Wave User Guide
XF^{e} Analyzer

October 2014

Seahorse Bioscience

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Chapter 1: Designing an XF^e Assay

Overview of the Process: Design, Run, and Analyze

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

Step 1: Design XF^e assay using Wave Desktop

Wave 2.2 can only be installed and operated on Desktop or laptop PCs, or Macintosh running Parallel 9. Wave 2.2 cannot be installed on an XF^e Controller. A new template can be designed using Wave Desktop (or using the Wave Controller 2.1 software). Start Wave Desktop by double clicking the shortcut  on the desktop computer.

Step 2: Run XF^e assay

Seahorse recommends designing assay templates using Wave Desktop; however, ***in order to run the assay on the XF^e Controller, the assay must be transferred to the XF^e first via USB or network drive.***

Run a template or design created on Wave Desktop if the XFe Controller is networked:

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**.

Run a template or design created on Wave Desktop if the XF^e Controller is not networked:

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**

Step 3: Analyze XF^e data using Wave Desktop

Seahorse recommends performing data analysis using Wave Desktop.

Using Assay Template file:

Transfer analysis files to Wave Desktop if the XF^e Controller is networked:

1. Load an assay template file (.asyt)
2. Click **Run**
3. Save template as an Assay Design file (.asyd) to network location
4. Perform assay on XF^e.

5. Wave Controller will automatically save Assay Results file (.asyr) to network location specified prior to running assay**.**
6. Start Wave Desktop > Click **Open** > Click **Browse** and locate the saved assay result file on network.

Transfer analysis files to Wave Desktop if the XFe Controller is not networked:

1. Load an Assay Template file (.asyt)
2. Click **Run**
3. Save template as an Assay Design file (.asyd) to USB or local XF^{e} Controller location.
4. Perform assay on XF^{e}.
5. XF^{e} software will automatically save Assay Results file (.asyr) to the location the Assay Design file (.asyd) was saved.
6. If Assay Result file (.asyr) was saved on the XF^{e} Controller local drive, transfer Assay Result file (.asyr) to USB flash drive.
7. Plug USB flash drive into desktop computer with Wave Desktop installed.
8. Start Wave Desktop > Click **Open** > Click **Browse** and locate the saved assay result file on the USB flash drive.

Using Assay Design file

Transfer analysis files to Wave Desktop if the XF^{e} Controller is networked:

1. Create a new Assay Design file (.asyd)
2. Click **Run**
3. Wave Controller will prompt to choose save location for Assay Design file (.asyd). Select a network location.
4. Perform assay on XF^{e}.
5. Wave Controller will automatically save Assay Results file (.asyr) to network location specified prior to running assay**.**
6. Start Wave Desktop > Click **Open** > Click **Browse** and locate the saved assay result file.

Transfer analysis files to desktop if the XF^{e} Controller is not networked:

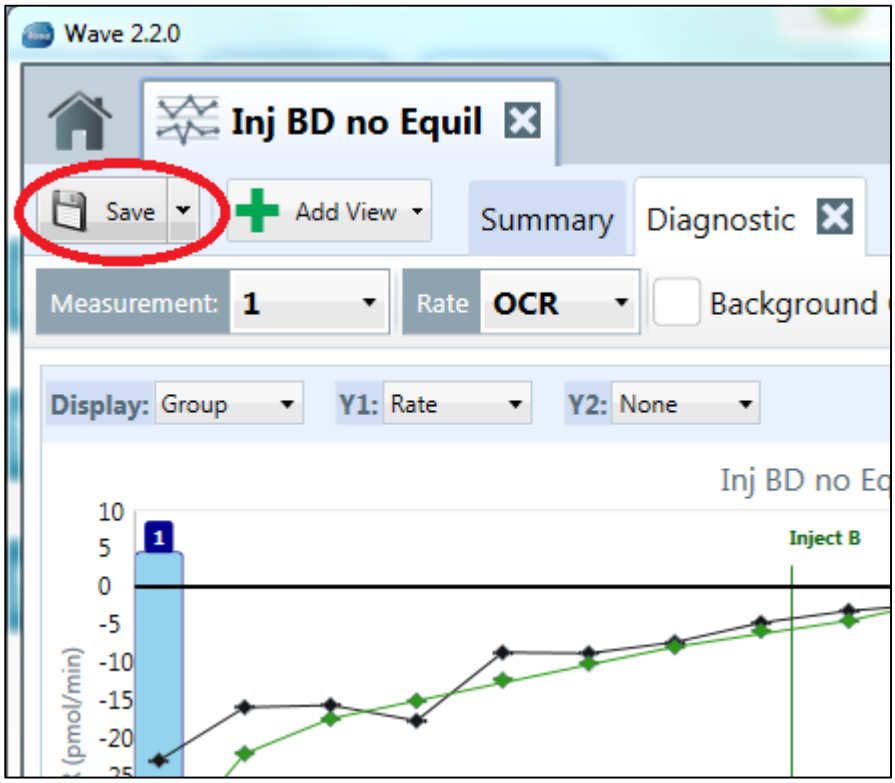
1. Create a new Assay Design file (.asyd)
2. Click **Run**
3. Wave Controller will prompt to choose save location for Assay Design file (.asyd). Select USB or local storage drive on XF^{e} Controller.
4. Perform assay on FX^{e}.
5. Wave Controller will automatically save Assay Results file (.asyr) to location specified prior to running assay**.**
6. Assay Results file (.asyr) must be transferred to computer running Wave Desktop.
7. Start Wave Desktop > Click **Open** > Click **Browse** and locate the saved assay result file.

Note: *XF^{e} software automatically saves a backup copy of the assay result file locally on the XF^{e}.*

Step 4: Transfer XF^{e} assay data to a Microsoft® Excel ™ Spreadsheet

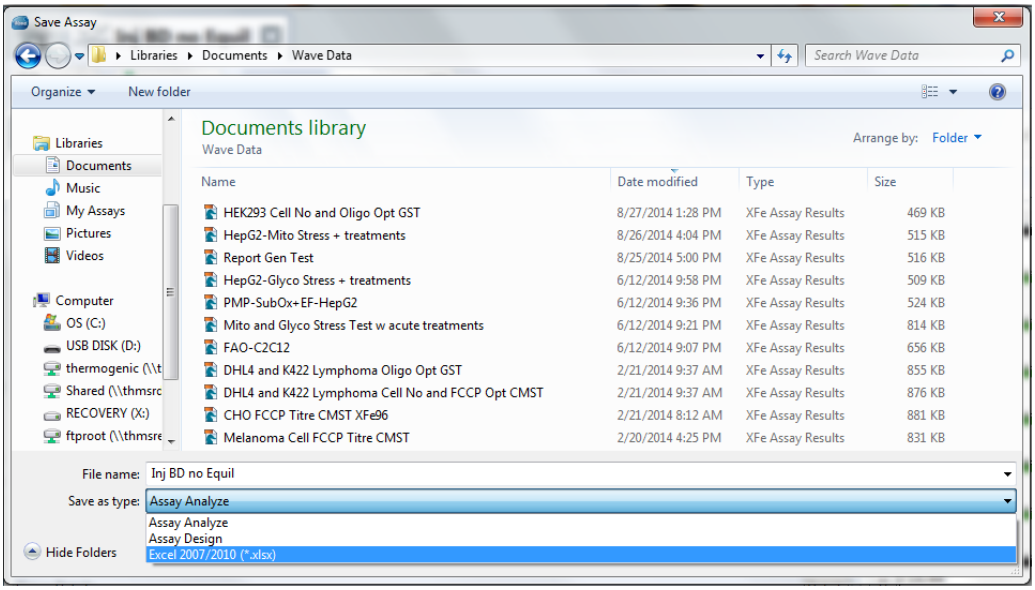
1. Press the downward-pointing arrow on the **Save** 

button in the top left corner of Wave Desktop analysis screen
2. Select **Save as** to bring up the Save Assay dialog box

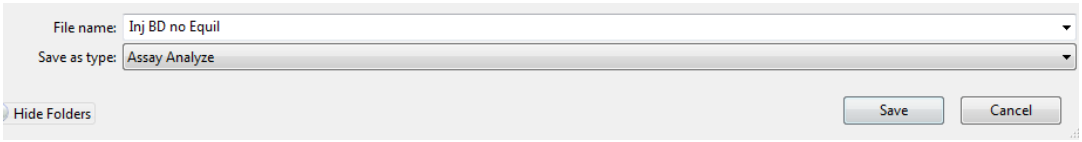


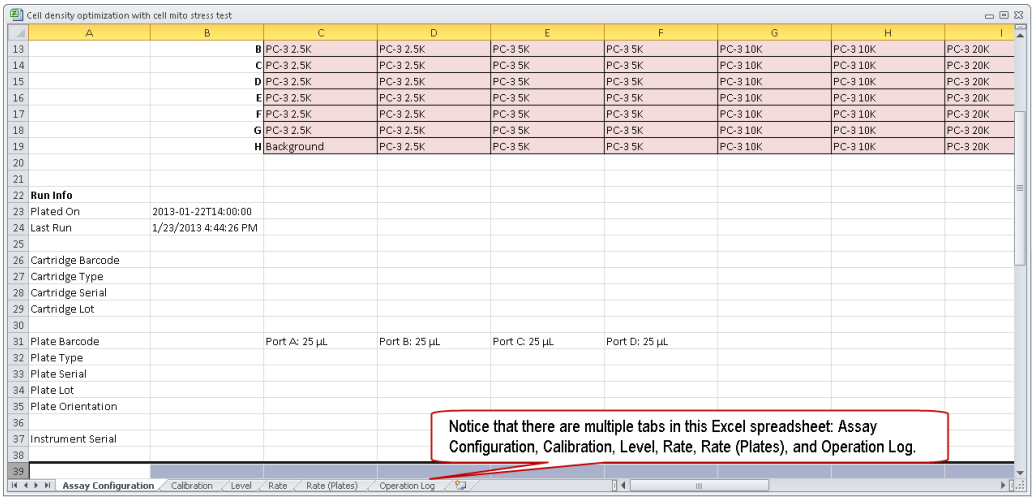
3. Click on the ***Save as type*** dropdown list at the bottom of the window. This list gives 3 options: Assay Analyze (.asyr), Assay Design (.asyd), and Excel 2007/2010 (*.xlsx).

4. Select Excel 2007/2010 (*.xlsx) and browse to desired save location for the Excel file.



1. Click the **Save** button in the lower right-hand corner.

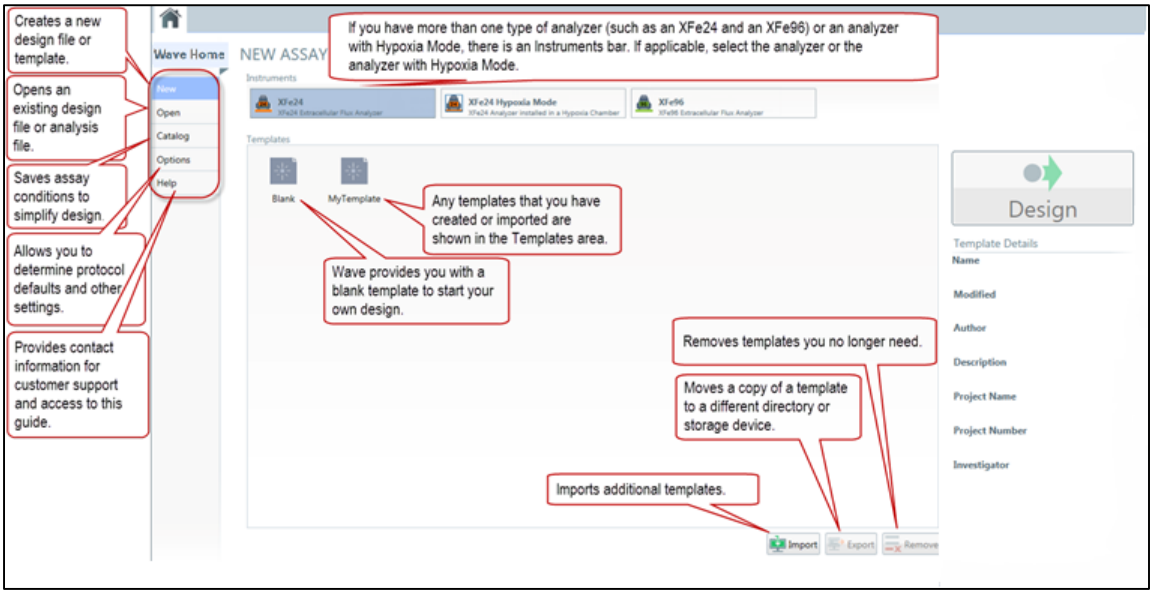




The following screenshot displays an example Excel spreadsheet that was generated by saving an analysis file in the Excel 2007/2010 (*.xlxs) format.

Wave Desktop Home Page

The screenshot below shows the Wave Desktop home page. The following illustration shows the purpose of most of the commands, buttons, and icons on the Wave Desktop home page.



Wave Desktop Home

On the left-hand side of the screen under Wave Desktop Home, there are 4 options for designing and managing default and customized assays:

New – Displays existing template files and option to create a new template or assay design file.

Open – Displays lists of recent analyses and design files and option to open an existing assay design file or analysis file.

Catalog - Saves frequently used assay conditions to simplify assay design. These conditions may include 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 and Wave Controller (XF^{e}, XF^{e}24, XF^{e}96, XF^{e}24 Hypoxia Mode or XF^{e}96 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.

Help –

User's Guide – Searchable User Guide. Click to a selected part of the guide using the Table of Contents. User Guide can also be printed with a connection to a printer.

Support – Support phone number, Support email address, link to Seahorse Bioscience Support web page.

Version – Displays the current version of Wave Desktop.

Wave Desktop Home: Instrument Modes

In Wave Desktop, under the heading NEW ASSAY, listed are all instruments selected during the initial setup of Wave Desktop. The instruments available should reflect the XF Analyzers present in the lab or that are routinely used.

The first step in creating a design file or template is to select the instrument type by clicking on the instrument tab at the top of Wave Desktop or to choose the instrument in Hypoxia Mode by clicking on the Hypoxia Mode tab:

XFp: Design and run assays on 8-well Miniplates.

XF^{e}24: Design and run assays on 24-well plates.

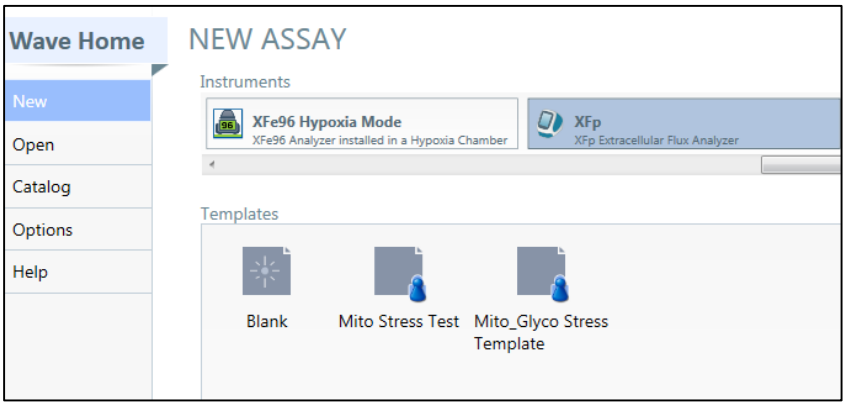
XF^{e}96: Design and run assays on 96-well plates.

XF^{e}24 Hypoxia Mode: Design and run assays in an XF^{e}24 installed in a hypoxia chamber.

XF^{e}96 Hypoxia Mode: Design and run assays in an XF^{e}96 installed in a hypoxia chamber.

Note: *If not all XF instruments in a specific lab are not available for use on Wave Desktop, the appropriate software can be installed using the Installation Guide or by contacting Seahorse Bioscience Support.*

Wave Desktop Home: New



Definitions for Templates and Analysis Files

View and manage files from the Wave Desktop Home page.

The **New** command provides access to a Templates window to view and manage all templates that have been created.

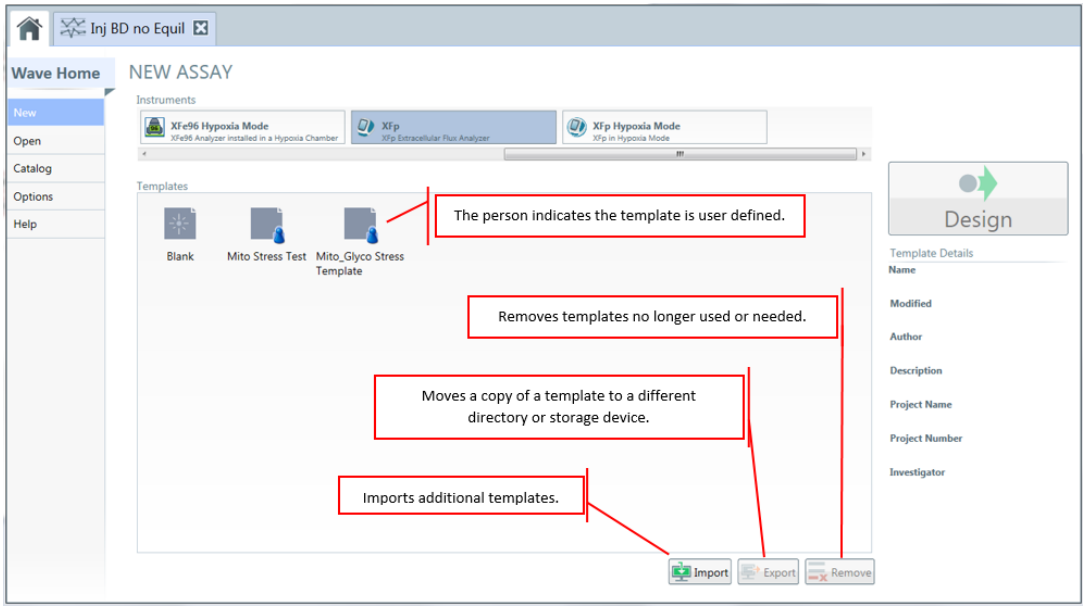
The **Open** command provides a view of all assay-related files: templates, design files, and analysis files.

Assay Template Files

A *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 re-edited for other experiments.

Note: *Templates have the file extension .asyt*.

When first opening Wave Desktop the only available template will be called “Blank”.
All customized templates will appear in the Templates pane. Each customized template that has an icon.  Manage templates by using the **Import**, **Export**, and **Remove** buttons below the Templates pane.



Import, Export, and Remove Buttons

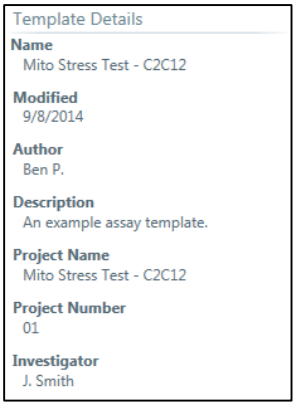
Click the **Export** button to export a template file in order to:

- Transfer template file to a flash drive, if XF^{e} is not networked.
- Transfer template file to a shared directory for multiple user access.
- Back up template file.
- Transfer template file from Wave Desktop to XFe Analyzer, if XF^{e} is networked.

To export a template, select the template and click or touch **Export**. To delete a template file, select or touch the template file and click **Remove**.

Template Details

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



Analysis Files

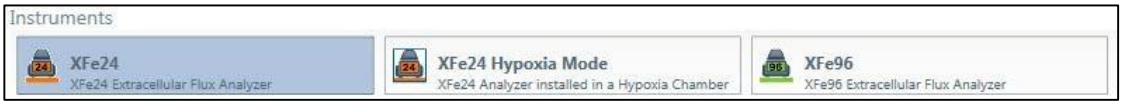
An *analysis file* (.asyr) contains data from a completed run, including all of the raw data and calculations, as well as information about the experiment's design. These files can either be used to analyze the assay data or saved as design files to start a new assay.

Assay Design Files

A *design file* (.asyd) contains all of the information needed to run an experiment, including groups and condition definitions, plate map configuration, and protocol definitions. Wave Controller on the XF^{e} Analyzer converts the design file to (.asyd) to an assay results file (.asyr) when the design is used to run samples. To reuse an assay design file, save it as a template.

Start a new Assay Template or Design:

1. If applicable, select the type of instrument on the instruments bar. The instruments bar will not be available for users with one instrument (no Hypoxia chamber). In this example, to run an assay on XF^{e}24 with Hypoxia Mode, select the XF^{e}24 Hypoxia Mode tab.



2. Select the Blank template from the Templates window.
3. Click the **Design** button to create a new Design tab 

4. Define groups and conditions. See **Define Groups and Conditions**.
5. Map groups to the plate. See Map Groups to the Plate Map.
6. Define the assay protocol. See Define a Protocol.
7. Review and run your assay. See Review and Run.
8. Open the analysis file (.asyr) to review and analyze assay results data. See Analysis.

Define Groups and Conditions

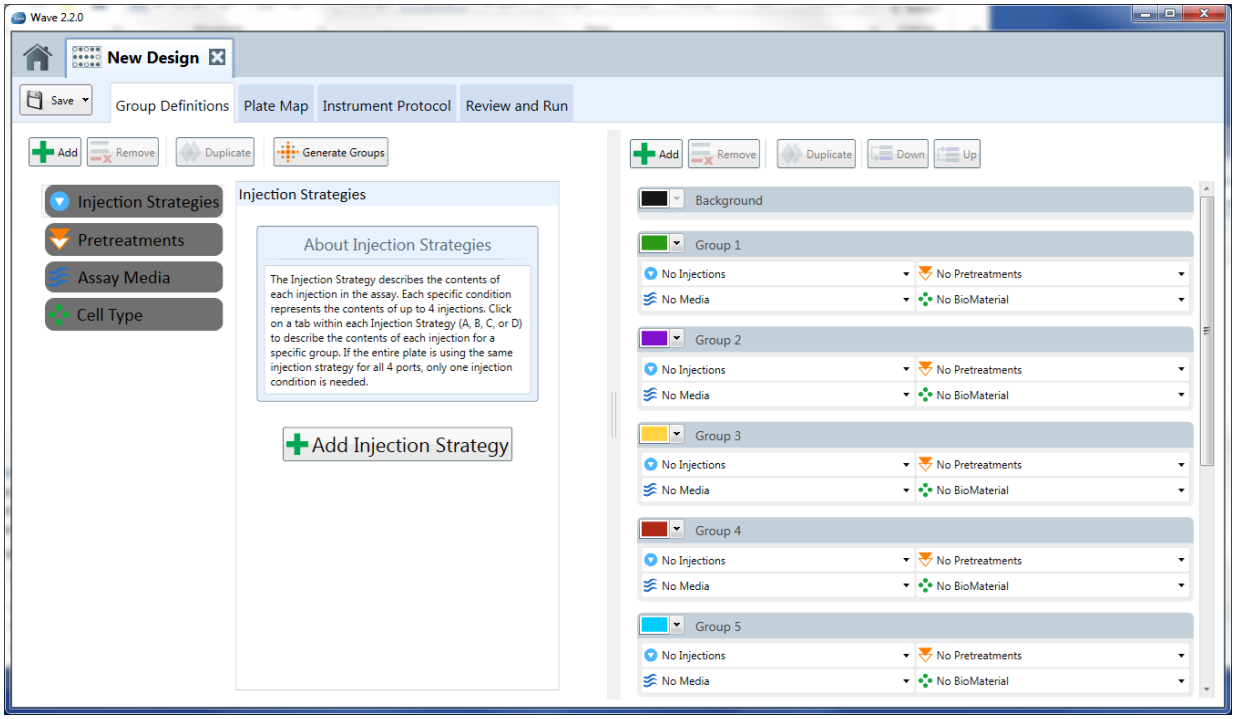
The **Group Definitions** tab is used to define Assay Conditions, Well Groups, and Plate Maps.

