SOP#: 004	Date Issued: 8/03/2021	Date Revised: 7/08/2021

TITLE:	XFe24 Analyzers
SCOPE:	Research Personnel
RESPONSIBILITY:	BORC staff
PURPOSE:	To outline the proper procedures for use and maintenance of the XFe24 Analyzers.

1   PURPOSE

This SOP explains how to use CFX Connect Real Time PCR to measure RNA expression level in cells and tissues.

2   RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3   BEFORE CONDUCTING YOUR EXPERIMENT

The flowchart illustrates the general procedure to carry out a XF assay. Please read it and plan your experiment in advance to prevent any unexpected problems.

General Assay Flow Chart for XF Assays
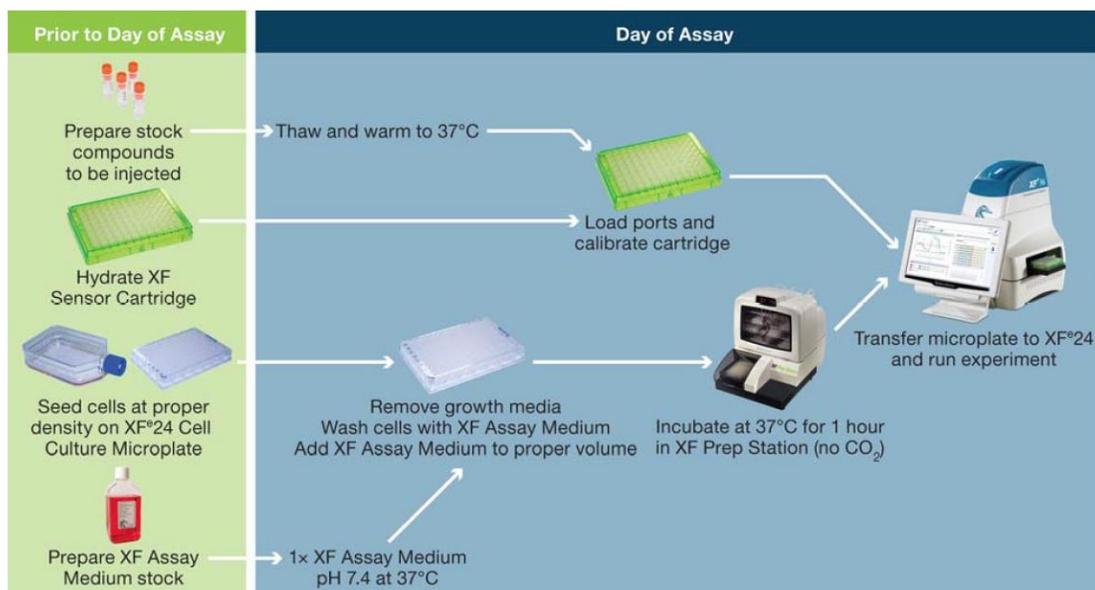
4   PROCEDURES

4.1 Power and Warm up

Turn the instrument on using the power switch on the rear panel. On the right side of the instrument near the access door, there is an LCD message screen. When the instrument is powered up the LCD message screen will show:

4.2 XFe Wave: Login

Launch XFe Wave and sign in. Allow the XFe Analyzer to warm to 37°C for at least two hours. Ideally, the instrument should be left on overnight to ensure consistent and accurate temperature. Note that the instrument will NOT warm to 37°C unless the software is launched (simply turning on the instrument only will NOT engage the heater). When XFe Wave software is launched on the controller, the LCD message screen will update and show the following display

4.3 Create/Edit Assay Templates

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

4.3.1 Open **Wave 2.4**.

4.3.2 Click **Templates** (below Wave Home).

4.3.3 Select the **Blank** template, a **Agilent Seahorse Default** template, or a **User-customized** template and click **Open** or double-click the template icon.

4.3.4 Add, remove, or modify group definitions; and create new assay groups. (See 4.4 Group Definitions.)

4.3.5 Assign groups to the Plate Map. (See "Plate Map".)

4.3.6 Review or modify Instrument Protocol. (See ": Instrument Protocol".)

4.3.7 Save the template, transfer it to XFe/XFp Analyzer. (See "Saving Assay Templates")

4.3.8 Run the assay. (See " Run Assay".)
4.4 Group Definitions
Group definitions is comprised of four components:
4.4.1 Injection Strategies
Injection strategies describe the contents of up to four injections (one for each of the four ports: A, B, C, and D), more than one injection strategy can be performed in an assay. For example, on the same plate, two injection strategies can be defined for the XF Cell Mito and XF Glycolysis Stress Test.

