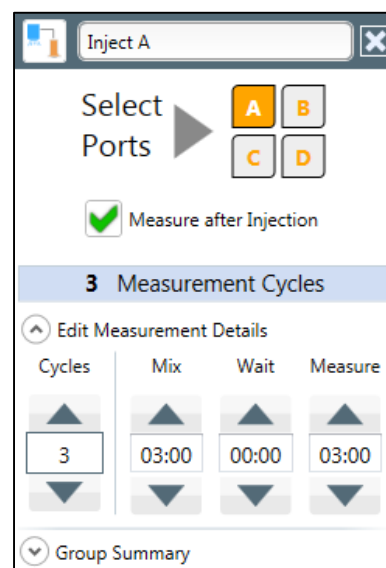


Click the **Injection** button to add a new injection command in the assay protocol. Injections

For each new injection, the software will automatically select the next available port. If a port is already assigned in the Instrument Protocol it will be greyed out.

Edit Measurement Details

After an injection is selected, the software will automatically add a set of measurement cycles after the injection. Default mix, wait, and measure times are provided on an instrument type-basis however the times and cycles are adjustable. If a measurement is not required following an injection, uncheck the **Measure after Injection** box.



Custom Cycles

Most XF assays require only measurement and injection cycles. In some situations, a custom cycle may be required. Press the **Custom** button to add a custom cycle to the assay. This allows for the incorporation of multiple *Mix and Wait* cycles as defined by your assay requirements.

Review and Run

General Information

The General Information section provides editable fields for information about the assay. This will be saved after the assay has completed and will be documented in the Assay Result file (*.asyr).

Choose to supply or edit any of the following fields:

- Assay Name
- Principal Investigator
- Project Name
- Group and Plate Layout
- Protocol Summary

Errors and Warnings

Any errors or warnings will be visible prior to transferring the Assay Template (or Assay Design) file to run on the XFp or XF^e Analyzer.

Advanced Settings

Email Recipient List

Note: This function requires an active internet connection on the XF^e96 or XF^e24 Analyzer.

To add email recipients:

1. Click on **Options**
2. Click **Advanced**
3. Type in each email address for users to receive the Assay Result file (.asyr) and click **Add**.

Add email address before starting an assay on the XF^e:

1. Go to **Review and Run** tab.
2. Go to **Email Recipient List** under **Advanced Settings**.
3. Type in email address and click **Add**.

Buffer Capacity

To record a background well buffer capacity:

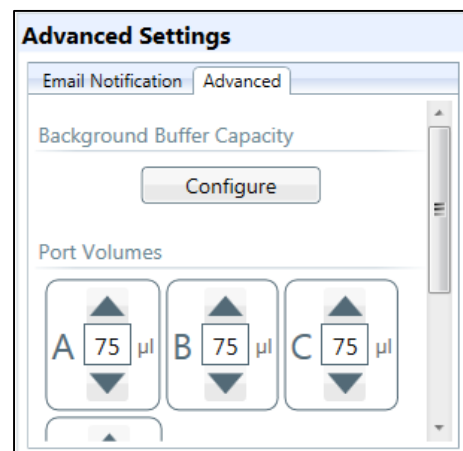
- Click **Configure** and type in the Buffer Capacity (mol/L) for each Background well specified on the **Plate Map**.

To record a different port volume for this assay:

- Type in a new value in the **Port Volume** field or use the up and down arrows to increase or lower the value to the desired amount.

To check the version number of the software and of template file:

- The Wave version number in addition to the template file version number is available under Version Information (Scroll to bottom of **Advanced Settings**).



Note: The displayed PPR kinetic rates do not reflect the custom Buffer Capacity per media until the assay has completed. Select the group to display in addition to the appropriate Background Well with matching Assay Media to apply the Buffer Capacity value.

Protocol Summary

Review the Instrument Protocol defined for an assay by selecting **Protocol**. To edit the Instrument Protocol, click on the **Instrument Protocol** tab prior to transferring or running the assay. The following illustration shows an example of an Instrument Protocol summary.

Summary	PROTOCOL Summary					Print Summary
Protocol						TOTAL TIME: 01:24:00
Group	Initialization	Basal	Inject A	Inject B	Inject C	
Port A	⚙️ Calibrate	⬇️ Mix: 00:03:00	⬆️ Inject Port: A	⬆️ Inject Port: B	⬆️ Inject Port: C	
Port B	📄 Equilibrate: 00:12:00	⌚ Wait: 00:00:00	⬇️ Mix: 00:03:00	⬇️ Mix: 00:03:00	⬇️ Mix: 00:03:00	
Port C		📊 Measure: 00:03:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	
Port D		⬆️ Mix: 00:03:00	📊 Measure: 00:03:00	📊 Measure: 00:03:00	📊 Measure: 00:03:00	
		⌚ Wait: 00:00:00	⬆️ Mix: 00:03:00	⬆️ Mix: 00:03:00	⬆️ Mix: 00:03:00	
		📊 Measure: 00:03:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	
		⬆️ Mix: 00:03:00	📊 Measure: 00:03:00	📊 Measure: 00:03:00	📊 Measure: 00:03:00	
		⌚ Wait: 00:00:00	⬆️ Mix: 00:03:00	⬆️ Mix: 00:03:00	⬆️ Mix: 00:03:00	
		📊 Measure: 00:03:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	⌚ Wait: 00:00:00	
			📊 Measure: 00:03:00	📊 Measure: 00:03:00	📊 Measure: 00:03:00	

Group Summary

Click the **Group** link to review the groups that have been defined for a specified assay. To change the group definitions:

1. Click on the **Group Definitions** tab.
2. Make any necessary changes to group conditions prior to running a design.

The following illustration shows an example of a Group Summary:

GROUP Summary Print Summary

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	1	1	1	1	1	5	5	5	5	5	B
B	2	2	2	2	2	2	6	6	6	6	6	6
C	3	3	3	3	3	3	7	7	7	7	7	7
D	4	4	4	4	4	4	8	8	8	8	8	8
E	9	9	9	9	9	9	13	13	13	13	13	13
F	10	10	10	10	10	10	14	14	14	14	14	14
G	11	11	11	11	11	11	15	15	15	15	15	15
H	B	12	12	12	12	12	16	16	16	16	16	B

Group	Injection	Pretreatment	Media	...
Background	N/A	N/A	N/A	N/A
1 0 fccp-No Pretreatments-cho-s 40	0 fccp	No Pretreatments	xf base	cho
2 0.5 fccp-No Pretreatments-cho-s 40	0.5 fccp	No Pretreatments	xf base	cho
3 1 fccp-No Pretreatments-cho-s 40	1 fccp	No Pretreatments	xf base	cho
4 2 fccp-No Pretreatments-cho-s 40	2 fccp	No Pretreatments	xf base	cho
5 0 fccp-No Pretreatments-cho-s 60	0 fccp	No Pretreatments	xf base	cho
6 0.5 fccp-No Pretreatments-cho-s 60	0.5 fccp	No Pretreatments	xf base	cho
7 1 fccp-No Pretreatments-cho-s 60	1 fccp	No Pretreatments	xf base	cho
8 2 fccp-No Pretreatments-cho-s 60	2 fccp	No Pretreatments	xf base	cho
9 0 fccp-No Pretreatments-cho-e 40	0 fccp	No Pretreatments	xf base	cho
10 0.5 fccp-No Pretreatments-cho-e 40	0.5 fccp	No Pretreatments	xf base	cho
11 1 fccp-No Pretreatments-cho-e 40	1 fccp	No Pretreatments	xf base	cho
12 2 fccp-No Pretreatments-cho-e 40	2 fccp	No Pretreatments	xf base	cho
13 0 fccp-No Pretreatments-cho-e 60	0 fccp	No Pretreatments	xf base	cho
14 0.5 fccp-No Pretreatments-cho-e 60	0.5 fccp	No Pretreatments	xf base	cho
15 1 fccp-No Pretreatments-cho-e 60	1 fccp	No Pretreatments	xf base	cho
16 2 fccp-No Pretreatments-cho-e 60	2 fccp	No Pretreatments	xf base	cho

Port Summary

Select Port A, B, C, or Port D links to review the description of each injection:

1. Injection concentration.
2. Name of the solvent (if specified).
3. Percentage of solvent used in the injection.
4. Background wells.
5. Port volume.

To change the *Injection Strategy*, click on the Group Definitions tab prior to running the design.

The following illustration shows an example of an injection port summary.

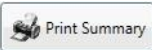
Port A Summary Print Summary

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

B Background Well 1 Volume: 25 µl
 Concentration:
 Solvent: %
 Oligo
 Concentration: 1 µM
 Solvent: %

Print Summary

The **Print Summary** button prints a summary of the design on a printer or to a PDF file. *To print a summary of a design:*

1. Click the **Print Summary** button. 
2. The print dialog box will appear.
3. Select the printer and press **Print**.

Save as Design & Save as Template

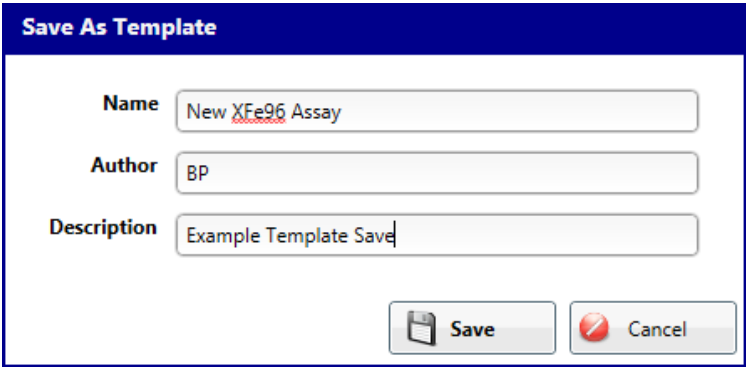
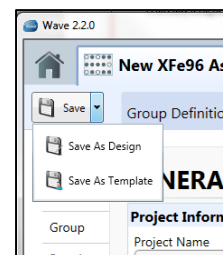
After designing the assay, save the file as either an Assay Design (.asyd) or Assay Template (.asyt) and transfer to the XFe^e or XFe Analyzer.

*For XFe Analyzers only – if the assay is created on the XFe Controller, the location specified for the Assay Design or Assay Template file will be the **same** location the Assay Result file is saved to.*

Note: Select “Save as Design” if the assay will be run immediately after designing the file. Select “Save as Template” if this assay will be run multiple times or expect to make minor adjustments for future assays. The XFe Analyzer does NOT support use of Assay Design files.

Save a new Assay Design as an Assay Template

1. 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.

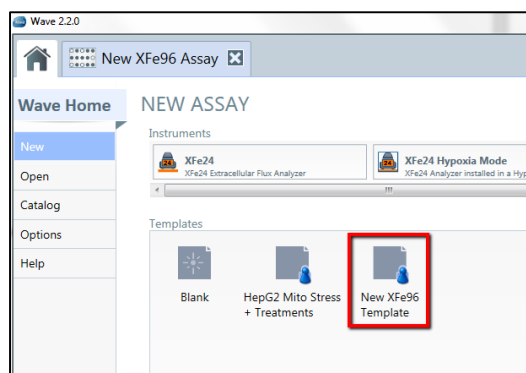



2. Enter the name of the assay template in the **Name** field (required).
3. Enter the **Author** name (optional).
4. Enter a description of the assay in the **Description** field (optional).
5. Click the **Save** button.

Locate all Seahorse Default Assay Templates and user-customized Assay Templates files from Wave Home by selecting **New**.

Note: Clicking **Save** will save an Assay Design file (.asyd), not an Assay Template file (.asyt).

Template files are stored permanently within the Templates directory on the C: drive. To view the default save location or edit the location of the Templates directory, click **Options** on the Wave Home screen.



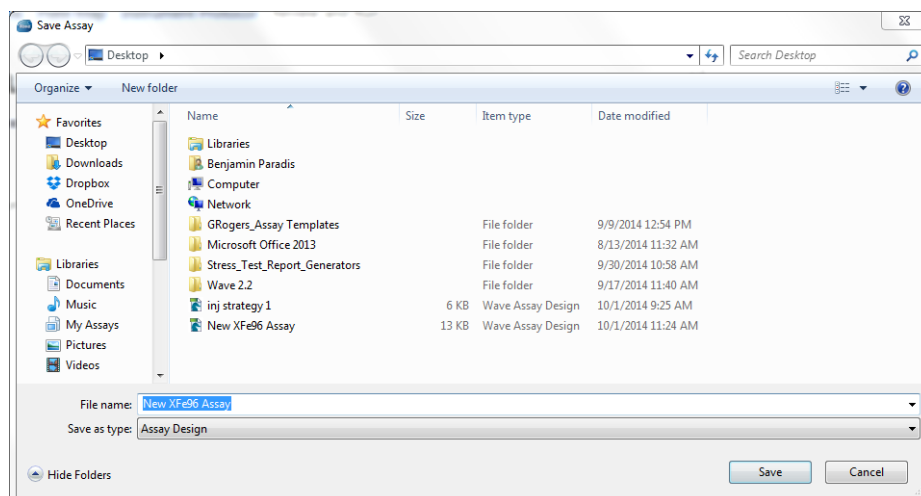
Open a Template in the Template Editor



To edit a template in the Template Editor, right-click on the template icon on the **New** screen. Click **Edit** after left-clicking the Assay Template to edit.

Save an Assay Design file (.asyd)

1. Click the **Save** floppy disc button or click the downward-pointing arrow on the **Save** button and choose the **Save as Design** option to save an Assay Design file (.asyd).
2. Type in the name of the Assay Design file (.asyd) in the **File Name**.
3. Click **Save**.



