Seahorse XF Cell Energy

Phenotype Test Report Generator

User Guide

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Introduction

The Seahorse XF Cell Energy Phenotype Test Report Generator is the Seahorse-recommended tool for analysis of your Seahorse XF Cell Energy Phenotype Test assay data. The Report Generator automatically calculates and report the key parameters of the Seahorse XF Cell Energy Phenotype Test: Baseline Phenotype, Stressed Phenotype, and Metabolic Potential.

Values are calculated as absolute oxygen consumption rate (OCR) in pmol O_2/min, or absolute extracellular acidification rate (ECAR) in mpH/min. The Seahorse XF Cell Energy Phenotype Test Report Generator supports assay results from all Seahorse XF Analyzers and provides a simple, standardized Summary Report displaying the Energy Phenotype for each group or condition analyzed in a just a few short steps.

Assay Parameter Calculations

The Seahorse XF Cell Energy Phenotype Test Report Generators uses the equations outlined in Table 1 below to calculate the parameters of the Seahorse XF Cell Energy Phenotype Test. Each parameter value calculated in the Report Generator represents the average of individual well calculations in an Assay Group on the Plate Map. Error bars are calculated based on the individual well calculations for each parameter. See the Calculations tab in the Report Generator for more information and example calculations.

Seahorse XF Cell Energy Phenotype Test Profile
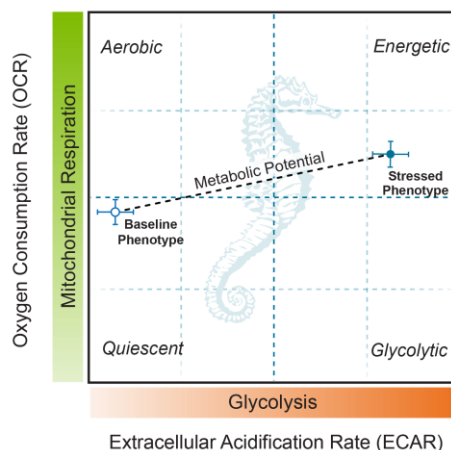
Table 1 | Seahorse XF Cell Energy Phenotype Test Parameter Equations

Parameter	Equation Used in Report Generator
Baseline OCR	Last OCR rate measurement before first injection
Baseline ECAR	Last ECAR rate measurement before first injection
Stressed OCR	Maximum OCR rate measurement after the first injection
Stressed ECAR	Maximum ECAR measurement after the first injection
Metabolic Potential (OCR)	(Stressed OCR Rate/Baseline OCR Rate) * 100%
Metabolic Potential (ECAR)	(Stressed ECAR Rate/Baseline ECAR Rate) * 100%

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Preparing Assay Result Files for Analysis

Workflow
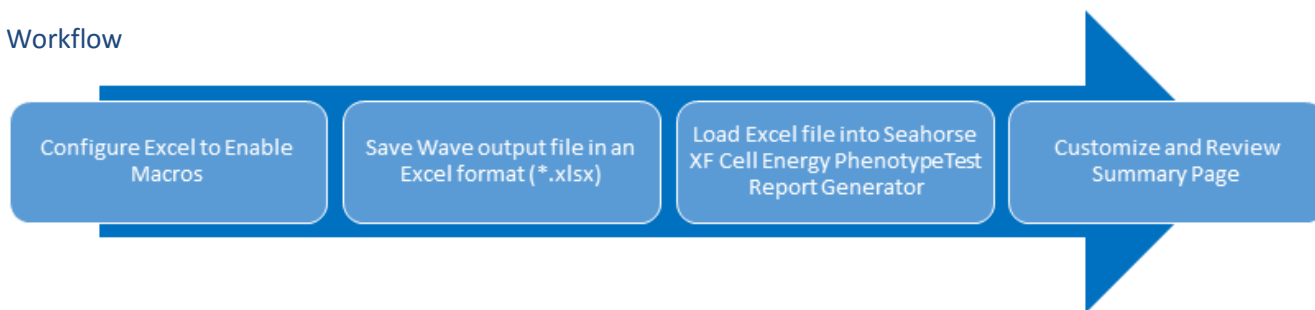
Configuring Microsoft Excel to Enable Macros

The Seahorse XF Cell Energy Phenotype Test Report Generator is compatible with Microsoft® Excel™ 2007, 2010, or 2013.