To add one or more injection strategies:
1 Click Add next to Injection Strategies. (See Figure 5.)
2 Enter a name for the injection strategy if desired. The default name when adding new injection strategies is Inj. Strategy # (# corresponds to how many injection strategies have already been added).
3 Next, specify the contents of each injection starting with Port A. Click Add Compound, and type in a compound name (for example oligomycin), or use the Compound Catalog drop-down menu to select a compound from the catalog.
4 If additional injections are called for from ports B, C, and D, repeat step 3.

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

To add one or more Pretreatments:
1 Click **Add** next to **Pretreatments**. (See Figure 8.)

2 Enter a name for the pretreatment or use the Pretreatment drop-down menu to select a pretreatment from the catalog. The default name when adding new pretreatments is **Pretreatment #** (# corresponds to how many pretreatments have already been added).
3 If desired, specify the type of pretreatment using the **Description** field.

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

To add one or more Assay Medium:

1 Click **Add** next to **Assay Media**.

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

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

4.4.4 Cell type

Cell Type describes the biological material/samples used, including information about the cell line, seeding density, and passage number.
To define one or more Cell Types:
**1** Click **Add** next to **Cell Type**.

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

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

4.5 Plate Map

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

4.5.1 Assign groups automatically

Click **Distribute Groups**. Wave automatically determines the maximum number of replicates per group, and assigns wells to each group starting with column 1.
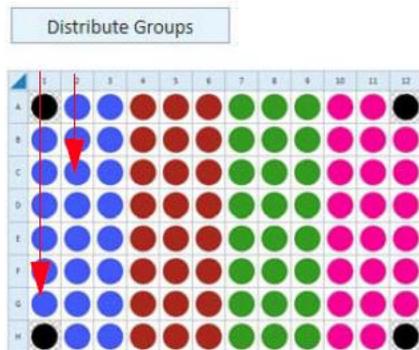
4.5.2 Assign groups manually
1 Click the group in the Groups list on the left.
2 Click the well on the Plate Map to assign that individual well to the selected group. You can assign an entire row or column to a group by clicking the row or column label, or drag-and-drop to select an area of the Plate Map to assign to the selected group.

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

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

4.6.1 Default protocol commands
Every Instrument Protocol includes the following steps:
1 Calibrate (always ON)
Calibrate is always the first step in a protocol and cannot be disabled. The Calibrate step reads the coefficients of the Sensor Cartridge and Cell Plate to ensure accurate data acquisition.
2 Equilibrate (highly recommended for every assay)
Equilibration ensures temperature stability before beginning an assay. The default setting for Equilibration is ON. Equilibrate can be disabled although this is strongly discouraged.
3 Baseline Measurement cycle
The Baseline measurement cycle is the starting point for every Agilent Seahorse assay and consists of three measurements before the first injection. Data acquired during the Baseline measurement cycle provide valuable information about the bioenergetic status of the cells under starting conditions.

4.6.2 Measurement cycles
Measurement cycles indicate the steps in the Instrument Protocol when rate data is collected for oxygen consumption rate (OCR) and extracellular

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

- **Mix**: The amount of time to raise/lower the sensor cartridge to ensure analytes and drug compounds are uniformly mixed in each well before/after an injection, as well as to reintroduce oxygen to the well after forming the microchamber for each measurement.
- **Wait**: The amount of time to delay the Measurement step after the Mix step. This command is required for the XFe24 Analyzers in every assay, but may be added to Instrument Protocol for any analyzer.
- **Measure**: The amount of time to record the flux of analytes in the transient microchamber once the sensor cartridge probes are lowered following a Mix (or Wait) command. The default measurement cycle times are: 3 minutes Mix, 2 minutes Wait, 3 minutes Measure.

4.6.3 Edit measurement details

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

4.6.4 Injections

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

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

4.6.5 Custom cycles

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

4.7 Saving Assay Templates

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

Click **Save As** to display the **Save As Template** window:
  1 Enter the name of the assay template in the **Name** field (required).
  2 Enter the **Author** name (optional).

3 Enter a description of the assay in the **Description** field (optional).
4 Click **Save**.

4.8 Run Assay

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

4.8.1 Click **Start Run**, and select a location to save the assay template file. The assay result file will be saved in the same location following assay completion. Assay template and result files can be saved to a USB Flash Drive, shared network directory, or locally on the XFe Controller. Wave Controller will also automatically save a backup assay result file locally on the XFe Controller in the location: C:\ProgramData\ Seahorse Bioscience, Inc\Seahorse Wave\Assays.