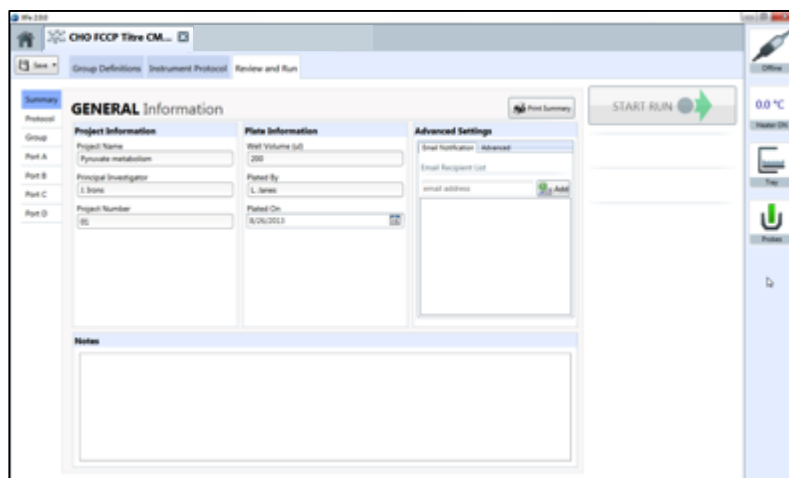
Chapter 2: Performing an XFe Assay

(Wave Controller ONLY)

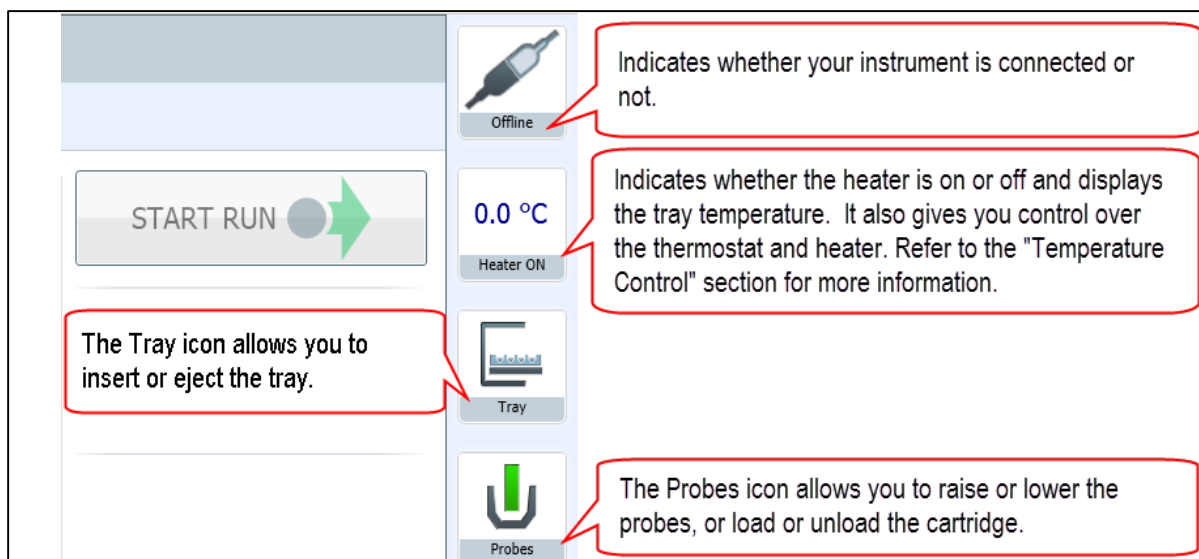
Note: This section applies to Wave Controller software only on the XFe^e96 and XFe^e24 Analyzer ONLY. The functions described in this chapter do NOT apply to Wave Desktop or XFe Controller software.

If the Assay Template file (.asyt) or Assay Design file (.asyd) was created using Wave Desktop, the template or design file must be imported to the Seahorse XFe^e Analyzer through an active network connection or USB flash drive. If the Seahorse XFe^e Analyzer is not networked, copy the template file or design file to a USB flash drive and import to the Seahorse XFe^e Analyzer.

Import the Assay Template file (.asyt) or Assay Design file (.asyd) to the Seahorse XFe^e Analyzer by selecting **Import** on the **Wave Home** screen. When ready to run the assay, navigate to the **Review and Run** tab.



Wave Controller ONLY: Widget icons located on the right side of the **Review and Run** tab display the XFe^e Analyzer status, current temperature, and control eject/insert the tray and to raise/lower probes.

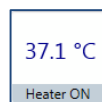


Temperature Settings

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

Temperature Widget is used to:

- Turn the heater ON/OFF.
- Set the desired target assay temperature.
- Set the tolerance range for temperature fluctuation. The temperature widget will change color if the temperature is above or below the acceptable tolerance range from the temperature set point (see below).



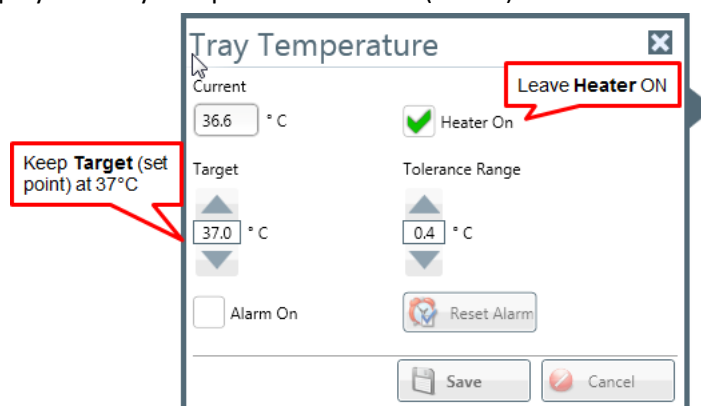
The temperature icon will indicate if the instrument temperature has exceeded the tolerance range. For XFe Analyzers with an active network connection, automatic email notification can be configured if a temperature exceeds the tolerance range.

Note: The Status Indicator on top of the Seahorse XF^e Analyzer will also change from blue to amber if the temperature has exceeded the tolerance range.

Set Target Temperature and Tolerance Range:

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

- Current instrument temperature
- Heater ON/OFF checkbox
- Target temperature (set point)
- Tolerance range
- Alarm ON/OFF checkbox



Click on the **up/down** arrows (or use a keyboard to type in values) to adjust the **Target Temperature (set point)** or the **Temperature Tolerance Range**.



Click **Save** to save any changes made on the Tray Temperature window.

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

Set Alarm for Temperature Tolerance Range:

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

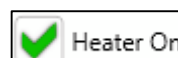
**Turn OFF Alarm:**

1. Uncheck the **Alarm On** box.
2. Press the **Save** button

To acknowledge the tray temperature alarm if the tray temperature exceeds the tolerance range and the alarm is activated, press the **Reset Alarm** button.



After resetting the alarm, the heater can be turned **ON** or **OFF** depending on the direction that the temperature exceeded the tolerance range by checking or unchecking the **Heater On** box.

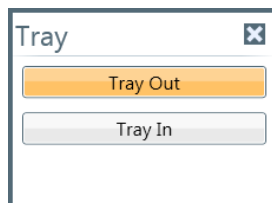


Seahorse recommends checking the temperature before each run. This ensures the tray temperature starts within the targeted range. For any suspected temperature issues or unexpected temperature fluctuations please contact Seahorse Bioscience Support.

Tray Control

The Tray widget allows for control of the tray in the XFe Analyzer. To eject the tray or a cell plate on the tray:

1. Press the **Tray** widget.
2. Click on **Tray Out** button to eject the tray.
3. Click the **Tray In** button to insert the tray.



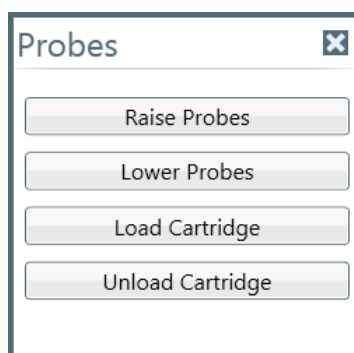
Probe Control

The **Probes** widget allows for control to raise/lower probes and load/unload a Cartridge. To raise or lower the probes, or load or unload a cartridge:

1. Click the **Probes** button.



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



Barcode Errors

The XFe Analyzer reads and logs the barcode for both the Cell Plate and Cartridge prior to starting an assay on the XFe Analyzer. On the rare occasion the XFe Analyzer is unable to read the barcode, a barcode error will be displayed on Wave Controller (see below). Please contact Seahorse Bioscience Support to assist with remedying this error in order to complete the assay.

Cartridge Barcode Read Failure:

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

- **Scan:** Retry scanning the Cartridge barcode.
- **Manual:** Manually input the Cartridge barcode information. Contact Seahorse Bioscience Support for this step.
- **Cancel:** Cancel the assay.



Manually enter Cartridge Barcode Information:

Contact Seahorse Bioscience Support immediately using the information provided below to obtain Cartridge Barcode information. After selecting **Manual**, the *Cartridge Barcode* window will appear (below):

For immediate assistance with a Cartridge Barcode read error, visit the [Seahorse Bioscience Support webpage](#) and call the appropriate technical support telephone number for your specific region. Seahorse Bioscience Technical Support will provide the information required to complete this form to proceed with the assay.

Cartridge Barcode

Contact Seahorse Bioscience Technical Support for assistance in proceeding with your assay. Support will guide you through the steps required to fill in the information below, and may be reached by phone or email:

US: 1 800 671 0633 #3
 Outside US: +1 978 671 1600 #3
 UK: 0800 096 7632
 Germany: 0800 180 6678
 Elsewhere in Europe: +45 32 36 96 78
 China: +0086 21 3390176
 Asia and Pacific Rim (outside China): Contact a Seahorse Distributor
 email (worldwide): Support@seahorsebio.com

Lot Number: Serial Number:

O2:
 O2_A O2_B

pH:
 PH_A PH_B PH_C

Cell Plate Barcode Read Failure

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

- **Manual:** Manually input the Cell Plate barcode information. Contact Seahorse Bioscience Support for this step.
- **Cancel:** Cancel the assay.

Plate barcode read failure

Unable to read plate barcode. Click Manual to enter the barcode manually or Cancel to cancel the Assay.

Manually enter Cell Plate Barcode Information:

1. Press the **Open Tray** button to open the tray along with the Cell Plate.
2. The Cell Plate barcode can be found on the side of the cell plate.
3. Write down the barcode information and press the **Close Tray** button.
4. On the *Plate Barcode Info* window type in the barcode information..
5. Press the **Accept** button.

Plate Barcode Info

Barcode:

Status Indicator

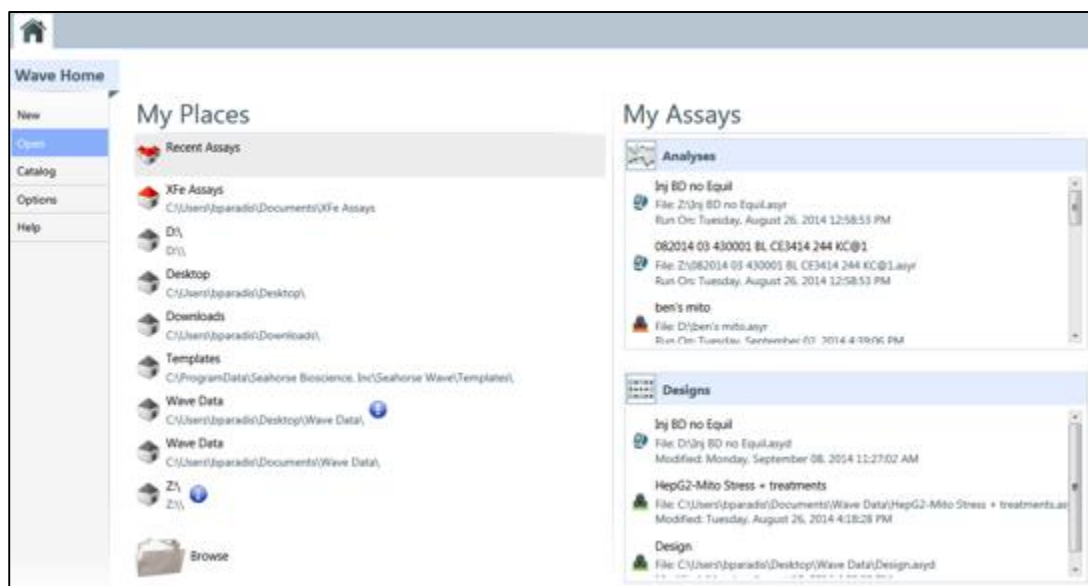
During an assay on the XF^e Analyzer the *Status Indicator* on the top of the Seahorse XF^e Analyzer will change color if a task requires user interaction or if an error has occurred.

Examples are:

1. To load a cartridge or cell plate.
2. Remove a used cartridge and cell plate.
3. Accept or cancel an assay if during calibration one or more wells did not calibrate properly.
4. Any Errors that occur during the run – Barcode read errors for the Cell Plate or Cartridge or if a protocol error is encountered.

Chapter 3: Managing Seahorse Assay Files

Wave Home: Open



My Places and My Assays

Select **Open** below **Wave Home** to access *My Places* and *My Assays*. *My Places* displays recently accessed file directories on the local computer, USB or network directory. Use *My Places* to select the desired file directory containing Seahorse Assay Design and/or Assay Result files. *My Assays* will display all Seahorse Assay Design/Assay Result files in the selected directory.

Recent Assays is the default directory and *My Assays* displays all recently opened Seahorse Assay Result files (**Analysis**) and Assay Design files (**Designs**). Configure the desired number of directories displayed below *My Places* as well as the number of Assay Result and Assay Design files displayed below *My Assays*.

