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# Release Notes: Wave 2.6.1 Software

Version 2.6.1

Released: April 2019

## Overview

Wave (Desktop and Controller) is the software for design, data acquisition and analysis of XF assays. This document provides information on new features and other updates in the latest release of Wave software: Wave *Desktop* 2.6 (PC only) and Wave *Controller* 2.6 (Agilent Seahorse XFe Controllers with the Windows 10 64-bit OS).

## New Features

### Updated XF Report Generators in Export menu

The new Agilent Seahorse XF Real-Time ATP Rate Assay Report Generator is now available in Wave's *Export* options. All other XF Report Generators have been updated for usability improvements, bug fixes and expanded language compatibility support.

# Fixed Bugs

## Log Files flushed automatically without restarting Wave Controller software

The Log File compilation function in Wave Controller no longer requires restarting Wave Controller software first.

## Opening *xfd files in Wave Desktop 2.6

The *xfd file type is the assay result file format for data acquired on XF Analyzers (XF96 & XF24). If the *xfd file extension was saved in capital letters (i.e. data.XFD) the file would not be recognized by Wave Desktop software. This has been fixed in this release of Wave Desktop 2.6.1

## Runtime tool tip only shows O2 value

While running an assay and hovering the mouse cursor on a well in the plate map, the tool tip only displayed O2 data. The tool tip has been updated in Wave Controller 2.6.1 to display both O2 and pH data.

## Shorter Injection Valve Open Time for Seahorse XFe96 Analyzers

For XFe96 Analyzers (only), the injection valve open time has been adjusted to reduce the risk of port contents leakage. The injection valve open time is not a user-configurable setting.

# Preloaded Content

The content listed below is included with the Wave 2.6 installation & update:

## 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
- XF Glycolytic Rate Assay (Induced Assay)
- XF $CO_2$ Contribution Factor Assay
- XF Buffer Factor Assay
- XF Mito Fuel Flex Test – Dependency
- XF Mito Fuel Flex Test – Flexibility
- XF Real-Time ATP Rate Assay
- XF Real-Time ATP Rate Assay (Induced)

## Catalog of Seahorse assay kit components (for template design):

- Compounds (i.e. Oligomycin, FCCP, Rotenone/antimycin-a)
- Pretreatments (i.e. Control, Experimental)
- Media (i.e. Seahorse XF Assay Medium) – 2 new assay media added to the Catalog - Seahorse XF DMEM Medium, pH 7.4 and
- Seahorse XF RPMI Medium, pH 7.4
- Cells (i.e. A549, MCF10A, RAW 264.7)
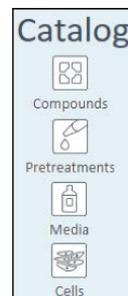
**Note:** *See Assay Templates & Assay Guides for more information on the cell types in the Wave 2.6 Catalog.*

## Agilent Seahorse XF Report Generators

- XF Real-Time ATP Rate Assay Report Generator
- 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
- XF Mito Fuel Flex Test Report Generator

## System Requirements & Compatibility

There are two configurations of Wave 2.6 software:

1. **Wave Desktop 2.6** – Used for XF data analysis & assay design. Supported by Windows Operating Systems (7 + later) only. Minimum computer specifications for Wave Desktop 2.6 are:

| Computer | Specifications |
|---|---|
| Windows PC | **Operating System:** Windows 7, 8.1, and 10<br>**Processor:** Intel Core i3 (or better)<br>**Hard Disk Space:** 175 GB<br>**System Memory (RAM)**: 4 GB (minimum*)<br>**Screen Resolution:** 1280 x 800 (minimum)<br>**Supported Excel Versions:** 2010, 2013 & 2016 |
| Macintosh PC<br><br>*(requires use of a virtual machine)* | **Operating System:** Mac OSx 10.11 or higher<br>**Virtual Machine:** Parallels 12 & Windows 7, 8.1 and 10<br>**Processor:** Intel Core i3 (or better)<br>**Hard Disk Space:** 175 GB<br>**System Memory (RAM):** 4 GB (minimum*)<br>**Screen Resolution:** 1280 x 800 (minimum)<br>**Supported Excel Versions:** 2011 & 2016 |

