Agilent Seahorse XF Cell Energy Phenotype Test

Report Generator User Guide

Agilent Technologies

Notices

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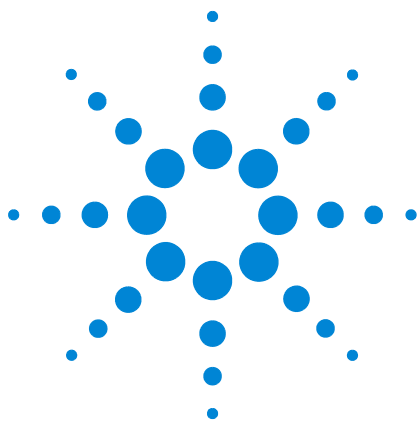
Contents

1

Introduction

Agilent Seahorse XF data analysis and interpretation – accelerated. The Agilent Seahorse XF Cell Energy Phenotype Report Generator is the recommended software tool for analysis of the Agilent Seahorse XF Cell Energy Phenotype Test. The Agilent Seahorse XF Cell Energy Phenotype Report Generator is a Microsoft Excel Macro that automatically calculates and reports assay parameters (Table 1 on page 6) of the Agilent Seahorse XF Cell Energy Phenotype Test: Baseline Phenotype, Stressed Phenotype, and Metabolic Potential.

All data are calculated and displayed as absolute oxygen consumption rate (OCR) in pmol O_2/min, and absolute extracellular acidification rate (ECAR) in mpH/min. The Report Generator supports assay result data from all Agilent Seahorse XF Analyzers.
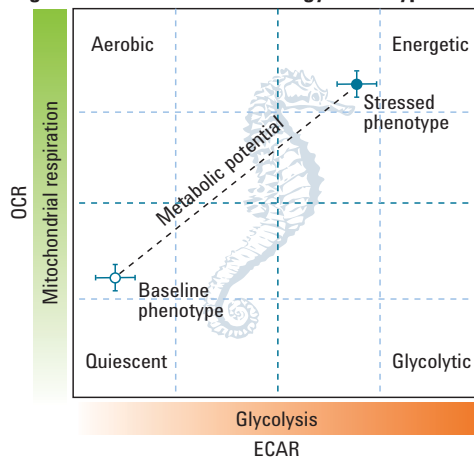
Agilent Seahorse XF Cell Energy Phenotype Profile

Figure 1   Agilent Seahorse XF Cell Energy Phenotype Profile displayed as a scatter plot of OCR (y-axis) and ECAR (x-axis).

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Parameter Calculations

Table 1 outlines the parameter equations used in the Agilent Seahorse XF Cell Energy Phenotype Report Generator. Each parameter value calculated represents the average of individual well calculations for each assay group on the Plate Map. Error bars are calculated based on the individual well calculations for each parameter.

Table 1   Agilent Seahorse XF Cell Energy Phenotype Test Parameter Equations

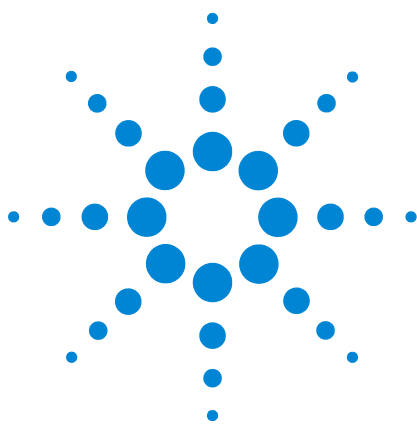| Parameter name	Parameter equation
Baseline OCR	Last OCR rate measurement before first injection
Baseline ECAR	Last ECAR rate measurement before first injection
Stressed OCR	Maximum OCR rate measurement after the first injection
Stressed ECAR	Maximum ECAR measurement after the first injection
Metabolic potential (OCR)	(Stressed OCR/Baseline OCR) × 100 %
Metabolic potential (ECAR)	(Stressed ECAR/Baseline ECAR) × 100 %

XF Report Generator Overview

The Agilent Seahorse XF Cell Energy Phenotype Report Generator displays data and assay-related other information on 5–6 tabs.