Groups can be added manually or define the assay conditions and have Wave generate groups automatically.

The 4 different types of assay conditions are:

- Injection Strategies
- Pretreatments
- Assay Media
- Cell Type(s)

To begin creating an Assay Design file (.asyd), click **Injection Strategies** then click **Add Injection Strategy**.



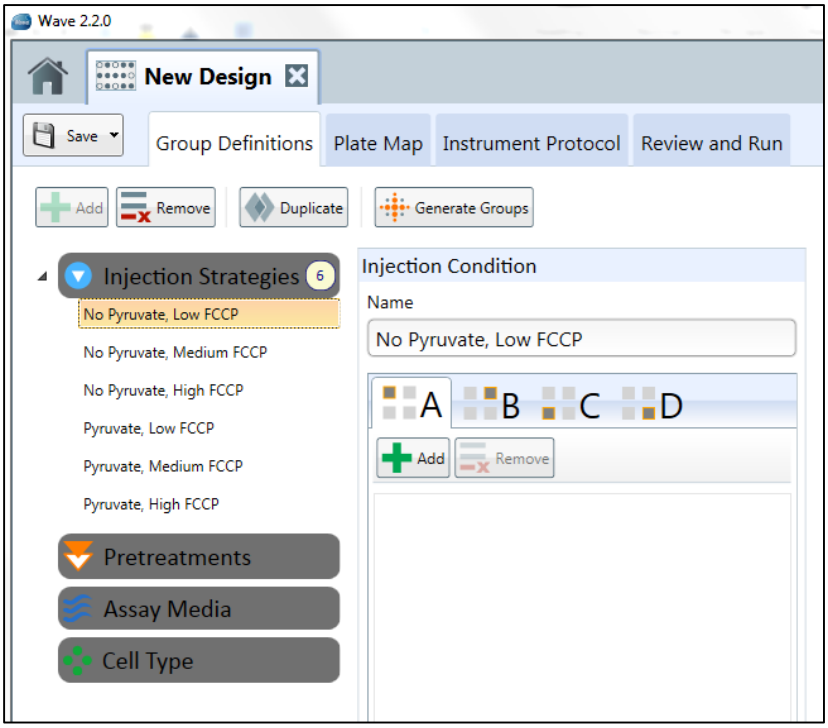
Assay Conditions: Injection Strategies

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).

To define an Injection Strategy:

1. Touch or click the **Injection Strategies** bar.
2. Touch or click the **Add Injection Strategy** button. 

3. Touch or click on the name of the new injection strategy on the left (default is **Inj. Strategy 1**) to display a dialog box. Complete the fields to name the strategy and describe the contents of each injection port. 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**.



4. Define injections for each port that will be used during the assay. Switch between ports A, B, C, and D by clicking or touching on the tabs in the Injection Condition window.

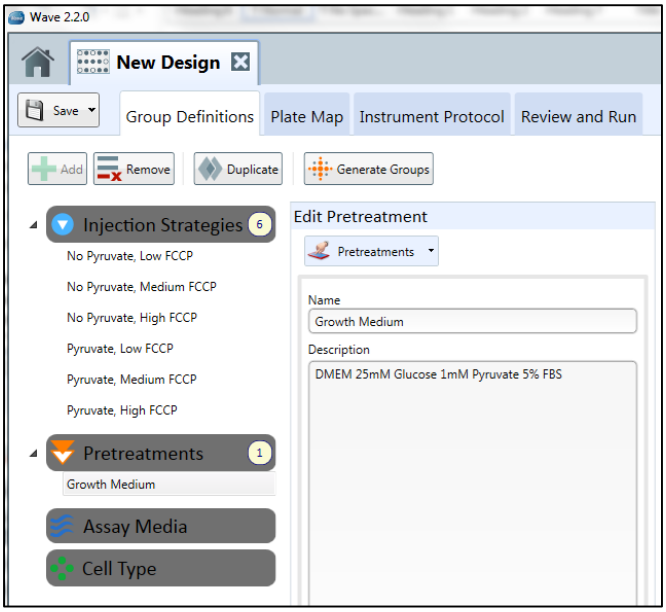
If the experiment uses the same injection strategy for all wells and all 4 ports, specify one injection strategy.

Assay Conditions: Pretreatments

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

1. To define pretreatments:
2. Touch or click the **Pretreatments** bar.
3. Click the **Add Pretreatment** button. 

4. Click **Pretreatment 1** to display the **Edit Pretreatment** dialog box. In the following example, **Pretreatment 1** has been renamed *Growth Medium*.



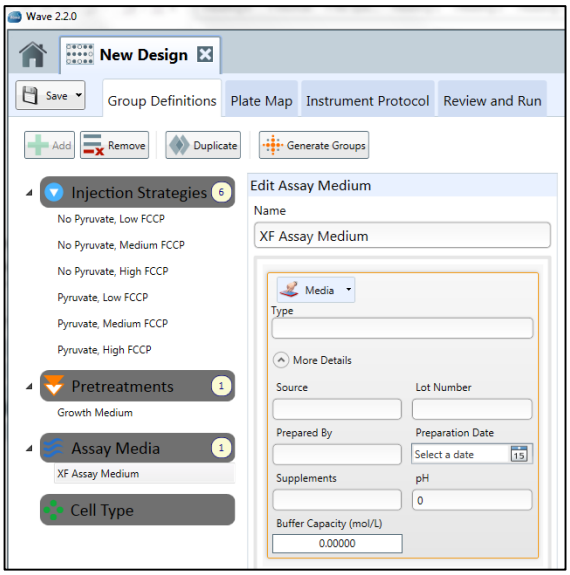
5. Enter the name of the pretreatment in the **Name** field and provide record-keeping details in the **Description** field.

Assay Conditions: Assay Media

The Assay Media condition describes the assay medium used in this assay. Record the Base medium and supplements for each medium used in the assay.

To define the medium:

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

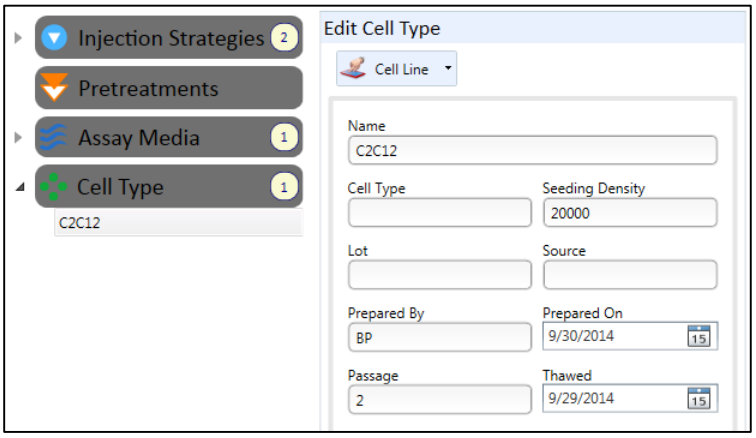


Assay Conditions: Cell Type

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

To define the cell type:

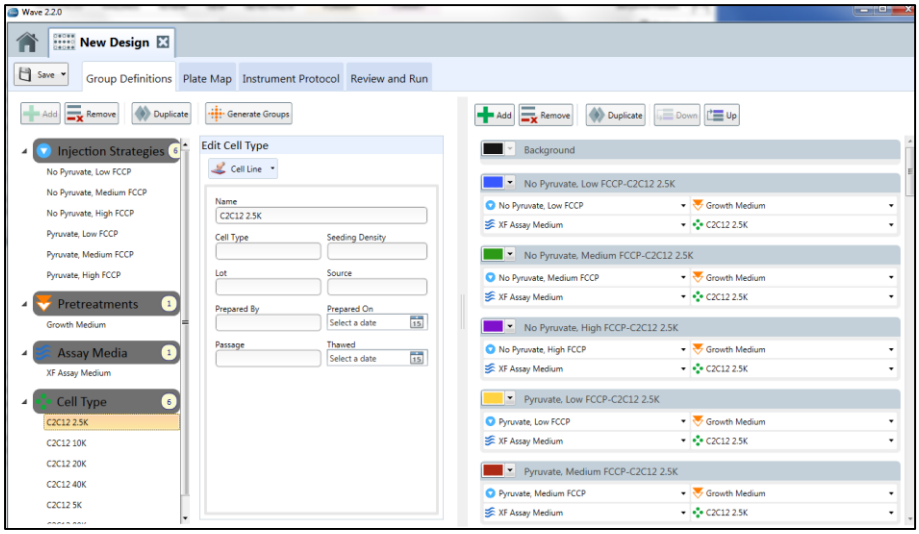
1. Touch or click the **Cell Type** bar, then click **Add Cell Type Condition** button.
2. Select **Cell Type 1** to display the Cell Type window.



3. Enter additional details in the appropriate fields

Automatically Generate Groups

Wave automatically generates groups based on the number of independent variables defined in the assay. 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, touch or click the **Generate Groups** button.

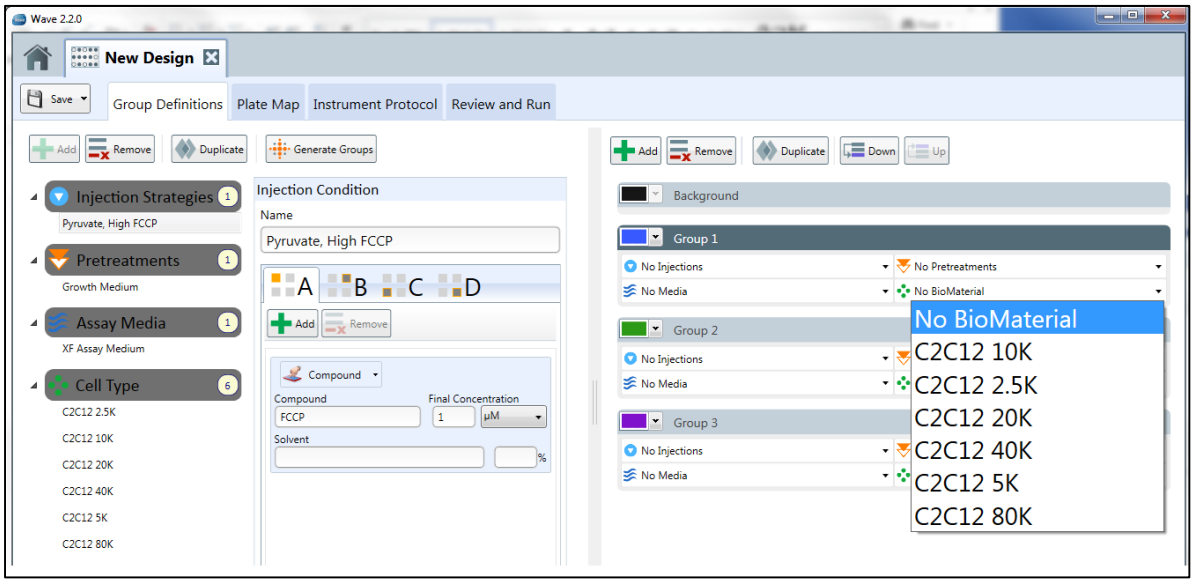


This example shows 6 cell types defined (C2C12 2.5K, C2C12 5K, C2C12 10K, C2C12 20K, C2C12 40K, C2C12 80K). There are 6 Injection Strategies and one XF Assay Medium were defined. Click **Generate Groups** and Wave generates 36 groups including every possible combination of Injection, XF Assay Medium, and Cell Type.

The combinatorial logic is 6 x 1 x 6, resulting in the generation of 36 groups. For example, if there were 3 Injection Strategies, 1 XF Assay Medium, and 6 Cell Types, the combinatorial logic would be 3 x 1 x 6, generating 18 groups.

Manually Generate Groups

Generate groups manually to select the conditions that distinguish one group from another.



To add a group:

1. Click the **Add** button circled under the Well Groups window.
2. Define the variables that make up the group by choosing from the dropdown list of conditions that have been defined.
3. Repeat steps 1 and 2 to add another group.

Once Groups have been defined, the groups must be mapped to the well plate.

Map Groups to the Plate Map

The **Plate Map** is where Wave displays information about each well in the experiment. Wave uses group assignments to calculate group statistics in the Analysis file.

Groups

There are 3 ways to determine the number of groups in each assay.

Use the **Distribute Groups** command, assign them manually, or add groups in the Plate Map tab. The quickest method is using the Distribute Groups command in Wave.

Distribute Groups

Wave can distribute groups across a plate by using the **Distribute Groups** command. This command determines the maximum number of replicates and distributes the groups in vertical sections across the plate.

When the **Well Groups** list is complete, click the **Distribute Groups** button.

Background Wells

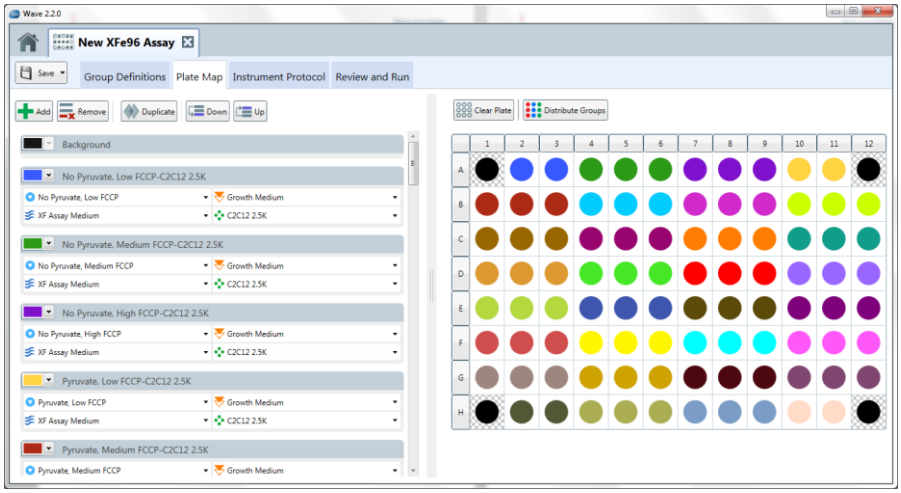
Wave will automatically default to Seahorse recommended background wells.

To change the number and locations of these background wells by clicking on the Background

tab in the Well Groups window, then click or tap on the desired well to assign it as a background well.

Define a Protocol

Click the **Instrument Protocol** tab to define the assay protocol (including measurement and injection cycles) once the groups, conditions and plate map have been determined.

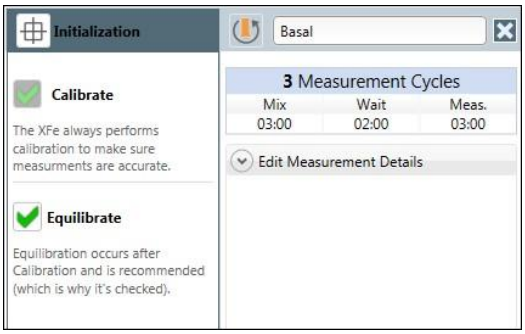


Default Commands and User-Specified Injection Strategies

The predefined assay protocol includes the following steps:

1. Calibrating
2. Equilibrating (changeable)
3. Defining the Basal Measurement Cycle (editable)

Note: *Specifying injections is entirely subjective. There are no defaults. However, if an injection is specified, the XF^e will typically take a measurement after the injection. Predefined measurement cycles are defined by default.*



Calibration

Calibration tests ensure the accuracy of the XF^e and are always the first step in a protocol; therefore, the Calibrate command cannot be omitted.

Equilibration

Equilibration ensures temperature stability before beginning an XF^e assay.

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 basal measurement prior to the first injection (predefined)
2. After each injection (injections are user-defined for each port.)

Seahorse recommends three measurement cycles for each metabolic rate (basal and post-injection). A measurement cycle consists of 3 commands: **"Mix"**, **"Wait"**, and **"Measure"**.

The **"Mix"** instruction directs how long the instrument is to raise and lower the sensor cartridge probes to ensure that analytes or compounds, following injections, are uniformly dispersed within the medium in each well.

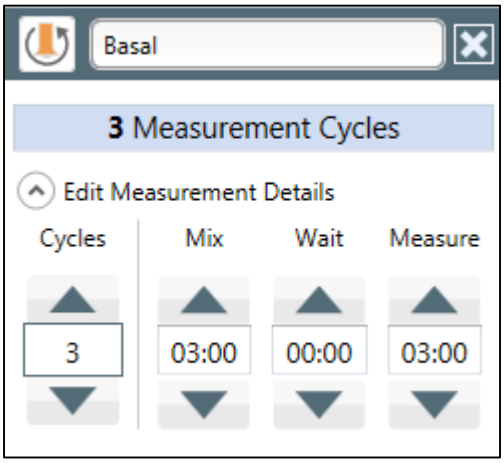
The **"Wait"** instruction (required for XF^{e}24 only) directs the instrument to delay a specified period of time after the "Mix" instruction before taking a measurement.

The **"Measure"** instruction directs the instrument how long to record the flux of analytes in the transient microchamber once the sensor cartridge probes are lowered.

Wave allows additional measurement cycles as needed.

Basal Measurement Cycle

The Basal Measurement Cycle consists of the first three measurements that occur 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.

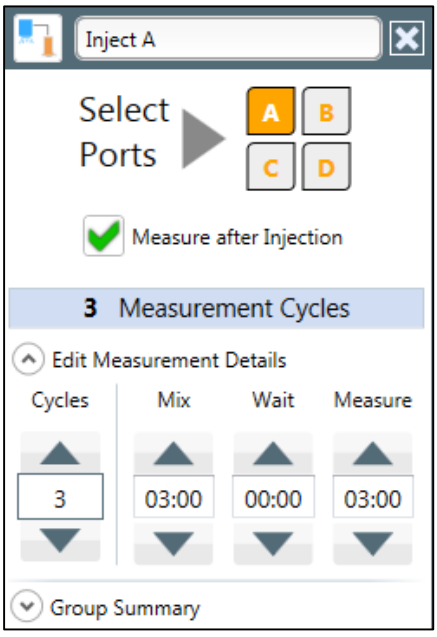


If necessary, use the up arrow  or the down arrow  to increase or decrease the number of **Cycles** for each measurement. Seahorse Bioscience recommends 3 cycles per measurement for optimal results.

Change the name of the Basal Measurement Cycle by selecting 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.



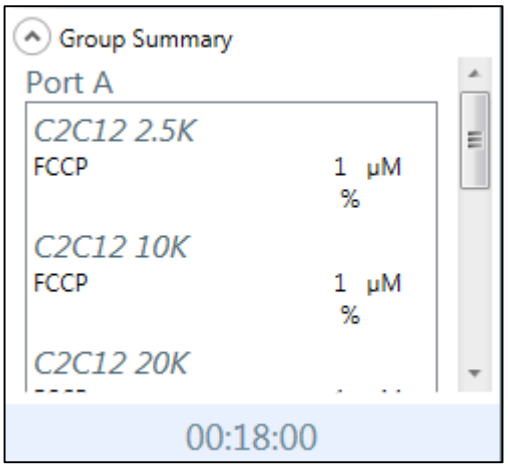
For each new injection, the software will automatically select the next available port. To change this default setting, select another port (A, B, C, or D). Only available ports are selectable; if a port is already assigned, it will be greyed out.

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; however these times and number of cycles are customizable. If a measurement is not required following an injection, uncheck the **"Measure after Injection"** box.

Group Summary

Select the **Group Summary** bar to view the compounds assigned to these ports.



Custom Sequence

Most XF assays require only measurement and injection cycles, but in some user-defined assays, a custom sequence of commands is required. In these cases, select the **Custom** button to add a custom sequence cycle to the assay. This allows for the addition of as many "mix and wait" cycles as defined by the custom protocol.

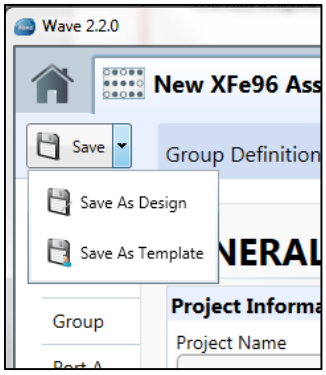
Save as Design and Save as Template

After designing the assay, save the file as either an Assay Design (.asyd) or Assay Template (.asyt).

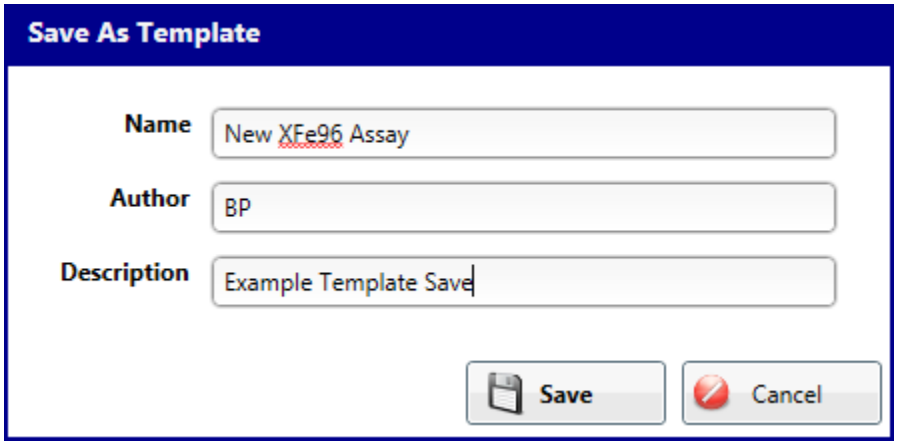
Note: *Select "Save as Template" if this assay will be run multiple times or expect to make minor adjustments for future assays. Select "Save as Design" if the assay will be run immediately after designing the file. The Assay Design file (.asyd) save location will be the same location the Assay Result file (.asyr) will be saved as well.*

To save new assay design as a template file:

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).