Browse

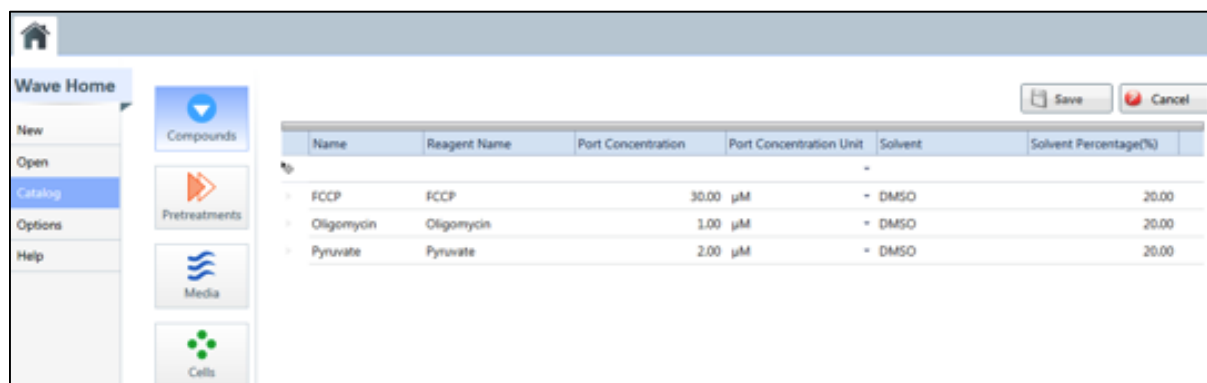
Click **Browse** to open a Seahorse assay file in Wave. Wave supports the following Seahorse file types:

- Assay Result (*.asyr)
- Assay Design (*.asyd)
- Assay Template (*.asyt)
- Wave 1.0 and 1.1 files (*.asy)
- XF Result Files (*.xfd)

Note: For complete step-by-step instructions to import *.xfd files into Wave Desktop, see [Converting & Analyzing XF-Generated Data Files with Wave](#).

Wave Home: Catalog

The **Catalog** can be used to save frequently used assay conditions, such as *Compounds*, *Pretreatments*, *Media* and *Cells*. All saved catalog entries can be selected when configuring the **Groups/Conditions** for an Assay Template or Assay Design.



Add a Catalog Entry:

1. Open the Catalog view.
2. Select a condition, such as Compounds.
3. Double-click below the **Name** column to enter the compound name. The **Name** field is required. All additional fields to describe the Catalog entry are optional.
4. Once finished with the Catalog entry, press **Enter**.
5. To add another Catalog entry, follow steps 1-3 above.
6. When finished entering all Catalog entries, press the **Save** button on the upper-right corner of the Catalog view.

Delete a Catalog entry:

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

Share Custom Catalog:

Customized Catalog entries can be uploaded to another user's Wave Desktop or to Wave Controller on the XF[®] Analyzer.

To share custom Catalog entries – Computer #1:

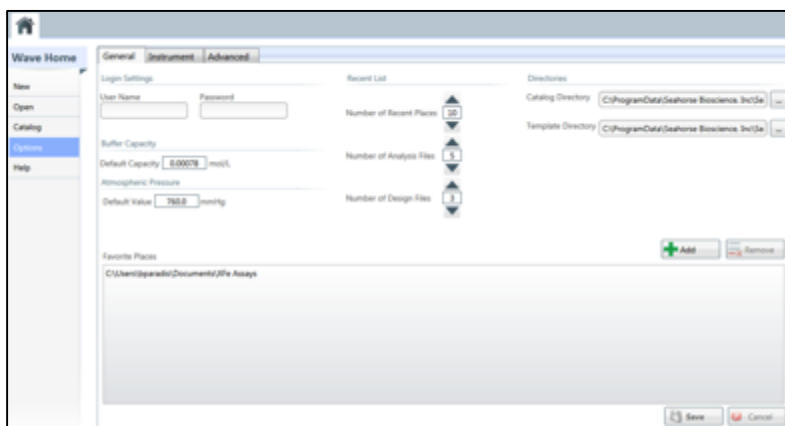
1. Start Wave and open the Catalog view.
2. The Catalog directory can be found in *General Options* under the *Directories* on the right side of the window.
3. Open Windows Explorer and navigate to the Catalog directory file location.
4. Copy/Paste all or select Catalog conditions to an available network drive or USB flash drive. Do NOT 'Cut' the Catalog entries.

Computer #2 or Wave Controller on the XF^e Controller:

1. Insert USB flash drive (or open the network directory) containing the saved Catalog entries.
2. Open Windows Explorer.
3. Navigate to the same file location the Catalog entries were saved from on Computer #1.
4. Paste the desired Catalog entries in the appropriate location.

Wave Home: Options

The **Options** view contains customizable options for various instrument and software settings for Wave Desktop and Wave Controller for the XF^e Analyzer. The 3 main option tabs are: **General**, **Instrument**, and **Advanced**.



General Settings

Options that can be modified under the **General** tab are:

- Wave Controller and XF^e Analyzer ONLY – Changing a password or user name under Login Settings
- View/edit default Buffer Capacity Value.
- Wave Controller and XF^e Analyzer ONLY – View/edit default Atmospheric Pressure.
Note: The **Atmospheric Pressure** only applies to the XF^e Controller settings.
- Add/remove file directories from *Favorite Places* on the Wave Home > Open view.
- Modify the number of *Favorite Places* displayed and the number of *Analysis* and *Design* files in the selected *Favorite Places*.
- File Directory in Windows Explorer to save *Catalog* entries and *Assay Templates*.

NOTE: Click the **Save** button at the bottom of the window to save any/all changes made to the General tab default settings.

Buffer Capacity

Seahorse Bioscience determined the default buffer capacity setting for XF Assay Medium and XF Base Medium. Default Capacity field allows for users to enter a custom **Default Capacity**. Wave uses the buffer capacity to translate ECAR (Extracellular Acidification Rate) readings to PPR (Proton Production Rate). The 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 the running media **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 the Assay Result file.
2. Click **Modify** and select **Groups/Conditions**.
3. Double-click **Assay Media** to display media used in the assay.
4. Select individual media and type in buffer capacity value.
5. Repeat Step 4 for all media listed.
6. Click **Apply** when finished.

Buffer Capacity (mol/L)
Only available in analysis mode
0.00000

Background Well Buffer Capacity

To record the buffer capacity for background wells:

1. Open the Assay Result file.
2. Click **Modify** and select **Assay Properties**.
3. Under **Advanced Settings** select **Advanced** to display Background Buffer Capacity.
4. Click **Configure** to enter background well buffer capacity.
 - a. Wells that have been assigned as background wells will automatically populate the list.

Advanced Settings
Email Notification Advanced
Background Buffer Capacity
Configure

Example below:

Row	Column	Buffer Capacity (mol/pH)
A	1	0.00078
A	12	0.00078
H	1	0.00078

Default Buffer Capacity = 0.00078 mol/pH

Save Cancel

Seahorse Bioscience has determined the buffer capacity for both **XF Base Medium** and **XF Assay Medium**. This information can be found on the second page of the Data Sheet for both products on the Seahorse Bioscience website. The hyperlinks are:

- <http://www.seahorsebio.com/resources/pdfs/XF-base-medium-data-sheet.pdf>
- <http://www.seahorsebio.com/resources/pdfs/XF-Assay-medium-v3.pdf>

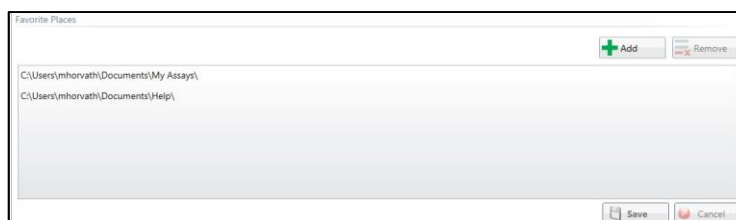
Atmospheric Pressure

This setting applies to Wave Controller on the XF^e Analyzer ONLY. Modify the atmospheric pressure when running assays at high altitudes or if the atmospheric pressure in the lab where the XF^e Analyzer is located differs from the default value of 760.0 mmHg.

Note: Modifying the atmospheric pressure setting does not change the atmospheric pressure in the XF^e Analyzer. Adjustments to the atmospheric pressure affect the calculation of the partial pressure of oxygen for each assay well.

Favorite Places

The **Favorite Places** section near the bottom of the page is for adding or removing directories from the **My Places** screen.



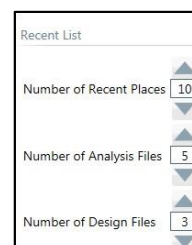
Add a folder:

1. Click the **Add** button.
2. Browse to the desired directory.
3. Click **Select Folder** to add the directory to *Favorite Places*.

To delete a folder from *Favorite Places*, simply select the directory from the list of displayed directories and click the **Remove** button.

Recent Lists

Use **Recent Lists** to change the number of *Favorite Places*, *Analysis* and *Design* files Wave displays on the Wave Home > Open view. Use the **up/down** arrows or type in the desired number of directories or Seahorse assay files. Click the **Save** button at the bottom of the screen to save all changes.



Directories

The **Directories** column displays the default location of the Catalog directory and Assay Template Directory. To change the location of either of these directories, click the button [...] to the right of the directory field to browse to the preferred directory.

Instrument Tab Default Settings

The **Instrument Tab** has 2 functions depending on which version of Wave used:

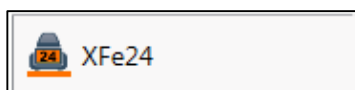
Wave Controller

Displays the default settings for the Instrument Protocol, Port Volume, and Well Volume for the XF^e Analyzer installed. There will be **one** instrument listed.

Wave Desktop

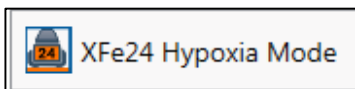
Displays the default settings for the Instrument Protocol, Port Volume, and Well Volume for the XF^e24, XF^e96, and XFp Analyzers installed.

Instrument Tab for each Analyzer:



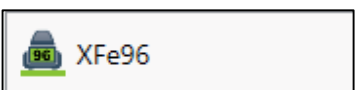
XF^e24

XF^e24 – Design, run, and analyze assays for the XF^e24 Analyzer.



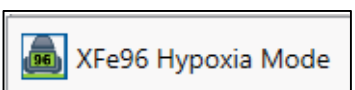
XF^e24 Hypoxia Mode

XF^e24 Hypoxia Mode – Design, run, and analyze assays for the XF^e24 Analyzer in a hypoxia chamber.



XF^e96

XF^e96 – Design, run, and analyze assays for the XF^e96 Analyzer.



XF^e96 Hypoxia Mode

XF^e96 Hypoxia Mode – Design, run, and analyze assays for the XF^e96 Analyzer in a hypoxia chamber.



XFp

XFp – Design and analyze assays for the XFp Analyzer.

To modify the default Instrument settings, first select the Seahorse XF^e or XFp Analyzer instrument tab list. Next modify default instrument settings for:

- Number of cycles in a protocol.
- Time for each for **Mix**, **Wait**, and **Measure** cycle.
- Port volume (notation purposes only)
- Well volume (notation purposes only)

Protocol Defaults

Seahorse recommends to start with the Protocol Defaults set in Wave. Seahorse XF^e and XFp Analyzers will perform the Protocol Default steps after each compound injection.

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.

Protocol Defaults

Cycles	Mix	Wait	Measure
3	03:00	00:00	03:00

Measure After Injection

Optional – Uncheck **Measure After Injection** to disable the configured Protocol Defaults (not recommended).

Ports and Wells

Seahorse Bioscience recommends using the default settings for Port and Well volumes however the default port volume and well volume for each instrument can be adjusted.

Note: *These settings do not change the function of the instrument; they are for record-keeping only. During the run, the entire contents of each port will be injected.*

Ports and Wells

Port Volume	Well Volume
25 µl	200 µl

To change the Port Volume or Well Volume:

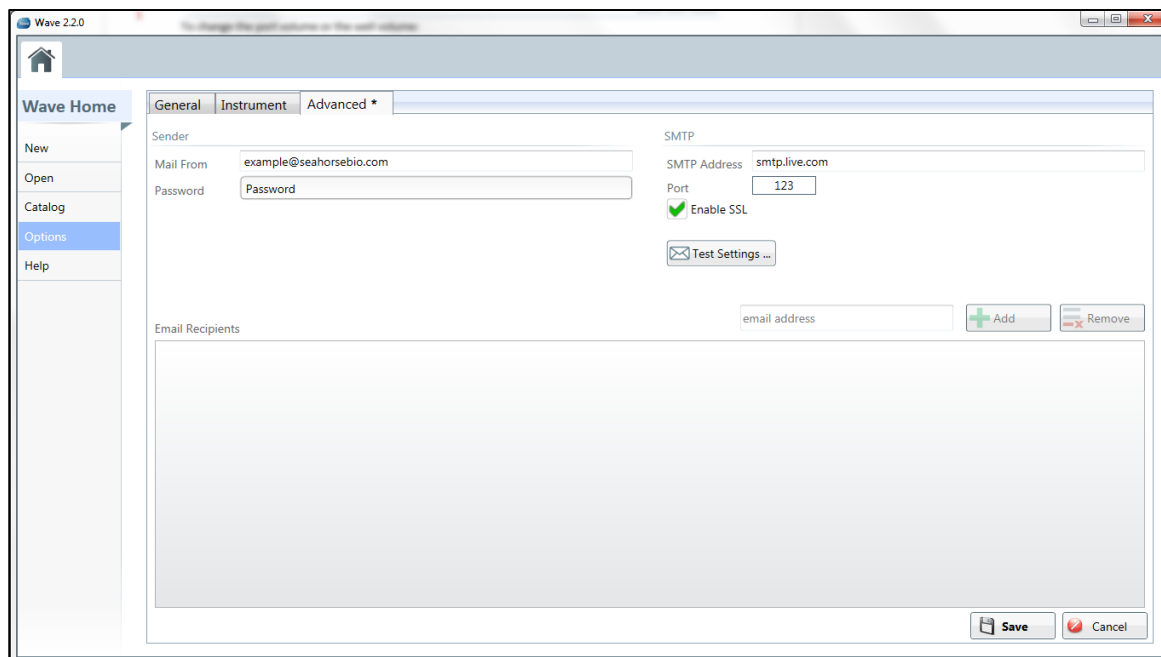
1. Use the **up/down** arrows or type in the new value in the **Port Volume** field or the **Well Volume** fields.
2. Click **Save** at the bottom of the screen to save changes.

