Agilent

Agilent Seahorse XF Real-Time ATP Rate Assay

Report Generator User Guide

Notices

Manual Part Number

S7888-10012

Edition

First edition, May 2018

Printed in USA

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Wilmington, DE 19808-1610 USA

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

1 Introduction

The Agilent Seahorse XF Real-Time ATP rate assay measures the rate of ATP (adenosine triphosphate) production from glycolysis and mitochondria simultaneously in live cells using label-free technology. This real-time kinetic quantification delivers a dynamic picture of cellular bioenergetics, providing researchers with unique insights into cellular phenotype and function.

The Agilent Seahorse XF Real time ATP rate assay Report Generator transforms XF OCR and ECAR data to mitoATP and glycoATP Production Rates. The assay uses metabolic modulators (oligomycin and a mix of rotenone and antimycin A) that when serially injected, allows the calculation of the mitochondrial and glycolytic ATP production as well as total ATP production rate.

The Agilent Seahorse XF Real-Time ATP Rate Assay Report Generator is a Microsoft Excel Macro that automatically transforms experimentally-derived OCR and ECAR data into ATP Production Rates, reported in pmol/min (**Table 1** on page 6). Figures and data tables in the Report Generator can be easily transferred to other software programs for additional graphing or statistical analysis. The XF Real-Time ATP Rate Assay Report Generator supports analysis of result data generated by Agilent Seahorse XFe96, XF96, XFe24 and XFp Analyzers.

Parameter Calculations

Table 1 outlines the parameter calculations performed in the XF Real-Time ATP 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 XF Real-Time ATP Rate Assay parameters equations (standard assay).

Parameter name	Parameter equation
mitoATP Production Rate (Basal)	[(Last OCR rate measurement before first injection - Minimum OCR rate measurement after oligomycin but before Rot/AA injection) x 2 x (P/O)]
glycoATP Production Rate (Basal)	Last glycoPER measurement before first injection.
Total ATP Production Rate (Basal)	(mitoATP Production Rate) + (glycoATP Production Rate)
XF ATP Rate Index (Basal)	(mitoATP Production Rate) / (glycoATP Production Rate)

NOTE

More information about XF Real-Time ATP Rate Assay parameter calculations can be found in the white paper called Quantifying Cellular ATP Production Rate Using Agilent Seahorse XF Technology.

NOTE

Additional parameters are reported for an induced XF Real-Time ATP Rate Assay in the section called "Induced Assays" on page 16.

XF Report Generator Overview

For the standard XF Real-Time ATP Rate Assay, the Report Generator displays result data and other assay-related information on the five tabs described below:

- **Summary Printout**: One-page Summary Report of the imported XF Real-Time ATP Rate Assay result data per group plotted as a stacked bar chart of basal mitoATP Production Rate and glycoATP Production Rate, energetic map of basal ATP rates, and XF ATP Rate Index.
- **Normalize**: Plate map of normalization values applied in the imported result file. The normalization values displayed in the Report Generator cannot be modified. See **"Normalize Assay Results"** on page 20 and the **Wave User Guide** for more info.
- **Measure Sheet**: OCR & PER kinetic graphs, data table of average assay parameters per group, and average rate measurement kinetic data per group.
- **Assay Parameter per Well**: Data tables organized by group containing parameter values for each well assigned to the group.
- **Project Information**: Software end-user license agreement, plate map layout showing group assignments and excluded wells, and other assay-related information.

Wave software version 2.4 or higher is required for data analysis using the XF Real-Time ATP Rate Assay Report Generator. It is recommended to download the latest version of Wave Desktop software for your PC, version 2.6 at **https://www.agilent.com/en/products/cell-analysis/software-download-for-wave-desktop**.

1 Introduction

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
- Normalize Assay Results
- Exclude Outlier Wells

Configure Microsoft Excel to Enable Macros

The XF Real-Time ATP Rate Assay Report Generator is a Microsoft Excel Macro-Enabled Template compatible with Microsoft Excel versions 2010, 2013, and 2016 for Windows PCs, and Microsoft Excel for Mac versions 2011 and 2016. To use this Report Generator, Excel must be configured to allow macros to run. To enable macros once, double-click the **Seahorse XF Real-Time ATP Rate Assay Report Generator.xltm** file, then click **Enable Editing** and **Enable Content** (yellow information bar) if prompted (**Figure 1**).

Figure 1. Enable macros using the **Enable Editing** button as seen on the yellow information bar. This needs to be performed once upon opening the XF Real-Time ATP 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 from Wave Software

1. Open your assay result file in Wave Desktop 2.6 software, then click **Export**.
2. Select Microsoft Excel and click **Save** (**Figure 2**). Optional:
3. Modify the default file name, and save location.
4. Double-click the file called: **Seahorse XF Real-Time ATP Rate Assay Report Generator.xltm**.
5. Click **Load New Data File**.
6. Locate the Microsoft Excel file (exported from Wave 2.6), and click **Open**.

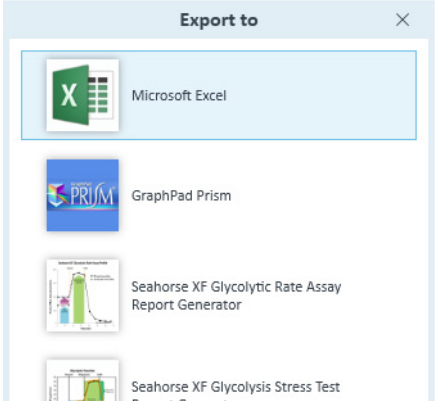


Figure 2. Wave 2.6 (Desktop & Controller) export options, highlighting the Microsoft Excel Workbook file export.

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 3**). The Report Generator will automatically calculate the parameters for each group selected and display results on the **Summary Printout** tab.

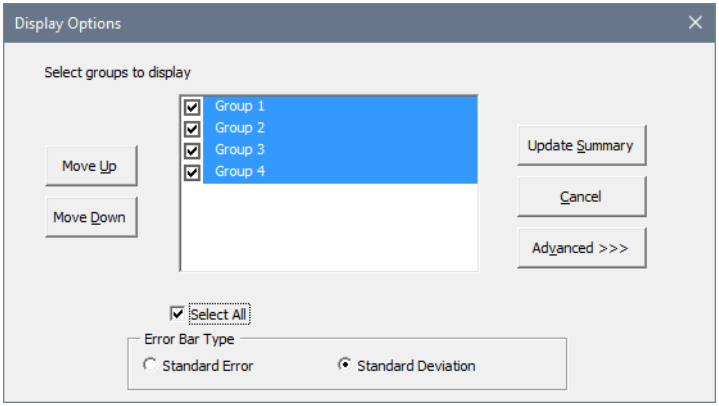


Figure 3. The Display Options window in the Report Generator showing groups for selection. Group names must be configured in Wave 2.6 before export.

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** dialog.

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 E**dit 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 for standard assays, and Table 2 for induced assays).
- 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 4**) can be accessed by clicking **Advanced** on the **Display Options** window. The **Advanced Options** displays values for CO_2 Contribution Factor (CCF) and P/O ratio per group (P/O). The CO_2 Contribution Factor should only be adjusted after assessing results from your first XF Real-Time ATP Rate Assay. See the **XF Glycolytic Rate Assay Kit User Guide** for more information.

