

Report Generator User Guide

Agilent Seahorse XF Glycolysis Stress Test

For research use only.

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Introduction

Seahorse XF data analysis and interpretation – **accelerated**. In a few simple steps, transform Seahorse XF Glycolysis Stress Test assay result data into interpretable, compelling data tables and figures of glycolytic function for each group analyzed using the Seahorse XF Glycolysis Stress Test Report Generator. Easily copy figures or data tables from the Report Generator to other software programs for additional graphing or statistical analysis and eliminate tedious, error-prone steps in any XF data analysis workflow.

The Seahorse XF Glycolysis Stress Test Report Generator is a Microsoft Excel Macro that automatically calculates and reports assay parameters of the Seahorse XF Glycolysis Stress Test (Table 1) as absolute Extracellular Acidification Rate (ECAR) in mpH/min and supports assay result data from all Agilent Seahorse XF Analyzers.

Parameter Calculations

Parameter calculations performed in the Seahorse XF Glycolysis Stress Test Report Generator are outlined in Table 1 below. 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. See the *Calculations* tab in the Report Generator for more information and example calculations.

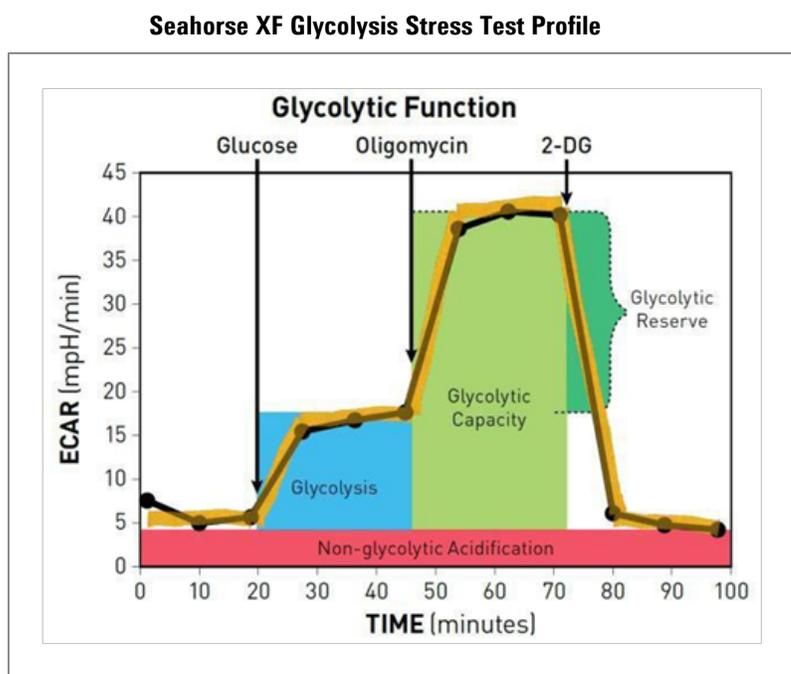


Table 1 | Seahorse XF Glycolysis Stress Test Parameter Equations

Parameter Value	Equation
Glycolysis	(Maximum rate measurement before Oligomycin injection) – (Last rate measurement before Glucose injection)
Glycolytic Capacity	(Maximum rate measurement after Oligomycin injection) – (Last rate measurement before Glucose injection)
Glycolytic Reserve	(Glycolytic Capacity) – (Glycolysis)
Glycolytic Reserve as a %	(Glycolytic Capacity Rate) / (Glycolysis) × 100
Non-glycolytic Acidification	Last rate measurement prior to glucose injection
Acute Response	(Last measurement rate before glucose injection – Last rate measurement before acute injection)

XF Report Generator Overview:

The Seahorse XF Glycolysis Stress Test Report Generator displays result data and other assay-related information on 4-5 tabs. For the optimal Report Generator data analysis experience, [update to Wave 2.3](#).

1. **Summary Printout:** One-page *Summary Report* of the imported Seahorse XF Glycolysis Stress Test assay result data presented as a kinetic graph and bar charts for select assay parameters.
2. **Bar Charts:** Assay parameters for each group in the imported result data displayed as bar charts.
3. **Normalize:** Plate map of normalization values applied to the result data. 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 display the **Normalize** tab.
4. **Measures Sheet:** Data table of assay parameters, data table and kinetic graph of ECAR values for each group displayed in the Report Generator, and coordinates for each group on the Plate Map.
5. **Calculations:** Overview of what rates are selected on the kinetic graph, equations, and how the well-to-well calculations are performed for each group analyzed.

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*
- Normalize Assay Results
- Exclude Outlier Wells

Configure Microsoft Excel to Enable Macros

The Seahorse XF Glycolysis Stress Test 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. In order to use the XF Report Generators, Excel must be configured to allow **macros** to run:

To enable macros once:

1. Double-click the *XF Glycolysis Stress Test Report Generator.xltn* file icon.
2. Click **Enable Editing** and **Enable Content** (located on the yellow information bar) if prompted to do so when opening the Report Generator.

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:

Recommended

Direct-export from Wave 2.3:

The recommended method to analyze assay result data in the Report Generator is using the **direct export** feature available in Wave 2.3:

1. Transfer the assay result file to a personal computer using a USB flash drive or shared network directory.
2. Double-click to open the assay result file in Wave Desktop 2.3.
3. Click **Export**.
4. Select the **Seahorse XF Glycolysis Stress Test Report Generator**.
Optional: Modify the default file name and save location.
5. Click **Save**.

Import Excel file manually:

To manually import assay result data to the Report Generator:

1. Click **Export**.
2. Select **Microsoft Excel**.
Optional: Modify the default file name and save location.
3. Click **Save**.
4. Download the Seahorse XF Glycolysis Stress Test Report Generator from the [Agilent website](#).
5. Unzip the compressed folder and open the Report Generator file.
6. Click **Load New Data File**.
7. Locate the Microsoft Excel file (exported from Wave 2.3) and click **Open**.

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 1). 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.

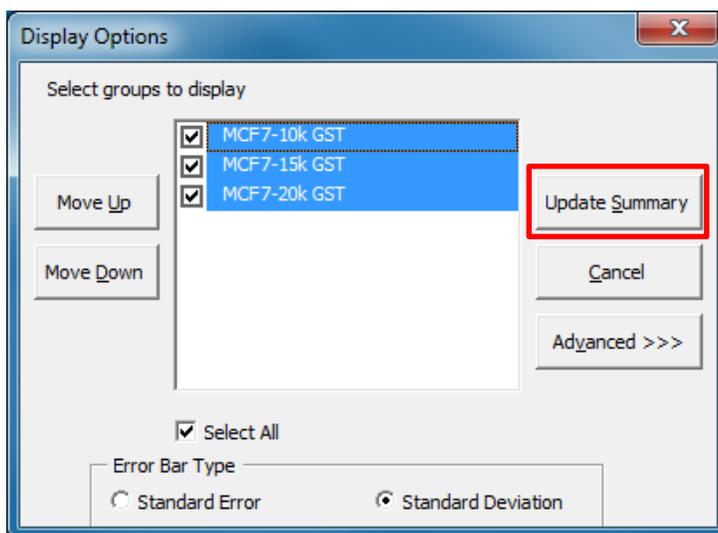


Figure 1 | Group names configured in Wave will be displayed on the *Display Options* window when selecting groups in the Report Generator.

Save a Summary Report:

Recommended

Direct-export from Wave 2.3:

Wave 2.3 automatically creates a unique *Summary Report* file (Microsoft Excel Macro-Enabled Worksheet) for each assay result file exported the Report Generator. Open the *Summary Report* file to view calculated parameters for selected groups, format/customize the appearance of graphs and figures, or select new groups from the assay result file to calculate assay parameters. Additional data files cannot be loaded into this Report Generator file as this file represents the Summary Report for *one* assay – create a *Summary Report* for each assay result file using the direct-export feature from Wave 2.3.

Import Excel file manually:

Options for saving a Summary Report of assay result data that has been manually imported to the Report Generator include:

- **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: *.xltm) will render the Report Generator macro inoperable – modifying the groups selected or importing additional assay result data is not supported in the Excel Workbook file type.

Advanced Options

Advanced Options is accessed on the *Display Options* window when selecting assay groups to display and displays the Instrument Protocol, which is automatically imported into the Report Generators from the Excel file. Instrument Protocol displays the number of:

- Injections performed in the imported Excel file.
- Measurements before injection (Baseline) and after each injection.

Acute Injections

An injection that occurs in the Seahorse XF Glycolysis Stress Test assay following the baseline measurements, but before the Glucose injection is called an *Acute Injection*. The default Seahorse assay template in Wave (Desktop or Controller) called “Seahorse XF Glycolysis Stress Test (Acute Injection)” has the specific Instrument Protocol for acute injections and should be used for every Seahorse XF Glycolysis Stress Test assay with an acute injection. For custom assays, this injection step must be manually added to the Instrument Protocol in Wave (Desktop or Controller) prior to the assay.

Note: An Instrument Protocol with Custom Cycle steps are not supported in XF Report Generators.

- An acute injection must be injected **before** Glucose from Port A on the Cartridge.
- The **Acute Injection** checkbox is enabled by default when a 4th injection is detected in the imported Excel result file.
- Parameter calculations in Table 1 are shifted by the number of acute response measurements for assays with an **Acute Injection**.

Error Bar 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, 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](#)).
- Standard Deviation is calculated using the Microsoft Excel function.
- Standard Error of the Mean is calculated using the equation: $\frac{(Standard\ Deviation\ of\ Group)}{\sqrt{(Number\ of\ Wells\ in\ Group)}}$

Normalize Assay Results:

It is highly recommended to analyze rate data that has been normalized to a cellular or mitochondrial parameter compared to non-normalized, raw rate data. Normalization data must be added to the assay result file on the **Normalize** view using Wave 2.3 (Figure 2a) before export, normalized rate data will be displayed by default for the kinetic rate data and assay parameter calculations in the XF Report Generator. Click the **Normalize** tab to view the normalization plate map, unit, and scale factor as entered in Wave 2.3. Use the **Normalize** button on the *Summary Printout* page to toggle the data displayed on each chart between normalized and non-normalized rate data (Figure 2c).

Normalization Unit: Cell Count	
	1
A	
B	20000
C	20000
D	20000
E	20000
F	20000
G	20000
H	

Figure 2a | Example of a normalization plate map for the Agilent Seahorse XFp Analyzer in Wave 2.3.

1	
A	0.00
B	20000.00
C	20000.00
D	20000.00
E	20000.00
F	20000.00
G	20000.00
H	0.00

Normalization Unit: Cell Count

Scale Factor: 1

Figure 2b | Example of a normalization plate map for the Agilent Seahorse XFp Analyzer in the Report Generator.

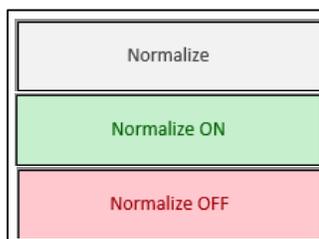


Figure 2c | **Normalize** button on the *Summary Printout* tab. Data exported from Wave 2.3 without normalized rate data will show a gray **Normalize** button. By default, normalized rate data will be displayed, as indicated by the *Normalize ON* button status, in the Report Generators. Click the *Normalize ON* button to toggle the data display to show non-normalized rate data.

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 XF Report Generator. If an XF Report Generator does not have the **Normalize** tab, this means the rate data was not normalized in Wave 2.3.

Exclude Assay Wells (Outliers) or Groups from Report Generator 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.

	1
A	Background
B	Control
C	Control
D	Control
E	2.5 uM
F	2.5 uM
G	2.5 uM
H	Background

Figure 2a | 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.

Frequently Asked Questions

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

Parameter equations are described in [Table 1](#) 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 section called [Exclude Outliers/Groups from Analysis](#) 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 [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 Seahorse XF Glycolysis Stress Test and not supported in Report Generator analysis. Please contact [Seahorse Technical Support](#) if you have any additional questions regarding Custom Cycles.

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

Normalized (or non-normalized) ECAR data is used for parameter calculations, parameter calculations using baseline rate data (%) is not supported at this time.

Where is my OCR data?

OCR (Oxygen Consumption Rate) data is displayed on the *Measures Sheet* tab in the Seahorse XF Glycolysis Stress Test Report Generator.

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

The Seahorse XF Glycolysis Stress Test Report Generator is designed for analysis of individual assay result files. Combining multiple result files in the Seahorse XF Glycolysis Stress Test Report Generator is not supported at this time.

Feedback

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

Glossary

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. OCR is a measure of the rate of glycolysis of the cells.

Glycolysis

The process of converting glucose to pyruvate. The Seahorse XF Glycolysis Stress Test presents the measure of Glycolysis as the ECAR rate reached by a given cell after the addition of saturating amounts of glucose.

Glycolytic Capacity

The measurement is the maximum ECAR rate reached by a cell following the addition of oligomycin, effectively shutting down oxidative phosphorylation and driving the cell to use glycolysis to its maximum capacity.

Glycolytic Reserve

This measure indicates the capability of a cell to respond to an energetic demand as well as how close the glycolytic function is to the cell's theoretical maximum.

Non-glycolytic Acidification

This measures other sources of extracellular acidification that are not attributed to glycolysis.