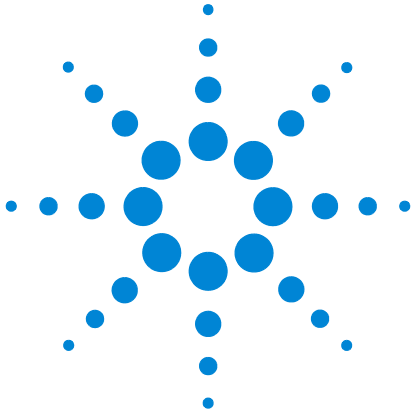

# Agilent Seahorse XF Glycolytic Rate Assay

## Report Generator User Guide

Agilent Technologies

# Notices



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# Contents

# 1 Introduction



The Agilent Seahorse XF Glycolytic Rate Assay provides accurate measurements of glycolytic rates for basal conditions and compensatory glycolysis following mitochondrial inhibition (Figure 1). The calculated glycolytic rates accounts for contribution of $CO_2$ to extracellular acidification derived from mitochondrial/TCA cycle activity, and are directly comparable to lactate assays.

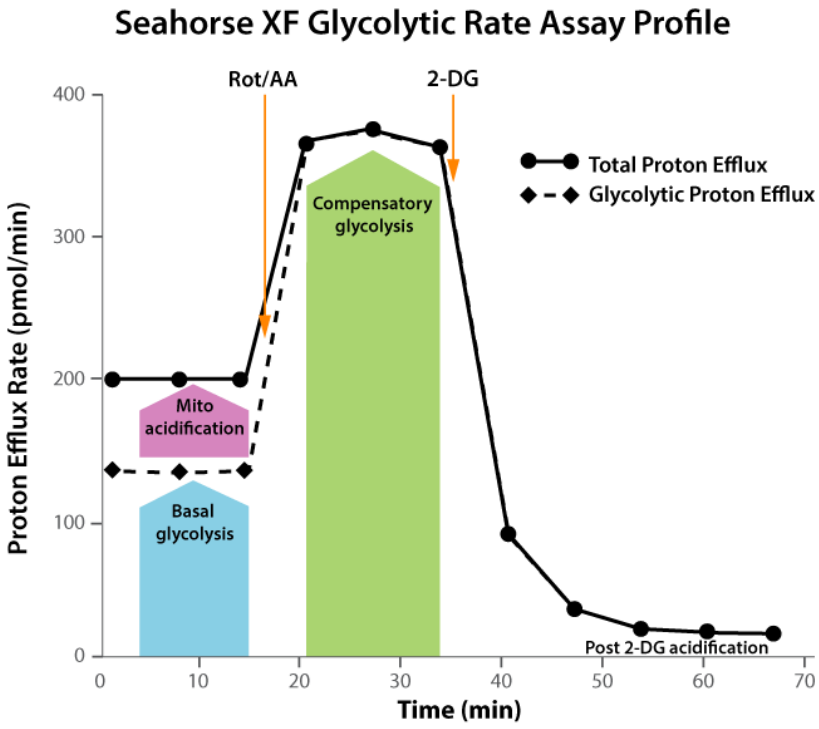


**Figure 1** Agilent Seahorse XF Glycolytic Rate Assay parameters and kinetic profile.

The Agilent Seahorse XF Glycolytic Rate Assay Report Generator is a Microsoft Excel Macro that automatically converts experimentally-derived OCR and ECAR data into Glycolytic Proton Efflux Rate (glycoPER), reported in pmol $H^+$/min (Table 1). Figures and data tables in the Report Generator can be easily transferred to other software programs for additional graphing or statistical analysis. The Seahorse XF Glycolytic Rate Assay Report Generator supports assay result data generated by Agilent Seahorse XFe96, XFe24, XFp and XF96 Analyzers.

## Parameter Calculations

Table 1 outlines the parameter calculations performed in the Seahorse XF Glycolytic Rate Assay 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 Glycolytic Rate Assay parameters equations (no induced assay).

| Parameter name | Parameter equation |
|---|---|
| Basal Glycolysis | Last glycoPER measurement before first injection. |
| Basal Proton Efflux Rate (PER) | Last PER measurement before first injection. |
| % PER from Glycolysis (Basal) | (Basal Glycolysis)/(Basal PER) x 100% |
| Compensatory Glycolysis | Maximum glycoPER measurement after Rot/AA injection. |
| mitoOCR/glycoPER (Basal) | [(Last OCR measurement before first injection) - (Minimum OCR after Rot/AA injection)] /(Basal Glycolysis) |
| Post 2-DG acidification | Minimum glycoPER measurement after 2-DG injection. |

**Note**: Definitions of PER and glycoPER can be found in the Agilent Seahorse XF Glycolytic Rate Assay Kit User Guide. Additional parameters are reported for an induced Seahorse XF Glycolytic Rate Assay in the section called Induced Assays.

# 2 Agilent Seahorse XF Report Generator Overview

The Seahorse XF Glycolytic Rate Assay Report Generator displays result data and other assay-related information on the four or five tabs described below.

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

- **Summary printout:** One-page Summary Report of the imported Agilent Seahorse XF Glycolytic Rate Assay data plotted as bar charts.
- **Normalize:** 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 display the **Normalize** tab.
- **Measures sheet:** Rate data plotted as kinetic graphs of ECAR, PER, OCR, and glycoPER, table of assay parameters and data table of measurement values for the kinetic graph.
- **Assay parameter per well:** Data table of assay parameters per well for each group in the imported file.
- **Project information:** Software License Terms, plate map layout displaying group positions, excluded wells, and other important assay-related information.

# 3 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 Glycolytic Rate Assay 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 Glycolytic Rate Assay 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 Agilent Seahorse XF Glycolytic Rate Assay 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

## Import Excel file

1. Open the result file in Wave 2.3, then click **Export**.
2. Select Microsoft Excel and click **Save** (Figure 3).
   Optional: Modify the default file name, and save location.
3. Download the Seahorse XF Glycolytic Rate Assay Report Generator from the Agilent website: http://www.agilent.com/en-us/support/cell-analysis-(seahorse)/seahorse-xf-report-generators.
4. Unzip the compressed folder, and double-click the file called: Seahorse XF Glycolytic Rate Assay Report Generator.xltm.
5. Click **Load New Data File**.
6. Locate the Microsoft Excel file (exported from Wave 2.3), and click **Open**.

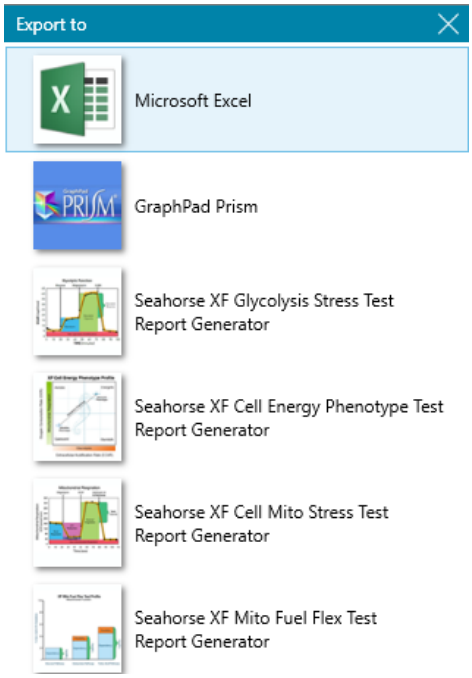


**Figure 3** Highlighting the Excel export in the list of Wave 2.3 (Desktop & Controller) export options.

## Select Groups and Display Results

After assay result data has been imported to the Report Generator, 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.

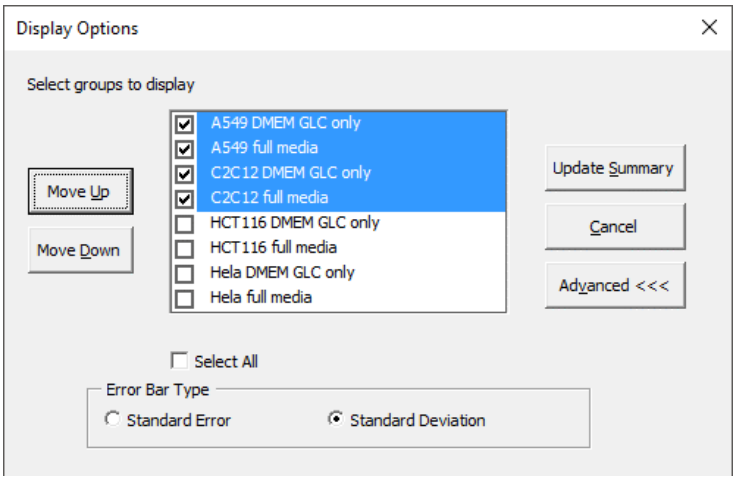


**Figure 4** The Display Options window in the Report Generator showing groups for selection. Group names must be configured in Wave 2.3 before exporting to Excel.

### 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.

# Save a Summary Report

### Save/Save as

1 Click the **Save** icon (small floppy disc) to display the **Save as** function.

2 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 is not supported as an Excel Workbook file type.

# 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 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 (Table 1 on page 6, and Table 2 on page 17).
- Standard deviation is calculated using the Microsoft Excel function.
- S.E.M. is calculated using the equation:

$$\frac{(Standard\ Deviation\ of\ Group)}{\sqrt{(Number\ of\ Wells\ in\ Group)}}$$

# Advanced Options

Advanced Options (Figure 5) can be accessed by clicking **Advanced** on the **Display Options** window. The **Advanced Options** displays the Buffer Factor and $CO_2$ Contribution Factor per group, which are automatically imported from Wave 2.3. For assays performed using the recommended Seahorse XF Glycolytic Rate Assay Medium formula, there is no need to modify the Buffer Factor. The $CO_2$ Contribution Factor should only be adjusted after assessing results from your first Seahorse XF Glycolytic Rate Assay. See the Buffer Factor Protocol User Guide or the $CO_2$ Contribution Factor Protocol User Guide for more information.

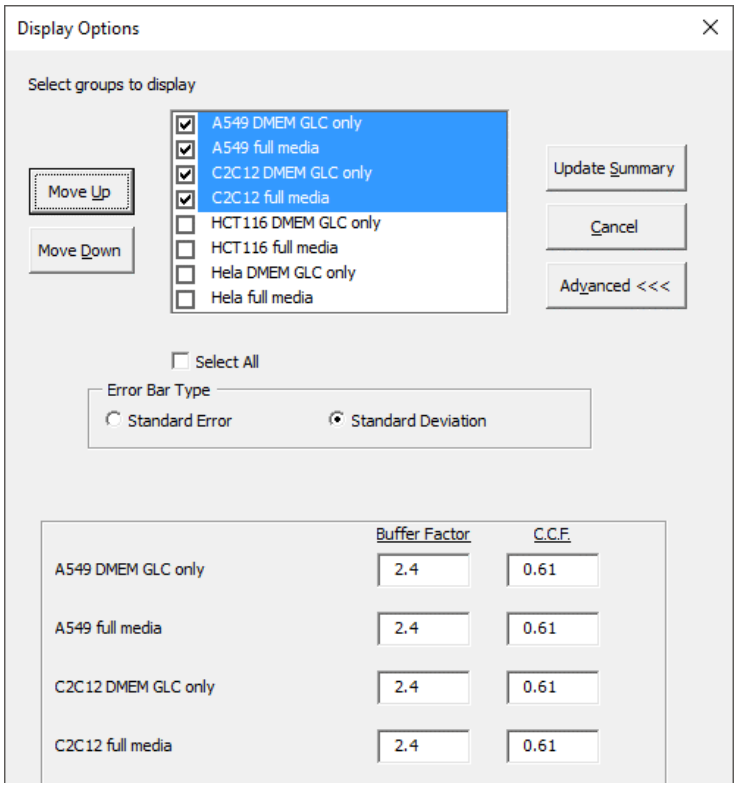


**Figure 5** Editable fields for Buffer Factor and $CO_2$ Contribution Factor (C.C.F.) displayed in Advanced Options.

# Induced Assays

A Seahorse XF Glycolytic Rate Assay with an acute injection is called an induced assay. An acute injection is an injection that occurs following the baseline measurements but before the Rotenone/Antimycin A (Rot/AA) injection, The Seahorse assay template called XF Glycolytic Rate Assay (Induced Assay) is specifically designed for this type of assay. For custom assays, the acute injection must be manually added to the Instrument Protocol prior to starting the assay.

- The acute injection must be injected before Rot/AA from Port A on the cartridge.
- The Report Generator automatically displays an additional field on the Display Options window called Injection Mapping (Figure 6). The acute injection must be identified using the Injection Mapping drop-down menu before generating a Summary Report. If **None** is selected, the position of the Rot/AA injection must be assigned.

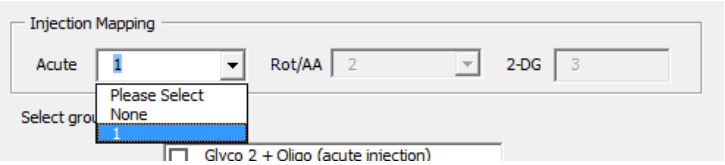


**Figure 6** **Injection Mapping** drop-down menu is displayed after importing an assay result file with more than two injections. After identifying the acute injection, the Report Generator automatically assigns the Rot/AA and 2-DG injections sequentially.

- The Report Generator will report additional assay parameters for an induced assay. Parameter names and equations are displayed in Table 2 on page 17.

**Table 2** Agilent Seahorse XF Glycolytic Rate Assay (Induced) parameter equations

| Parameter name | Parameter equation |
|---|---|
| Induced Glycolysis | Average of the glycoPER measurements after the induced assay injection and before Rot/AA injection. |
| Induced PER | Average of the PER measurements after the induced assay injection and before next injection. |
| % PER from Glycolysis (Induced) | (Induced Glycolysis)/(Induced PER) x 100% |
| Induced mitoOCR* | (Average of the OCR measurements after induced injection and before next injection) - (Minimum OCR after Rot/AA injection) |
| mitoOCR/glycoPER (Induced) | (Induced mitoOCR)/(Induced Glycolysis) |

* Induced mitoOCR is not reported separately in the Agileny Seahorse XF Glycolytic Rate Assay Report Generator.

# 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 7).

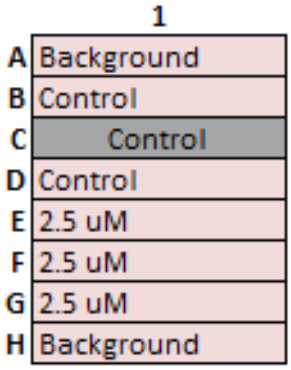


**Figure 7** XFp Plate Map on the Project Information tab. Assay well C has been turned off in Wave 2.3 prior to export, therefore the Control group parameter calculations are based on assay wells B and D only.

## Normalize Assay Results

It is highly recommended to analyze rate data that has been normalized to a cellular parameter as opposed to non-normalized, raw rate data. Normalization data must be added to the **Normalize** view in Wave 2.3 (Figure 8) before export - normalized rate data is 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 9 on page 20). 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 10 on page 20).

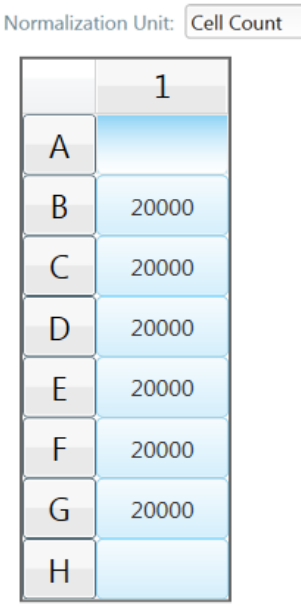


**Figure 8** Example of a normalization plate map for the Agilent Seahorse XFp Analyzer in Wave 2.3.

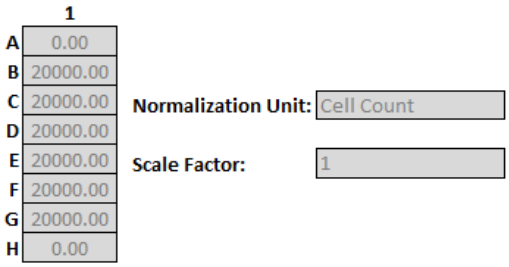


**Figure 9** The same normalization plate map from Wave 2.3 (Figure 8) displayed in the Agilent Seahorse XF Glycolytic Rate Assay Report Generator.

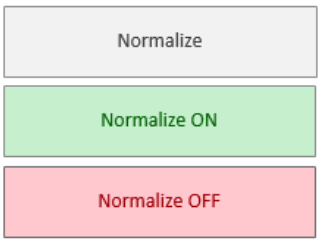


**Figure 10** **Normalize** button on the **Summary Printout** tab. By default, normalized rate data exported from Wave will be displayed as indicated by the **Normalize ON** button status (green). Click the **Normalize ON** button to toggle the data display to show non-normalized rate data (**Normalize OFF** - red). Data exported from Wave 2.3 without normalized rate data shows 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 the result data has not been normalized, the Report Generator will not display the **Normalize** tab.

# 4 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 and Table 2 on page 17 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 section from Analysis, or the Wave User Guide for more information.

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

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 only)

Custom Cycles are not part of the standardized assay template for the Seahorse XF Glycolytic Rate Assay and not supported in Report Generator analysis. A Custom Cycle refers to an additional 'Mix' or 'Wait' command step in the Instrument Protocol an assay. 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 non-normalized) ECAR data is used for parameter calculations, parameter calculations using baseline rate data (%) is not supported at this time.

### Warning message(s) when the % PER from Glycolysis is less than 50% and less than 10%

When the % of PER from Glycolysis for a selected group is less than 50% or less than 10%, the Report Generator displays a warning message in the legend on the Summary Printout tab (below the Normalize button). For more information, see the Advanced Options section.

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

The Seahorse XF Glycolytic Rate Assay Report Generator enables the import and analysis of individual XF Glycolytic Rate Assay result files. Import and analysis of multiple result files in this 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 Agilent Seahorse Technical Support at: seahorse.support@agilent.com

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