Excel must be configured to allow **macros** to run:

To enable macros once:
1. Double-click the *Seahorse XF Cell Energy Phenotype Test Report Generator* file.
2. Click **Enable Editing** and **Enable Content** (located on the yellow information bar) if prompted to do so when opening the Report Generator file.

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

Saving Wave Assay Result File in Excel Format (*.xlsx)

The Seahorse XF Cell Energy Phenotype Test Report Generator uses Assay Result data exported as an Excel file (*.xlsx) from Wave (Controller or Desktop) or from the XFp Analyzer to populate the groups for selection and calculate the parameters of the Seahorse XF Cell Energy Phenotype Test.

1. **Open** the XF Cell Energy Phenotype Assay Result file (*.asyr) in Wave (Desktop of Controller)[†].
2. Click **Save As...** and select **Excel 2007/2010 (*.xlsx)** as the file type and follow the prompts to select a name and location for the Excel output file.

[†]***Note***: For XF24 and XF96 users *.xfd files will need to be converted to *.asyr before using the Seahorse XF Cell Energy Phenotype Test Report Generator. To convert files:
- Open **Wave** > Browse and select the **.xfd result file** > Click **Open.**
- Click **Save as...** and select Assay Analyze (*.asyr) as the file type > Rename (if necessary) and select location to save the file.
- Save the .xfd file as an *.asyr > Proceed to **Step 2** above.

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Analyzing XF Assay Results

To begin using the Seahorse XF Cell Energy Phenotype Test Report Generator, first unzip the folder downloaded from the Seahorse Bioscience website. Contained in this folder is the Report Generator as well as instrument-specific Assay Template. The Assay Template can be imported to your XF^e or XFp Analyzer to quickly set up and run the XF Cell Energy Phenotype assay. To begin using the Report Generator, double-click the file called: *Seahorse XF Cell Energy Phenotype Test Report Generator.xltm*

> **TIP!**
> Can't find the zipped folder containing the Report Generator? Check your **Downloads** folder, which can be accessed through your internet browser or using Windows Explorer (PC). Alternatively, you can visit the Seahorse Bioscience website to download as many times as desired!

Import Data into the Report Generator

1. Click the **Load New Data File** button in the upper-right corner of the Summary Printout page.

2. Use the Windows dialogue box to locate the Excel result file and click **Ok**.

3. Select the desired Groups from the assay to display in the Report Generator and click **Update Summary** (Figure 1).
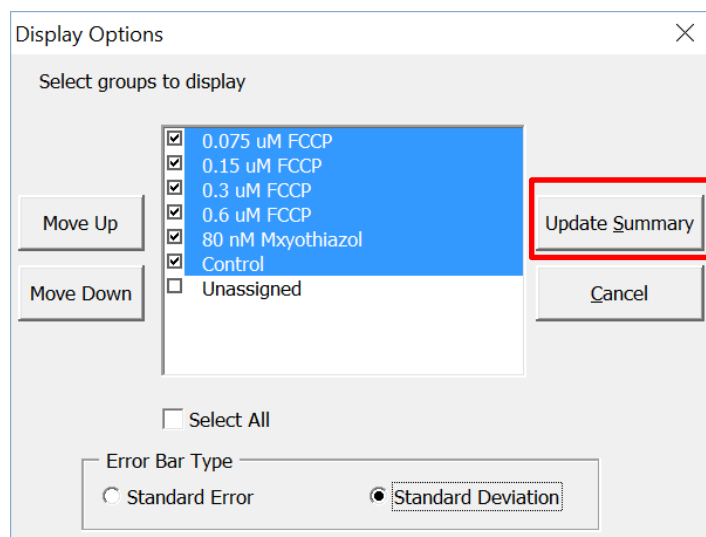
Figure 1 | Group names populated from the Wave Assay Result file appear in the group selection window.