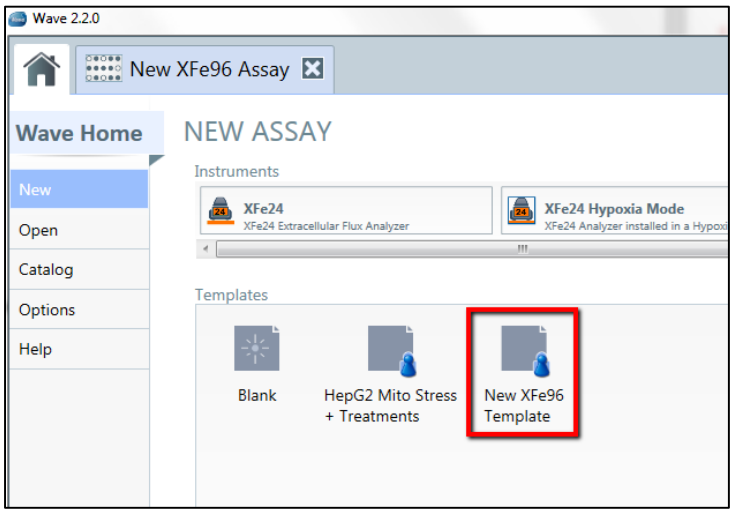
The **Save as Template** browser is displayed.



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.

Locate and access custom template files from the **New** section of Wave Home. All the custom templates created and saved appear under the **Templates** pane on that window. The following example shows how to locate template files within Wave Desktop.

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



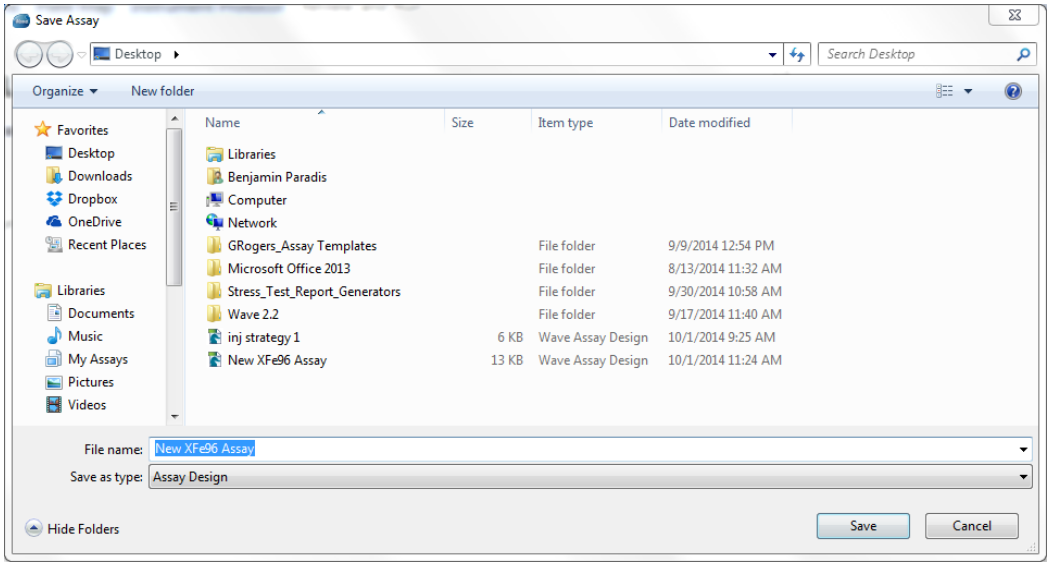
Template files are stored permanently within the Templates directory on the C: drive. View and edit the location of the Templates directory in the **Options** section of XF^{e} Home, on the **General** tab.

Open a Template in the Template Editor

To open a template in the Template Editor, right-click on the template icon  on the XF^{e} Home > New screen. Select **Edit** by left-clicking the **Edit** pop-up item.

To save a custom design as an Assay Design file (.asyd):

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



Note: *If using Wave Desktop, upload custom template file to a flash drive and transfer it to XF^{e} for use.*

Review Assay Template/Design on the XF^{e} Wave Controller

General Information

The General Information section provides editable fields for information about the assay. First the Assay Template file (.asyt) or Assay Design file (.asyd) must be transferred to Wave Controller on the XF^{e} Analyzer.

Choose to supply or edit any of the following fields:

General Information

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

Errors and Warnings
Any errors or warnings will be visible prior to running the assay on the XF^{e}

Notes/Email Recipient List

Email Recipient List

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**

Also add an email address before starting an assay on the XF^{e}. To do this:

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

Advanced

The **Advanced** tab of **Advanced Settings** allows for recording the background well buffer capacity, set port volumes for an assay and can check the software version and file version numbers.

To record a background well buffer capacity:

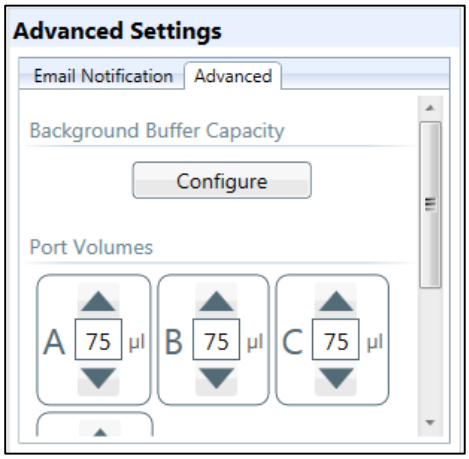
- Click **Configure** and type in the Buffer Capacity (mol/L) for each Background well specified on the **Plate Map**.

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.

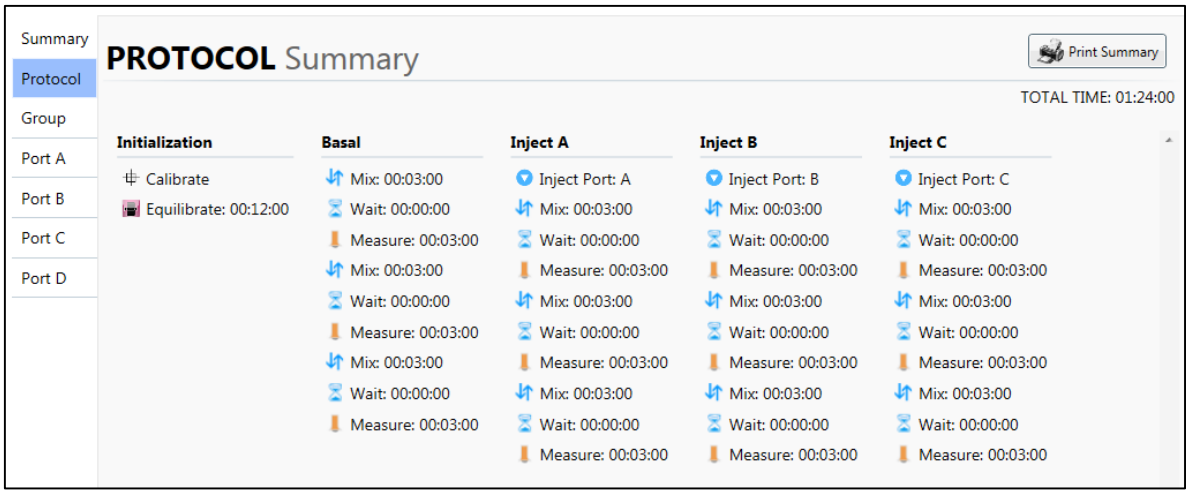
To check the version number of the software and of template file:

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



Protocol Summary

Review the protocol defined for an assay by selecting the **Protocol** link. To change the protocol, click on the Instrument Protocol tab to edit the protocol prior to running the assay design. The following illustration shows an example of a protocol summary that the Protocol link could display.



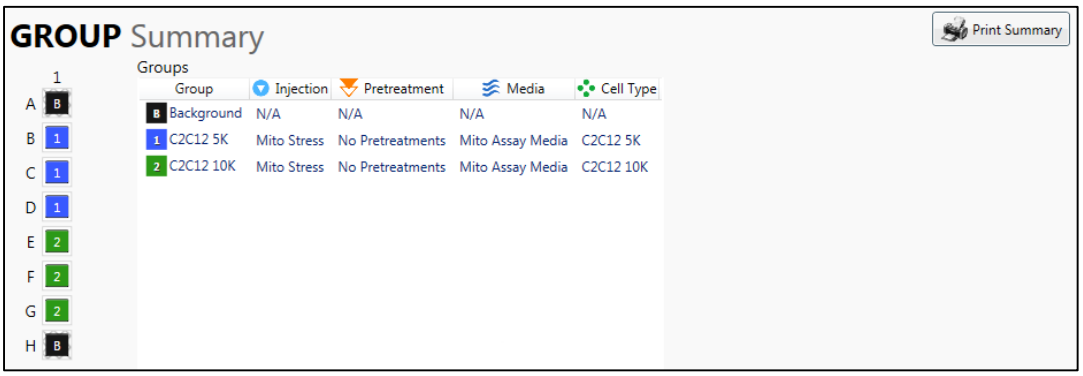
Group Summary

Click the **Group** link to review the groups that have been defined for a specified assay.

To change these group definitions:

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

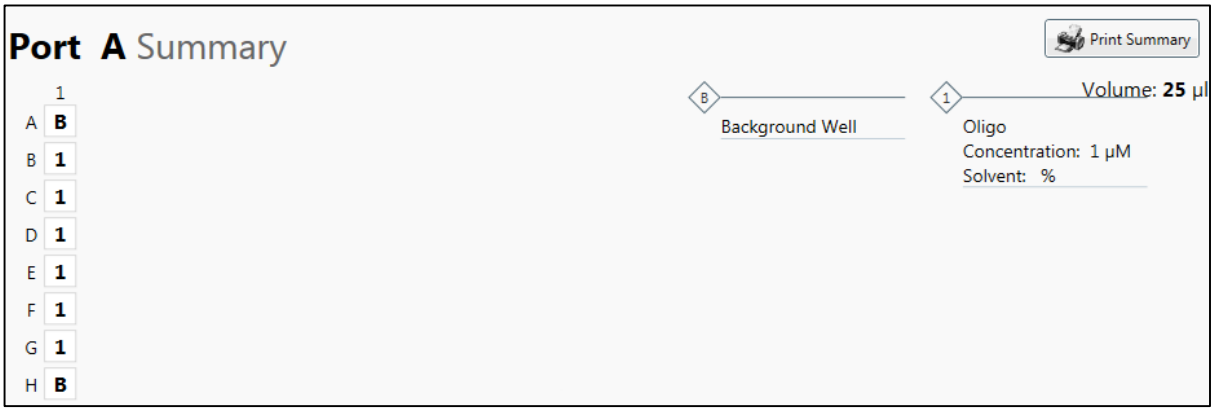
The following illustration shows an example of a group summary.



Port Summary

Select Port A, Port B, Port C, and Port D links to review the description of each injection administered through a port along with the following details:

- Injection concentration
- Name of the solvent (if specified)
- Percentage of solvent used in the injection
- Background wells
- Port volume



To change these injections, click on the Group Definitions tab prior to running the design. The following illustration shows an example of an injection port summary.

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 

button.

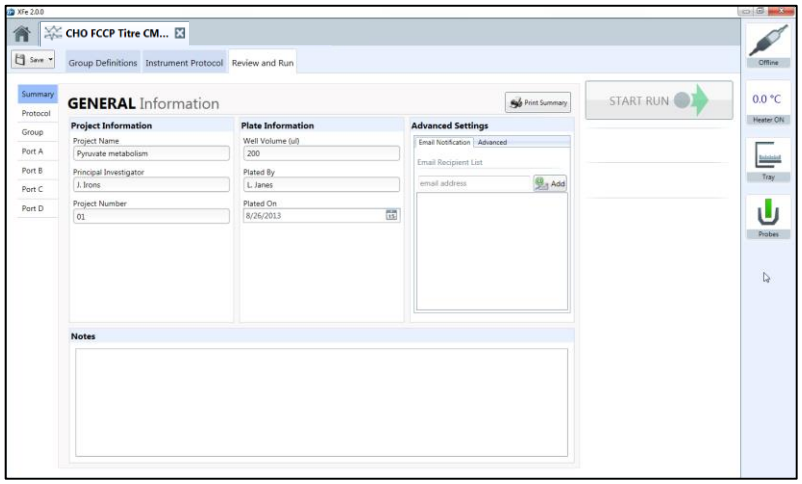
   It displays the Print dialog box.

2. Select the printer. (Use the scroll bar, circled in red, to find the printers that are available via network connection.)
3. Select the Number of copies.
4. Press the **Print** button.

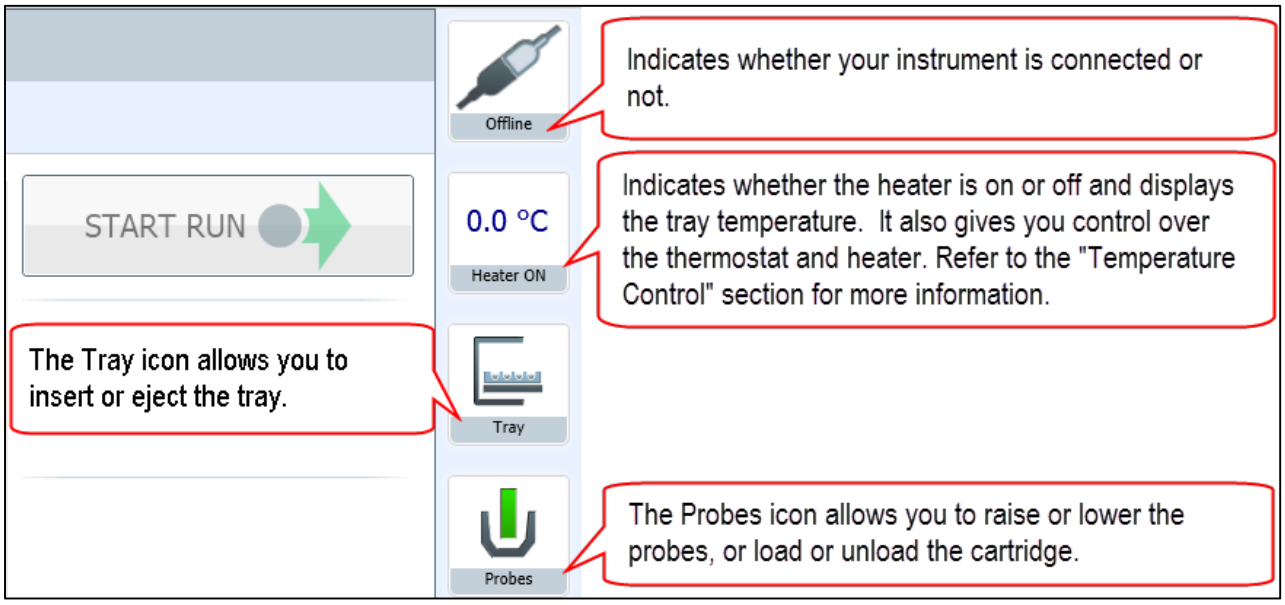
Chapter 2: Performing an XF^{e} Assay

Before running an assay on the XF^{e} Controller, the Assay Template file (.asyt) or Assay Design file (.asyd) must be moved to the XF^{e} Controller if the design or template was created using Wave Desktop. If the XF^{e} Controller is not networked, copy the template file or design file to a USB flash drive and transfer to the XF^{e} Controller.

Once the Assay Template file (.asyt) or Assay Design file (.asyd) has been transferred to the XF^{e} Controller, start the Wave Controller on the XF^{e}. When ready, Wave will display the Review and Run window as shown in the example below.

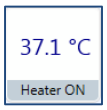


The following illustration provides a brief explanation of the icons located on the right side of the **Review and Run** tab.



Temperature Control

The Temperature icon is used for:



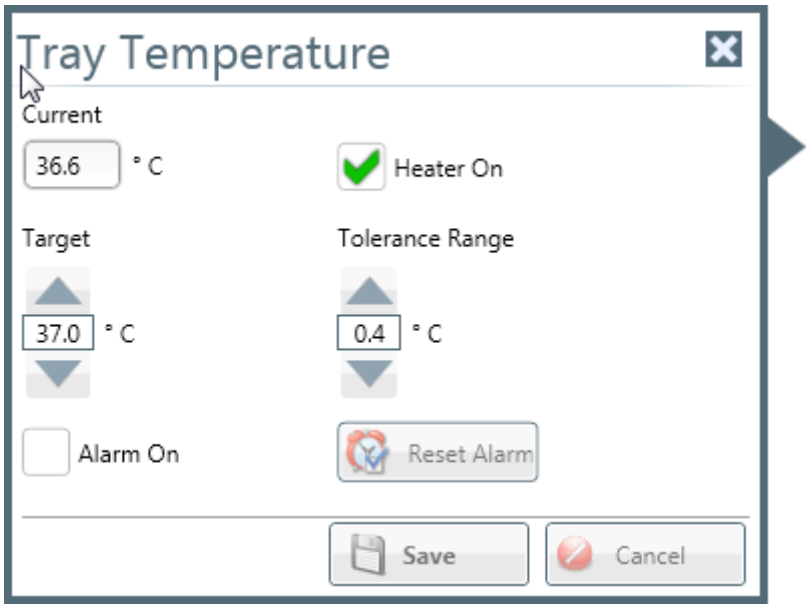
- Turning the heater ON/OFF
- Setting the target temperature.
- Setting the tolerance range. If the temperature veers from the target temperature by too much, the icon will change color.
- Automatic email notification if the temperature exceeds the tolerance range.

The following example shows how the Temperature control icon looks if the temperature has exceeded the tolerance range.

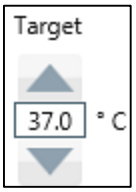
Note: *The Status Indicator on top of the XF^{e} Analyzer will also change from blue to amber if the temperature has exceeded the tolerance range.*

Specify Target Temperature and Tolerance Range:

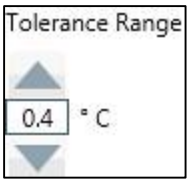
1. Click on the **Temperature** icon. The Tray Temperature window shows the current temperature, the target temperature, and the tolerance range.



2. Click on the **up** and **down** arrows to raise or lower the target temperature or type in a new number in the temperature field.



3. Click on the **up** and **down** arrows to raise or lower the tolerance range or type in a new number in this field.



4. Press the **Save** button.

Turn ON Alarm for Temperature Tolerance Range:

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

2. Press the **Save** button.

Checking the Alarm On box will cause Wave Controller to send an email notification when the tray temperature exceeds the tolerance range to all recipients specified. In order to receive this notification, specify all email addresses within **Options** (Wave Controller Home page > Options).

Turn OFF Alarm:

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

To reset the alarm after receiving notification that the tray temperature has exceeded the tolerance range:

1. Check the **Reset Alarm** button.
2. Press the **Save** 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. If the temperature exceeds the targeted range during an assay, please contact Seahorse Bioscience Support.

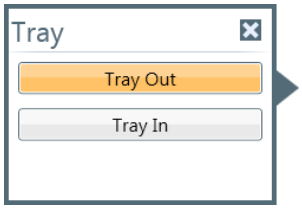
Tray Control

Prior to a run, after a run is complete, or after a run has terminated due to an unexpected error, it is sometimes necessary to eject or insert a tray.

To eject or insert the tray:

1. Click on the **Tray** icon. 

2. Click on the **Tray Out** button to eject the tray or click on the **Tray In** button to insert the tray.



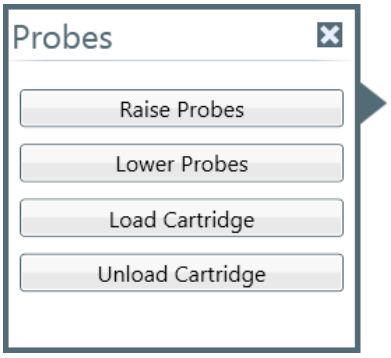
Probe Control

Prior to a run, after a run is complete, or after a run has terminated due to an unexpected error, it is sometimes necessary to raise or lower the probes, or load or unload a cartridge.

To raise or lower the probes, or load or unload a cartridge:

1. Click the **Probes** button. 

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

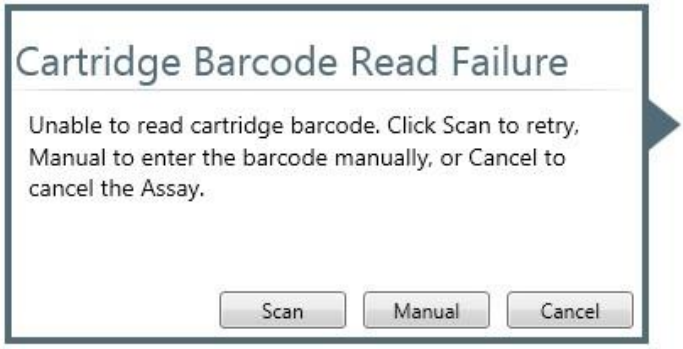


Barcode Errors

On the rare occasion that the XF^{e} Controller encounters a barcode error, Wave Controller and Seahorse Bioscience Support will assist in correcting the error to complete the run.

Cartridge Barcode Read Failure:

Wave Controller will present the following message if a barcode error occurs:

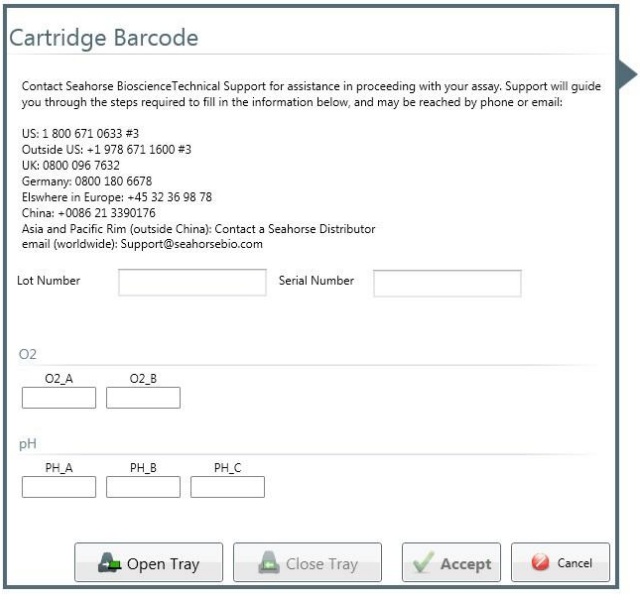


It provides 3 options:

- Scan: Click the **Scan** button to retry scanning the barcode. No other action is necessary.
- Manual: Click **Manual** to enter the barcode information manually.
- Cancel: Click **Cancel** to end the assay.

