Wave 2.4 Release Notes

Overview

Wave (Desktop and Controller) is the primary software tool for Agilent Seahorse assay design, data acquisition, analysis, and interpretation. This document provides information on new features and other changes in Wave Desktop 2.4 (PC only) and Wave Controller 2.4 (Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Analyzers only).

Key New Features

Same workflow, streamlined Agilent interface

The new color palette, button styles and icons deliver a more efficient, organized, streamlined experience but preserve the same overall workflow for designing assays and analyzing result data.

Proton Efflux Rate - A new, quantitative measure of acidification

Proton efflux rate (PER) is a new, standardized metric for reporting Agilent Seahorse XF acidification data, and provides a more accurate, reproducible measure of acidification as it accounts for system buffering effects. PER calculates the number of protons exported by cells in assay medium over time (analysis only). Display PER data by selecting PER from the Rate Measurement Drop-down Menu in an assay result file.

Extended Assay Temperature Range[*]

Wave Controller 2.4 delivers stable, accurate temperature performance for Seahorse XF assay temperatures between 16 - 42 °C. This enables analysis of a wider variety of sample types and conditions. Refer to the Wave User Guide for experimental guidelines for non-37 °C operation to maximize system performance.

[*] For Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Analyzers only. This feature does not apply to Wave Desktop 2.4 as this program does not operate the Agilent Seahorse XFe Analyzers.

Agilent Technologies

Modify instrument protocol in real-time[*]

Seahorse acquires and displays result data in real-time. Now with *Wave Controller 2.4*, you can add and remove measurement cycles based on real-time result data as it is acquired during an assay.

Agilent Seahorse XF Glycolytic Rate Assay preloaded content

The Wave 2.4 software update provides several critical components for the newest Seahorse kit: the XF Glycolytic Rate Assay. Four new assay templates are installed and available for selection on the **Templates** view on Wave Home. These templates provide the simplest, fastest way to setup and run the XF Glycolytic Rate Assay or optional protocols for XF Buffer Factor or XF CO_2 Contribution Factor. The Agilent Seahorse XF Glycolytic Rate Assay Report Generator is available in the list of **Export** options. Every XF Glycolytic Rate Assay result file must be analyzed using this XF Report Generator to calculate glycoPER: the proton efflux rate derived from glycolysis (discounting the effect of CO_2-dependent acidification). This measurement is highly correlated with extracellular lactate production rate.

Default Preloaded Content

The content listed below is automatically installed with Wave 2.4.

Agilent Seahorse assay templates:

- XF Cell Energy Phenotype Test
- XF Cell Mito Stress Test
- XF Cell Mito Stress Test (Acute Injection)
- XF Glycolysis Stress Test
- XF Glycolysis Stress Test (Acute Injection)
- XF Glycolytic Rate Assay *NEW*
- XF Glycolytic Rate Assay (Induced Assay) *NEW*
- XF CO_2 Contribution Factor Assay *NEW*
- XF Buffer Factor Assay *NEW*
- XF Mito Fuel Flex Test - Dependency Workflow
- XF Mito Fuel Flex Test - Flexibility Workflow

[*] For Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Analyzers only. This feature does not apply to Wave Desktop 2.4 as this program does not operate the Agilent Seahorse XFe Analyzers.

Catalog of Seahorse assay kit components (for experiment design):

- Compounds (for example, Oligomycin, FCCP, Rotenone/antimycin-a)
- Pretreatments (for example, Control, Experimental)
- Media (for example, Seahorse XF Assay Medium)
- Cells (for example, A549, MCF10A, RAW 264.7)

NOTE   See Assay Templates & Assay Guides for more information on the cell types listed in the Wave 2.4 Catalog.