Advanced Tab Default Settings

(Wave Controller only – requires active internet connection on XF^e Analyzer)

Use the **Advanced** tab to configure email notifications from the XF^e Analyzer to specific recipients when:

- Calibration has completed and the Calibration Plate must be replaced with the Cell Plate.
- Completion of the assay. The Assay Result file will be attached to this email notification.



To add email recipients:

1. Enter 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 in the 'From' field when receiving an email notification from the XFe Analyzer in your personal email account.
2. Specify an SMTP address in the **SMTP Address** field and the access port in the **Port** field.
3. Check **Enable SSL** if your IT configuration requires it.
4. Type the email address for a single recipient in the **Email Address** field.
5. Click the **Add** button.
6. Repeat Steps 4 and 5 for each recipient.
7. When finished adding all email addresses, press the **Save** button.

Test email notification settings:

1. Click **Test Settings**.
2. All email recipients will receive a test notification email. If email is not in the Inbox, check your Junk Mailbox.

Remove names from the email recipient list by first selecting the email address then click the Remove button.

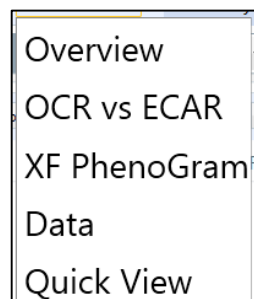
Note: Active internet connection on the XF[®] Controller required for use of the emailing features.

Chapter 4: Analyzing Assay Results with Wave

Seahorse Bioscience recommends analyzing Assay Result files using Wave Desktop. After an assay has completed, save the Assay Result file to a USB flash drive or network directory location to open the assay result file in Wave Desktop. Assay Result files may be analyzed using Wave Controller on the XFe Analyzer however for optimal performance, Wave Desktop should be used. The analysis views described below must be added to the individual Assay Result file.

The 6 data analysis views in Wave are:

- Quick View
- Overview
- OCR vs. ECAR
- XF PhenoGram
- Data
- Summary (not listed – always displayed for each Assay Result file).

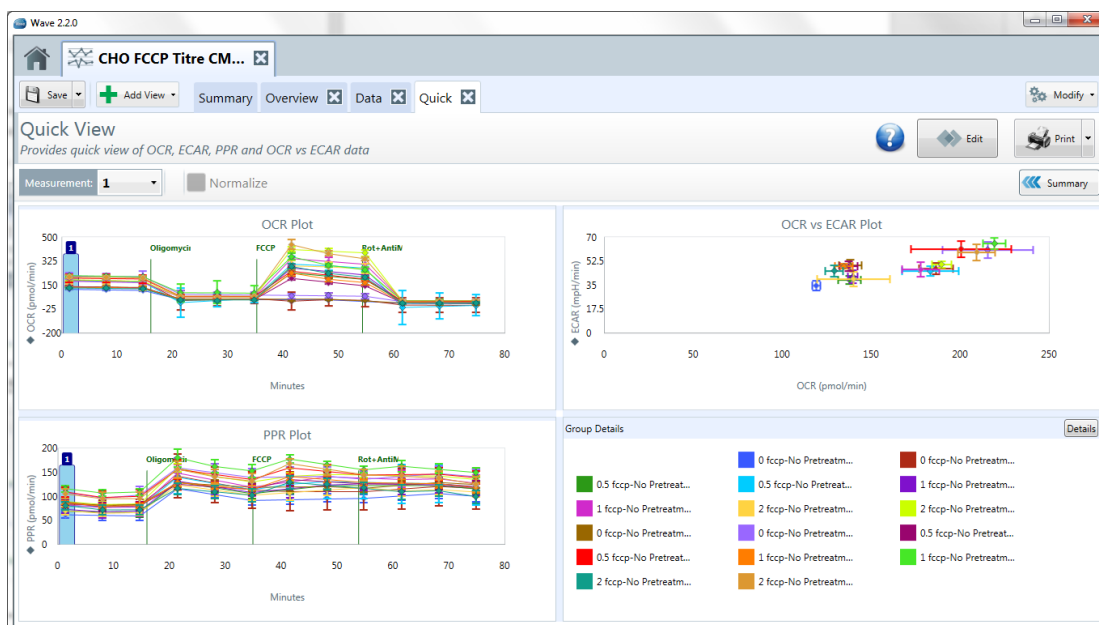


Analysis Views in Wave:

Quick View:

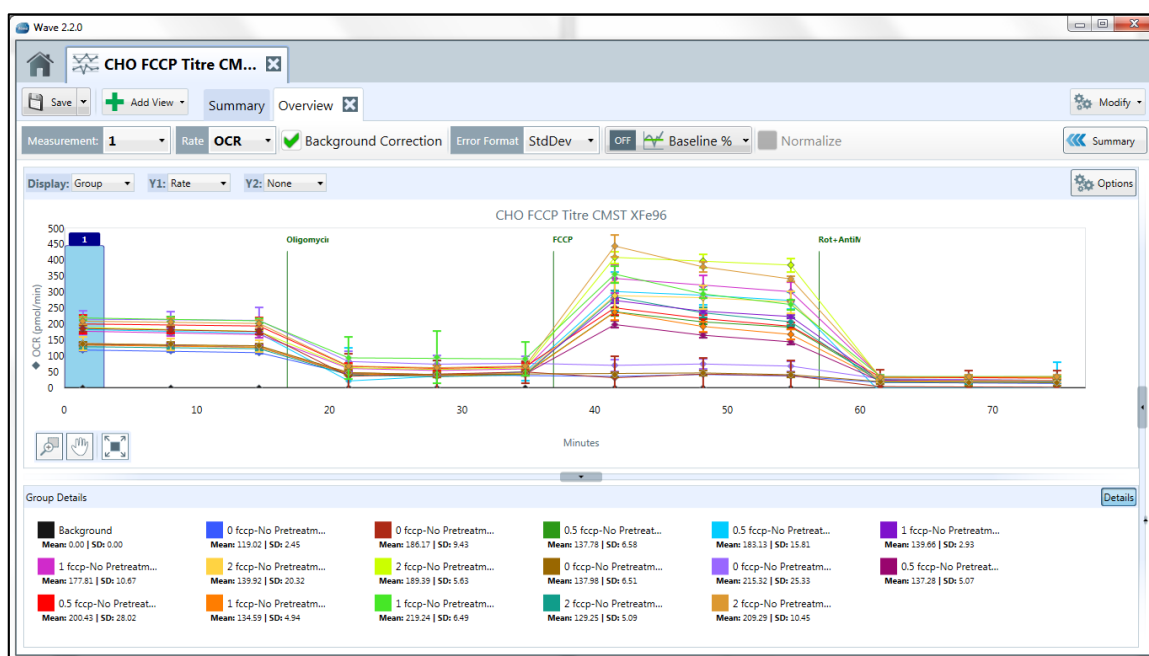
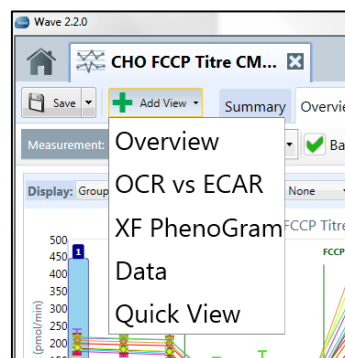
Quick View is the first graphical analysis view displayed when opening a new assay result file. Quick View simultaneously displays OCR, ECAR, OCR vs. ECAR (or PPR^{*}).

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



Overview:

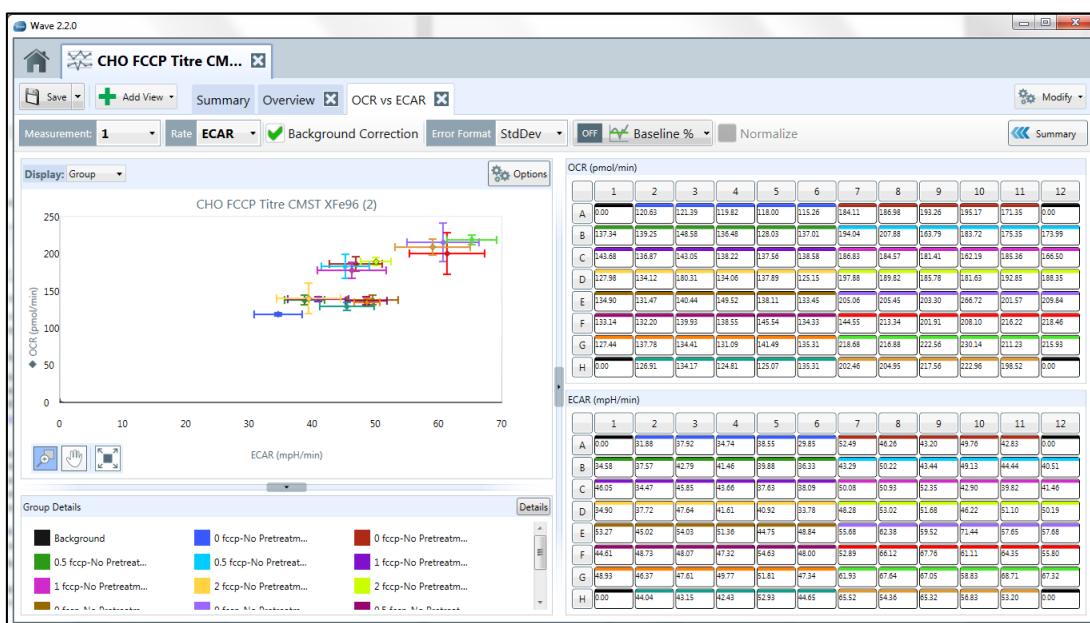
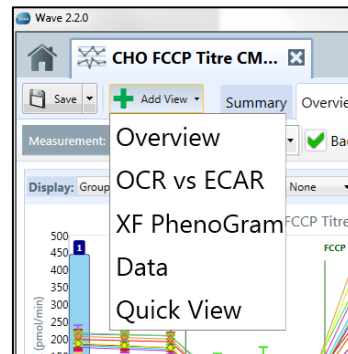
Overview displays kinetic graphs for all rates (OCR, ECAR, PPR). Group statistics are also calculated and displayed within this view. **Overview** is the primary analysis view for analyzing Assay Result data. To display **Overview**, click **Add View** in the top left region of the screen and click **Overview** from the dropdown menu.



Note: Add any number of **Overview** tabs by selecting the **Add View** button in the top left region of the screen and clicking **Overview** from the dropdown menu. The Plate Map (typically displayed) is hidden in the screen shot above.

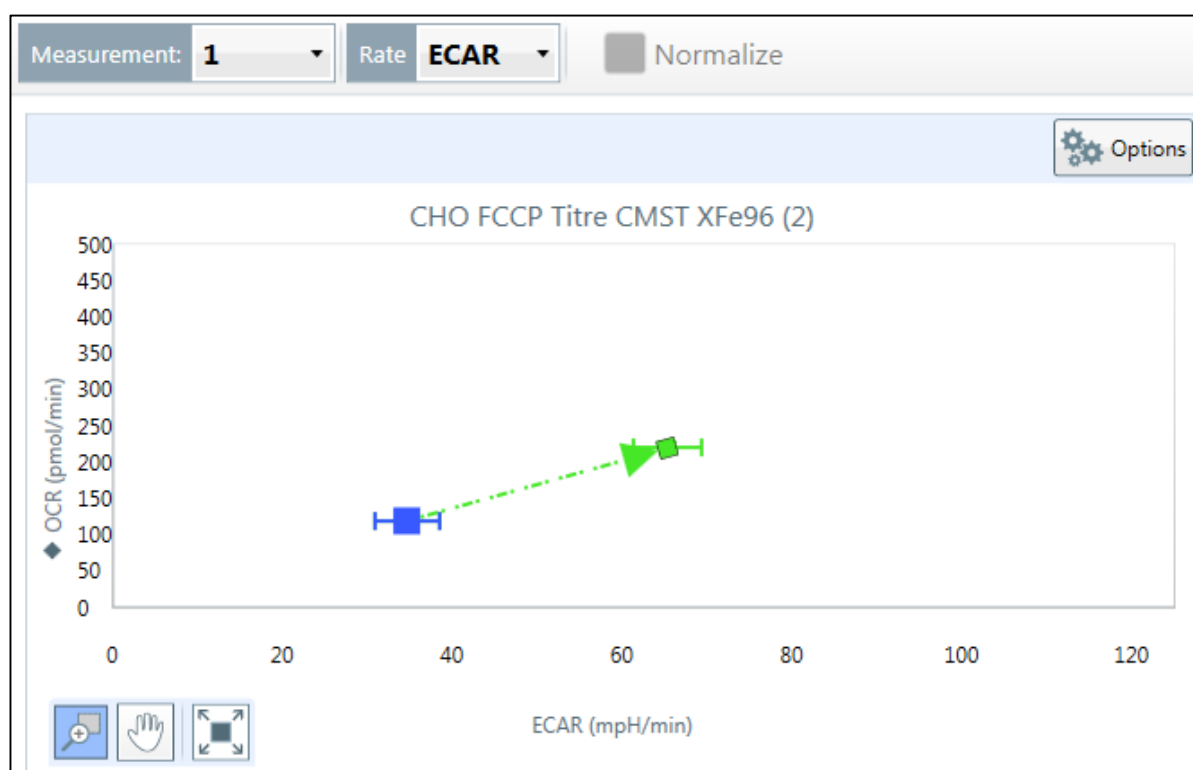
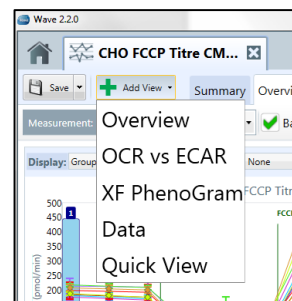
OCR vs. ECAR:

The **OCR vs. ECAR** view displays the OCR on the Y1-axis and ECAR on the X-axis. To display the **OCR vs. ECAR** view select **Add View** in the top left region of the screen and choose **OCR vs ECAR** from the dropdown menu.