Entering the Cartridge Barcode Manually:

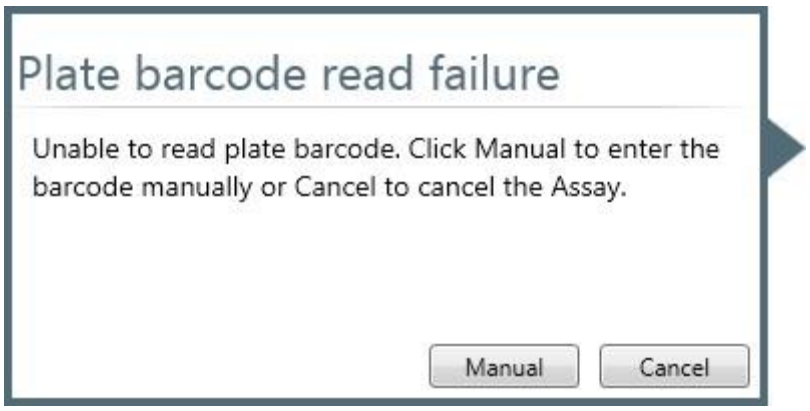
Click **Manual** to enter in the barcode manually, the window below will appear:



Contact support@seahorsebio.com or visit http://www.seahorsebio.com/support/tech-support.php for telephone numbers by region and time of day. Technical Support will provide the information required to complete this form to proceed with the assay.

Plate Barcode Read Failure

The following message will appear if a plate barcode read error occurs:



Click:

- **Manual** to enter the barcode manually.
- **Cancel** to end the assay.

Entering the Plate Barcode Manually

Plate outside the XF^{e} Controller:

1. Enter the plate barcode in the **Barcode** field. The plate barcode is on the side of the plate.

Plate inside the XF^{e} Controller:

1. Open the tray by pushing the **Open Tray** button.
2. Check the sides of the plate for the barcode.
3. Write down the number.
4. Press the **Close Tray** button.
5. Complete the Barcode Information form.
6. Press the **Accept** button.

Status Indicator

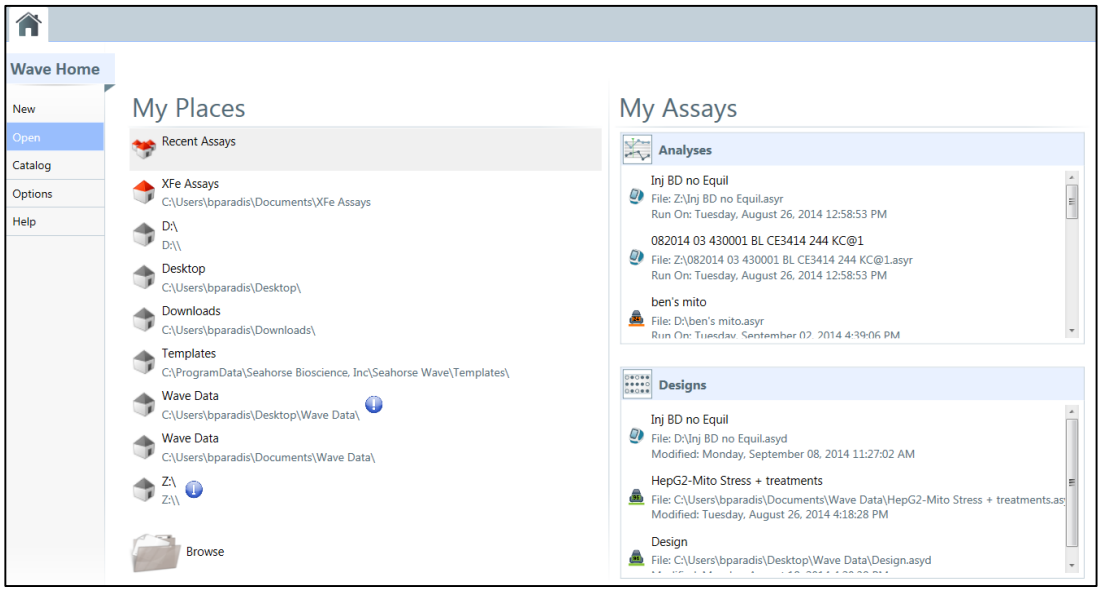
When running an assay, the Status Indicator light on top of the XF^{e} Analyzer will change color if an error has occurred or if a task requires user interaction:

- The LED will change from blue to amber, which indicates any other the following:
- Load a cartridge or plate.
- Remote a used plate and cartridge.
- To accept or refuse a calibration after one or more wells did not calibrate properly.
- Any errors that occur during the run, including cartridge barcode errors, plate barcode errors, or protocol error.

Chapter 3: Managing Assays

Access Directories, Analyses, and Designs

Select the Open command under Wave Home; the My Places and My Assays allow for quick access to recently performed and custom assays and designs.



My Places and My Assays

My Places keeps track of the directories for saved or opened assays, designs, and templates. Select **Recent Assays**, **My Assays**, or any other directory visited while running Wave, the assays and designs stored there will be visible under the **My Assays** pane on the right- hand side of the window. Recent Assays is the default directory and are in color, as shown in the following table. Those directories accessed through Wave, but are not defaults, are shown in grey scale.

Click on Recent Assays to access the most recent analyses and templates.

Click on Templates to access any assays or analyses that are stored under personal Templates directory.

Hover over an analysis or design file to find the most recent modification date, device name, project name, project number, and investigator's name.

Set the maximum number of files listed by selecting **Recent Places** in **Options**. The maximum number of files listed under Analyses and Designs in Options can also be set in **Options**.

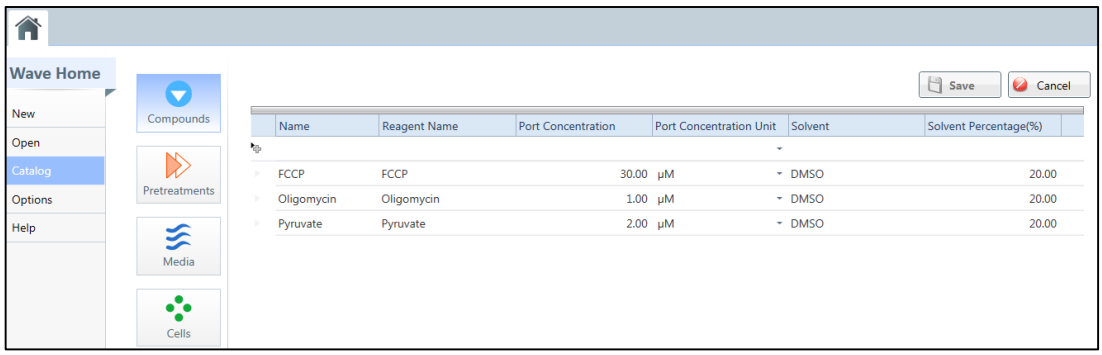
Browse

Navigate to additional assay design files and assay analysis files by using the **Browse** button at the bottom of the **My Places** pane. Open current assay files (.asyr), Wave 1.0/1.1 files (.asy), and XF files (.xfd) from the **Open Assay** window.

Note: *Although Wave will convert XF data from previous versions, check the converted files to ensure that the information is complete. See* ***Converting & Analyzing XF-Generated Data Files with Wave****, available on Seahorsebio.com for complete instructions.*

Access to Predefined Conditions

Define conditions in the Catalog. When beginning a new assay design, these predefined conditions will be available for selection from the dropdown list for each condition. Define **Compounds** (used in Injection Strategies), **Pretreatments**, **Assay Media**, and **Cell Types**.



To add a specific condition:

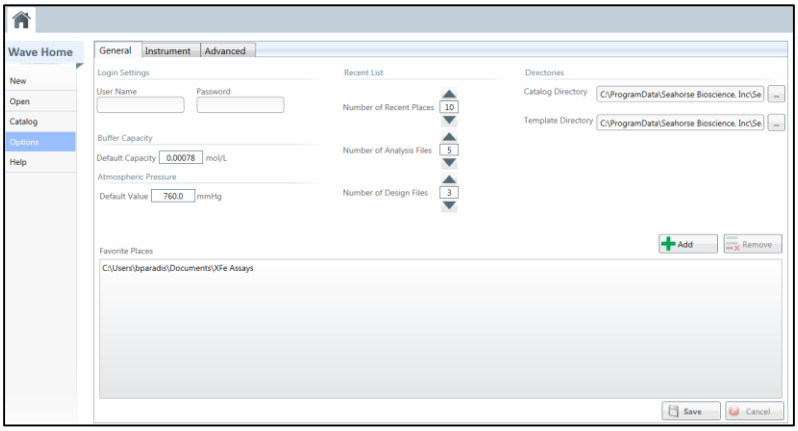
1. Choose a condition, such as Compounds.
2. Double click under **Name** to start typing.
3. Fill out the appropriate fields describing this condition. The **Name** field is required; the other fields are optional.
4. Press **Enter**.
5. Add the next condition by following steps 1-4.
6. When finished entering condition definitions, press the **Save** button on the top right- hand corner of the page.

Manage the conditions available on Wave by adding and removing them from the list. To find the location of a specific user catalog directory, use **Options**.

Share these definitions by copying the catalog files to a USB flash drive and then pasting them into the Catalog directory of a different computer or a different XF instrument.

Customizable Default Settings

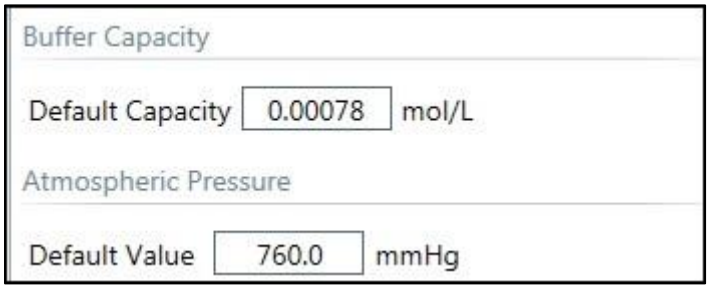
The **Options** screen contains default settings on 3 different tabs: **General**, **Instrument**, and **Advanced**.



General Tab

General allows for:

- Changing a password or user name under Login Settings
- View default settings for buffer capacity and atmospheric pressure.
  - ***Note:*** *The **Atmospheric Pressure** can also be changed on the XF^e Controller by clicking **Options** (Wave Home Screen) > **General** tab*



Buffer Capacity

Seahorse Bioscience determined the default buffer capacity setting for XF Assay 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 can also be adjusted in the assay design file.

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 determined.*

Atmospheric Pressure

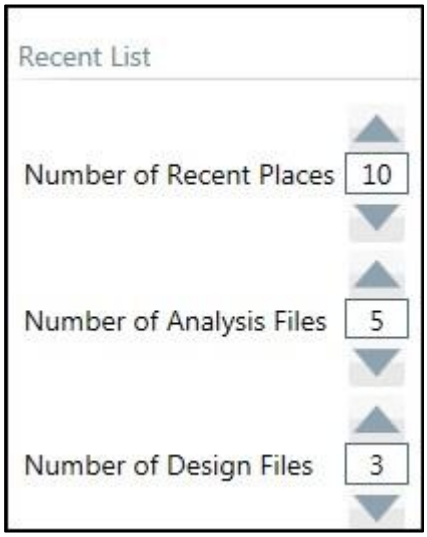
Change the atmospheric pressure when running an assay at high altitudes or if the atmospheric pressure in the lab differs from the default value of 760.0 mmHg.

Note: *Changes to this value do not cause changes in the atmospheric pressure within the instrument. Rather, this field is used to record the atmospheric pressure in a laboratory for purposes of calculating the partial pressure of oxygen in the assay wells.*

These are settings that can change from one assay to the next but that are usually defaulted to specific values.

Recent Lists

With **Recent Lists**, change the maximum number of directories and files that Wave displays under **My Places** and **My Assays** by typing in the new values or using the up and down arrows.



After specifying the maximum number under each setting, click the **Save** button at the bottom of the screen

Directories

The **Directories** column shows the default location of the Catalog directory and Template Directory.

To change the location of either of these directories, click on the  button to the right of the directory field to browse to the preferred directory or type the path name of the directory in the directory field.

After specifying a new directory, click the **Save** button at the bottom of the screen.

Favorite Places

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

To add a folder to the list of folders that Wave displays under My Places, click the **Add** button to browse to the location of the folder on the Select Folder dialog box, and then click the **Select Folder** button.

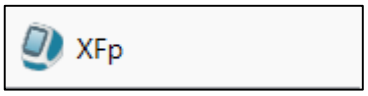
To remove a folder from the list of folders under My Places, select the folder and press the **Remove** button.

After adding or removing a directory, click the **Save** button at the bottom of the screen.

Instrument Tab

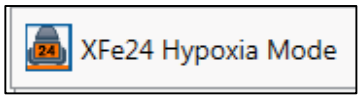
The **Instrument** tab shows the instrument or instruments that are available for data acquisition and analysis and customization of the default values for protocols, port volumes, and well volumes.

For XF^{e} there are four possible instrument configurations:

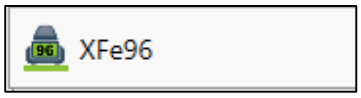


XFp – Perform assays on 8-well plates.

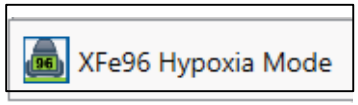
XF^{e}24 – Perform assays on 24-well plates.



XF^{e}24 Hypoxia Mode – Perform 24- well assays in a hypoxia chamber.



XF^{e}96 – Perform assays on 96-well plates.



XF^{e}96 Hypoxia Mode – Perform 96- well assays in a hypoxia chamber.

Select the type of XF instrument, then select the other default values for this instrument within the Instrument window.

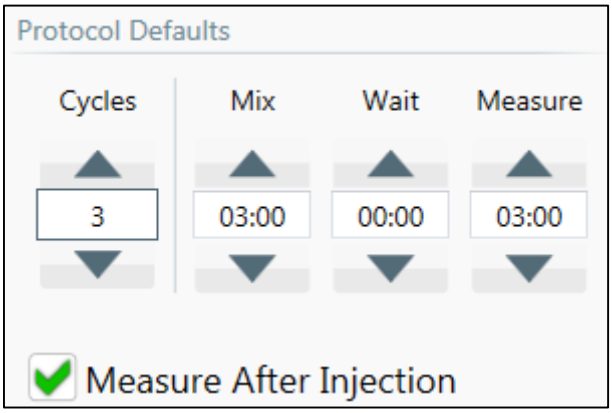
Enter default values for:

- Number of cycles in a protocol
- Number of minutes and seconds for **Mix**, **Wait**, and **Measure**
- Port volume (recording only)
- Well volume (recording only)

The following sections describe how to enter these values.

Protocol Defaults

Seahorse recommends to start with the default settings for Cycle count as well as Mix, Wait, and Measure times. To change the default times for mix, wait, and measure, and number of cycles, type in the new values or use the up and down arrows.

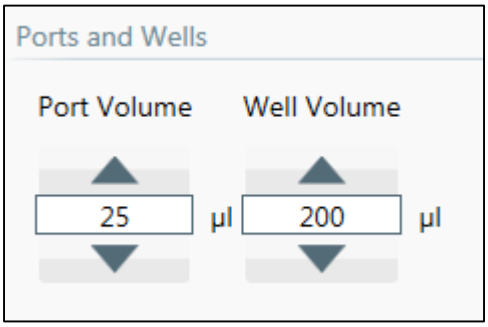


By default, XF^{e} will measure after each injection. To change this, uncheck **Measure After Injection**. After making changes, click the **Save** button at the bottom of the screen.

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.*



To change the port volume or the well volume:

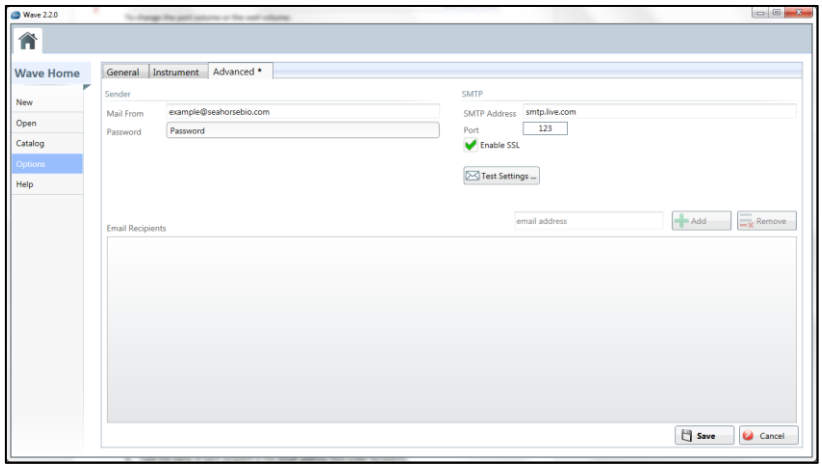
1. Type in the new value in the **Port Volume** field or the **Well Volume** field or use the up and down arrows to increase or lower the value to the correct amount.
2. Click **Save** at the bottom of the screen to save changes.

Advanced Tab

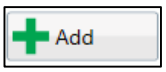
Enable email notification within the Advanced tab for the following occurrences:

- After calibration has completed
- After successfully finishing the run (this email notification includes the results file as an attachment.)

The example below shows the configuration for sending notification through Outlook or Hotmail.



In order to have XF^{e} notify email accounts of the status of every assay that is performed:

1. Supply an email address in the **Mail From** field and supply a password for this address in the **Password** field.
2. Specify an SMTP address in the **SMTP Address** field and the access port in the **Port** field.
3. Check Enable SSL if the IT configuration requires it.
4. Type the name of each recipient in the **email address** field under Recipients.
5. Click the **Add** 

button.
6. Repeat Steps 2 and 3 for each recipient.

When finished adding recipients, press the **Save** button.

To check that personal mail settings are valid:

1. Click the **Test Settings** button after adding a personal email address to the Email recipients
2. Check email to ensure that notification has been received.
3. Check Junk E-mail if email is not in the Inbox.

To take names off the list of recipients, select the name and press the **Remove** button.

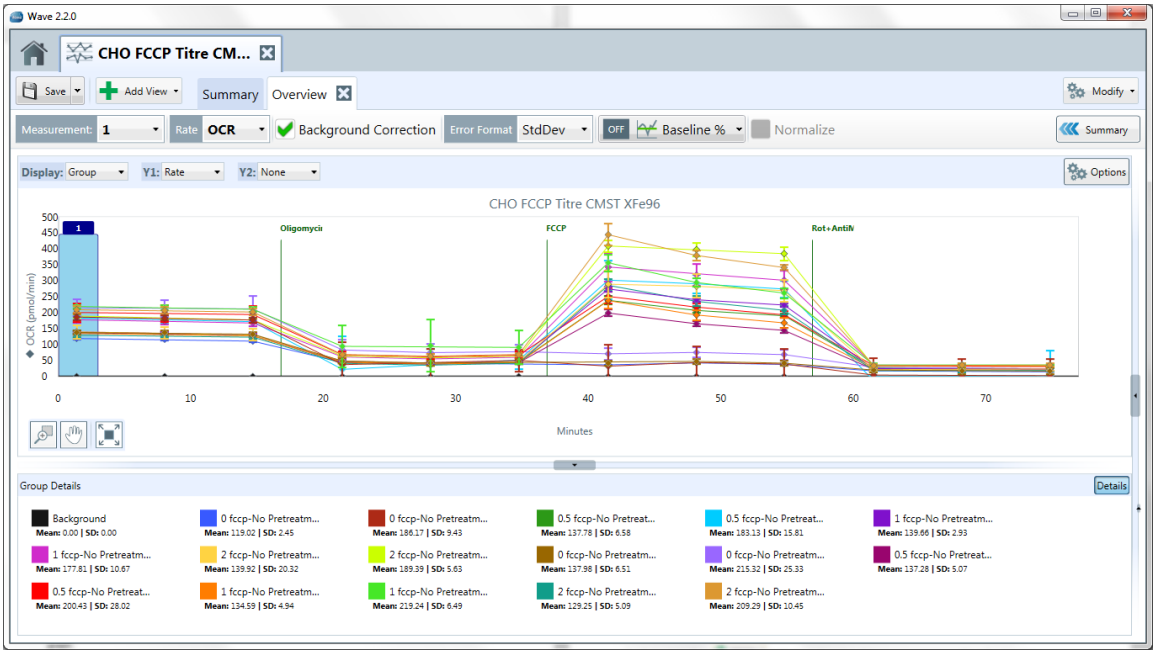
Chapter 4: Analyzing an XF^{e} Assay

This chapter describes the 4 different views available and explains how to customize these views. The 6 task-oriented views that to aid in analysis are:

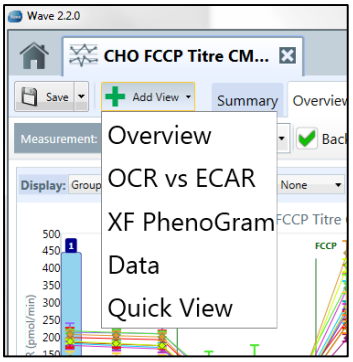
- Overview
- OCR vs. ECAR
- Metabolic Switch
- Data
- Quick View
- Summary

Analysis: Overview

Overview displays kinetic graphs for all rates, and where to perform common tasks, such as excluding outlier wells. Group statistics are also calculated and displayed within this view.



To display **Overview** select **Add View**  in the top left region of the screen and choose **Overview** from the dropdown menu.