For the optimal Report Generator data analysis experience, update to Wave 2.3.

*   **Summary printout:** A one-page summary report of the imported assay result data presented as an XF Cell energy phenotype, metabolic potential, OCR, and ECAR bar charts.

*   **Normalize**: A plate map of normalization values applied in the imported result file. This tab is only displayed for result files that have been normalized in Wave 2.3 before Excel export. See the Wave User Guide for more info.

*   **Note**: Assay result data that has not been normalized in Wave 2.3 will not be displayed in the Normalize tab

*   **Measures sheet**: OCR and ECAR rate data plotted as kinetic graphs and as data tables for each group selected

*   **Assay parameter per well**: A data table of assay parameters per well for each group in the imported file

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

*   **Project information**: Software License Terms, detailed assay information, and plate map layout displaying group positions and excluded wells

2

How To

The following sections describe how to perform routine functions in the Report Generator:

- Analyze data in the Report Generator

- Save a Summary Report

- Generate an Energy Phenotype using XF Cell Mito Stress result data

- Normalize assay results

- Exclude outlier wells

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Configure Microsoft Excel to Enable Macros

The Agilent Seahorse XF Cell Energy Phenotype Report Generator is a Microsoft Excel Macro-Enabled Template, and is compatible with Microsoft Excel versions 2010, 2013, and 2016 for Windows PCs, and Microsoft Excel for Mac versions 2011 and 2015. To use this Report Generator, Excel must be configured to allow macros to run. To enable macros once, double-click the **Seahorse XF Cell Energy Phenotype Report Generator.xltm** file, then click **Enable Editing** and **Enable Content** (yellow information bar) if prompted (Figure 2).
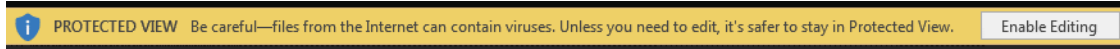

Figure 2   Enable macros using the **Enable Editing** button, as seen on the yellow information bar. This needs to be performed once upon opening the Report Generator for the first time after download.

To always enable macros (recommended for the best experience using Report Generators):

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**.

Analyze Data in the Report Generator

Direct-export from Wave 2.3 *Recommended*

1 The recommended method to analyze assay result data in the Report Generator is using direct export from Wave 2.3 (Figure 3 on page 12).

2 Transfer the assay result file to a personal computer using a USB flash drive or shared network directory.

3 Double-click to open the assay result file in Wave Desktop 2.3.

4 Click **Export**.

5 Select the **Seahorse XF Cell Energy Phenotype Test Report Generator**. *Optional:* Modify the default file name and save location.

6 Click **Save**.

Import Excel file manually

1 In Wave 2.3, click **Export**.

2 Select **Microsoft Excel** and click **Save**. *Optional:* Modify the default file name and save location.

3 Download the Agilent Seahorse XF Cell Energy Phenotype Report Generator from the Agilent website.

4 Unzip the compressed folder and open the Report Generator file.

5 Click **Load New Data File**.

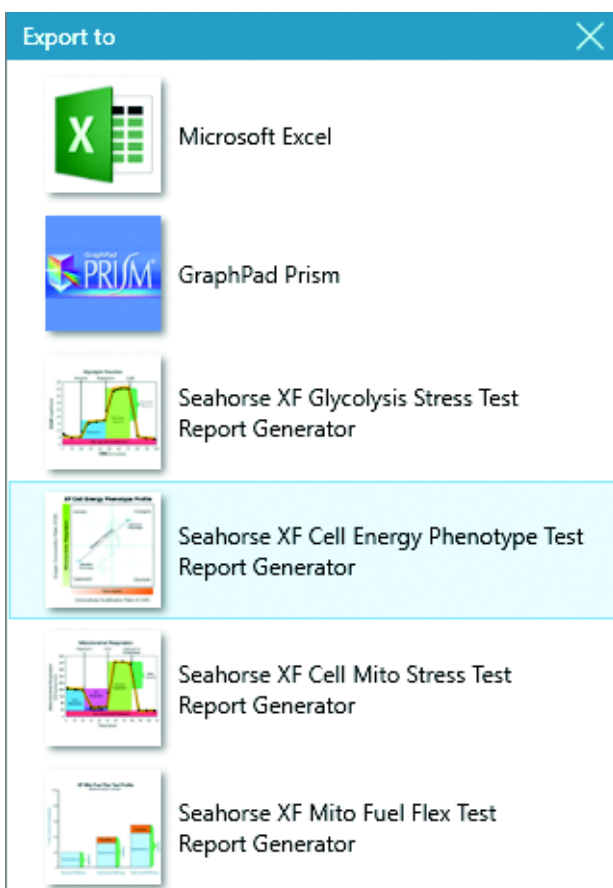6 Locate the Microsoft Excel file (exported from Wave 2.3) and click **Open**.