4.8.2 After selecting a save location, the tray door on the XFe Analyzer will open. Place the Sensor Cartridge (hydrated and loaded with compounds) along with the Utility Plate onto the tray. Ensure the Cartridge fits properly on the Utility Plate, the lid is removed from the Cartridge, and the direction of the Cartridge is in the proper orientation.

4.8.3 After loading the Sensor Cartridge and Utility Plate, press **I'm Ready** to initiate Sensor Cartridge Calibration. The time to complete Calibration for assays at 37 °C is approximately 10-20 minutes. For assays performed at temperatures other than 37 °C, an additional 30 minutes of PreCalibration time will be added to ensure accurate data acquisition.

Load Calibrant Utility Plate

Are you ready to load the **Calibrant Utility Plate** and **Sensor Cartridge**?

| I'm Ready | Cancel Assay |

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

Load Cell Plate

Calibration is complete, are you ready to load the **Cell Plate**?

| Open Tray | Cancel Assay |

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

Load Cell Plate

Remove the **Calibrant Utility Plate** and place the
Cell Plate on the thermal tray.

| Load Cell Plate | Cancel Assay |

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

Unload Sensor Cartridge

Are you ready to eject the used **Sensor Cartridge** and
Cell Plate?

| Eject |

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

Assay Complete!

The assay is complete and will now close.

Where do you want to go next?

| View Results | Wave Home |

4.9 Add and Remove Measurement Cycles in Runtime
During a Agilent Seahorse XF assay, data is acquired, calculated, and presented in
the real-time. For certain assays, it may be necessary to add or remove
measurement cycles in real-time.
4.9.1 Wave Controller 2.4 enables real-time Instrument Protocol modification for
commands that have not yet been executed. To display the Instrument Protocol
for the selected command, click on an **Instrument Protocol** step above the data.
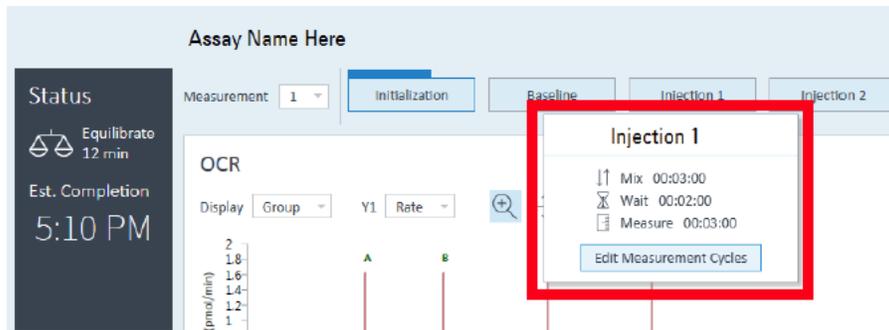
4.9.2 Click Edit Measurement Cycles to display the Edit Number of Cycles window. Use the up/down arrows (or manually type a number) to add/remove measurement cycles from the selected command.
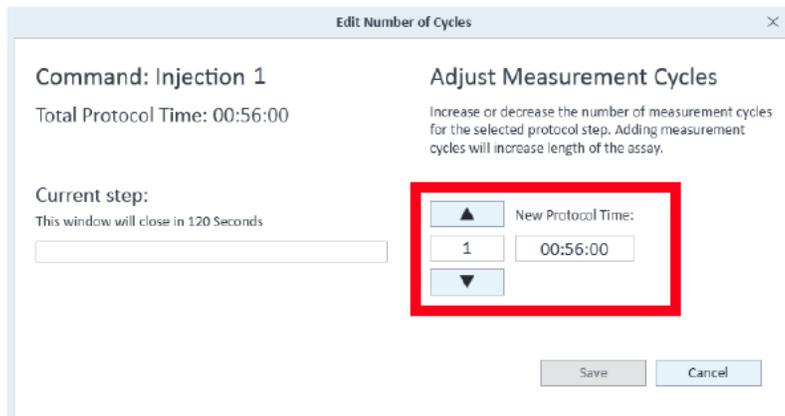
4.10 Analyzing Assay Results

Assay result files (*.asyr) are generated by XFe and contain all data acquired during an assay. Examples of what type of data is acquired and stored in assay result files include:
   • Rate data (OCR, ECAR, PER, and PPR)
   • Level data (mmHg or mpH)
   • Calibration results
   • Consumable information from the barcodes on the cell plate and sensor cartridge used in the assay

In addition, any details added to the assay template file about the experiment, such as groups, compound, pretreatments, is migrated and saved in the assay result file.