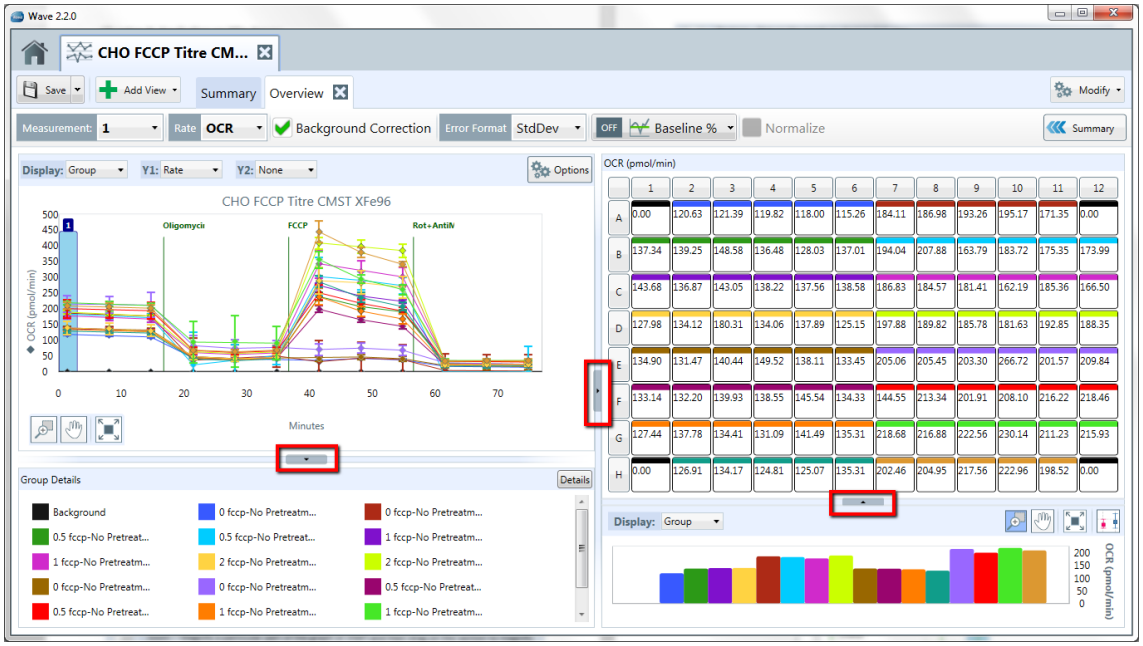
Add any number of **Overview** tabs by selecting the **Add View** button in the top left region of the screen and choosing **Overview** from the dropdown menu.

This section describes the different parts of the **Overview** screen and explains how to customize each part for a specific experiment:

- Kinetic graph
- Rate details
- Bar graph

Arrows for Resizing a Graph or Chart

To resize a graph or chart, click the resize arrow as depicted in the following illustration (arrows are circled in red).



To expand the kinetic graph horizontally, click the arrow circled in the middle of the screen, as shown in the following illustration.

To decrease the size of the graph, click the same arrow circled on the right-hand side of the graph.

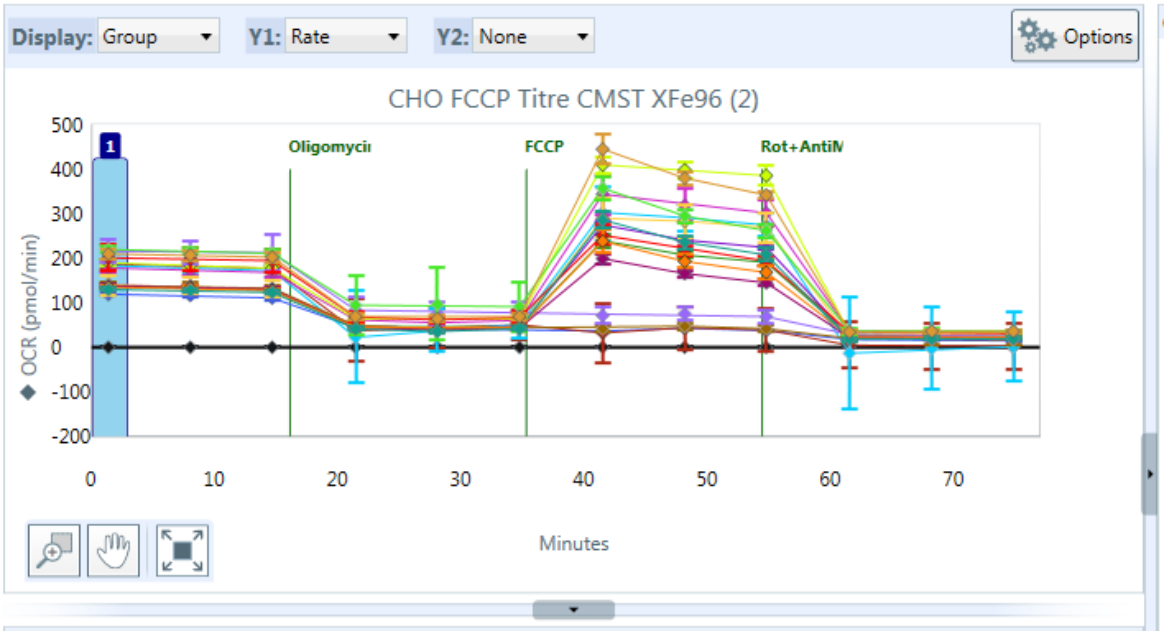
Zoom, Pan, and Restore Buttons

There are 3 buttons available on each graph and chart in the **Overview** and the OCR versus ECAR view.

	Zoom – Magnify a particular part of the graph or chart and then drag on the section to magnify.
	Pan – Move around the magnified area and move the cursor around the graph or chart.
	Restore – Return the graph or chart to full view.

Kinetic Graph

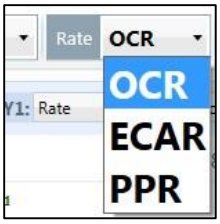
A *kinetic graph* is the most common way to display data from the XF^{e} Controller.



OCR, ECAR, or PPR

There are three rates measured by the XF^{e} Controller :

- Oxygen Consumption Rate (OCR)
- Extracellular Acidification Rate (ECAR)
- Proton Production Rate (PPR)



The default view displays time on the X-axis (in minutes) and rate on the Y-axis. Change the displayed rate (OCR, ECAR, or PPR) by selecting it from the Rate dropdown menu above the graph.

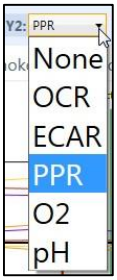
- *OCR*: Oxygen Consumption Rate data (pmol/minute) will be displayed.
- *ECAR*: Extracellular Acidification Rate data (mpH/minute) will be displayed.
- *PPR*: Proton Production rate (pmol/minute) will be displayed.

Raw Level Data

Display the O_2 or pH raw level data used to calculate the rates. To view the raw level data, select "**Level**" from the **Y1:** dropdown menu.

OCR, ECAR, PPR, O_2, or pH Overlay

To overlay two rates on the same graph, select a different rate or measurement from the **Y2:** dropdown menu.



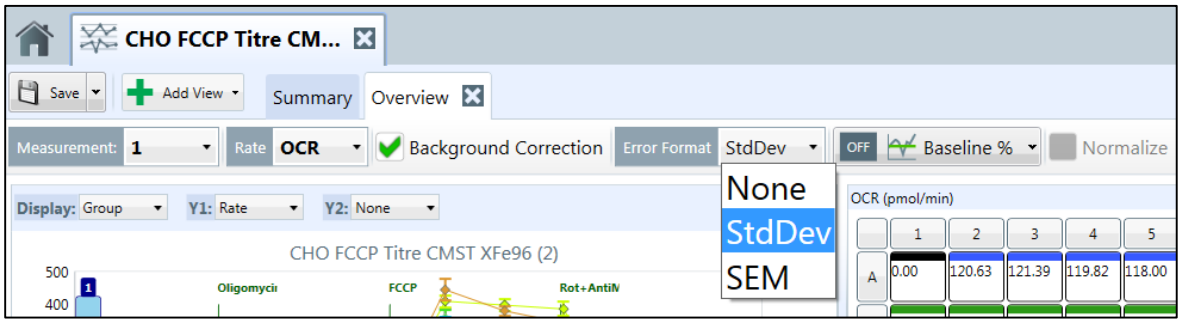
When a rate is selected, Wave will display the rate for the Y2 axis and the rate for the Y1 axis on the same kinetic graph so the rates can be compared.

When O_2, is selected, Wave will display the O_2 (mmHg) measurement level for each group. The software will show the exact numeric measurement hovering the mouse cursor over the group line on the kinetic graph.
When pH is selected, Wave will display the pH measurement for each group. Similarly, the software will show the exact pH by hovering the mouse cursor over the group line on the graph.

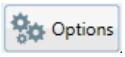
Data for O_2, pH level, or rate data, can be viewed for each well rather than the group. To view data or each well on the graph, select **Well** from the **Display** drop down menu.

Standard Deviation and Standard Error of the Mean

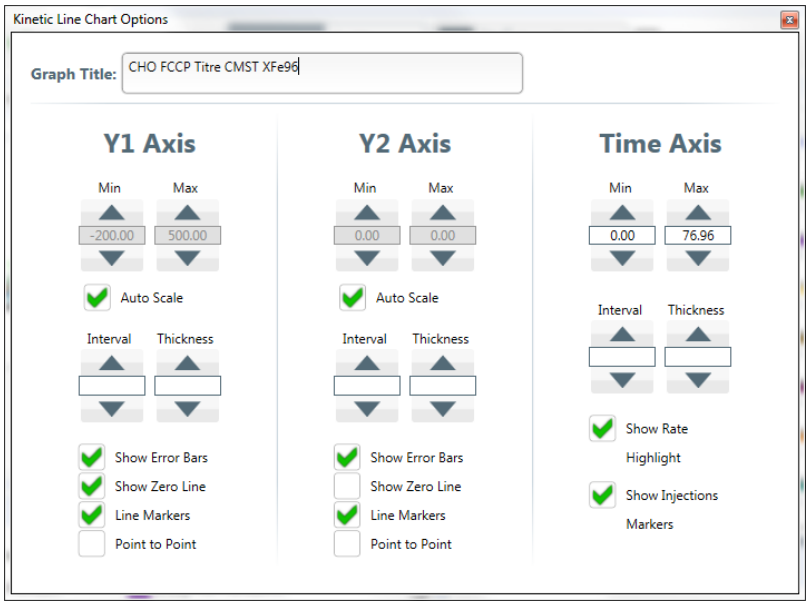
Select the Error Format dropdown list to view the standard deviation (Std Dev) or the standard error of the mean (SEM).



Kinetic Graph Customization

Select **Options** 

(located in the upper right-hand corner of the pane) to manipulate the kinetic graph visualization.



Configure specific settings for each axis using the window above:

- The Minimum, Maximum, and Interval fields sets the scale of each axis.
- The Thickness field allows for visualization of lines on the graph (1–thinnest, 5–thickest) that delineate each interval.
- The Show Error Bars box to toggle error bars on or off.
- The **Show Zero Line** box displays a horizontal line originating at the zero point on the x- axis for reference.

- The **Point-to-Point** check box shows point-to-point rates for a particular axis.
- The **Show Rate Highlight** check box highlights the selected measurement with a blue vertical marker.
- The **Show Injections Marker** check box shows when each injection occurred.

Append to Summary

Overview content can be appended to the summary tab in two ways:

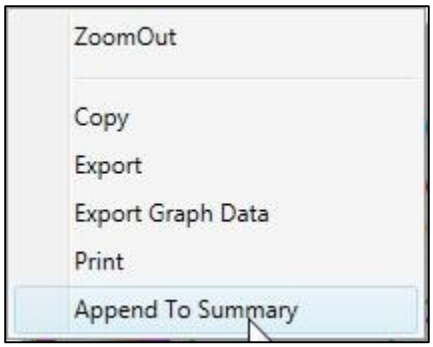
Add all the information in the Overview tab to the Summary tab:

Click **Summary** 

in the top left-hand corner of the **Overview** screen. This will append all of the graphs and charts on the screen onto the **Summary**.

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

Right click the graph and select **Append to Summary** from the dropdown list. To view appended content, select the **Summary** tab.



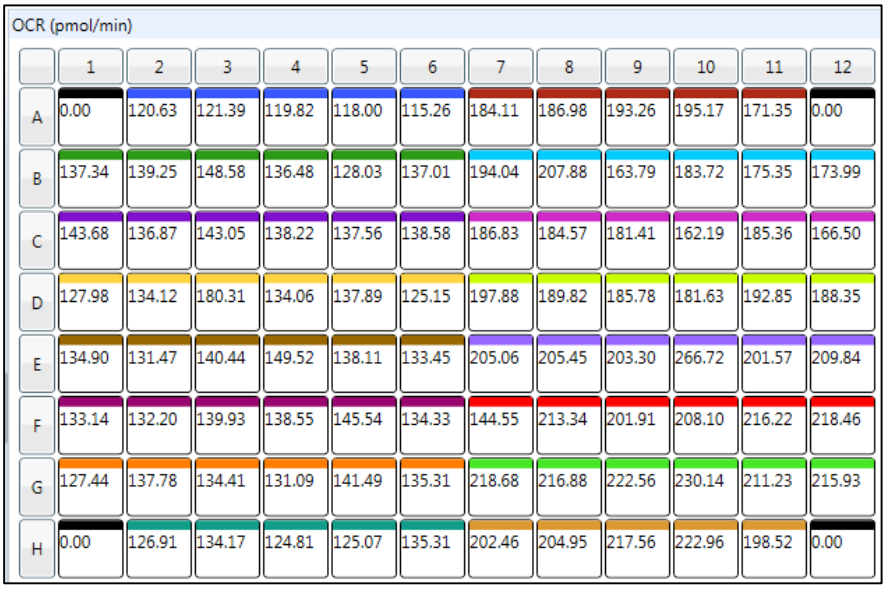
Rate Details and Bar Graph

The following sections explain how to understand and manipulate data in the following parts of the screen:

- Rate details
- Bar graph
- Group details

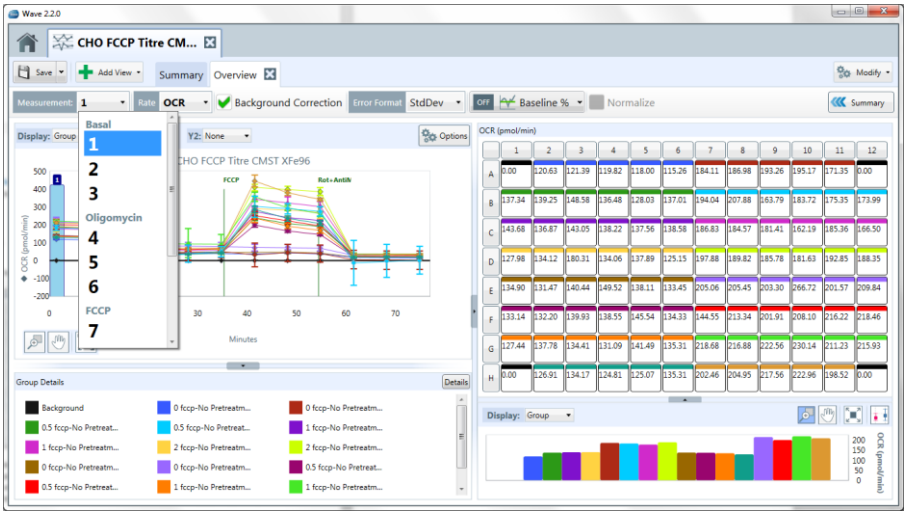
Rate Details

In the upper right-hand corner of the **Overview** tab, the **Rate Details** grid is displayed.



	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

The image above shows individual rate data points for each well at a specific measurement point. To change the measurement point displayed in the **Rate Details** grid, choose from one of the points in the **Measurement** dropdown menu, which is on the top left-hand side of the screen as shown below. To exclude a well from analysis, simply click the well and it will change from a white background to gray background (see above image).

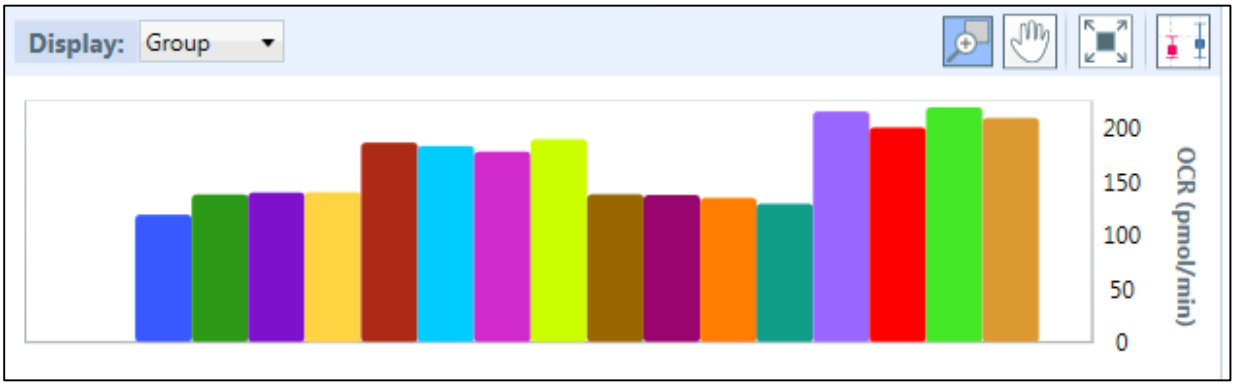


The measurement point that is selected will be highlighted in a blue column on the kinetic graph as a reminder of which rate data, bar graph, and group details are being displayed in the **Rate Details** grid. If OCR is selected in the Rate dropdown menu, Wave will display OCR in the Rate Details grid. In the previous illustration, Injection 1 is selected; therefore, this measurement is highlighted in blue on the kinetic graph, and the OCR rate measurements are reflected in the Rate Details graph.

Exclude individual wells from the Rate Details grid so that Wave excludes them from the kinetic graph and the group statistics data. When a well is excluded, Wave will not show it on the graph and will exclude the well from group statistics calculations. Use the column (numbers) and row (letters) buttons to exclude entire sections of the grid.

Bar Graph

Rates can be viewed in the Details bar chart found below the Rate Details grid. The rate data can be displayed by group or by well.



To see the rate data by well, use the **Display** dropdown menu, circled in red on the preceding illustration and select **Well**. Use this tool to view group differences for a particular measurement (group mode), or to help identify outliers within specific groups (well mode).

Plate Adjustments

The **Plate** layout shows two types of information:

- Grid of the individual rate data points for each well at a specific measurement point
- Group details that include the mean and standard deviation for the selected rate

Plate View is useful for searching for outliers to eliminate them from the calculations. An *outlier* is an observation that is numerically distant from the rest of the data.

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

The plate layout is visible on the right side of the **Overview** tab. The following illustration shows an example of the Plate view for an XF^{e} Cell Mito Stress test.

OCR (pmol/min)

	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

To eliminate outliers:

- Click on any well that is an outlier. The background color of the well will change to gray 

to reflect that it is no longer part of the rate calculation on this tab.

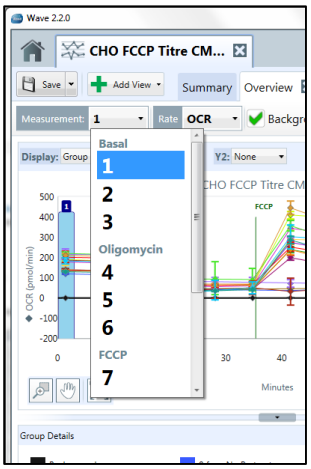
To include a previously eliminated well:

- Click on the well again, and Wave will include it in the rate calculation and its background color 

will return to white.

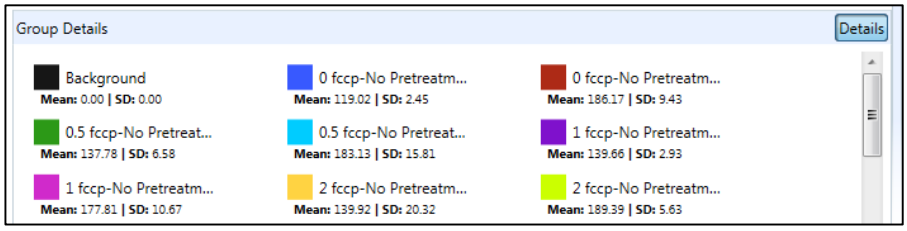
To change the measurement point:

- Click on the downward-pointing arrow to the right of the Measurement field and select the measurement point from the dropdown list.



Group Details

The final display found in the **Overview** tab is the **Group Details** pane (lower left side of the screen).



Group Details has two functions:

1. Legend for both the kinetic graph and the rate details grid and bar chart. The group colors shown in these visualizations correspond with the group names listed in the Group Details pane.
2. Display group statistics.

To view the group statistics, select the **Details** button in the upper-right corner of the window, which displays the mean and standard deviation for each group in the experiment. To see the standard error of the mean (SEM), change the display from standard deviation to **SEM**.

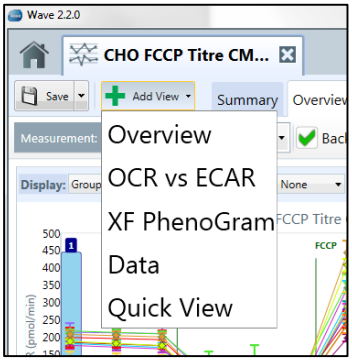
Note: *Wells that have been previously excluded in the Rate Details grid will automatically be excluded from the calculation of these metrics. Double-clicking on the colored square for the group will prevent the group from being displayed on the kinetic graph but will not exclude the wells from these calculations. However, the mean and standard deviation of wells in excluded groups will change to Mean: 0.00 and Standard Deviation: 0:00. Double click the square again to turn a group back on.*

Analysis: OCR vs. ECAR View

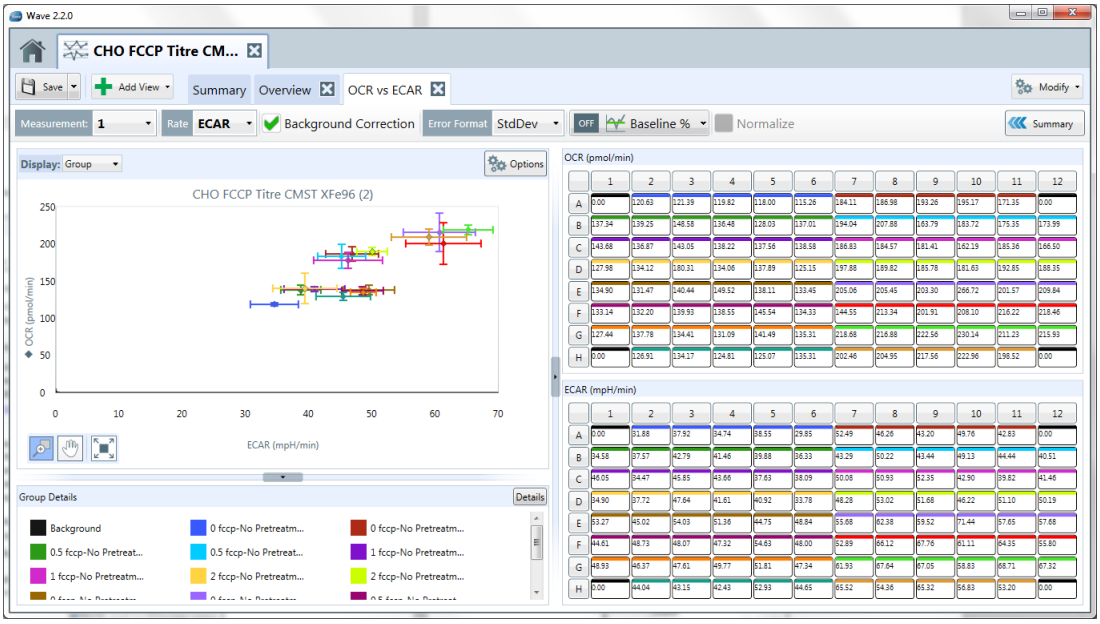
The **OCR vs. ECAR** view displays the OCR on the Y1-axis and ECAR on the X-axis.