The Report Generator will automatically calculate the Baseline Phenotype, Stressed Phenotype, and Metabolic Potential on the Summary Printout page for all groups selected in the *Load Options* window (Figure 1). See *Navigating the Seahorse XF Cell Energy Phenotype Test Report Generator* (next page) for more information about the Summary Printout and accompanying tabs, how to normalize, remove outliers, and other useful information about this Report Generator.

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Navigating the Seahorse XF Cell Energy Phenotype Test Report Generator

The Seahorse XF Cell Energy Phenotype Test Report Generator contains 5 tabs:

1. **Summary Printout:** One-page graphical summary of Assay Result presented as an XF Cell Energy Phenotype, Metabolic Potential, OCR and ECAR Bar Charts.

2. **Normalize**: Copy/Paste normalization data (protein content, cell count, etc.) to normalize XF Cell Energy Phenotype assay data to a cellular parameter.

3. **Data**: OCR and ECAR kinetic graphs, data table, and baseline OCR/ECAR ratio.

4. **Glossary**: Definition of terms used in the Seahorse XF Cell Energy Phenotype Test.

5. **How To**: Instructions on how to use the Seahorse XF Cell Energy Phenotype Test Report Generator (same content as this User Guide).

How To

The following sections describe common functions within the Report Generator and how to perform each function:

- Generate an Energy Phenotype from Seahorse XF Cell Mito Stress Test Data
- Error Bar Type and Calculations
- Save a Summary Report
- Normalize Data in the Report Generator
- Remove Outlier Wells in Wave

Generate an Energy Phenotype from Seahorse XF Cell Mito Stress Test Data

The Seahorse XF Cell Energy Phenotype Test Report Generator can generate an Energy Phenotype using Assay Result data from the Seahorse XF Cell Mito Stress Test:

1. **Open** the XF Cell Mito Stress Test Assay Result file (*.asyr) in Wave (Desktop or Controller).
2. Click **Save As…** and select **Excel 2007/2010 (*.xlsx)** as the file type and follow the prompts to select a name and location for the Excel output file.
3. Import the Excel file into the Seahorse XF Cell Energy Phenotype Test Report Generator following the same steps on page 5.

Using the imported XF Cell Mito Stress Test Excel file, the Seahorse XF Cell Energy Report Generator automatically plots the Baseline Phenotype (OCR and ECAR rate measurement before any injection) and the Stressed Phenotype (maximum OCR and ECAR rate measurement) on the XF Energy Map for each group selected. See Table 1 for all calculations in the Seahorse XF Cell Energy Phenotype Test Report Generator.

Note: An Instrument Protocol with Custom Cycle steps are not supported in the Seahorse XF Cell Energy Phenotype Test Report Generator. See the Frequently Asked Questions section for more information.

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Error Bar Type and Calculations

Error Bar Type is a universal setting and applies to ALL graphs and charts in the Report Generator. Standard Deviation is selected as the default error bar type. To change the error bar type to Standard Error, click Edit Current Group Selection and select Standard Error.

- Standard Deviation is calculated using the Microsoft Excel function.

- Standard Error of the Mean is calculated using the equation: $\frac{(Standard\ Deviation\ of\ Group)}{\sqrt{(Number\ of\ Wells\ in\ Group)}}$

- Error Bars are calculated from each replicate of the rate measurement used to determine the XF Cell Energy Phenotype parameter (See Table 1 on page 3 for Parameter Equations).