XF PhenoGram:

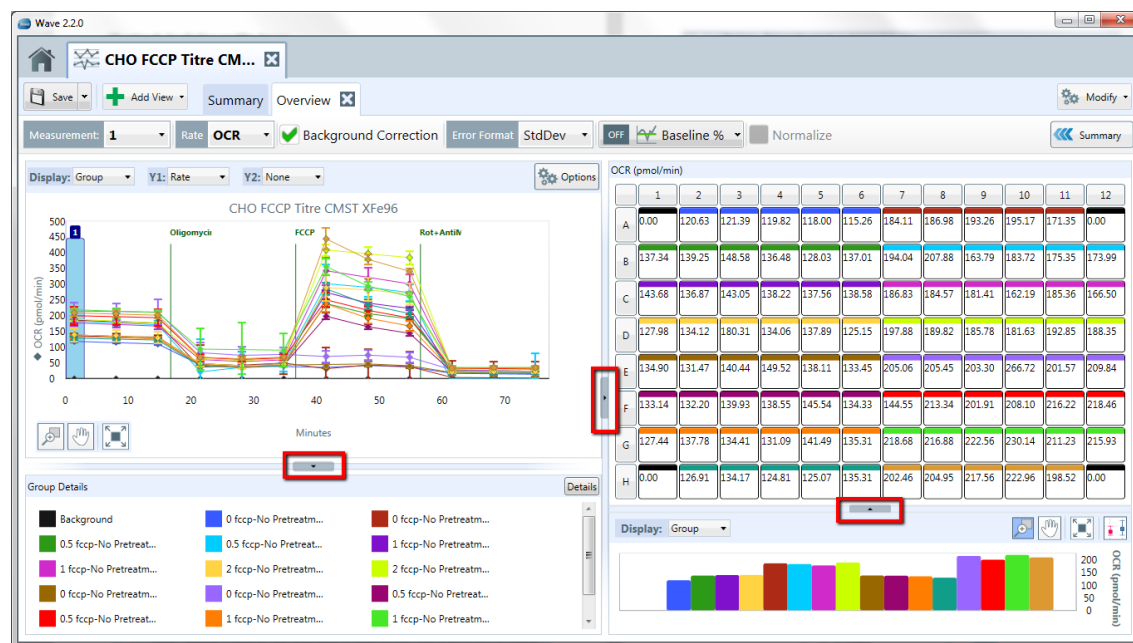
The **XF PhenoGram** view allows for comparing metabolic phenotypes between two cell populations. To display the **XF PhenoGram** view select **Add View** in the top left region of the screen and choose **XF PhenoGram** from the dropdown menu.



Customizing Analysis Views and Graphs

Resize a Graph or Plate Map

To resize a graph or chart, click the resize arrow as depicted in the following illustration (arrows outlined in red below for the Overview analysis view).



To expand/shrink the kinetic graph, use the outlined arrow between the kinetic graph and plate map and slide horizontally. Hide the Group Details using the highlighted arrow between the kinetic graph and Group Details. Hide the bar chart using the highlighted arrow between the plate map and bar chart.

Zoom, Pan, and Restore

There are 3 buttons available on each graph and chart in the **Overview**, **OCR vs ECAR**, and **XF PhenoGram** analysis views.



Zoom – Magnify a particular part of the graph or chart.



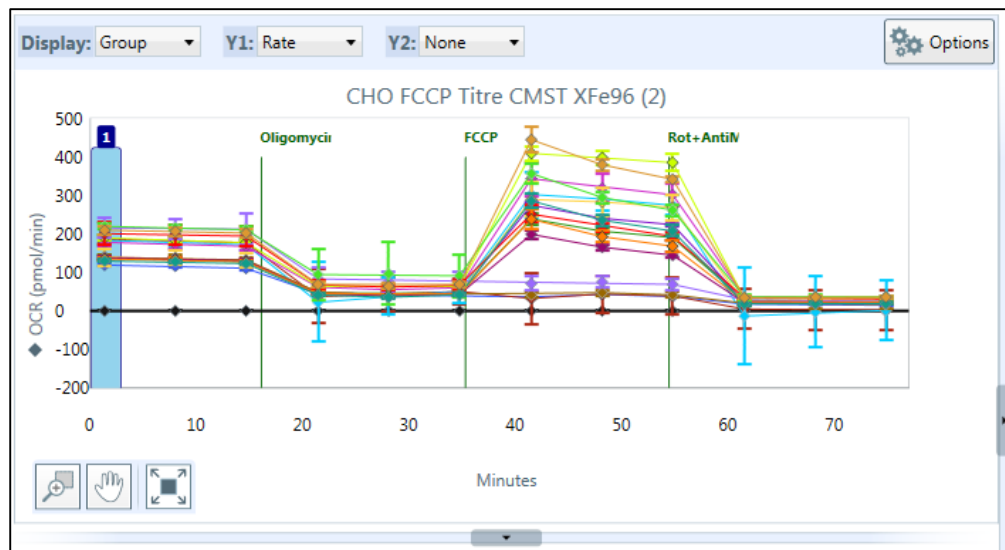
Pan – Move around the magnified area of the graph or chart.



Restore – Return the graph or chart to full view

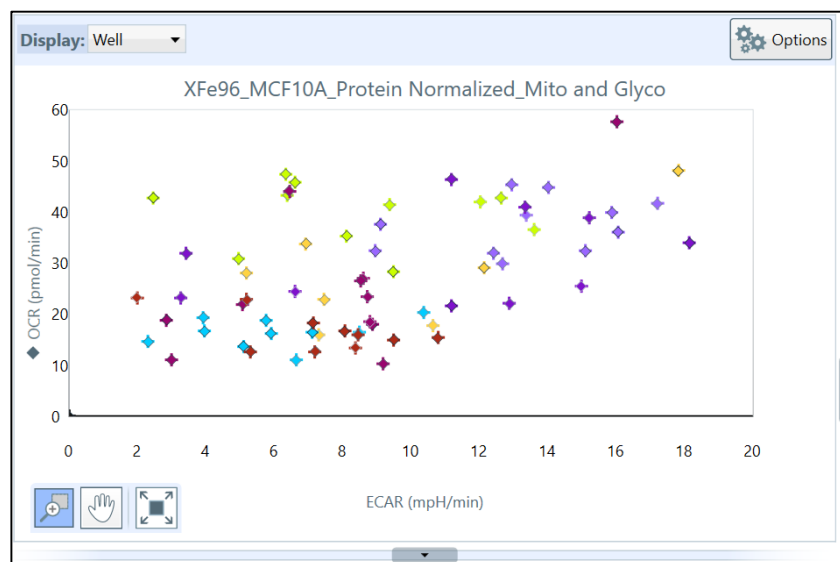
Kinetic Graph (Rate vs. Time)

A *kinetic graph* is the most common way to display data from the Seahorse XF[®] 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.



Rate Data: OCR, ECAR, or PPR

There are three rates measured by the Seahorse XF^e Analyzer:

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



The default view displays time on the X-axis (in minutes) and rate on the Y-axis. Use the rate drop-down menu above the kinetic graph to select a different rate to display (OCR, ECAR, or PPR).

Level Data: O₂ and pH

Display the O₂ or pH raw level data used to calculate the rates. Level data for OCR is displayed as oxygen tension in mmHg. Level data for ECAR and PPR is displayed as pH. To view the raw level data, select “**Level**” from the **Y1:** dropdown menu. Level data is available on the **Overview** and the **Data** analysis views only.



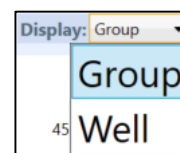
Rate Overlay: OCR, ECAR, PPR, O₂, or pH

To overlay two rates on the same kinetic graph, use the **Y2:** dropdown menu. When a rate is selected in the Y2 drop-down menu, Wave will display the rate for the Y2 axis and the rate for the Y1 axis on the same kinetic graph to facilitate comparison of both rates.



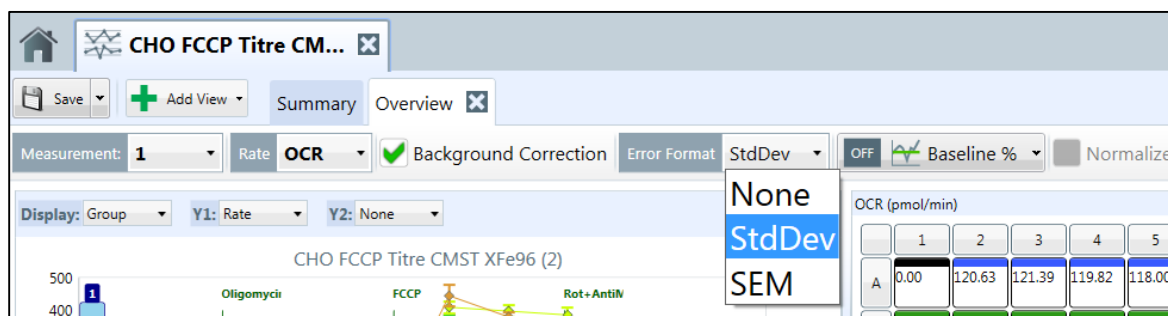
Display Modes: Group and Well

Rate data is graphically displayed in *Group* mode on all analysis views by default. *Group* mode displays rate data as the average of all wells in each group for each measurement. On the **Overview** tab, only 1 kinetic trace for each group will be displayed. The **OCR vs ECAR** tab will display a single point per group for each rate measurement. In *Group* mode, error within each group is displayed as Standard Deviation or Standard Error of the Mean. To display individual kinetic traces or data points for each assay well in each group, change the **Display** to *Well* mode using the drop-down menu. Error bars are *not* displayed when in **Well** mode.



Standard Deviation and Standard Error of the Mean

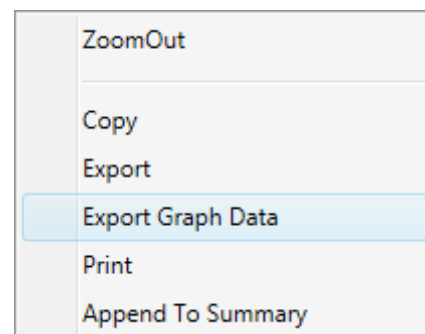
Choose the desired the error bar type using the *Error Format* dropdown menu. Error bar types are Standard Deviation (StdDev) or the Standard Error of the Mean (SEM). Choose **None** to hide error bars from the data graph. In **Well** mode, error bars are *not* displayed.



Save Custom Graph Data in Microsoft Excel from Analysis View

Data displayed on each analysis view in Wave can be exported to Microsoft® Excel.

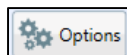
1. Right click anywhere on a graph or plate map to display the option menu and select **Export Graph Data**:
Note: For touch screen use, hold your finger on any part of the graph or plate map for three seconds.
2. The Windows save dialog box will appear displayed.
3. Browse to the desired save location, type in the file name for the export and click **Save**.



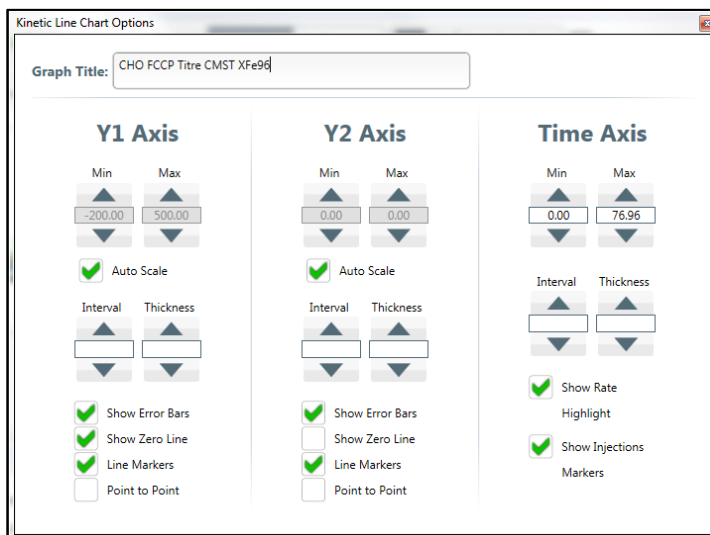
Data will be exported to Microsoft® Excel will exactly as it appears in the graph or plate map for additional analysis.

Note: This MS Excel file export is **not** compatible for analysis with a Seahorse XF Report Generator. The Assay Result file must be saved as a MS Excel file using the *Save as...* drop-down in the upper-right corner of Wave. For more information, see section: **Export Data to Microsoft Excel**

Format Graph Options



Select **Options** (located in the upper right-hand corner of the graph on the analysis view) to modify additional graph display settings. Configure specific settings for each graph/data display using the options displayed in the window below.



- **Minimum, Maximum, and Interval:** Set the scale for the Y1, Y2 and X axis.
- **Thickness:** Increase/decrease the width of the kinetic traces (1–thinnest, 5–thickest).
- **Show Error Bars:** Toggle error bars ON/OFF.
- **Show Zero Line:** Display a horizontal reference line at the zero point on the x-axis.
- **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 indicating the selected measurement.
- **Show Injections Marker:** Toggle ON/OFF display of the vertical injection lines on the kinetic graph.