Figure 1   Catalog

Agilent Seahorse XF Report Generators:

- XF Cell Energy Phenotype Report Generator
- XF Cell Mito Stress Test Report Generator
- XF Glycolysis Stress Test Report Generator
- XF Glycolytic Rate Assay Report Generator *NEW*
- XF Mito Fuel Flex Test Report Generator
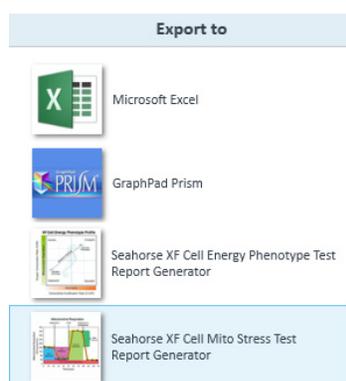
Figure 2   Export dialog

System Requirements and Compatibility

There are two configurations of Wave 2.4 software:

1 **Wave Desktop 2.4:** Installed on a PC or laptop only; For assay design, data analysis and interpretation.

Table 1   System requirements

Computer	Specifications
Windows PC	**Operating system:** Windows 7, 8.1, and 10 **Processor:** Intel Core i3 (or better) **Hard disk space:** 175 GB **System memory (RAM):** 2 GB minimum **Screen resolution:** 1280 x 800 minimum **Supported Excel versions:** 2010, 2013 and 2016
Macintosh PC	**Operating system:** Mac OS w. Parallels 9 (or higher) and Windows 7, 10 **Processor:** Intel Core i3 (or better) **Hard disk space:** 175 GB **System memory (RAM):** 2 GB minimum **Screen resolution:** 1280 x 800 minimum **Supported Excel versions:** 2011 and 2016

2 **Wave Controller 2.4:** Standalone on the Agilent Seahorse XFe24 and Agilent Seahorse XFe96 Controller only. The XFe Controller is the computer that controls the analyzer, acquires data, and enables assay design and (minimal) data analysis.

Table 2   Instrument specifications

Instrument type	Specifications	Software name
Seahorse XFe96 Analyzer Seahorse XFe24 Analyzer	**Operating System:** Windows 7, 32-bit **Processor:** Intel Core i3-3220 (latest) **System Memory:** 4 GB RAM	Seahorse Wave Controller

NOTE   Installing third-party software programs on the Seahorse XFe Controller (for example, Microsoft Excel or GraphPad Prism) is not supported, and may void the instrument warranty. Transfer any files exported from Wave Controller 2.4 to a personal computer or laptop for data analysis.

Fixed Bugs and Other Info

Accessing Agilent Seahorse files during an assay

Access to **Wave Home** and any open template or result files is disabled while running an assay on Agilent Seahorse XFe96 and Agilent Seahorse XFe24 Analyzers. Prior to starting an assay, transfer any result files to a personal computer or laptop with Wave Desktop 2.4, or wait until the assay is finished.

Correct injection line position for XFd result files

The injection lines displayed on the kinetic graph are displayed in the correct position on the x-axis for XFd data files (XF96 and XF24 analyzer result files).

Saving assay template files

For custom assay templates, the **Save** function overwrites the existing template content with all new changes. Click the **Save as Template** button to create a new template to modify, but preserve the content in the original template file.

Maximum time to run an Agilent Seahorse experiment

The maximum length of time (Instrument Protocol) for an assay on the XFe Analyzer is 12 hours. Assays cannot be created with an Instrument Protocol longer than 12 hours. Assay templates created with older versions of Wave software may have an Instrument Protocol greater than 12 hours, however to run the assay, the Instrument Protocol time must be reduced to less than 12 hours.

XFe Controller (computer) best practices

- Restart the XFe Controller at least once per week.
- Prior to starting a Seahorse assay, save and close any open assay template and result files.
- During an assay, do not insert or eject USB flash drives (or network cable).
- Reduce the amount of 'analysis' performed, do not analyze runtime data if it is not necessary.