Save a Summary Report

There are several options for saving your customized Seahorse XF Cell Energy Phenotype Test Report Generator:

Simplest + Recommended

- **Save As + Save – Excel Macro**:
  After importing your assay data into the *Macro-Enabled Template* file, save your customized report: Click *File > Save As* or click the *Save* icon (small floppy disc). Open the saved Summary Report to view results, format/customize the appearance of graphs and figures, modify group selection, or normalize results to a cellular or mitochondrial parameter.

  The Report Generator default file type is called an *Excel Macro-Enabled Template (*.xltm)*. This file cannot be overwritten. Seahorse Bioscience recommends using this file for analysis of each Seahorse XF Cell Energy Phenotype Test performed.

- **Save As – Excel Workbook**: Use the *Save As* function to save the customized Summary Report as an Excel Workbook file format (*.xlsx). Saving as an Excel workbook or any other file type than the default file type (Excel Macro: *.xlsm) will render the Report Generator inoperable – Additional assay data cannot be imported into an Excel Workbook file format.

- **Save As – PDF**: Use the *Save As* function to save the customized Summary Report as a PDF file format (*.pdf). Saving as a PDF or any other file type than the default file type (Excel Macro: *.xlsm) will render the Report Generator inoperable – Additional assay data cannot be imported into a PDF file format.

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Normalize Data in the Report Generator

Normalize Assay Result data to a cellular or mitochondrial parameter in the Seahorse XF Cell Energy Phenotype Test Report Generator. Data can be copied and pasted from any source into the Report Generator, or typed manually into the **Normalization Plate Map**.

1. Open the Report Generator.
2. Import the Excel result file. The Normalization button on the Summary Printout tab will be inactive until normalization data has been applied to the Report Generator (Figure 2).
3. Click the **Normalize** tab.
4. *Copy* and *Paste* normalization values into the **Normalization Plate Map** on the Normalization tab.
5. Type in a *Normalization Unit* (required).
6. Press **Apply**.

Figure 2 | Normalized data has not been applied to the Report Generator.

How do I know my data is normalized?

- **Normalization Button**: Located on the Summary Printout tab, use the Normalization button to toggle ON/OFF displaying normalized and non-normalized XF Cell Energy Phenotype assay data.

  Normalize ON: Normalization values have been applied to the report generator and the displayed data has been recalculated using the applied normalization values (Figure 3).
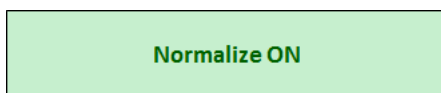
Figure 3 | Data displayed in the Report Generator is normalized.

  Normalize OFF: Rate data displayed in the kinetic graph and assay parameter bar charts do **not** have normalization values applied (Figure 4).
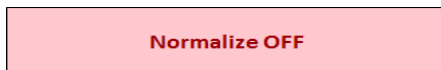
Figure 4 | Data displayed in the Report Generator is not normalized.

- **Y-axis Label**: Before applying normalizing data in the Report Generator, the y-axis label will be displayed as OCR (pmol/min) or ECAR (mpH/min) (Figure 5). After applying normalization data, the y-axis for all graphs in the Report Generator will display the normalization parameter (Figure 6).
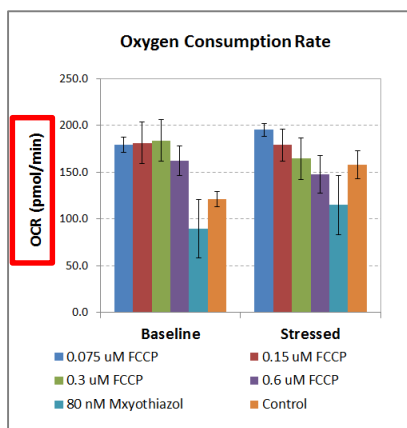
Figure 5 | Default Y-axis label, normalization data has not been applied to the data in the Report Generator.
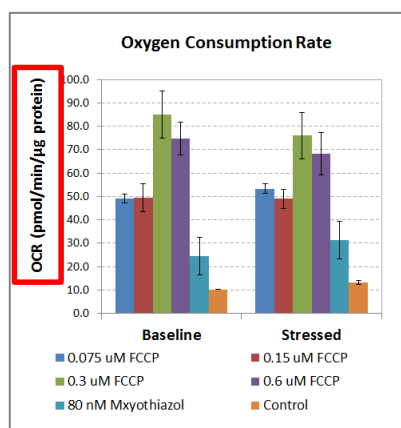
Figure 6 | Y-axis label with after applying protein normalization data in the Report Generator.