The default analysis view for every new assay result file is called **Quick View**. Reopening a saved result file always displays the last-modified or viewed analysis view. Each analysis view can be added to an assay result file using the **Add View** button. The four analysis views in Wave software are: • Quick View • Overview • OCR vs. ECAR • Data

4.10.1 Quick View

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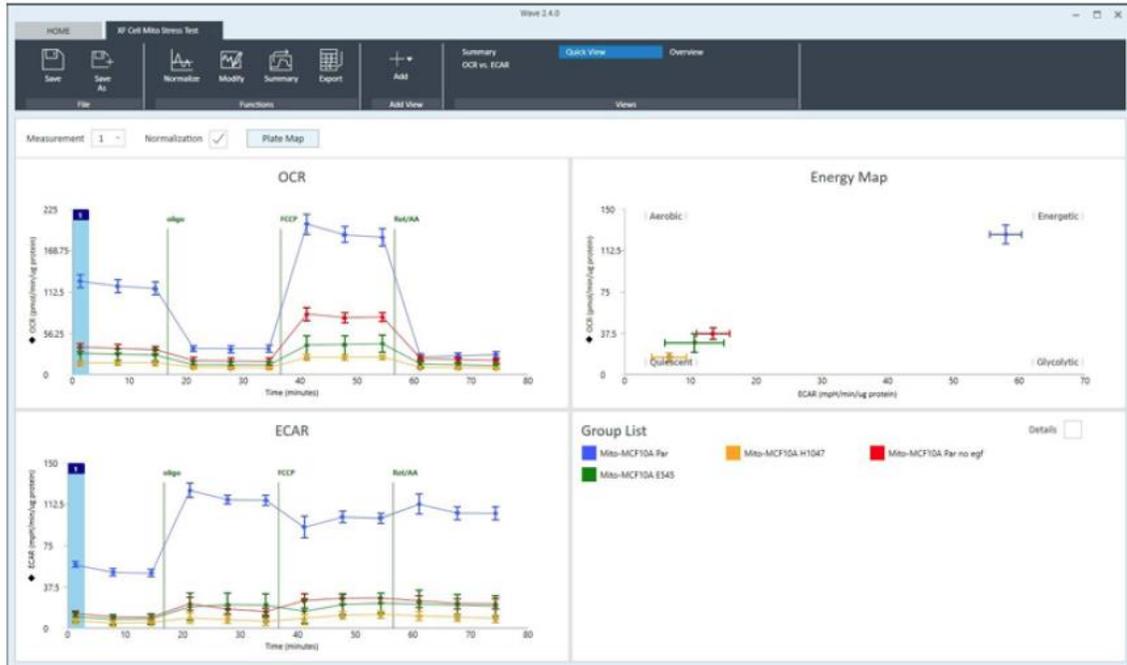

4.10.2 Overview

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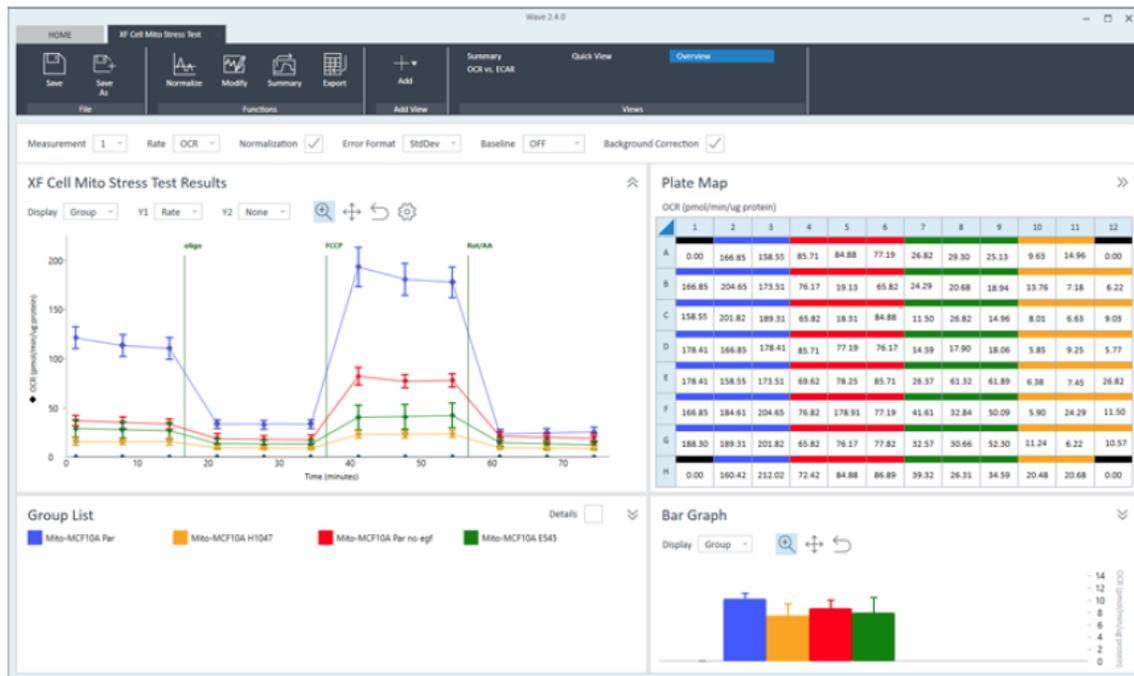
4.10.3 OCR vs. ECAR