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.

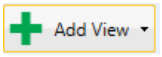


The following illustration shows an example of the OCR versus ECAR view.

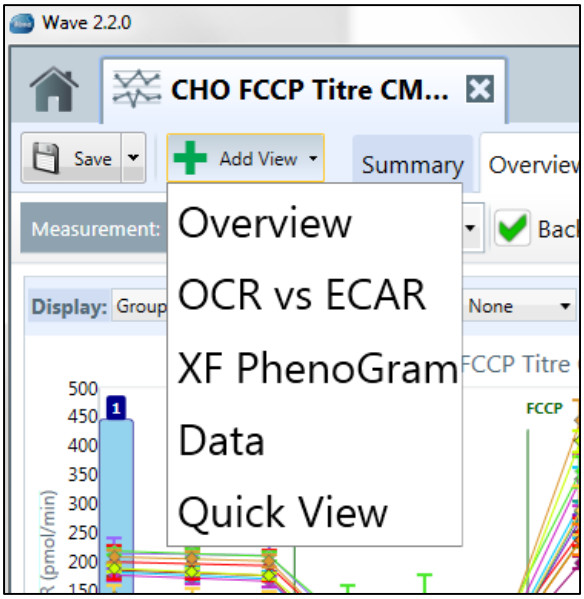


Analysis: XF PhenoGram View

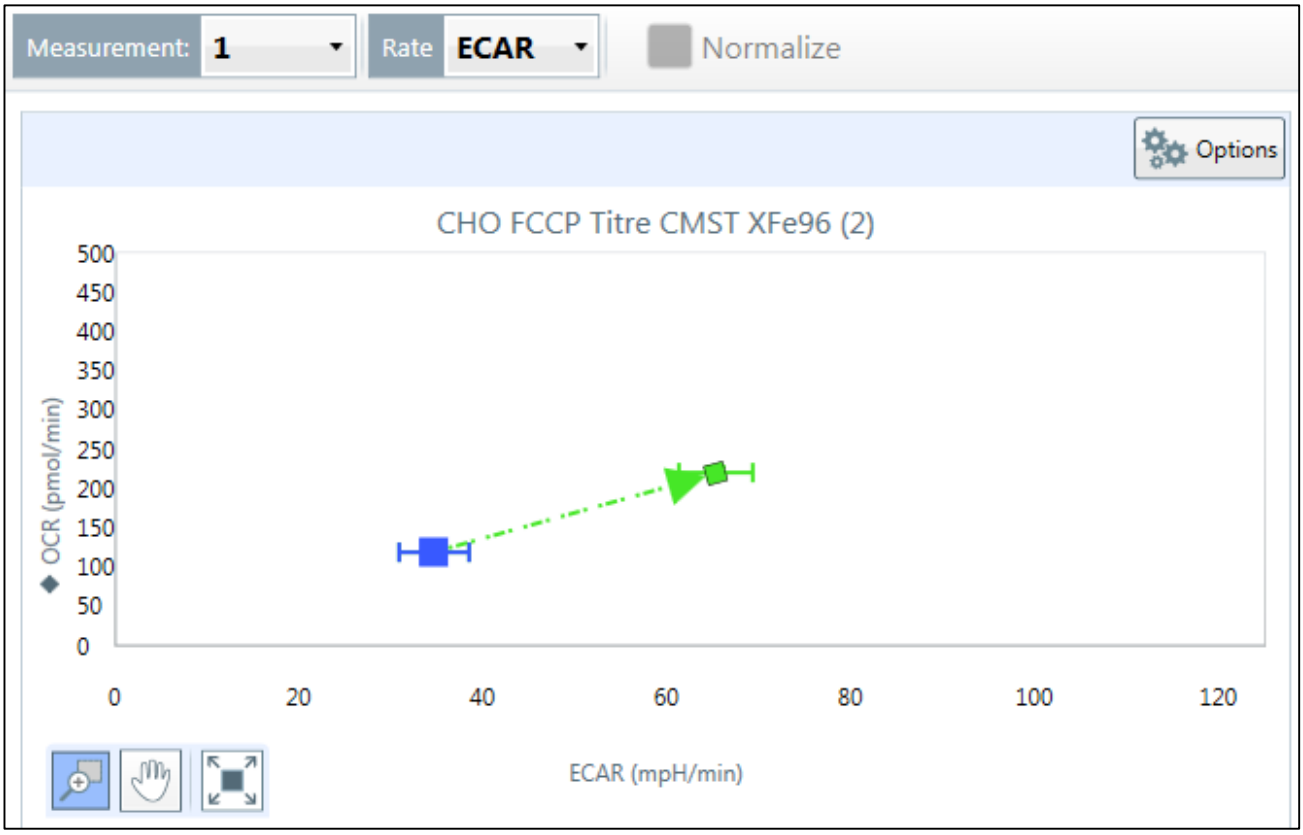
The Metabolic Switch view allows for comparing metabolic phenotypes between two cell populations.

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.



The following image shows an example of the **XF PhenoGram** view.

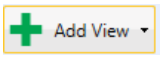


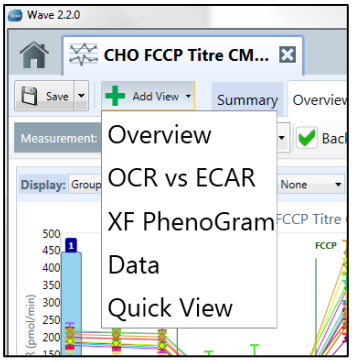
Analysis: Data View

Data view provides many different non-graphical views of the data and the run, including the following:

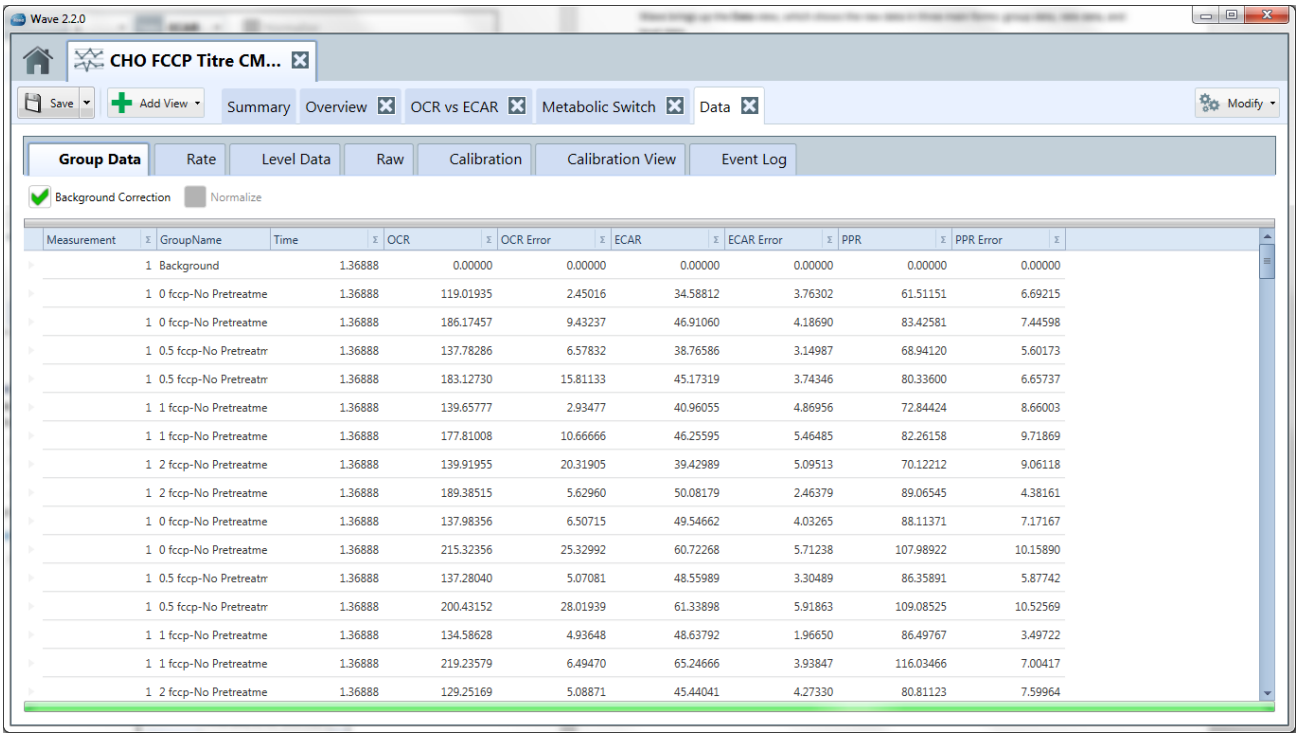
- Raw data, level data, group data, and well data
- Rates: OCR, ECAR, and PPR
- Calibration for each well and group
- An event log of the run

Each tab in the data view is exportable to an Excel file.

To display the Data view, click on **Add View**  and choose **Data**.

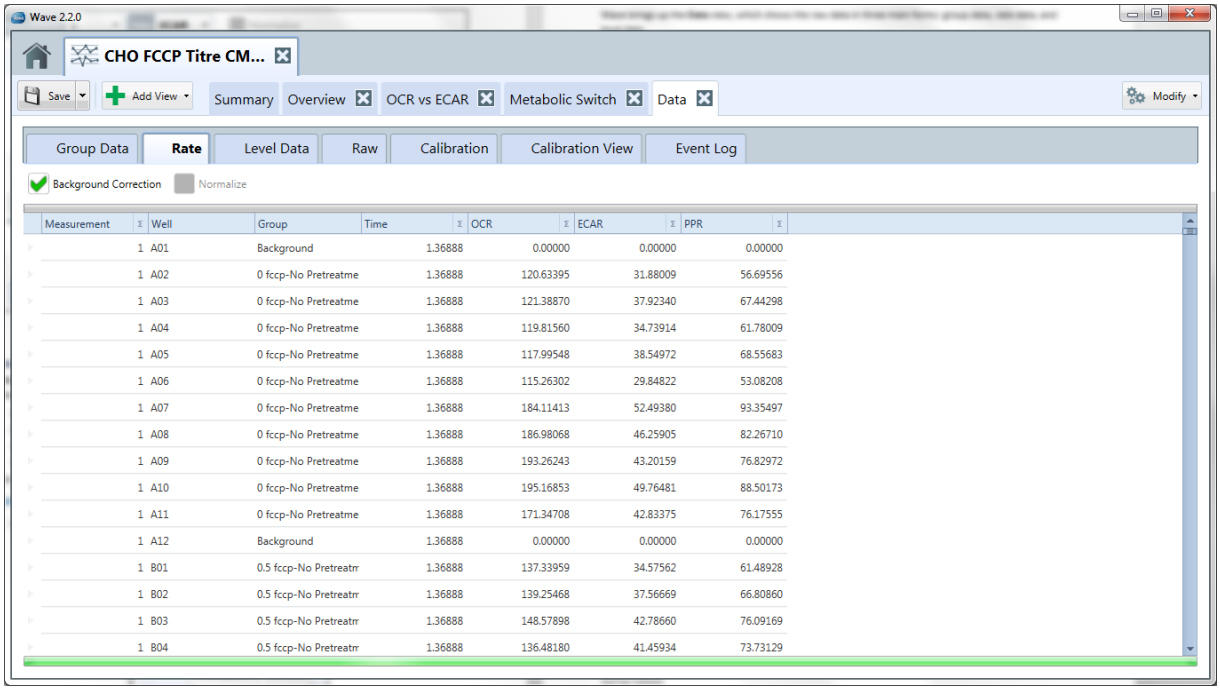


Wave brings up the **Data** view, which shows the raw data in three main forms: group data, rate data, and level data.



Measurement	GroupName	Time	OCR	OCR Error	ECAR	ECAR Error	PPR	PPR Error
1	Background	1.36888	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
1	0 fccp-No Pretreatme	1.36888	119.01935	2.45016	34.58812	3.76302	61.51151	6.69215
1	0 fccp-No Pretreatme	1.36888	186.17457	9.43237	46.91060	4.18690	83.42581	7.44598
1	0.5 fccp-No Pretreatr	1.36888	137.78286	6.57832	38.76586	3.14987	68.94120	5.60173
1	0.5 fccp-No Pretreatr	1.36888	183.12730	15.81133	45.17319	3.74346	80.33600	6.65737
1	1 fccp-No Pretreatme	1.36888	139.65777	2.93477	40.96055	4.86956	72.84424	8.66003
1	1 fccp-No Pretreatme	1.36888	177.81008	10.66666	46.25595	5.46485	82.26158	9.71869
1	2 fccp-No Pretreatme	1.36888	139.91955	20.31905	39.42989	5.09513	70.12212	9.06118
1	2 fccp-No Pretreatme	1.36888	189.38515	5.62960	50.08179	2.46379	89.06545	4.38161
1	0 fccp-No Pretreatme	1.36888	137.98356	6.50715	49.54662	4.03265	88.11371	7.17167
1	0 fccp-No Pretreatme	1.36888	215.32356	25.32992	60.72268	5.71238	107.98922	10.15890
1	0.5 fccp-No Pretreatr	1.36888	137.28040	5.07081	48.55989	3.30489	86.35891	5.87742
1	0.5 fccp-No Pretreatr	1.36888	200.43152	28.01939	61.33898	5.91863	109.08525	10.52569
1	1 fccp-No Pretreatme	1.36888	134.58628	4.93648	48.63792	1.96650	86.49767	3.49722
1	1 fccp-No Pretreatme	1.36888	219.23579	6.49470	65.24666	3.93847	116.03466	7.00417
1	2 fccp-No Pretreatme	1.36888	129.25169	5.08871	45.44041	4.27330	80.81123	7.59964

Both the raw fluorescent data and the calibration data can be reviewed in the **Data** tab. To view a particular data set, click on the specific tab within the window. In the following example, the **Rate** tab shows the rate data in a grid format.



Measurement	Well	Group	Time	OCR	ECAR	PPR
1	A01	Background	1.36888	0.00000	0.00000	0.00000
1	A02	0 fccp-No Pretreatme	1.36888	120.63395	31.88009	56.69556
1	A03	0 fccp-No Pretreatme	1.36888	121.38870	37.92340	67.44298
1	A04	0 fccp-No Pretreatme	1.36888	119.81560	34.73914	61.78009
1	A05	0 fccp-No Pretreatme	1.36888	117.99548	38.54972	68.55683
1	A06	0 fccp-No Pretreatme	1.36888	115.26302	29.84822	53.08208
1	A07	0 fccp-No Pretreatme	1.36888	184.11413	52.49380	93.35497
1	A08	0 fccp-No Pretreatme	1.36888	186.98068	46.25905	82.26710
1	A09	0 fccp-No Pretreatme	1.36888	193.26243	43.20159	76.82972
1	A10	0 fccp-No Pretreatme	1.36888	195.16853	49.76481	88.50173
1	A11	0 fccp-No Pretreatme	1.36888	171.34708	42.83375	76.17555
1	A12	Background	1.36888	0.00000	0.00000	0.00000
1	B01	0.5 fccp-No Pretreatm	1.36888	137.33959	34.57562	61.48928
1	B02	0.5 fccp-No Pretreatm	1.36888	139.25468	37.56669	66.80860
1	B03	0.5 fccp-No Pretreatm	1.36888	148.57898	42.78660	76.09169
1	B04	0.5 fccp-No Pretreatm	1.36888	136.48180	41.45934	73.73129

Each data point is identified with a well column and a group column, so that the data can be displayed in a number of different formats.

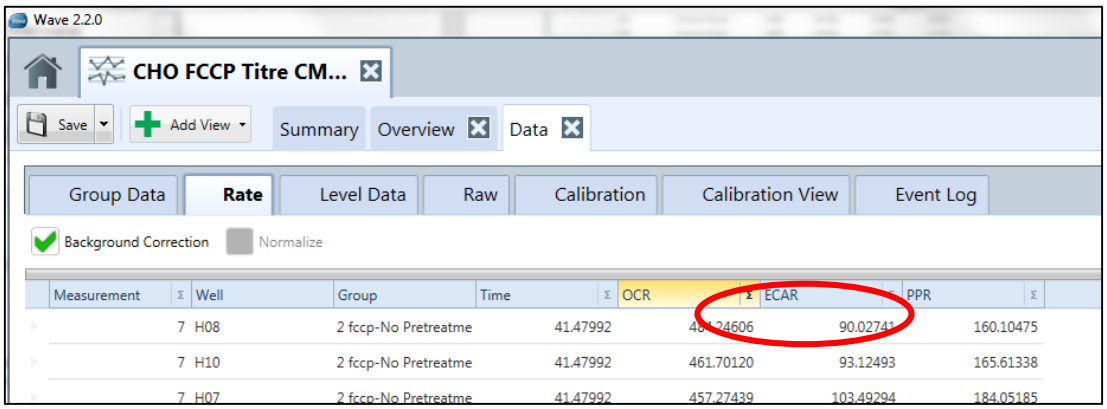
Sort by Column

To sort the data by a specific column:

- Click on the column header: Measurement, Well, Group, Time, OCR, ECAR, or PPR.
- Wave will sort the data in ascending or descending order in the selected column.

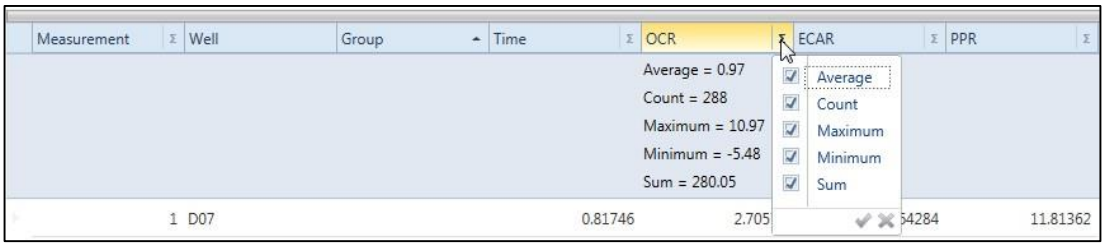
Display in Ascending or Descending Order

To change from ascending to descending order, click the arrow to the right of the column name. In the following illustration, Wave lists OCR data in ascending order. Click the up arrow to the right of the cursor to change to descending order.



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

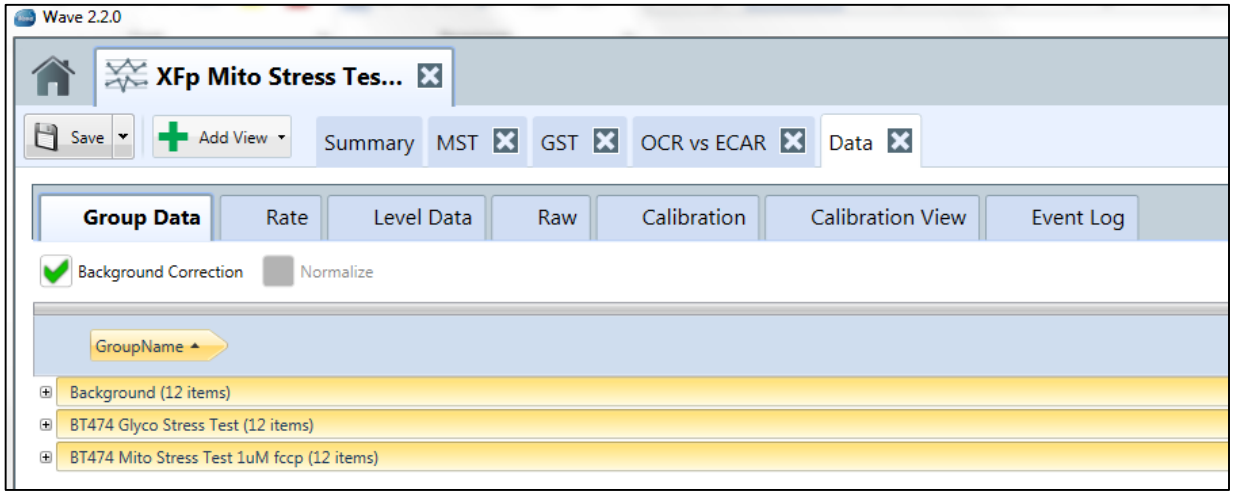
To display the Average, Count, Maximum, Minimum, or Sum, or any combination of these values, click on the Sigma sign to the right of the arrow and check the applicable check boxes. In the following example, all of these values are checked for OCR data; therefore, Wave shows the average, count, maximum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



Group the Data by Field

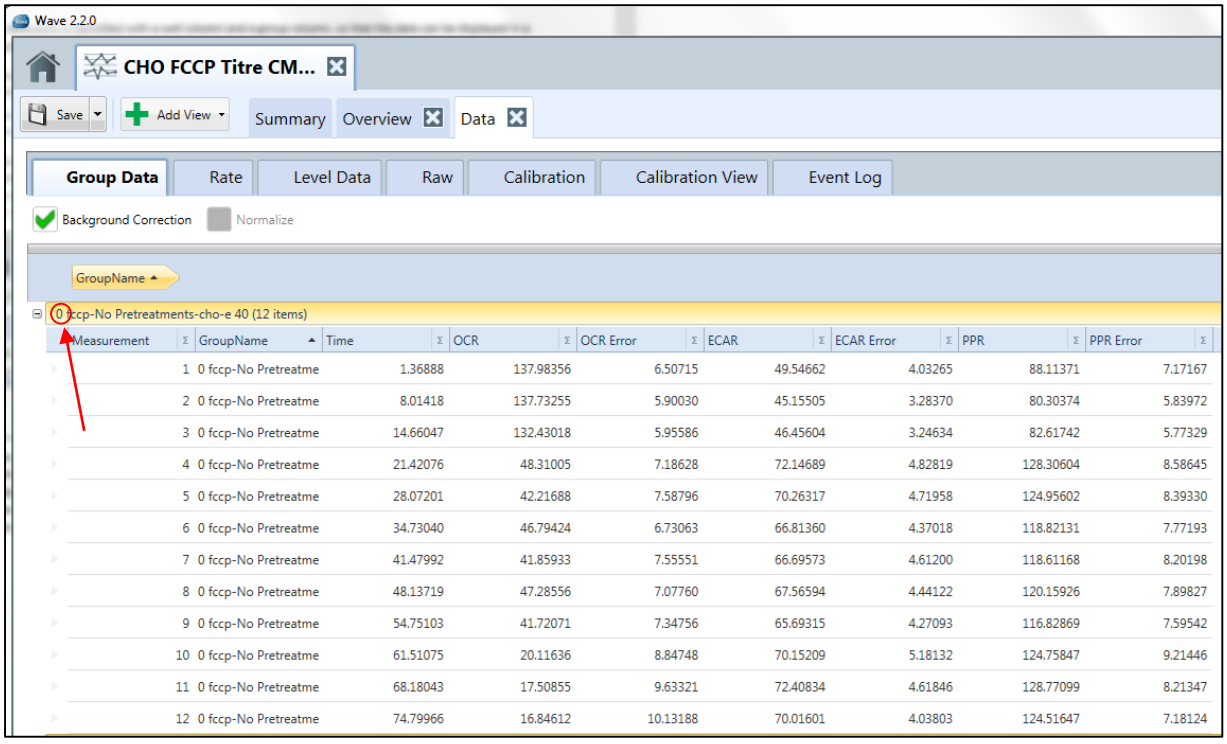
To group the data by field:

- 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

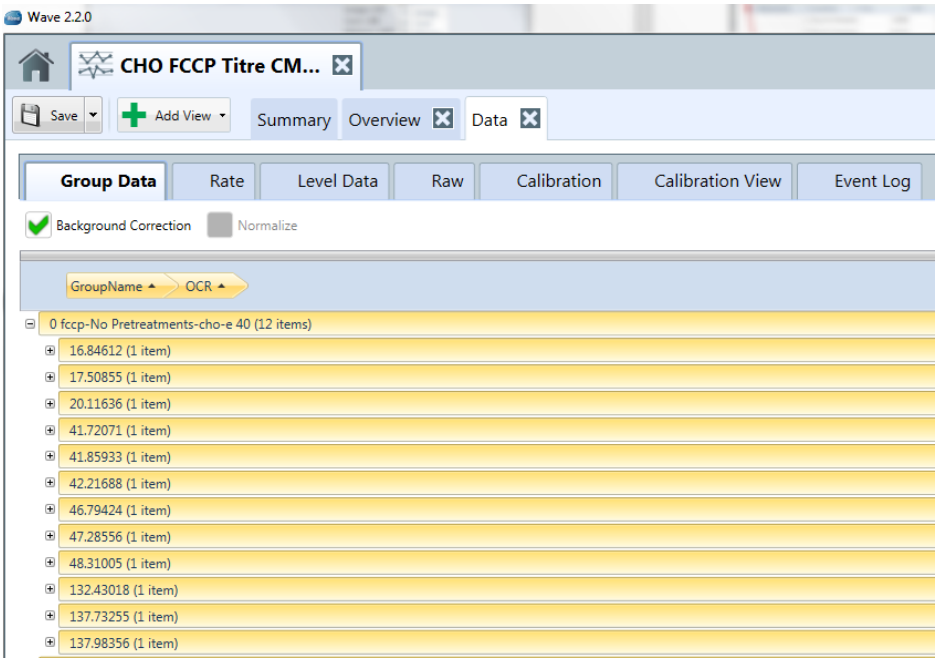


To expand a group:

- Click on the **+** (plus sign) to the left of a group to expand it.



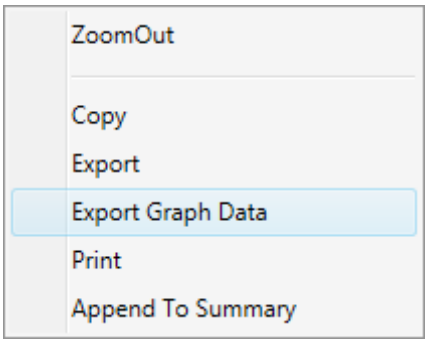
After expanding a group, select another column heading to define the groupings further. In the following example, the heading OCR is dragged to the grey bar after GroupName.



Export to Excel from any View

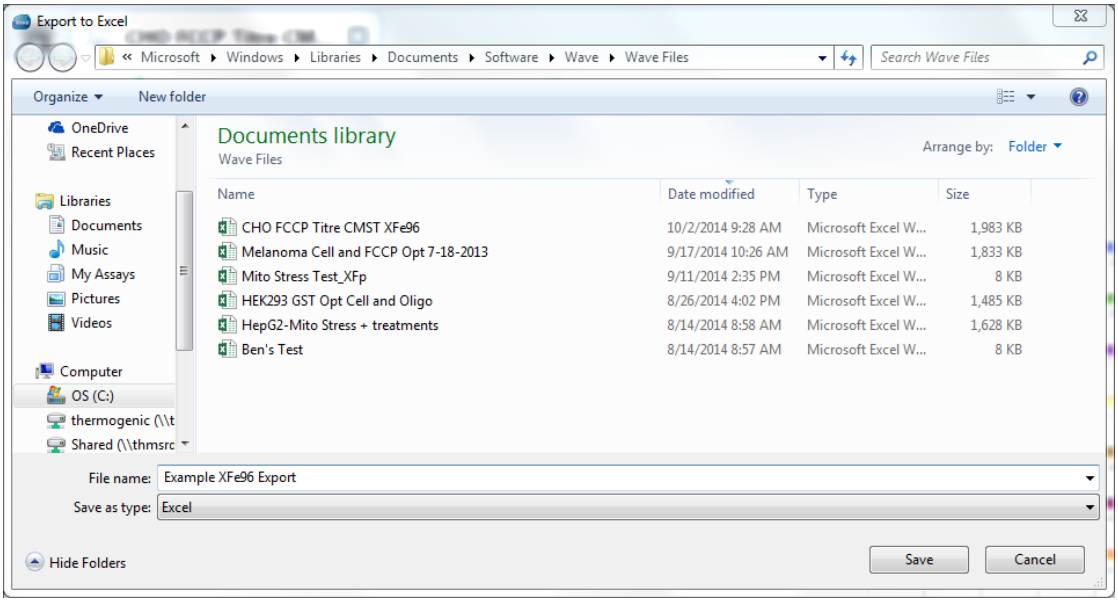
Export the data to Microsoft® Excel from any view.

To export data from here or from any analysis view:



1. Right click anywhere on the graph (for touch screen, hold finger on any part of the grid for three seconds). Wave displays the following menu.

2. Choose **Export Graph Data**. The Save dialog box is displayed.



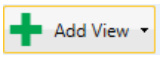
3. Browse to a directory where the Excel file will be saved.
4. Type a name for the file in the **File name** field.
5. Select Excel as the type in the **Save as type** field.
6. Click the **Save** button.

Wave will export the data grid exactly as it appears in the data window so that further analysis can be performed in Excel.

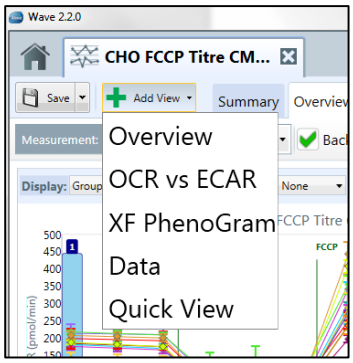
Analysis: Quick View

Quick View displays provides a simultaneous view of OCR, ECAR, OCR vs. ECAR and PPR*.

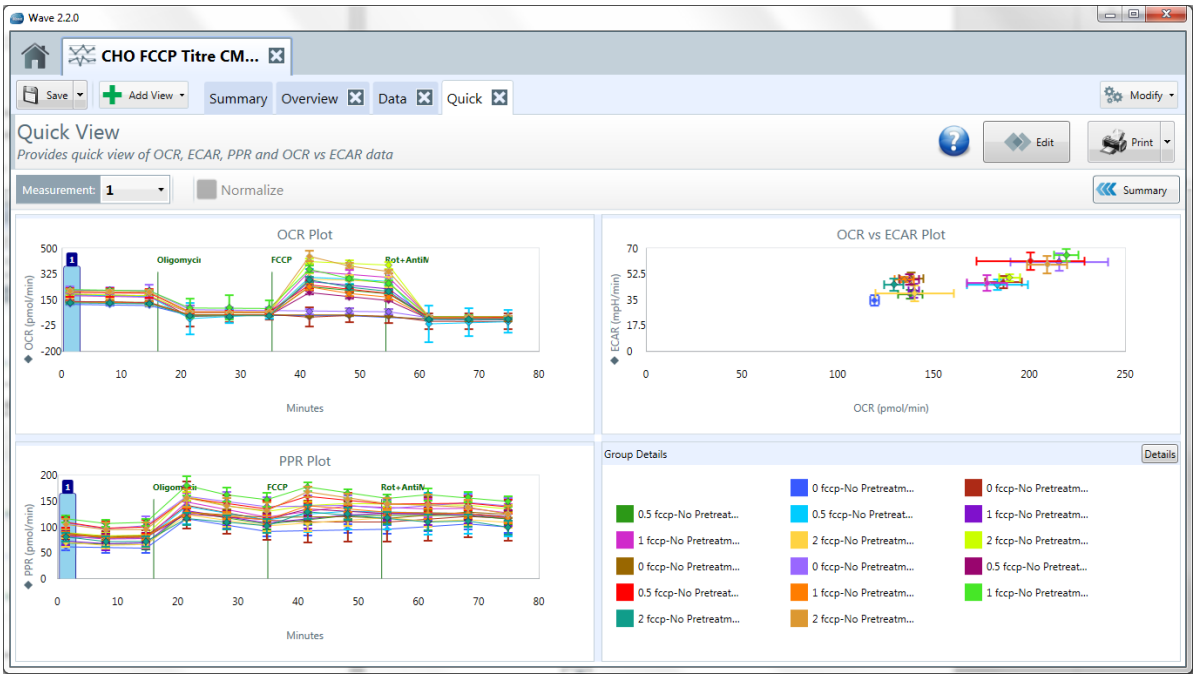
** PPR is only displayed when the Assay Media buffer capacity is specified within the Groups/Conditions setup view.*

To view the **Quick View** tab, click **Add View** 

and select **Quick View**



The image below shows an example of the **Quick View** tab.

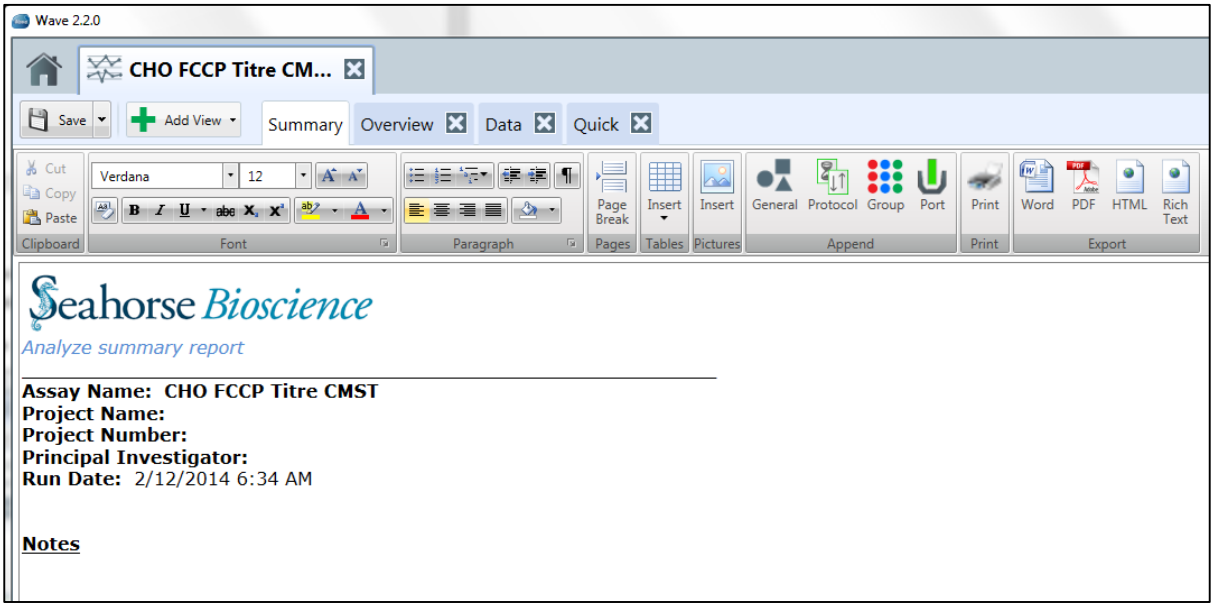


Analysis: Summary View

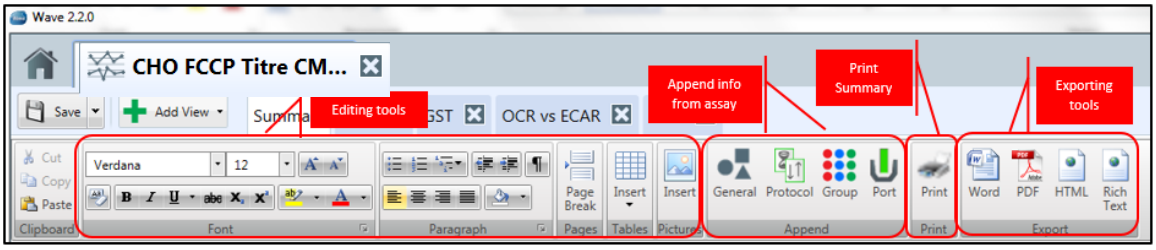
The **Summary** tab is the first view when opening an assay result file (.asyr). The **Summary** tab allows for customizing notes and adding graphs/charts in other views. The following can be performed in the **Summary** tab:

- Document a summary of the run, complete with kinetic graphs, bar charts, and group and protocol definitions
- Print the summary
- Export the summary to Microsoft Word, Adobe PDF, HTML, or Rich Text

The **Summary** tab displays an editable version of the protocol summary that can also be printed.



This window provides a multi-faceted tool bar.



Edit

The bar above is contains editing tools for editing the summary:

- Cut, copy, and paste to the clipboard
- Change the font, its size, color, and style
- Subscript or superscript a character or characters
- Highlight text or insert a table, picture or page break.

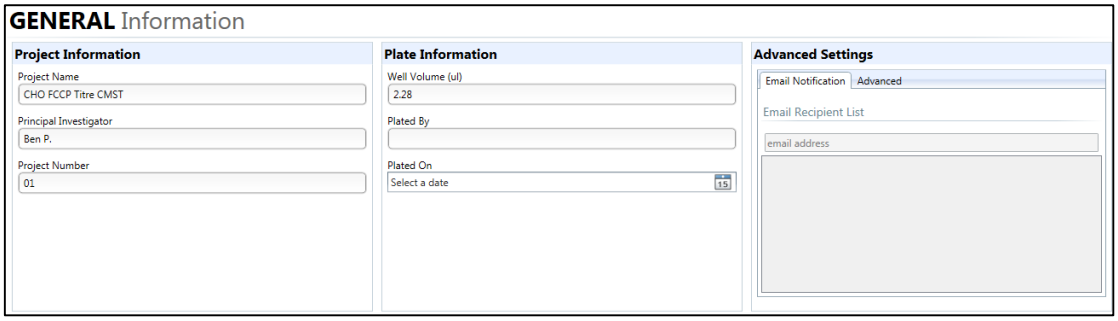
Append information from assay results

Add General information recorded as part of the assay design, which is from the Review and Run screen of the design file.

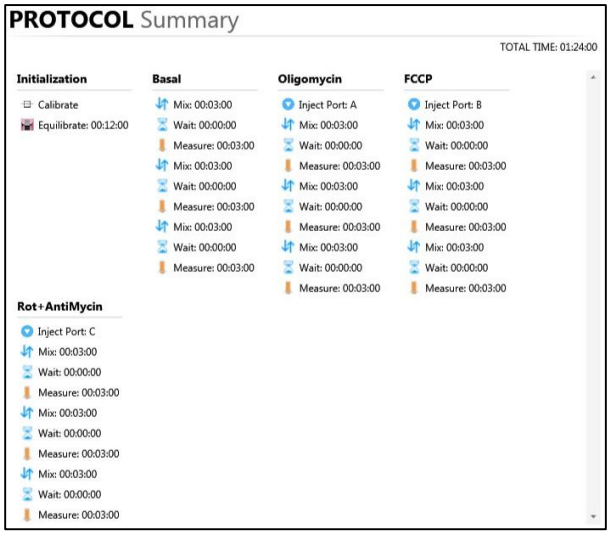
To append **General Information** to the **Summary** view, click the **General** button.

The following illustration shows an example of the General Information section that Wave appends into the summary tab.

To append the **Protocol Summary** to the **Summary** view, click the **Protocol** button.



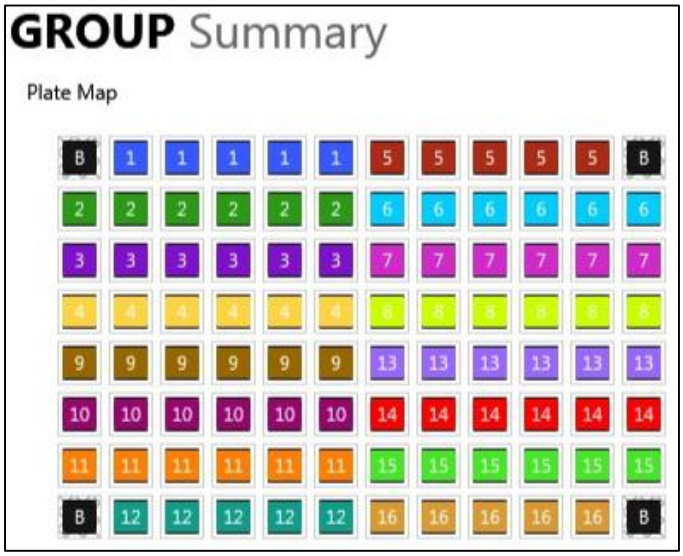
The following illustration shows an example of a protocol summary.



To append the **Group Summary** to the **Summary** view, click the **Group** 

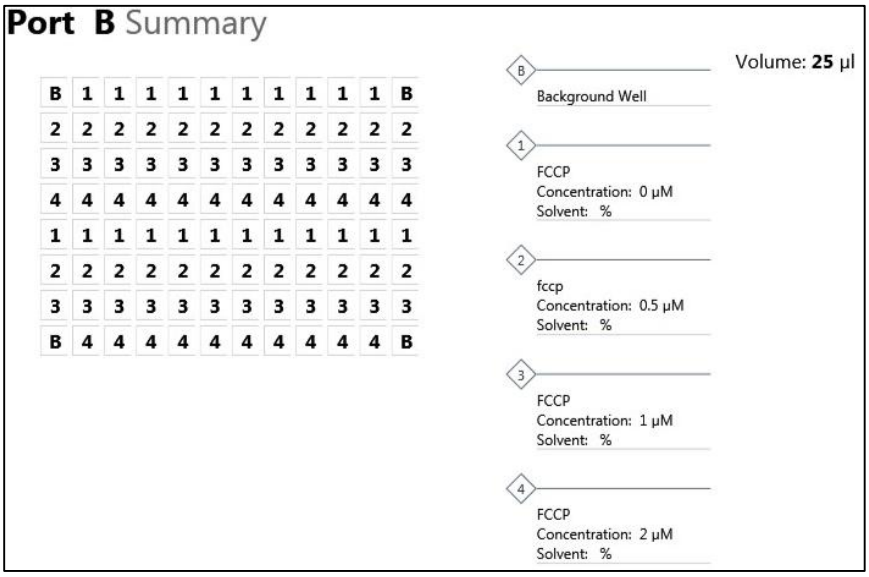
button.

The following illustration shows an example of a Group summary.



To append the **Port Summary** to the **Summary** view, click the **Port** button. 

The following illustration shows a part of a Port summary.



Print

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.

The **Summary** tab is always accessible when an analysis file is open. This information is used to record how the assay was run (mix, wait, and measure times, along with injection sequences are shown), as well as information about where the groups are located within the plate.

Export

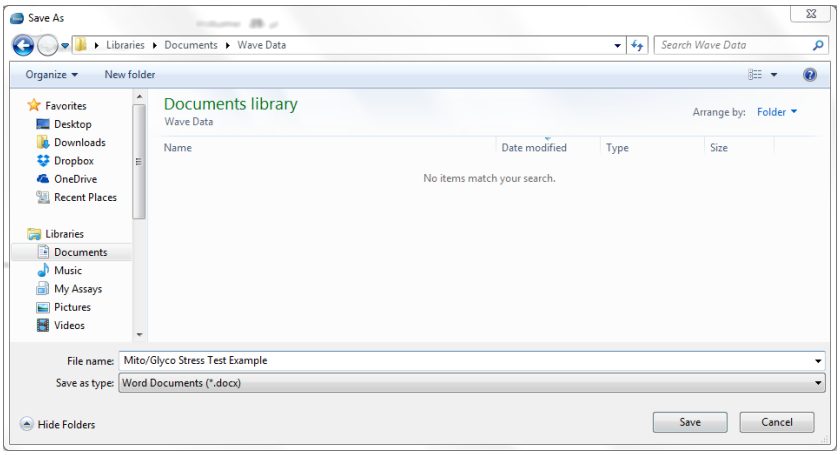
Export the assay summary document into 4 different formats:

- Word
- PDF
- HTML
- Rich Text

To export summary document:

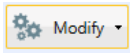
Click the appropriate icon from the Export section of the tool bar:

1. The **Save As** dialog box comes up.

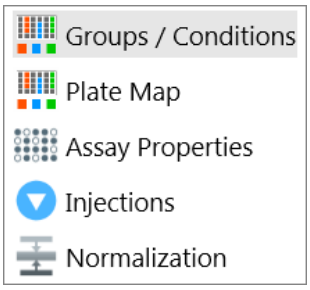


2. Browse to the location to save the summary document.
3. Type the name of the summary file in the **File name** field.
4. Select the type of file from the **Save as type** dropdown list.
5. Press the **Save** button to save file.

Modifying Analysis Views

The **Modify** 

button in the upper right-hand corner of the **Summary** screen provides a menu of actions to take as shown in the following illustration.