*For the optimal experience, 8 GB (or higher) of System Memory (RAM) is recommended.*

2. **Wave Controller 2.6** – Used for instrument control & data acquisition. Compatible with new XFe96 and XFe24 Controllers with Windows 10, 64-bit OS only.

| Instrument Type | Specifications | Software Name |
|---|---|---|
| Seahorse XFe96 Analyzer<br>Seahorse XFe24 Analyzer | **Operating System:** Windows 10 64-bit<br>**Processor:** Intel Core i5<br>**System Memory:** 16 GB RAM | "Seahorse Wave Controller" |

**Note:** *Files exported from Wave Controller 2.6 software (XF Report Generator, Excel and GraphPad Prism) should be transferred to a personal computer or laptop for data analysis.*

## General Info

### Wave Desktop & Windows OS language compatibility

Wave Desktop is validated for compatibility with Windows OS languages for: English, German, French, Chinese (Traditional) and Japanese.

### Saving assay template files

For custom assay templates, the **Save** function will overwrite the existing template content with all changes in the file. To create an editable template file without modifying the original template file, click the **Save as Template** button.

### Analyzing hypoxia result data from the Agilent Seahorse XF24 & XF96 Analyzers

Proper analysis of hypoxia result data from a Seahorse XF24 or XF96 Analyzer requires use of specific software called the "Hypoxia Rate Calculator". Data modifications using the "Hypoxia Rate Calculator" are not applied to the rate data in the original XFd result file, therefore Wave Desktop 2.6 cannot be used for analysis of hypoxia result data from an XF24 and XF96 Analyzer.

### Save prompt and closing result & template files

Clicking the small red 'X' to close an assay result file tab and assay template file tab will always display a save prompt, even if all changes have been saved.

### 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.

### Maximum time to run a Seahorse experiment *(Wave Controller only)*

The maximum Instrument Protocol time for an assay on the XFe Analyzers is 12 hours. Assay template files cannot be saved with an Instrument Protocol exceeding 12 hours. Assay template files 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.

### Accessing Seahorse files during an assay *(Wave Controller only)*

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

### Cancelling an assay while it's running *(Wave Controller only)*

Assays can be cancelled while they're running however to ensure the data is saved correctly, do **NOT** close Wave Controller software until you can open and view data in the cancelled 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.

### XFe Controller (computer) Best Practices *(Wave Controller only)*

- Restart the XFe Controller at least once per week.
- Save & close open assay template files and assay result files prior to starting an XFe assay.
- Do not insert or eject USB flash drives (or insert/remove network cable) during an XFe assay.
- Reduce the amount of 'analysis' performed on the XFe Controller.
- If you don't need to interact with Wave Controller software during an assay, then don't!

# Known Issues

## Opening *.asy files in Wave 2.6

The *.asy file type is a file format generated by previous versions of Seahorse Wave software. This file type is no longer supported in Wave software (Wave 2.4 software and later). Please contact Seahorse Technical Support if you need to convert *.asy files to the current supported file type for analysis in Wave 2.6.

## Use of a comma decimal separator in Windows OS (English)

Comma are not recognized as a decimal separator in Wave Desktop 2.6 when the Windows OS language is set to *English*, use a period as a decimal separator.

## Bar chart outline for well data on the Overview analysis view

For larger data sets, highlighting an assay group (using the Group List) will make the selected group color appear black while viewing the Bar Chart data in *well mode*. 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.

## Appending a Protocol image on the Summary view

The *Protocol* image sent 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.

## 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 is *not* saved and will be reset to the default scaling view when reopening the assay result file.

## Opening Seahorse assay template files for different XFe Analyzers

Seahorse assay template files automatically installed with Wave Desktop software have identical file names for both XFe96 and XFe24 Analyzers. An error will appear when if an assay template file with the same name is opened (i.e. "XF Cell Mito Stress Test" assay template).

## 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.

## Save issues with Buffer Capacity & 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).

## Text display when adding and/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.

## 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.

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

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