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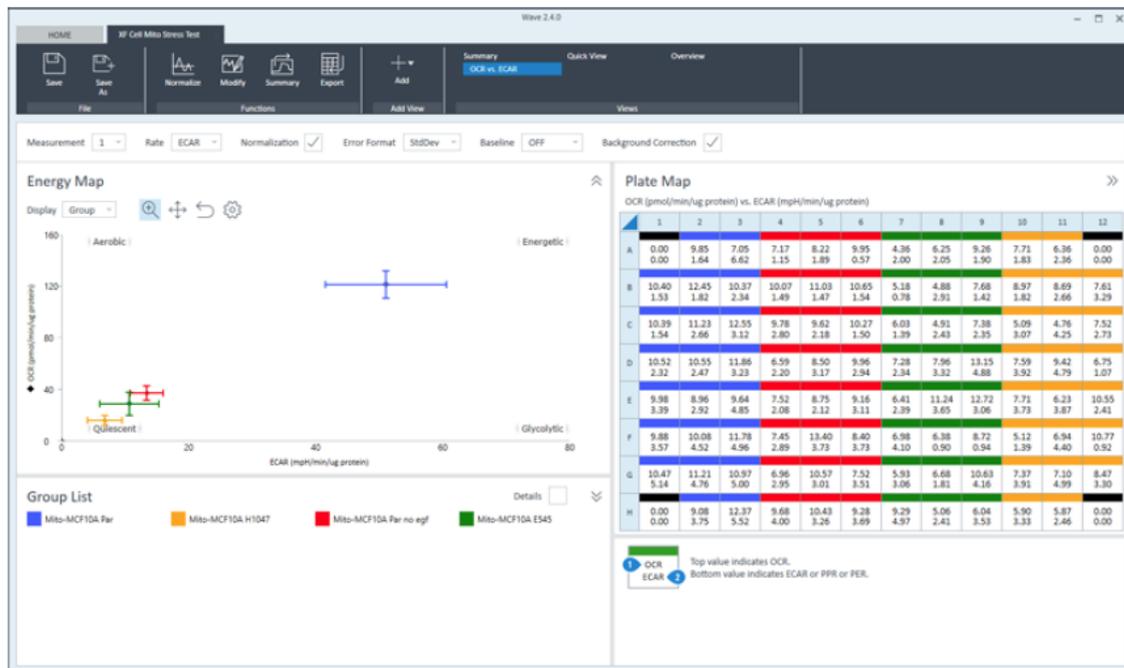
4.11.1 Data Export

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

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

2 Click **Microsoft Excel**.

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

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

5   MAINTENANCE

The XFe instrument was designed for minimal cleaning and maintenance by the user. Note all consumables are disposable and none of the instrument components come into contact with the cell plate at any time, preventing cross-contamination of biological or chemical materials. However, there are some minor tasks that can be performed by the user if necessary.

5.1 Probe Cleaning:

If cell culture plate wells are over-filled, or over a long period of time, the optical surface on the bottom of the measurement probes may require cleaning. Use a cotton-tipped stick and 95% ethanol to clean these optical windows beneath each probe. Take care not to scratch the surface and do not use a cleaner that leaves a residue.

5.2 Air filter replacement:

At approximately one year intervals, the air filter at the rear of the analyzer should be replaced. The filter retainer snaps away from the housing- use a screwdriver or wooden stick to remove it.

6   REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT