Notices

© Agilent Technologies, Inc. 2017

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Manual Part Number
S7888-10002

Edition
First edition, February 2017
Printed in USA
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610 USA

Warranty

The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as “Commercial computer software” as defined in DFAR 252.227-7014 (June 1995), or as a “commercial item” as defined in FAR 2.101(a) or as “Restricted computer software” as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies’ standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

Safety Notices

CAUTION

A CAUTION 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 damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

WARNING

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

Introduction
Parameter Calculations  6
XF Report Generator Overview  7

How To
Configure Microsoft Excel to Enable Macros  10
Analyze Data in the Report Generator  11
Select Groups and Display Results  13
Save a Summary Report  14
Error Bars and Calculations  16
Normalize Assay Results  17
Exclude Outliers/Groups from Analysis  19

Frequently Asked Questions
1
Introduction

Parameter Calculations 6
XF Report Generator Overview 7

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 O₂/min, and absolute extracellular acidification rate (ECAR) in mpH/min. The Report Generator supports assay result data from all Agilent Seahorse XF Analyzers.

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

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline OCR</td>
<td>Last OCR rate measurement before first injection</td>
</tr>
<tr>
<td>Baseline ECAR</td>
<td>Last ECAR rate measurement before first injection</td>
</tr>
<tr>
<td>Stressed OCR</td>
<td>Maximum OCR rate measurement after the first injection</td>
</tr>
<tr>
<td>Stressed ECAR</td>
<td>Maximum ECAR measurement after the first injection</td>
</tr>
<tr>
<td>Metabolic potential (OCR)</td>
<td>(Stressed OCR/Baseline OCR) × 100 %</td>
</tr>
<tr>
<td>Metabolic potential (ECAR)</td>
<td>(Stressed ECAR/Baseline ECAR) × 100 %</td>
</tr>
</tbody>
</table>
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
Introduction
2 How To

Configure Microsoft Excel to Enable Macros 10
Analyze Data in the Report Generator 11
Select Groups and Display Results 13
Save a Summary Report 14
Error Bars and Calculations 16
Normalize Assay Results 17
Exclude Outliers/Groups from Analysis 19

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
How To

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).

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.
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.

![Display Options](image)

**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 in the 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.
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.
Frequently Asked Questions

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?


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