Known Issues

Agilent Seahorse XF24 and Agilent Seahorse XF96 hypoxia result files

Proper analysis of a hypoxia assay on Agilent Seahorse XF24/XF96 Analyzers require use of the "Hypoxia Rate Calculator" software. Rate data in the XFd result file will still reflect the original data after importing to Wave Desktop 2.4. Any data modifications after use of the "Hypoxia Rate Calculator" do not persist in Wave Desktop 2.4.

Opening *.asy files in Wave 2.4

The *.asy file type is a file format generated by very early versions of Seahorse Wave software, these files cannot be imported to Wave 2.4. Please contact Agilent Seahorse Technical Support for assistance in converting *.asy files to the current supported file type.

Use of a comma decimal separator

Wave does not recognize a decimal point separator in select text fields for PC language settings configured to French or German. Using a comma as a separator is supported by Wave, and should be used if your PC language is set to French or German.

Save issues with Buffer Capacity and Buffer Factor when adding an Assay Media to the Catalog

The Catalog (below Wave Home) allows any input value for Buffer Factor (PER) and Buffer Capacity (PPR) when adding or editing an Assay Media. The appropriate input value range for Buffer Factor (PER) is 1.8-4 (mmol/L/pH). The appropriate input value range for Buffer Capacity (PPR) is 0-1 (mol/L/pH).

Canceling an assay while it is running

Assays can be canceled while they are running, however to ensure the data is saved correctly, do not close Wave Controller software until you can open and view data in the canceled assay result file. Closing (and force-quitting) Wave Controller before viewing the result file may result in a complete loss of the data from that assay.

Viewing well data on the Overview > Bar Chart black group outline in *well* mode

For larger data sets, highlighting an assay group (using the Group List) while viewing the Bar Chart data in well mode will make the selected group color appear black. The black color is the outline of the individual well Bar Chart for the selected group, Wave did not change the color of the group.

Home tab always returns to Templates view

The Home tab displayed the upper-left corner of Wave software when an assay template or result file is opened always returns to the Templates view by default.

Text display when adding or removing measurement cycles during an assay

Longer Instrument Protocol command names can be partially cut-off (not displayed) on the window for adding and removing measurement cycles while running an assay.

Appending a *Protocol* image on the Summary view

The *Protocol* image applied to the Summary view using the *Append Image* function (Summary view > tool bar) is poorly formatted. Copy/paste the image to another software program, and manually resize as necessary.

Custom Cycle Instrument Protocols "Move Up/Down"

The Move Up and Move Down buttons do not function correctly for the Custom Cycle Instrument Protocol command (only) and will not take effect on the Instrument Protocol when running an assay. Do not use the Move Up and Move Down buttons for the Custom Cycle feature.

Runtime tool-tip only shows O_2 Value

While running an assay, hovering over a select well on the plate map will display relevant data about that well. The Value field only displays O_2 data for the well, pH data is not displayed in the tool-tip.

Save prompt and closing result and template files

Click the small red **X** to close an assay result file tab, an assay template file tab always displays a save prompt, even if all changes have been saved.

***Group List* statistics while running an assay**

While running a Seahorse assay, the Group List Details checkbox shows the average and error (per group) for completed measurement cycles. For an active measurement cycle, the average and error values displayed in the Group List are those values of the previous measurement. These values do not get updated until the active measurement has completed.

Opening Seahorse assay template files for different XFe Analyzers

Seahorse assay template files are installed automatically with Wave Desktop software, and have consistent file names across both Seahorse XFe96 and XFe24 Analyzers (for example, *XF Cell Mito Stress Test* assay template). Opening a template with the same name but for different Seahorse XFe

Agilent Technologies

Analyzers (or the same assay template file twice) will display an error. Open the individual template file in a separate instance of Wave Desktop if both need to be open simultaneously.

Adjusting view scaling in a result file

Size and scale adjustments to a graph or plate map on an analysis view using the arrows or border lines are not saved, and will be reset to the default scaling view when reopening the assay result file.

If any software bugs not listed above are encountered, please contact Agilent Seahorse Technical Support at: seahorse.support@agilent.com

S7894-10000