Figure 3   Wave 2.3 (Desktop and Controller) export options.

Select Groups and Display Results

After exporting assay result data from Wave 2.3 to the Report Generator (or manually importing Excel data), use the Display Options dialogue window to select groups from the assay to display, and click **Update Summary** (Figure 4). The Report Generator will automatically calculate the parameters for each group selected and display results on the **Summary Printout** tab.

Error Bar Type

Standard Deviation is selected as the default **Error Bar Type** for all graphs. The **Error Bar Type** applies to all graphs in the Report Generator.
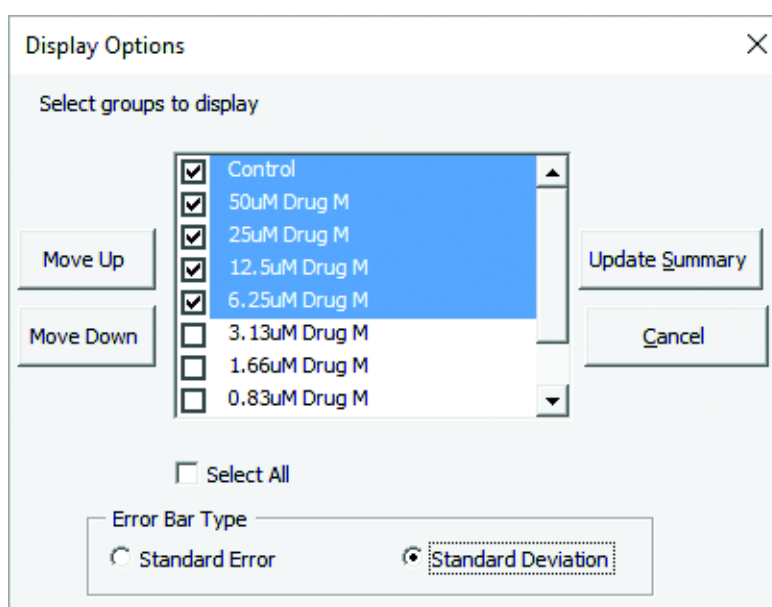

Figure 4   Group names configured in Wave will be displayed on the Display Options window when selecting groups in the Report Generator.

Save a Summary Report

After exporting from Wave 2.3

When exporting data from Wave 2.3 to the Report Generator, Wave will automatically create a unique Summary Report file (Microsoft Excel Macro-Enabled Worksheet) for each exported result file. Additional data files cannot be loaded into this Report Generator file, and must be created using the direct-export from Wave 2.3.

- **Save/Save as:** Click the **Save** icon (small floppy disc) to display the Save As function. Select a file location, and enter a custom file name if desired. The saved Summary Report can be re-opened to view the calculated parameters for the selected groups, format/customize the appearance of graphs and figures, or select new groups from the assay to run through the Report Generator. The Report Generator default file type is a Microsoft Excel Macro-Enabled Template (*.xltm) – this file cannot be overwritten.

- **Save As – Excel Workbook:** Use the **Save As** function to save the customized Summary Report as an Excel Workbook file format (*.xlsx).