Append to Summary

After customizing the kinetic graph and data displays on the **Overview** tab, content can be sent to the **Summary** tab in two ways:

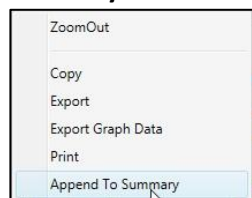
Add all the information in the Overview tab to the Summary tab:



Click **Summary** in the top left-hand corner of the **Overview** screen.

This will send all content on the **Overview** tab to the **Summary** tab.

Add only one item from the page, such as the kinetic graph:



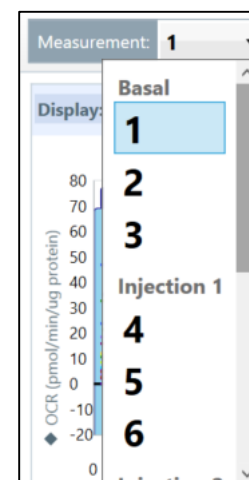
Right-click the portion of the **Overview** tab and select **Append to Summary** from the dropdown list. To view appended content, click the **Summary** tab.

Plate Map

In the upper right-hand corner of the **Overview** tab, the **Plate Map** is displayed.

The image to the right shows a Plate Map of the rate data for each assay well at a specific measurement time point. By default, OCR for rate measurement 1 is displayed on the Plate Map. Use the **Rate** drop-down menu to change the Plate Map rates displayed (OCR, ECAR, or PPR). To change the measurement displayed in the **Plate Map**, use the **Measurement** dropdown menu above the kinetic graph. 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 and check OFF **Show Rate Highlight**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.00	120.63	121.39	119.82	118.00	115.26	184.11	186.98	193.26	195.17	171.35	0.00
B	137.34	139.25	148.58	136.48	128.03	137.01	194.04	207.88	163.79	183.72	175.35	173.99
C	143.68	136.87	143.05	138.22	137.56	138.58	186.83	184.57	181.41	162.19	185.36	166.50
D	127.98	134.12	180.31	134.06	137.89	125.15	197.88	189.82	185.78	181.63	192.85	188.35
E	134.90	131.47	140.44	149.52	138.11	133.45	205.06	205.45	203.30	266.72	201.57	209.84
F	133.14	132.20	139.93	138.55	145.54	134.33	144.55	213.34	201.91	208.10	216.22	218.46
G	127.44	137.78	134.41	131.09	141.49	135.31	218.68	216.88	222.56	230.14	211.23	215.93
H	0.00	126.91	134.17	124.81	125.07	135.31	202.46	204.95	217.56	222.96	198.52	0.00



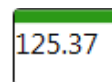
Exclude Outlier Wells

To exclude an assay well from the graph/data display simply click on the assay well. The assay well will change from a white background to gray background, indicating the well is excluded from the calculation/graphical display (Example – error bar for each rate measurement). Column (numbers) and row (letters) buttons can also be used to exclude entire sections of the grid, or click the upper-right corner to turn OFF the entire Plate Map.

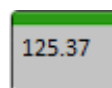
Note: Outliers eliminated in this view will not apply to other views, manually omit them in the **Plate Map** for each additional tab.

Turn ON/OFF Assay Well(s):

By default, all assay wells are turned ON and Wave includes all assay wells in the graphical data displays. Assay wells that are included in the graphs and in the rate/error calculations have a white background (see image to right).



Turn OFF an assay well from the rate calculations and graphs by clicking on the individual well. Assay wells that are turned OFF from one analysis view are not removed from additional analysis views.

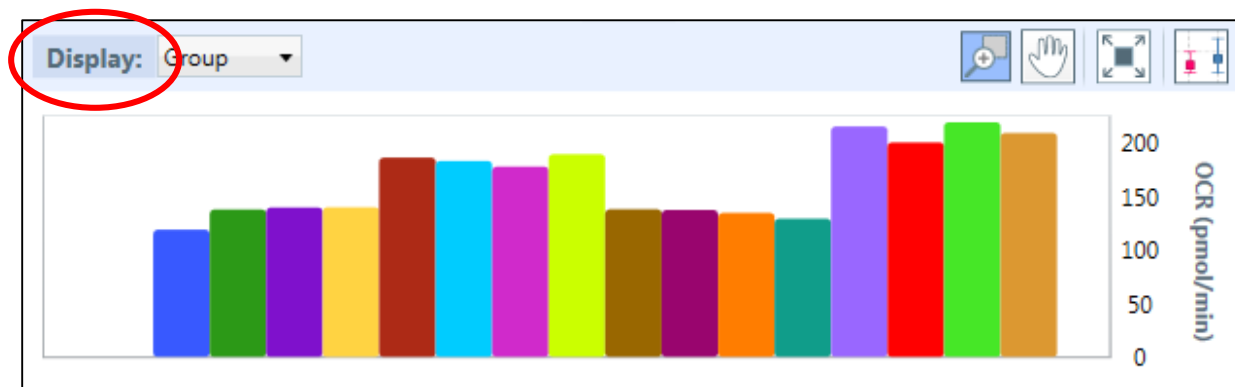
**Exclude Assay Well(s) from Data Export:**

When exporting data to GraphPad or a Report Generator, create an 'Outlier' Group (Modify > Groups/Conditions) and assign outlier wells to this group (Modify > Plate Map) to remove these wells from the groups in the data export from Wave.

Alternatively, the user may unassign these wells (Modify > Plate Map), which will remove the wells from the rate calculation visible on the current tab view as well as assign these wells to an 'Unassigned' group when exporting data from Wave to Excel or GraphPad.

Bar Graph

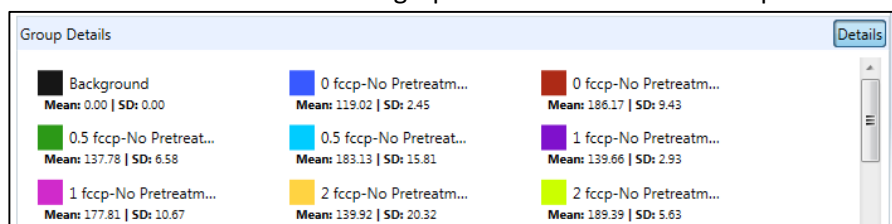
Individual rate measurement data is displayed in *Group* mode (average) as bar charts below the **Plate Map**. Use the **Display:** drop-down menu to change the rate data display from *Group* (average) to *Well* (individual well) mode.



Bar Charts can be useful for displaying group data to see differences between a control group and experimental treatment groups for a specific rate measurement. Displaying data in *Well* mode provides a simple data display for outlier identification for each group.

Group Details

The **Group Details** located below the kinetic graph or OCR vs. ECAR scatter plot functions as:



1. A group legend for the displayed graph data. The group names and group colors shown in the **Group Details** correspond with the data displayed on the kinetic graph or scatter plot. 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.

Note: The mean and standard deviation of hidden groups will change to **Mean: 0.00 and Standard Deviation: 0:00**.

2. Display group statistics for each group for the selected rate measurement by clicking the **Details** button in the upper-right corner of **Group Details** (average when in *Group* mode and error bar value based on type of error bar displayed – standard deviation or SEM). Change the rate measurement to recalculate and display the average and error bar value for the selected rate. Wells that have been excluded on the **Plate Map** will automatically be excluded from the calculation of the average and error displayed.


Data:

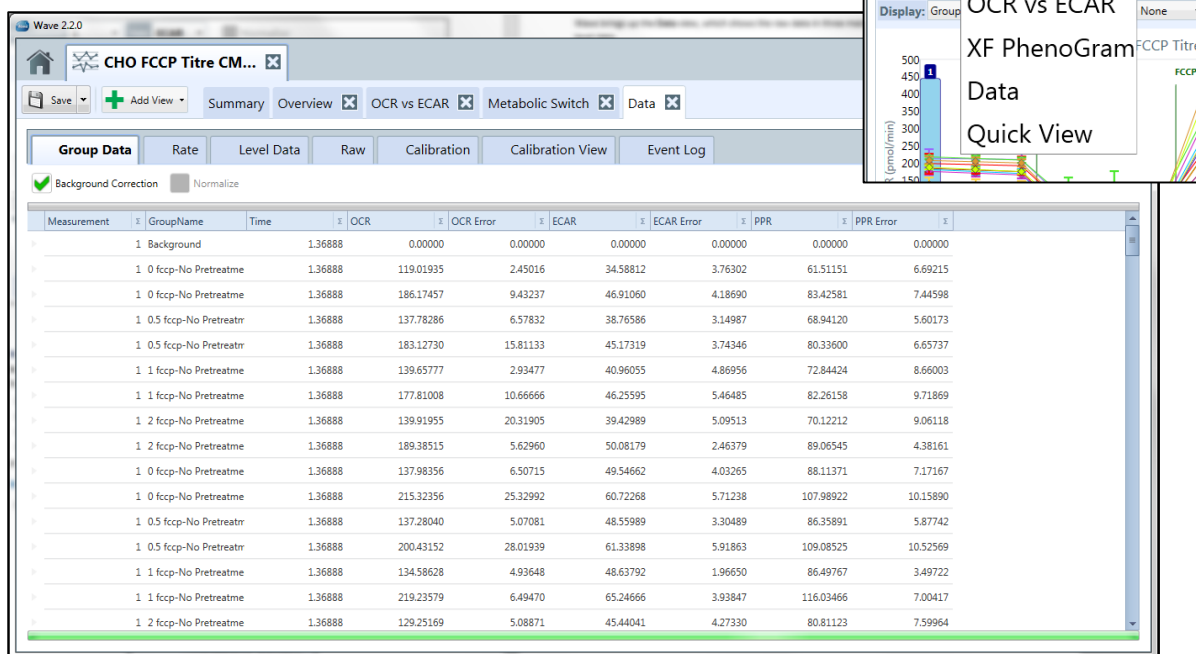
The **Data** analysis view is a comprehensive data log of every detail about the assay performed. Each tab in the **Data** view can be exported to Microsoft Excel. The 7 data tabs in the **Data** view are outlined below:

- **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.

The remaining **Data** view tabs below are used primarily for internal Seahorse purposes, including QC, validation processes, and Seahorse Technical Support:

- **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 XF^e / XFp Analyzer.
- **Event Log:** XF Analyzer instrument serial number, consumable lot numbers used during assay and a command log for processes performed during assay and whether they were successful or failed.

To display the Data view, click on **Add View**  and choose **Data**. The **Group Data** tab is displayed by default when opening the **Data** view. The **Group Data** tab is displayed below:



Sort by Column

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

Measurement	Well	Group	Time	OCR	ECAR	PPR
7 H08		2 fccp-No Pretreatme	41.47992	464.24606	90.02741	160.10475
7 H10		2 fccp-No Pretreatme	41.47992	461.70120	93.12493	165.61338
7 H07		2 fccp-No Pretreatme	41.47992	457.27439	103.49294	184.05185

Display the Average, Count, Maximum, Minimum, or Sum:

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

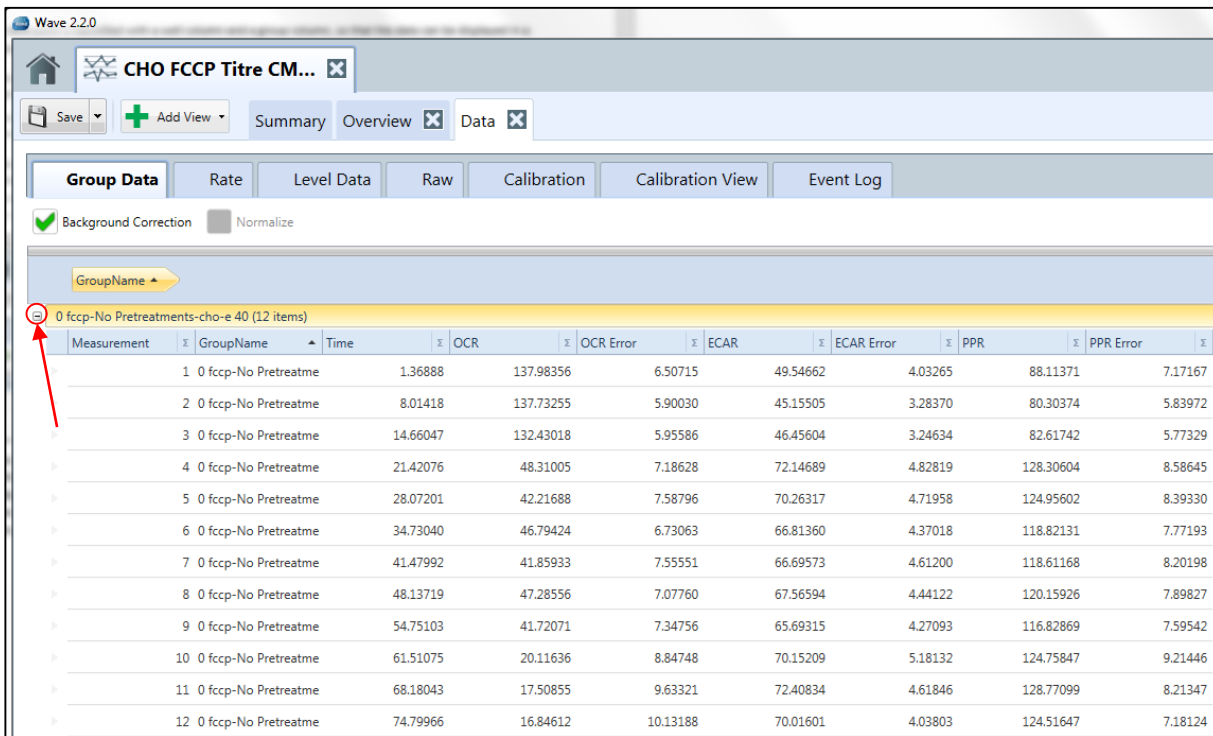
Measurement	Well	Group	Time	OCR	ECAR	PPR
1 D07			0.81746	2.705	4284	11.81362

Group the Data by Field

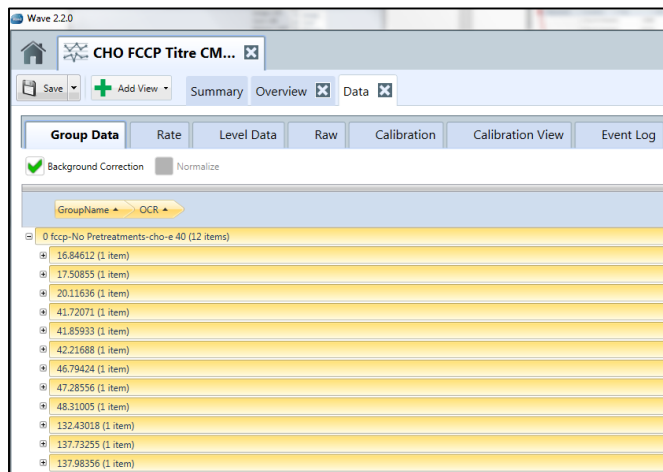
To group the data by field click on a column heading and drag it up to the gray bar. In the following example, the *GroupName* heading was dragged to the gray bar.

GroupName
Background (12 items)
Control (12 items)
Experimental (12 items)

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

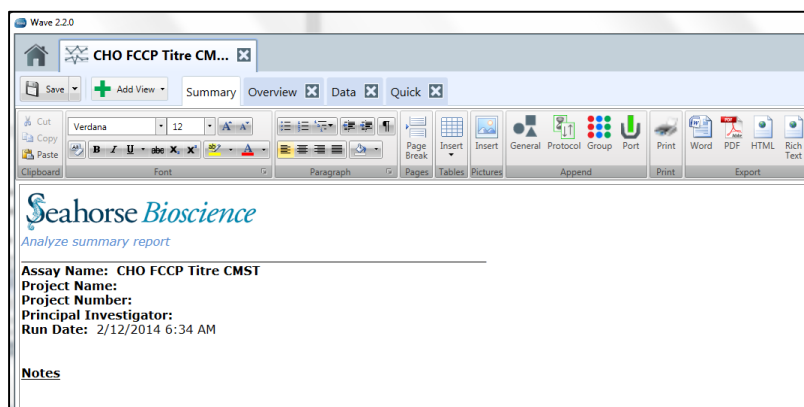


After expanding a group, select another column heading to define the groupings further. In the following example, the heading OCR is dragged to the grey bar after GroupName.



Summary:

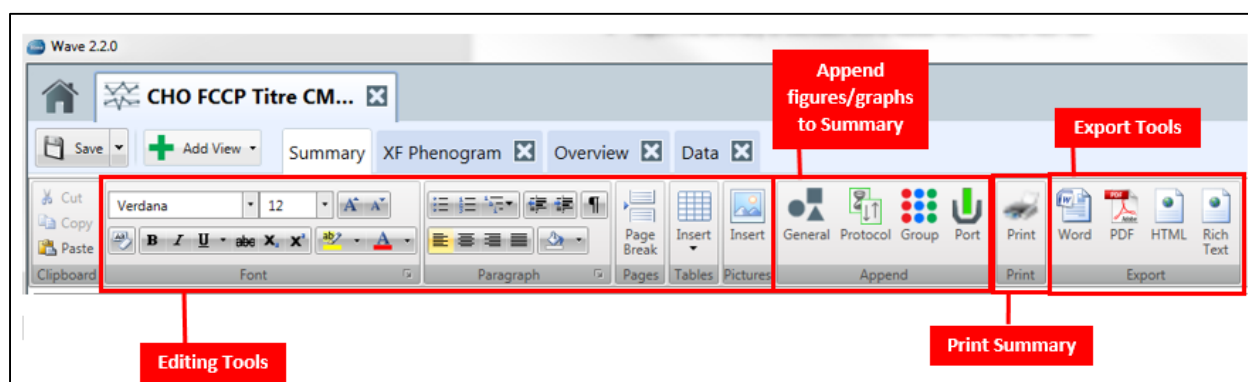
The **Summary** tab is always displayed and is the first tab when viewing an assay result file. The **Summary** tab cannot be closed or deleted. The **Summary** tab provides a place for adding custom notes or valuable information about the assay performed, appending graphs, charts, and/or plate maps 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.



Customize the Summary:

Similar to Microsoft Word, the **Summary** tab has a fully functional toolbar to edit the content, including:

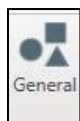
- Cut, copy, and paste to the clipboard.
- Change the font type, size, text and highlight color, and style.
- Subscript or superscript a character or characters.
- Add bullet points or numbers.
- Insert a table, picture or page break.



Append Assay Information to the Summary:

Append details from each analysis view in Wave to the Summary tab using the <<< Summary button.

The Summary tab toolbar also displays an *Append* sections appended to directly add information contained in the assay design.



General Information: Append General information to the summary tab. General information about the assay includes project and plate information.

Example **General Information** image:



Protocol Summary: Append the Protocol Summary to the Summary tab. The Protocol Summary displays the Mix, Wait, and Measure protocol for each step along with the time and number of cycles for each measurement.

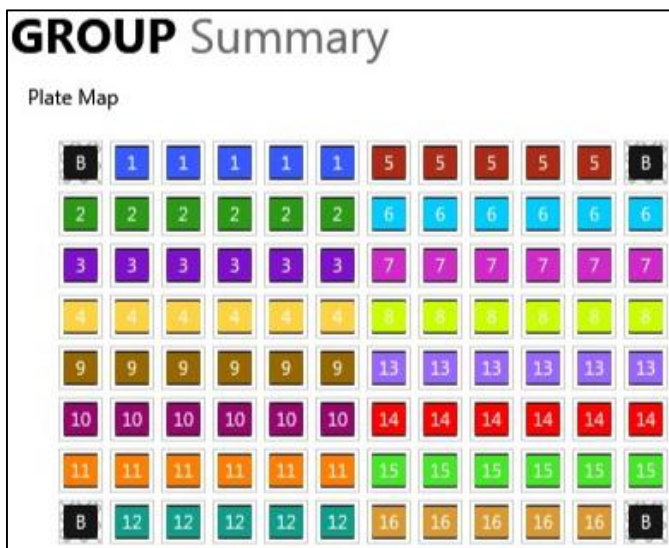
Example **Protocol Summary** image:

Initialization	Basal	Oligomycin	FCCP
Calibrate	Mix: 00:03:00	Inject Port: A	Inject Port: B
Equilibrate: 00:12:00	Wait: 00:00:00	Mix: 00:03:00	Mix: 00:03:00
	Measure: 00:03:00	Wait: 00:00:00	Wait: 00:00:00
	Mix: 00:03:00	Measure: 00:03:00	Measure: 00:03:00
	Wait: 00:00:00	Mix: 00:03:00	Mix: 00:03:00
	Measure: 00:03:00	Wait: 00:00:00	Wait: 00:00:00
	Mix: 00:03:00	Measure: 00:03:00	Measure: 00:03:00
	Wait: 00:00:00	Mix: 00:03:00	Mix: 00:03:00
	Measure: 00:03:00	Wait: 00:00:00	Wait: 00:00:00
		Measure: 00:03:00	Measure: 00:03:00
Rot+ AntiMycin			
Inject Port: C			
Mix: 00:03:00			
Wait: 00:00:00			
Measure: 00:03:00			
Mix: 00:03:00			
Wait: 00:00:00			
Measure: 00:03:00			
Mix: 00:03:00			
Wait: 00:00:00			
Measure: 00:03:00			



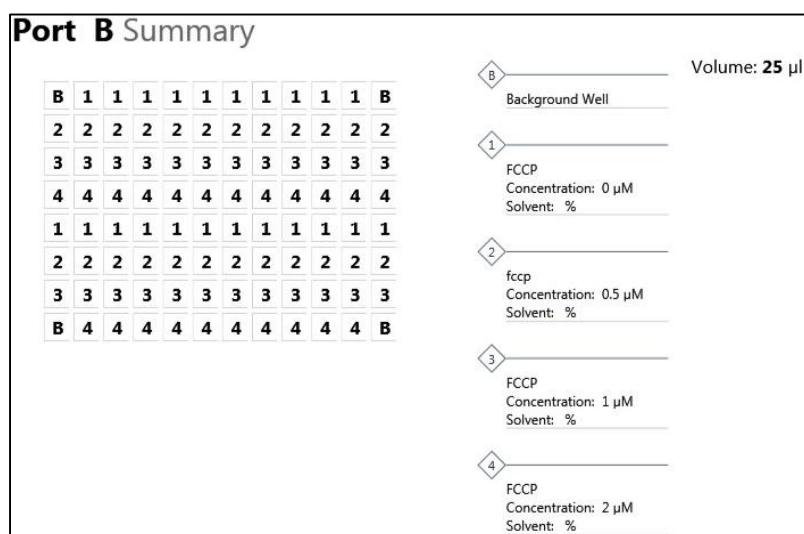
Group Summary: Append the Group Summary to the Summary tab. The Group Summary displays the number of groups along with the location of each group within the plate map.

Example **Group Summary** image:



Port Summary: Append the Port Summary to the Summary tab. The Port Summary contains information about the contents of each drug port that were injected during the assay.

Example **Port Summary** image:



Print the Summary:

1. Select the printer to use.
2. Select the pages to print.
3. Select the number of copies to print.
4. Press the Print button to print out the summary.

Export the Summary:

The Summary tab can be exported to 4 different file formats:

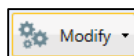
- Word
- PDF
- HTML
- Rich Text

Export Summary to alternate format:

1. Click the appropriate icon from the *Export* section of the tool bar:
2. Browse to the desired file location to save to.
3. Type the name of the file in the **File name** field, then press the **Save** button.



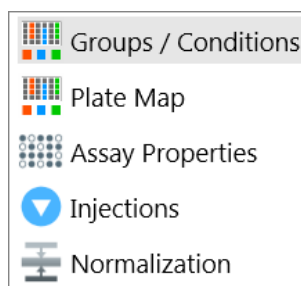
Modifying Assay Result Files



Each analysis view in Wave contains a **Modify** button in the upper right-hand corner of the view.

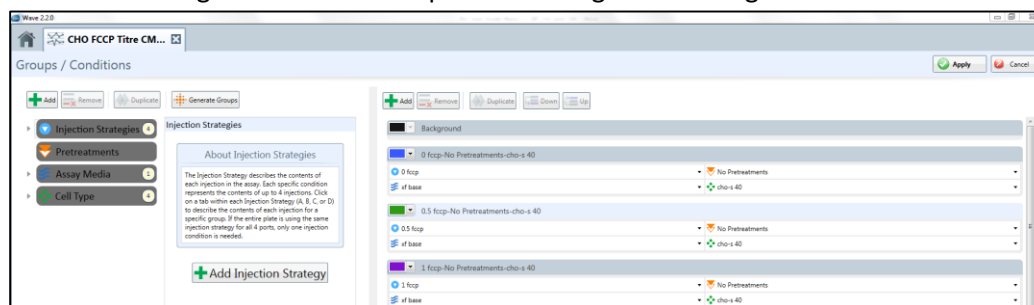
Modify provides a way to add and/or modify assay details after the assay has been completed. Select the part of the assay to modify from the drop-down list:

- Groups/Conditions
- Plate Map
- Assay Properties
- Injections
- Normalization



Modify: Groups/Conditions

1. Click **Modify** and select **Groups /Conditions** from the dropdown list. Wave displays the same Groups/Conditions window found in the assay design section of the software. This window enables any changes to be made to the group conditions for an experiment that has been run, such as correcting mistakes in the experiment design or entering additional details.

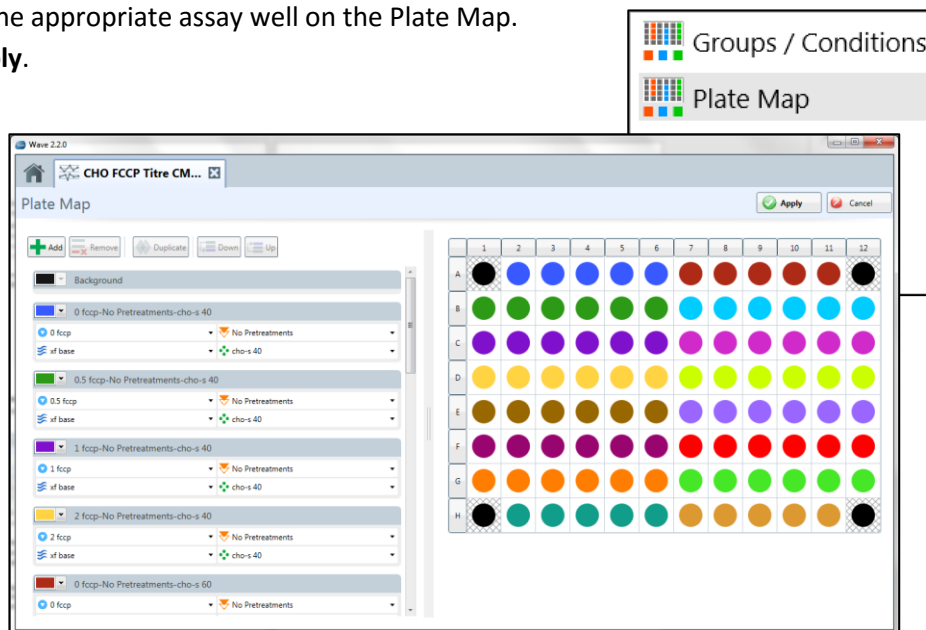


2. Once finished editing click **Apply** in the upper right-hand corner of the window to make changes to the Groups/Conditions details in the assay result file. Cancel out of this window to prevent any changes from taking effect

Note: The actual rate values in each well that were captured during the XF assay cannot be changed, only the Group/Condition naming and details.

Modify: Plate Map

1. Click **Modify** and select **Plate Map** from the drop-down menu.
2. The group properties along with the plate map with group assignments will be displayed. Modify the assay wells and groups the wells are assigned to by first selecting the group name on the left, then clicking the appropriate assay well on the Plate Map.
3. Click **Apply**.



Modify: Assay Properties

1. Click **Modify** and select **Assay Properties** from the dropdown menu. Wave will display the Assay properties window below:

GENERAL Information

Project Information	Plate Information	Advanced Settings
Project Name CHO FCCP Titre CMST	Well Volume (ul) 2.28	Email Notification Advanced
Principal Investigator Ben P.	Plated By	Email Recipient List
Project Number 01	Plated On Select a date	email address

2. Change or add text to:
 - a. Project Information
 - b. Plate Information
 - c. Advanced Settings
 - d. Notes
3. Click **Apply**.

Modify: Injections

1. Click **Modify** and select **Injections** from the dropdown menu. Change the label for each compound injection performed during the assay. The name displayed above each injection line on the kinetic graph will be updated to reflect the injected compound.

Wave 2.2.0

CHO FCCP Titre CM...

Injections

Change the name of your injections

- 1 Oligomycin
- 2 FCCP
- 3 Rot+AntiMycin
- 4

Normalization

Normalization is to scale data such that data from different microarrays can be compared. In some cases, it is useful to normalize rate data using results from another assay or laboratory technique in order to report a response in relation to another measurement, such as per cell or per quantity of protein. This function is performed by selecting **Normalization** in the tools button and adding or pasting in outside normalization data.

There are two ways to add normalization data to the Normalization window:

- Copying and pasting from a secondary source.
- Enter the data manually.

Copy and paste from a secondary source:

1. Copy the secondary source data from a spreadsheet or similar grid format to the Clipboard by using the key sequence Ctrl-C.
2. Click **Modify** and select **Normalization** from the dropdown menu.

The screenshot shows the 'Normalization' window in Wave 2.20. At the top, there is a 'Normalization Unit' input field. Below it is a grid for 'Normalization Values' with 12 columns (1-12) and 8 rows (A-H). To the right of the grid is a dropdown menu with the following options: 'Groups / Conditions', 'Plate Map', 'Assay Properties', 'Injections', and 'Normalization'. Below the dropdown menu are 'Select All' and 'Paste' buttons.

3. Click **Select All** to select all of the wells in the normalization plate map.
4. Click **Paste**.

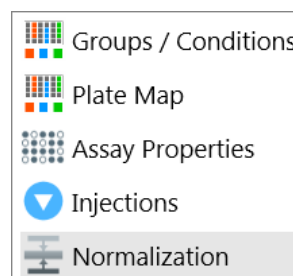
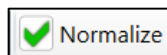
Note: This will paste the data from the clipboard into the normalization grid. Check the numbers in the grid to be sure they are correct. Data can also be entered manually by selecting a well in the grid and typing in the value.

5. Click **Apply**.

Note: A *Normalization Unit* **must** be entered in the field at the top of the screen to apply normalization data to the kinetic measurements. This unit will then be displayed in the Y1 axis title on the kinetic graph when normalization is selected. For example, type “ $\mu\text{g Protein}$ ” into the **Normalization Unit** field, and normalized OCR will be displayed on the kinetic graph as “ $\text{pmol}/\text{min}/\mu\text{g Protein}$ ”.

Enter normalization values manually:

1. Click **Modify** and select **Normalization** from the dropdown menu.
2. Enter values into the Normalization grid manually.
3. Follow Steps 5 and 6 from the previous procedure.

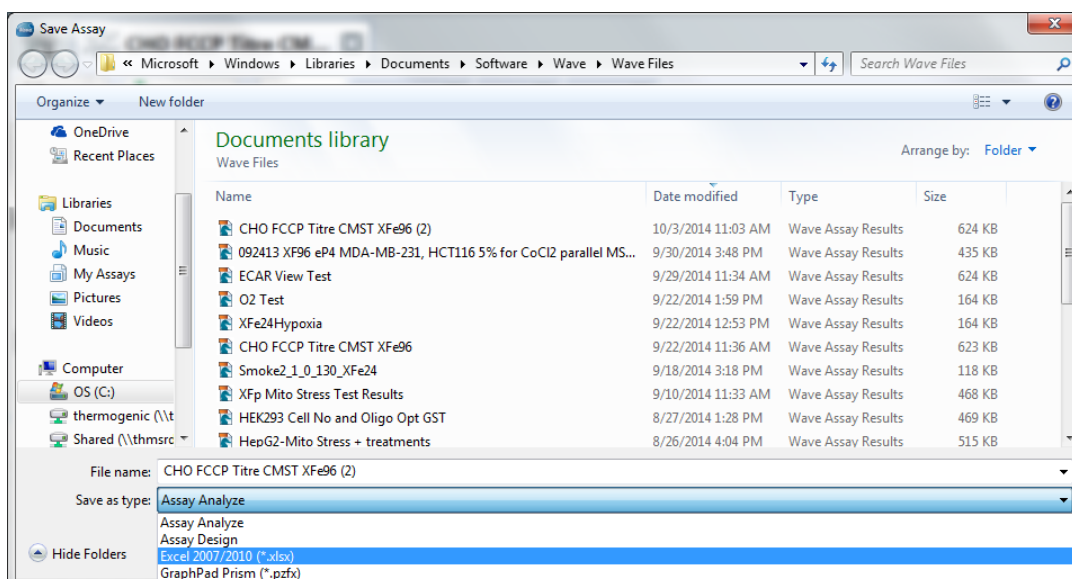


After data has been normalized, the **Normalize** checkbox will become active on all analysis tabs To toggle ON/OFF using the normalization data to recalculate the kinetic rates in the plate map and on the kinetic graph or scatter plot, click the checkbox on and off.

Export Data to Microsoft Excel

To use a Seahorse XF Report Generator, an Assay Result file must first be exported to Microsoft Excel from Wave (Desktop or Controller). Use Wave to add or customize the details of the Assay Result file or exclude outliers then follow the steps below to save the Assay Result file in a Microsoft Excel file format:

1. Press the downward-pointing arrow on the **Save** button (upper-left corner of Wave).
2. Select **Save as** and choose location to save Excel file.

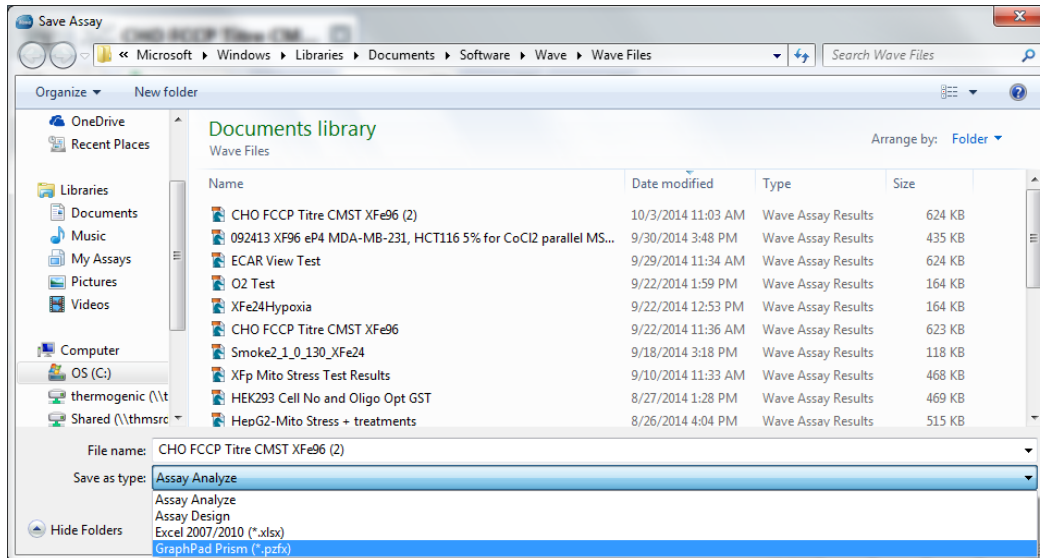


3. Click the drop-down *Save as type*: and select Excel 2007/2010 (*.xlsx).
4. Click **Save**.

Export Data to GraphPad Prism 6*

*Note: Wave export compatibility has been validated using GraphPad Prism 6.

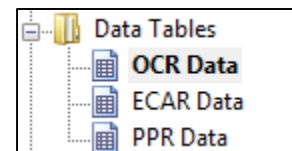
1. Press the downward-pointing arrow on the **Save** button located in the upper-left corner of Wave.
2. Select **Save as** and choose location to export the GraphPad file.



3. Click the drop-down *Save as type:* and select GraphPad Prism (*.pzfx).
4. Click **Save**.

GraphPad Data Views

GraphPad will automatically export the raw data for OCR, ECAR and PPR into the GraphPad analysis file (.pzfx). The image below shows the options to view raw rate data in GraphPad.

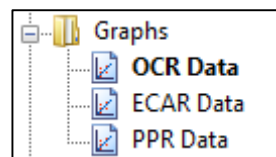


Raw data view in GraphPad:

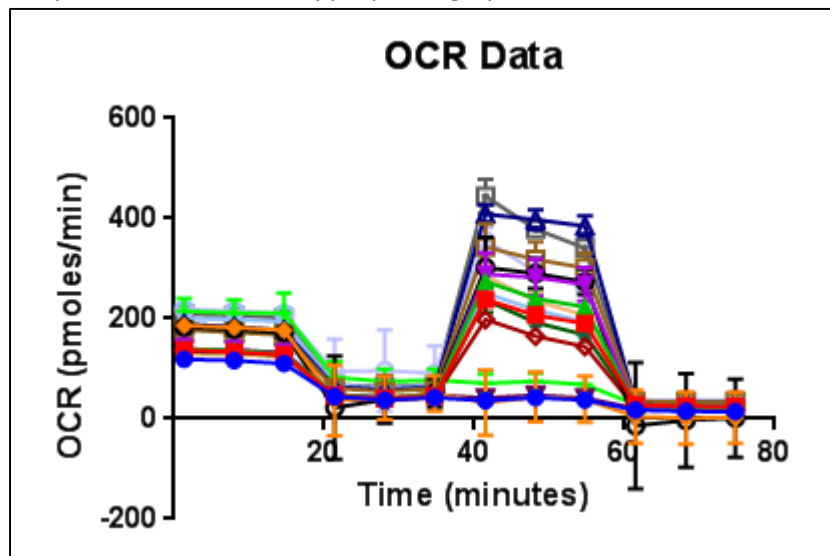
Time (minutes)	A:Y1	A:Y2	A:Y3	A:Y4	A:Y5	A:Y6	B:Y1	B:Y2	B:Y3	B:Y4	B:Y5	B:Y6	C:Y1	C:Y2	C:Y3	C:Y4	C:Y5	C:Y6	D:Y1
1	120.53390	121.38870	119.81560	117.95500	115.26300		137.33960	139.25470	148.57900	136.43180	128.82740	137.81470	143.67670	136.86520	143.04690	138.21950	137.56370	138.57530	127.98490
2	116.97800	119.52670	118.73280	114.27900	112.38800		134.26870	134.69790	142.85100	132.16900	126.09500	131.37180	138.33880	134.88810	139.58010	135.75200	133.97560	133.73340	125.55500
3	109.54780	114.42160	114.06130	108.96900	106.21790		127.33570	129.31800	135.85600	126.97980	120.85040	126.62350	129.55380	128.28350	135.83710	128.57610	128.81720	127.30740	117.27800
4	42.97560	43.69730	42.91730	45.03420	45.59140		46.54240	46.18310	50.58910	46.58190	45.74170	44.351470	48.771360	54.842270	48.045870	42.72970	46.580730	47.427110	40.329770
5	37.096970	37.584370	36.845830	38.525480	38.679490		41.317050	40.691020	44.06010	39.581070	39.260850	38.648970	41.429100	49.703670	42.558560	38.297650	39.693840	39.766990	34.810320
6	41.421080	41.244600	42.381420	44.702220	43.544590		47.947190	44.563310	49.188770	44.722270	43.833320	44.063640	46.871990	51.158520	49.192840	44.839290	44.854310	40.098780	
7	35.88950	36.51960	37.43980	37.67750	34.895770		219.64960	253.67160	249.54650	231.63020	229.48800	246.86780	268.56840	265.01320	289.95670	40.20280	276.64180	270.65310	272.54170
8	41.88450	42.70570	42.16990	44.08540	42.387090		197.90490	217.47360	212.14390	205.34260	198.63900	212.03610	233.90240	237.13660	244.41290	245.79910	241.92590	239.27780	273.73360
9	36.95190	38.53420	38.71210	38.70630	36.431680		184.42290	198.11800	194.74500	189.04550	181.47530	194.26130	218.87170	221.48700	224.35190	231.21410	223.83410	222.10900	264.19670
10	16.919570	20.002830	15.106970	16.939630	18.247830		27.737720	26.779870	32.852350	23.978990	26.173940	23.864590	21.994230	25.548520	19.473790	22.795730	25.366450	17.626350	
11	15.32260	17.011950	13.618160	14.181470	15.272920		25.788830	26.172870	31.419600	21.189820	24.863030	20.619330	21.204740	28.842540	26.663390	19.933580	21.395300	24.504400	18.950460
12	14.177230	15.826880	13.460830	14.064160	15.158540		25.081910	25.526790	31.097320	20.356600	23.439560	19.259920	19.550450	29.974750	25.834830	17.357740	20.616670	22.892960	19.386810

GraphPad Kinetic Graph Views

In addition to raw data, GraphPad also exports the OCR, ECAR (and PPR) kinetic graphs. Select the graph/rate to view or edit from the list on the left-side of GraphPad.



GraphPad will show the appropriate graph based on the selection above:



Note: Excluded wells and normalized rate data does not export from Wave Controller or Desktop. See sections on removing outliers and normalization for more information.