Remove Outlier Wells in Wave

Before exporting Assay Results to Excel from Wave (Desktop or Controller), outliers and unwanted wells must be reassigned to an 'Outlier Group' or unassigned from the Plate Map.

> **TIP!**
> Record Coordinates: After reviewing data but before modifying **Groups/Conditions** or **Plate Map**, write down the coordinates of the outliers on a piece of paper. Example: The yellow outlier well coordinates in Figure # below are: B3, B6, E4, E8, and E9.

Within Wave (Desktop or Controller):

1. Create 'Outlier' Group:
    a. Open the Assay Result file in Wave (Desktop or Controller).
    b. Click **Modify** (upper-right corner).
    c. Click **Groups/Conditions**.
    d. On the **Groups/Conditions** page, press **Add** to add a new Group. Name the new group 'Outliers'.
    e. Click **Apply**.

2. Assign Outlier Wells to 'Outlier' Group:
    a. Click **Modify** (upper-right corner).
    b. Click **Plate Map**.
    c. Locate the group name 'Outlier' from the list of groups on the left, then click the group name to select the 'Outlier' group.

> **TIP!**
> Unassign Assay Wells in Wave**:** A quicker method to remove outliers for Report Generator analysis is to unassign assay wells. To do this, open the Assay Result file in Wave and click **Modify**. Next, click **Plate Map** and simply click each assay well that you want to *unassign*. Unassigned assay wells will turn gray. Click **Apply** and Wave will automatically create an *Unassigned* group. Proceed to *Step f.*

    d. Click the wells on the Plate Map to assign to them to the 'Outlier' group. Below is an example of Outlier wells assigned to the Plate Map outlined in red (Figure 7).

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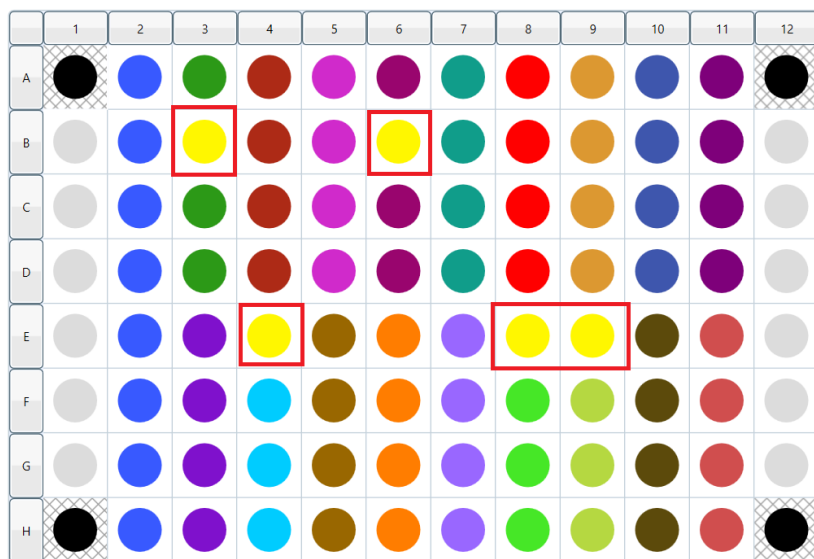
Figure 7 | Plate Map view in Wave.
The wells with a red outline have been reassigned to the 'Outlier' group (denoted by the yellow group color).

e. Click **Apply**.

f. Click **Save As** to save the Assay Result data as a MS Excel Workbook (*.xlsx). See section Save Wave Assay Result File in Excel Format for steps on saving data as an Excel Workbook.