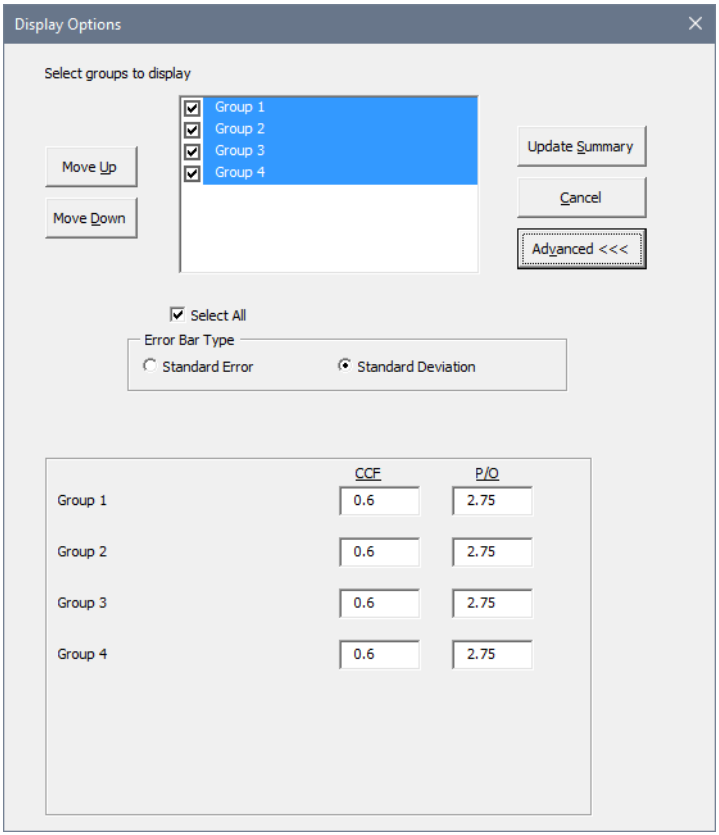


Figure 4. Editable fields for CO_2 Contribution Factor (C.C.F.) and P/O ratio displayed in Advanced Options.

Induced Assays

A XF Real-Time ATP Rate Assay with an acute injection is called an induced assay. An acute injection is an injection that occurs after the baseline measurements but before the oligomycin injection. The Seahorse assay template called XF Real-Time ATP Rate Assay (Induced Assay) is specifically designed for this type of experiment. For custom assays, the acute injection must be manually added to the Instrument Protocol prior to starting the experiment.

- The acute injection must be injected before oligomycin.
- The Report Generator automatically displays an additional field on the Display Options window called Injection Mapping (**Figure 5**). 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 oligomycin injection must be assigned.

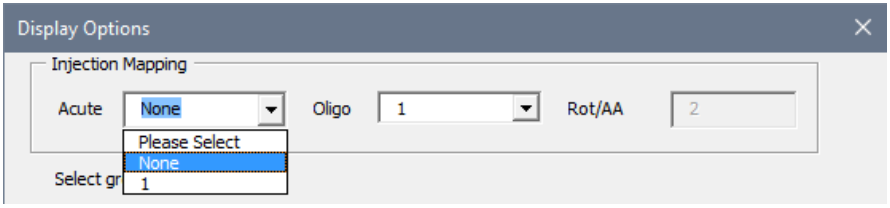


Figure 5. **Injection Mapping** drop-down menu is required for identifying the acute injection. After identifying the acute injection, the Report Generator automatically assigns the oligomycin and Rot/AA injections sequentially. If **None** is selected, the position of oligomycin injection must be assigned.

- The XF Real-Time ATP Rate Assay Report Generator will calculate and report additional parameters for an induced assay. Parameter names and equations are displayed in **Table 2** on page 17.

Table 2 XF Real-Time ATP Rate Assay parameter equations (Induced)

Parameter name	Parameter equation
mitoATP Production Rate (Induced)	(Average OCR rate measurement after acute injection and before oligomycin injection) - (Minimum OCR rate measurement after oligo & before Rot/AA injection)] x 2 x (P/O)
glycoATP Production Rate (Induced)	Average of the glycoATP Production Rate measurements after the acute injection and before next injection.
Total ATP Production Rate (Induced)	mitoATP Production Rate (Induced) + glycoATP Production Rate (Induced)
XF ATP Rate Index (Induced)	mitoATP Production Rate (Induced) / glycoATP Production Rate (Induced)

NOTE

More information about XF Real-Time ATP Rate Assay parameter calculations can be found in the white paper called Quantifying Cellular ATP Production Rate Using Agilent Seahorse XF Technology.

Data displayed in the Summary Report for induced XF Real-Time ATP Rate Assays is slightly different than the standard assay Summary Report. The Report Generator displays result data on six tabs:

- **Summary Printout**: One-page Summary Report of the imported XF Real-Time ATP Rate Assay result data per group plotted as kinetic graphs of mitoATP Production Rate, glycoATP Production Rate, total ATP Production Rate, energetic map of induced ATP rates, XF ATP Rate Index (basal and induced), and stacked bar chart of induced mitoATP and glycoATP rates.
- **Basal**: Stacked bar chart of basal mitoATP Production Rate and glycoATP Production Rate per group, and an energetic map of basal ATP rates.
- **Normalize**: Plate map of normalization values applied in the imported result file. The normalization values displayed in the Report Generator cannot be modified. See **"Normalize Assay Results"** on page 20 and the **Wave User Guide** for more info.
- **Measure Sheet**: OCR & PER kinetic graphs, data table of average assay parameters per group (basal and induced), and average rate measurement kinetic data per group.

- **Assay Parameter per Well**: Data tables organized by group containing parameter values for each well assigned to the group.
- **Project Information**: Software end-user license agreement, plate map layout showing group assignments and excluded wells, and other assay-related information.

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 2.6, 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**).

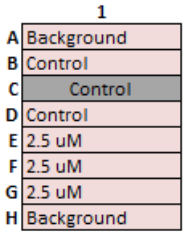
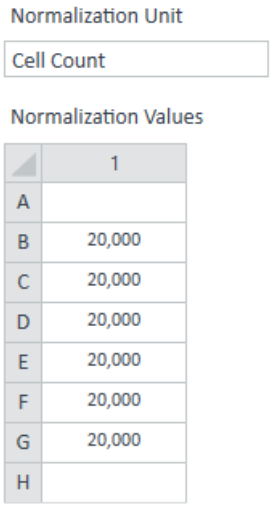


Figure 6. XFp Plate Map on the Project Information tab. Assay well C has been turned off in Wave 2.6 prior to export, therefore the Control group parameter calculations are based on assay wells B and D only.

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 2.6 (**Figure 7**) 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 2.6 (**Figure 8** on page 21). 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 9** on page 21).



	1
A	
B	20,000
C	20,000
D	20,000
E	20,000
F	20,000
G	20,000
H	

Figure 7. Example of a normalization plate map for the Agilent Seahorse XFp Analyzer in Wave 2.6.

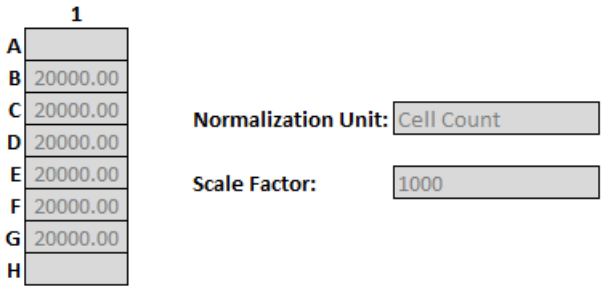


Figure 8. The same normalization plate map from Wave 2.6 (**Figure 7** on page 20) displayed in the XF Real-Time ATP Rate Assay Report Generator.

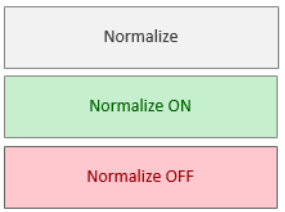


Figure 9. **Normalize** button on the **Summary Printout** tab. By default, normalized rate data exported from Wave will be displayed as indicated by the **Normalize ON** button status (green). Click the **Normalize ON** button to toggle the data display to show non-normalized rate data (**Normalize OFF** - red). Data exported from Wave 2.6 without normalized rate data shows a gray **Normalize** button.

NOTE

To preserve data integrity between Wave 2.6 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 2.6 and then re-exported to the Report Generator.

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 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 2.6 prior to Excel export. See **"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 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 XF Real-Time ATP 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 currently supported.

Why is there no glycoATP Production Rate data?

The XF Real-Time ATP Rate Assay Report Generator uses PER data exported from Wave 2.6 to calculate glycoATP Production Rates. If Buffer Factor is not assigned to assay media in Wave 2.6 before export, then the Report Generator will not calculate glycoATP Production Rates. Open your assay result file in Wave 2.6 and assign buffer factor to the assay media used and then re-export data to Excel. For instructions on assigning buffer factor to assay media in Wave 2.6 software, see the **Quick Reference Guide: Calculating PER Data**.

Can I analyze multiple result files in this Report Generator?

No, the XF Real-Time ATP Rate Assay Report Generator enables the import and analysis of individual assay result files at this time. Analysis of multiple result files must be done manually.

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: cellanalysis.support@agilent.com.

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First edition, May 2018

S7888-10012

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