- **Save As PDF:** Use the **Save As** function to save the customized Summary Report as a PDF file format (*.pdf).

| **NOTE** | Saving the Report Generator as an Excel Workbook or any other file type than the default file type (Excel Macro: *.xlsm) will render the Report Generator macro inoperable. Modifying the groups selected or importing additional assay result data are not supported as an Excel Workbook file type. |

Generate an Energy Phenotype from the Agilent Seahorse XF Cell Mito Stress Data

The Agilent Seahorse XF Cell Energy Phenotype Report Generator can generate an Energy Phenotype using assay result data from the Agilent Seahorse XF Cell Mito Stress Test. Simply export data from Wave 2.3 (or manually import Excel data) to the Report Generator, and select groups to display. The XF Cell Energy Phenotype Report Generator will plot the OCR/ECAR rate measurement before the first injection (Baseline Phenotype) and the maximum OCR/ECAR rate measurement after the first injection (Stressed Phenotype). See Table 1 on page 6 for more information about the calculations in this Report Generator.

NOTE Instrument protocols with Custom Cycle steps are not supported in the XF Cell Energy Phenotype Report Generator. See "Frequently Asked Questions" on page 21 for more information.

Error Bars and Calculations

Error Bar Type is a universal setting, and applies to all graphs and charts in the Report Generator. **Standard Deviation** is the default error bar type. To change the error bar type to Standard Error of the Mean (S.E.M.), click **Edit Current Group Selection** and select **Standard Error**.

- Error bars are calculated from each replicate of the rate measurement used to determine the assay parameter (see Table 1 on page 6).

- Standard deviation is calculated using the Microsoft Excel function.

- S.E.M. is calculated using Equation 1:

$$\frac{\text{(Standard deviation of group)}}{\sqrt{\text{(Number of wells in group)}}}$$

Equation 1.

Normalize Assay Results

It is highly recommended to analyze rate data that has been normalized to a cellular or mitochondrial parameter compared to nonnormalized, raw rate data. Normalization data must be added to the Normalize view in Wave 2.3 (Figure 5-A) before export. Normalized rate data will be used for parameter calculations and displayed by default in all kinetic graphs and bar charts in the Report Generator. Click the Normalize tab to view the normalization plate map, unit, and scale factor as entered in Wave 2.3 (Figure 5-B). Use the Normalize button on the Summary Printout page to toggle the data displayed on each chart between normalized and nonnormalized rate data (Figure 5-C).
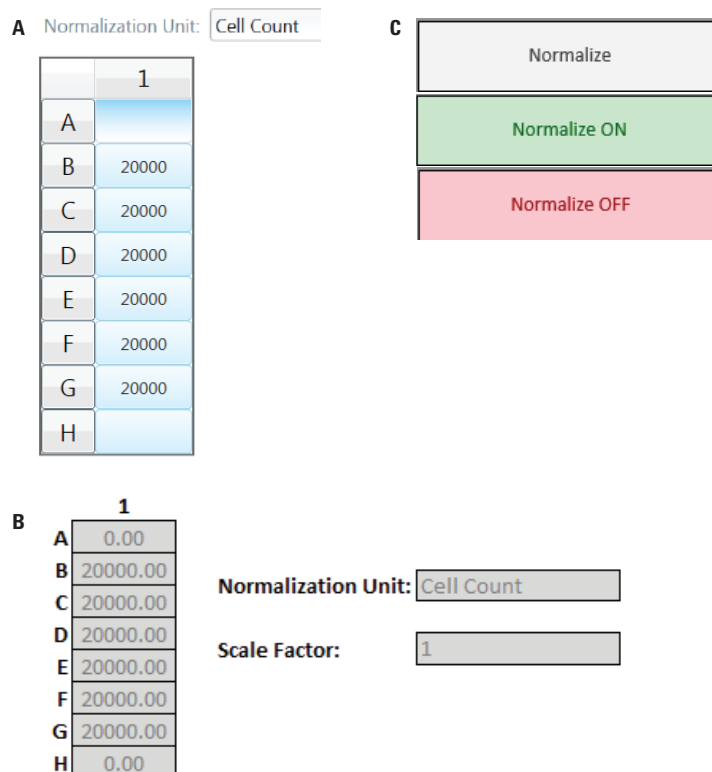

Figure 5   A) Example of a normalization plate map for the Agilent Seahorse XFp Analyzer in Wave 2.3.