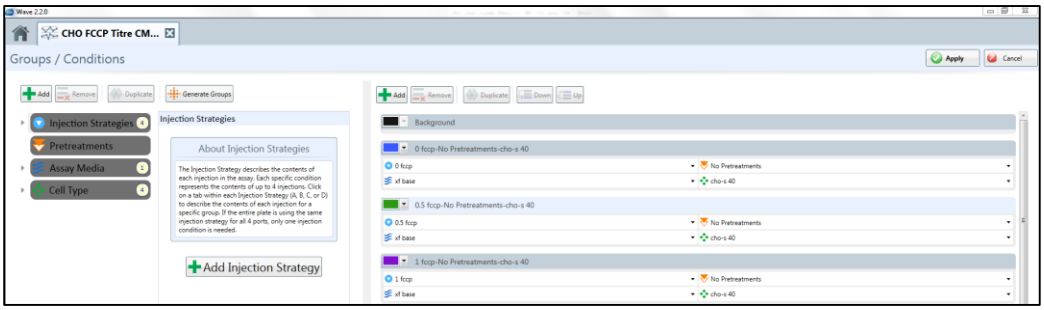


Groups and Conditions

Edit Groups and Conditions:

1. Click **Modify** and select **Groups /Conditions** from the dropdown list.

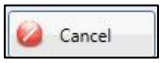
   Wave displays a reproduction of the Groups and Conditions window found in the Assay Design section of the software.



2. This window enables any changes to be made to the group conditions for an experiment that has been run. This allows for correcting mistakes in the experiment design or entering additional details.

3. Once completed with editing the appropriate fields, touch or click the **Apply** 

button (located in the upper right-hand corner of the window) to make changes to the analysis file. Cancel out of this window to prevent any changes from taking effect.

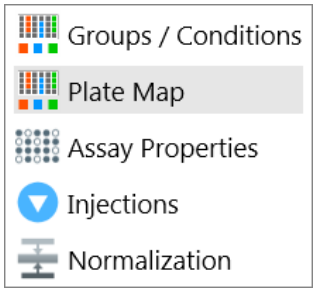
4. Click the **Cancel** 

button to cancel changes.

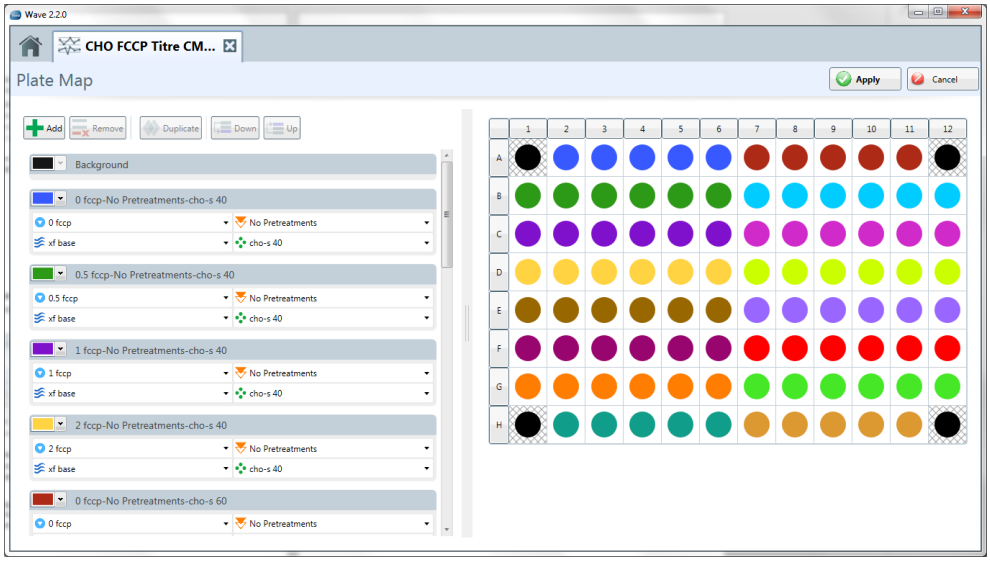
Plate Map

To modify the Plate Map:

1. Click **Modify** and select **Plate Map** from the drop down menu.



2. Edit group properties and the 8-well plate map for the groups being tested in the assay.

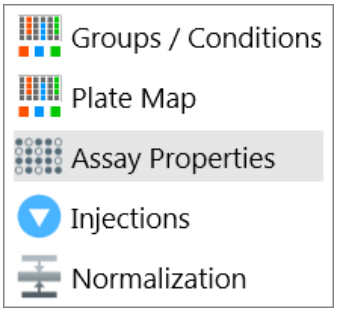


Assay Properties

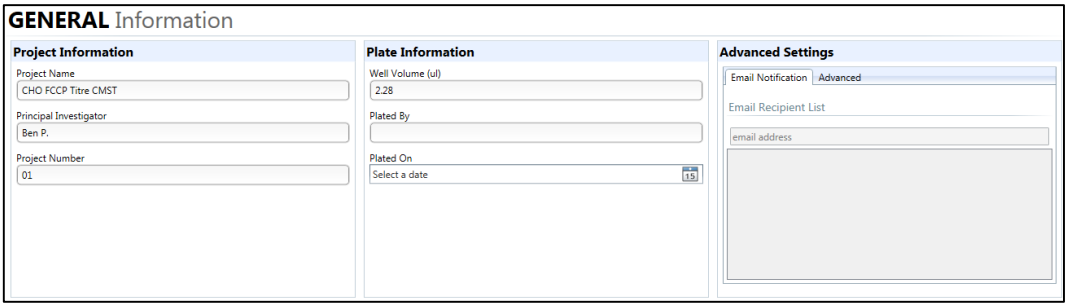
Modify and add information after completing an assay in the Assay Properties window.

To modify the assay properties:

1. Click **Modify** and select **Assay Properties** from the dropdown menu.



Wave now displays the Assay Properties window.

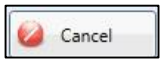


2. If necessary, change or add to fields under each of the headings:

   - Project Information
   - Plate Information
   - Advanced Settings
   - Notes

3. Once finished editing the appropriate fields, touch or click **Apply** 

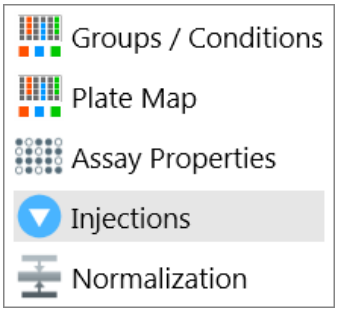
(located in the upper right-hand corner of the window) to make changes to the analysis file. Cancel out of this window to cancel any changes that have been made.

Click **Cancel** to cancel changes. 

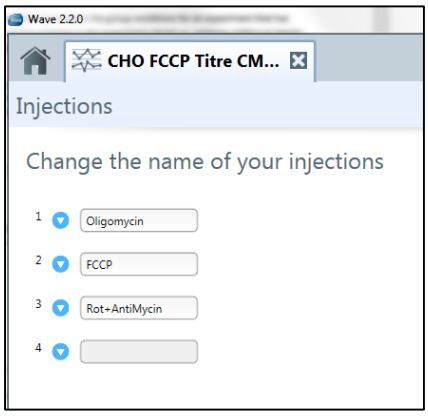
Injections

To change the names of injections:

1. Click **Modify** and select **Injections** from the dropdown menu.

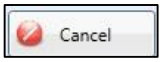


2. This window allows for changing the name of each injection.



3. Once finished with editing the appropriate fields, touch or click **Apply** 

(located in the upper right-hand corner of the window) to make changes to the analysis file. Cancel out of this window to cancel any changes that have been made.

4. Click **Cancel** 

to cancel changes.

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.

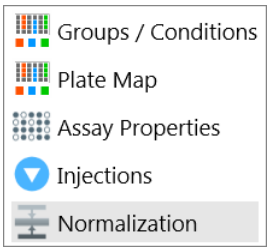
There are two ways to add normalization data to the Normalization window:

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

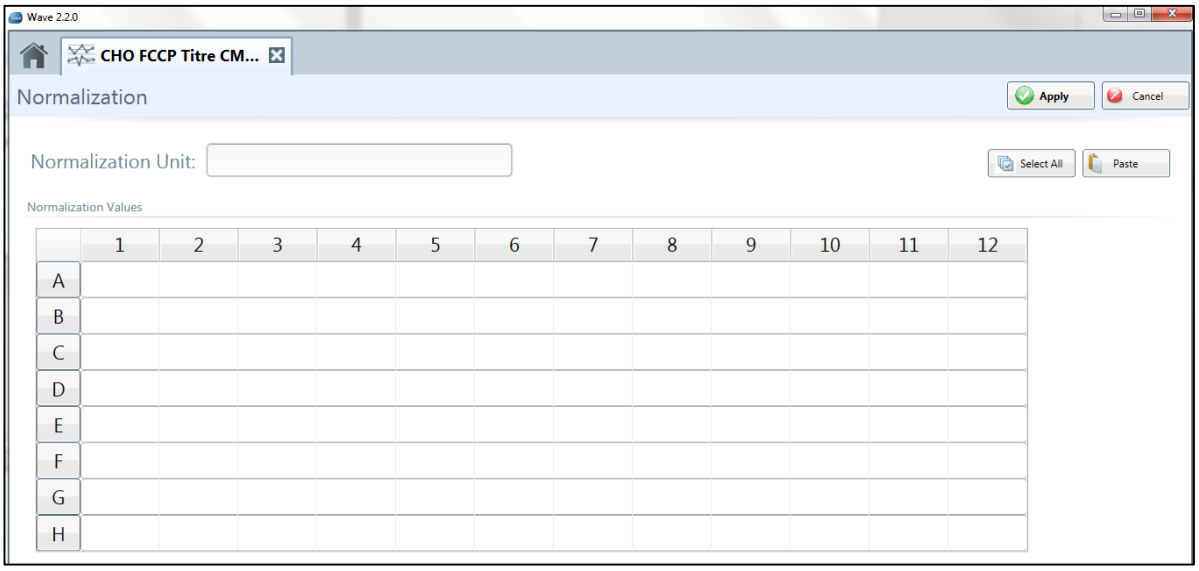
Copy and paste from a secondary source:

Copy the secondary source data from a spreadsheet or similar grid format to the Clipboard by using the key sequence Ctrl-C.

Click **Modify** and select **Normalization** from the dropdown menu.



Wave will display the Normalization screen.



Click **Select All.**  This will select all of the wells in the grid.

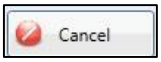
Click **Paste** 

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.*

Type in a Normalization Unit in the **Normalization** field at the top of the screen.

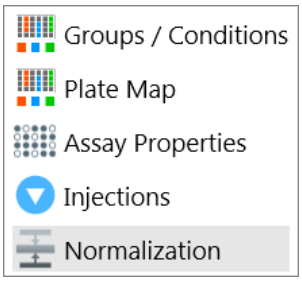
Note: *This unit will then be displayed in the Y1 axis title on the kinetic graph when normalization is selected. For example, type "Cell" into the* ***Normalization Unit*** *field, and normalized OCR will be displayed on the kinetic graph as "pMoles/min/Cell".*

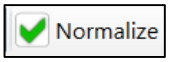
When finished editing the appropriate fields, touch or click **Apply**  (located in the upper right-hand corner of the window) to make changes to the analysis file.

Click **Cancel**  to cancel any changes.

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.

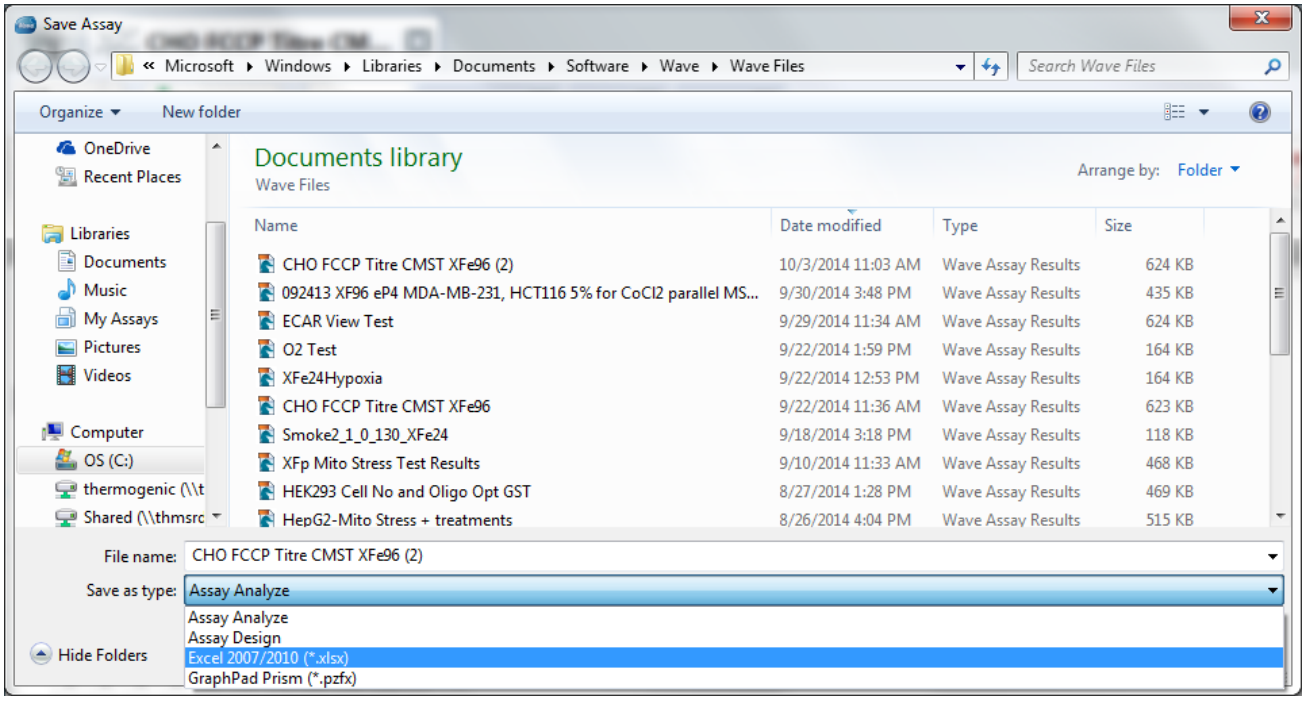


After data has been normalized, the "**Normalize**"  checkbox will appear in the **Overview** tab and the **Data** tab. To toggle the data between normalized and not normalized, click the checkbox on and off.

Analysis with Excel

Transfer the entire results file (.asyr) to Excel for further analysis:

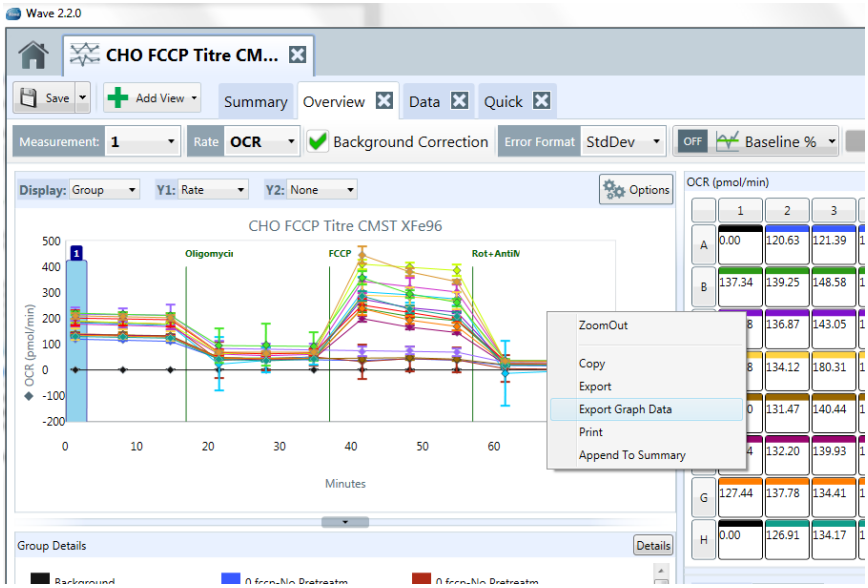
1. Press the downward-pointing arrow on the **Save**  button in the top left corner of the screen.
2. Select **Save as** to bring up the Save Assay dialog box.



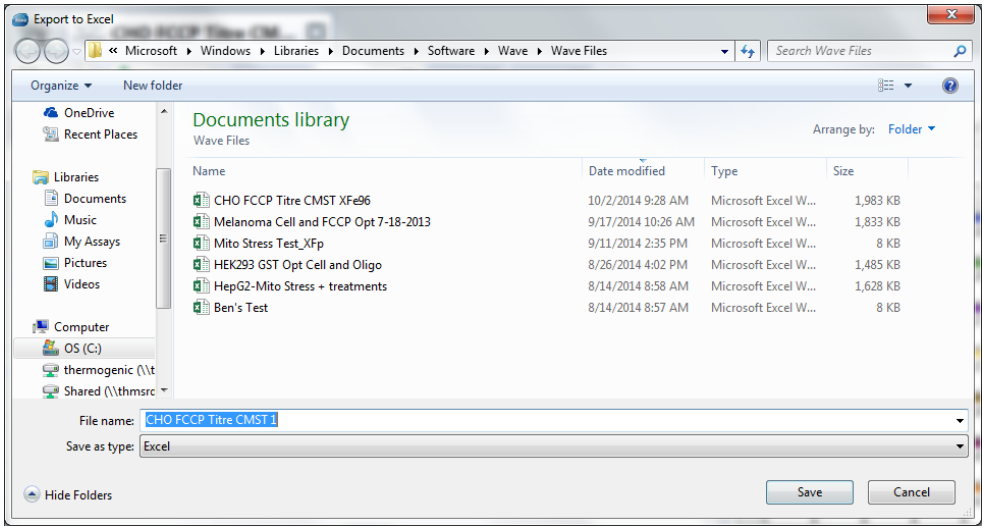
3. Navigate to location to save Excel file.
4. Type the file name in the **File name** field.
5. Click on the **Save as type** dropdown list at the bottom of the dialog box. Select Excel 2007/2010 (*.xlsx).
6. Click the **Save** button in the bottom right-hand corner of the dialog box.

Transfer data from a graph or chart to Excel for further analysis:

1. Right click on the graph and select **Export Graph Data** from the dropdown list.



2. Select a directory for the graph data.



3. Type a file name in the File name field.
4. Save data by pressing the **Save** button.

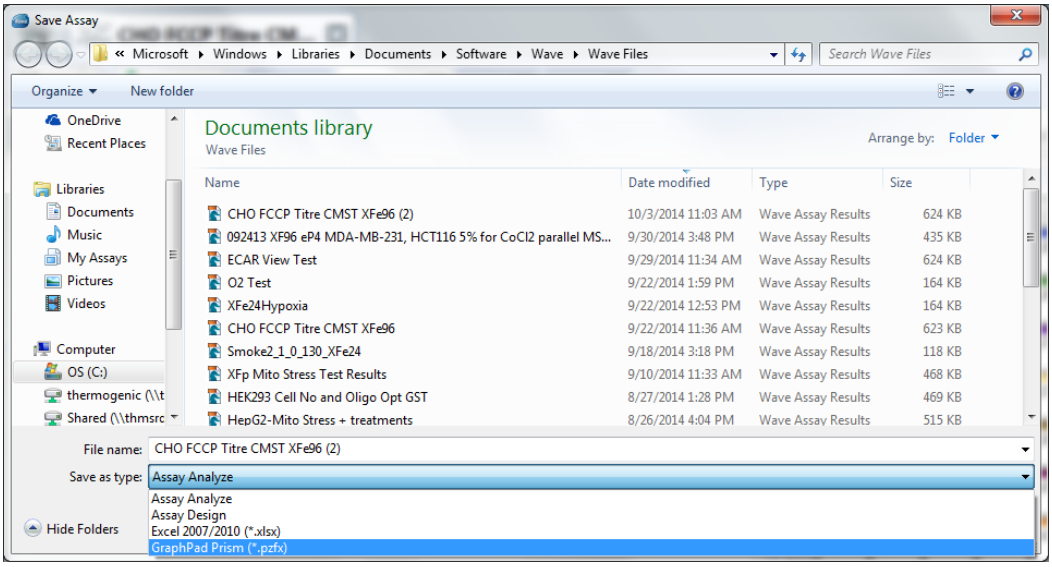
*Analysis with GraphPad Prism 6**

**Note: Wave Desktop compatibility was validated using GraphPad Prism 6*

Transfer results file (.asyr) to GraphPad Prism 6 for further analysis:

1. Press the downward-pointing arrow on the **Save** 

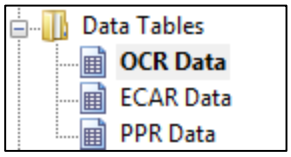
button in the top left corner of the screen.
2. Select **Save as…** and choose location to export GraphPad file



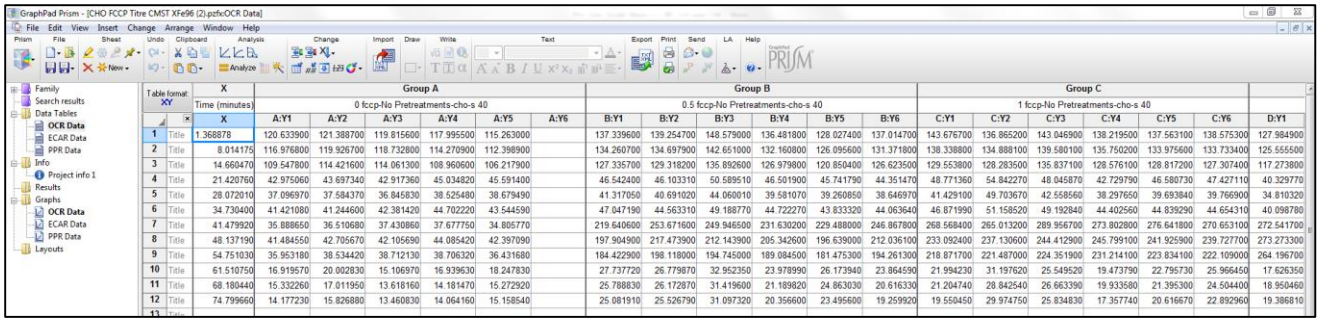
3. Type the file name in the **File name:** field.
4. Select GraphPad Prism (*.pzfx) in the **Save as type** drop-down list.
5. Click **Save** in the bottom-right corner of the dialog box.

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 three options to view raw data in GraphPad.



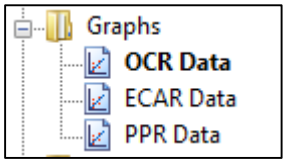
Raw data view in GraphPad:



GraphPad Kinetic Graph Views

In addition to raw data, GraphPad also contains the kinetic graphs for OCR, ECAR and PPR to edit the appearance and other features of the data for publication or presentation purposes.

First select the graph to review or edit from the list on the left-side of GraphPad.



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