Optional: Copy Normalization Values from Wave Desktop:
If the kinetic data has been normalized in Wave Desktop or Wave Controller, Copy/Paste the normalization values into the Report Generator

In Wave (Desktop or Controller):
1. Open the Assay Result file (*.asyr)
2. Click **Modify** (on any Analysis View), then click **Normalization**.
3. Press **Select All** to highlight the Plate Map in Wave.
4. Press **Ctrl + C** to copy the normalization values from the Wave > Normalization View.

*Note: Wave (Desktop and Controller) exports **raw** data only – Normalized rate data is **not** exported in the MS Excel output file.*

In Seahorse XF Cell Energy Phenotype Report Generator:
1. Click the **Normalization** tab.
2. Click and highlight all wells in the Normalization Plate Map.
3. Press **Ctrl + V** to paste the copied normalization values from Wave into the Normalization Plate Map.
4. Type in a **Normalization Unit** (required).
5. Press **Apply**.

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Glossary

Seahorse XF Cell Energy Phenotype Test

Oxygen Consumption Rate (OCR) – The rate of decrease of oxygen concentration in the assay medium. OCR is a measure of the rate of mitochondrial respiration of the cells.

Extracellular Acidification Rate (ECAR) – The rate of increase in proton concentration [or decrease in pH] in the assay medium. ECAR is a measure of the rate of glycolysis of the cells.

Baseline Phenotype – OCR and ECAR of cells at starting assay conditions (specifically, in the presence of non-limiting quantity of substrates).

Stressed Phenotype – OCR and ECAR of cells under an induced energy demand (specifically, in the presence of stressor compounds).

Metabolic Potential – Percentage increase of stressed OCR over baseline OCR, and stressed ECAR over baseline ECAR. Metabolic Potential is the measure of cells' ability to meet an energy demand via respiration and glycolysis.

Frequently Asked Questions

How do I remove outlier wells in the Report Generator?

Outliers must be re-assigned to an 'Outlier' group in Wave (Desktop or Controller). See page 9 for more information.

I'm unable to import my data as an Excel file into the Report Generator for analysis, why?

Ensure the file has been exported from Wave (Desktop or Controller) or from XFp 1.1 software as an Excel file (*.xlsx). Assay Result files must be saved as an Excel Workbook (*.xlsx) file format before importing into any Report Generator. If the Excel file has been exported from Wave but still cannot be imported into the Report Generator, please contact Seahorse Technical Support via email: support@seahorsebio.com

If you receive an error message about Instrument Protocol (XF^{e}96; XF^{e}24; XF96; XF24 only)

Errors upon data import into the Report Generator are likely caused by a c*ustom cycle* in your Instrument Protocol. A *Custom Cycle* refers to an additional 'Mix' or 'Wait' command step in the Instrument Protocol an assay. Custom Cycles are not part of the standardized Assay Template for the Seahorse XF Cell Energy Phenotype Test and are not supported in Report Generator analysis. Please contact Seahorse Technical Support if you have any additional questions regarding Custom Cycles.

How do I combine multiple result files in this Report Generator?

The Seahorse XF Cell Energy Phenotype Test Report Generator supports analysis of a single Assay Result file (exported from Wave as an Excel Workbook) at a time. Combining multiple Excel files on one graph in the Report Generator is not supported at this time.

What rate measurements are used to calculate the parameters in this Report Generator?

Rate measurements and equations used to calculate the parameters of the Seahorse XF Cell Energy Phenotype Test are outlined in the Assay Parameter Calculations on page 3 of this User Guide.

Feedback

Any questions, concerns or suggestions should be directed to Seahorse Technical Support via email: support@seahorsebio.com

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