B) The same normalization plate map from Wave 2.3 (Figure 5-A) displayed int he Report Generator.

C) **Normalize** button on the **Summary Printout** tab. By default, normalized rate data exported from Wave will be displayed as indicated by the Normalize ON button status (green). Click the **Normalize ON** button to toggle the data display to show nonnormalized rate data (Normalize OFF – red). Data exported from Wave 2.3 without normalized rate data will show a gray Normalize button.

NOTE	To preserve data integrity between Wave 2.3 and Report Generators, normalized data exported to a Report Generator is locked for editing. To modify the normalization values used in the Report Generator, they first must be edited in Wave 2.3, and then re-exported to the Report Generator. If result data has not been normalized, the Report Generator will not display the Normalize tab.

Exclude Outliers/Groups from Analysis

Individual assay wells or entire groups/conditions can be excluded from parameter calculations in the Report Generator. Before exporting data from Wave 2.3, click the assay well(s) on the plate map, or double-click the group name(s) on the **Group List** to exclude those wells or groups from data export. The **Project Information** tab displays the plate map layout and any assay wells or groups that have been excluded from the Excel export (Figure 6).

Figure 6   XFp Plate Map on the **Project Information** tab. Assay well C has been turned off in Wave 2.3 prior to export, therefore the Control group parameter calculations are based on assay wells B and D only.
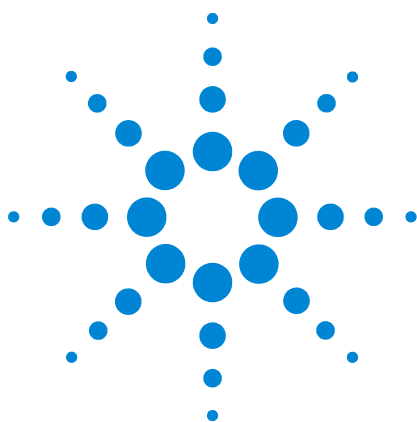
3

Frequently Asked Questions

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

Parameter equations are described in Table 1 on page 6 of this User Guide.

How do I remove outlier wells in the Report Generator?

Outlier assay wells must be turned OFF, or reassigned in Wave 2.3 prior to Excel export. See the "Exclude Outliers/Groups from Analysis" on page 19 or the Wave User Guide for more information.

Can I use the Excel file exported from the XFp, XF96, or XF24 Analyzer?

It is recommended to export assay result data directly to the XF Report Generator from Wave 2.3 (Desktop or Controller), however data can also be manually imported as an Excel file (*.xlsx). Excel files exported from earlier versions of Wave (Desktop or Controller) and XF96/XF24 software are not compatible. If the Excel file has been exported from Wave but cannot be imported to the Report Generator, please contact Agilent Seahorse Technical Support.

If you receive an error message about Instrument Protocol (XFe96; XFe24; XF96; XF24 only)

Errors upon data import into the Report Generator are likely caused by a custom cycle in the 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 Agilent Seahorse XF Cell Energy Phenotype Test and not supported in Report Generator analysis. Please contact Agilent Seahorse Technical Support if you have any additional questions regarding Custom Cycles.

Can I use baseline rate data (%) to calculate assay parameters?

Normalized (or nonnormalized) OCR data are used for parameter calculations; parameter calculations using baseline rate data (%) are not supported at this time.

Where can I find the OCR and ECAR kinetic graphs?

Both OCR and ECAR kinetic graph data are displayed on the Measures Sheet tab in the Agilent Seahorse XF Cell Energy Phenotype Report Generator.

How do I analyze multiple result files in a Report Generator?

The Agilent Seahorse Multi-File XF Cell Energy Phenotype Report Generator enables the import and analysis of multiple XF Cell Energy Phenotype Test (and XF Cell Mito Stress) result files. Find out more and download the Multi-File XF Report Generator.

Feedback

Feedback for the Report Generator or other products is always encouraged. Please direct any questions, concerns or suggestions to Agilent Seahorse Technical Support at: seahorse.support@agilent.com

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