Agilent

Agilent MitoXpress Xtra & pH-Xtra Assay

Data Visualization Tool User Guide

Notices

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2850 Centerville Road
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Contents

1 Introduction

The Agilent MitoXpress-Xtra and pH-Xtra products are designed to facilitate convenient microplate-based analysis of aerobic and glycolytic metabolism. Measurements are performed on fluorescence plate readers and can be applied to isolated mitochondria, bacteria and yeast, as well as to suspension and adherent cell models. The assays are also compatible with commonly used 3D models.

The **MitoXpress-Xtra Oxygen Consumption Assay** enables convenient microplate-based interrogations of mitochondrial function and broader metabolic perturbation. It enables metabolic characterization or both prokaryotic and eukaryotic cell types and provides a rapid, sensitivity high-throughput measure of mitochondrial function and convenient dose response analysis.

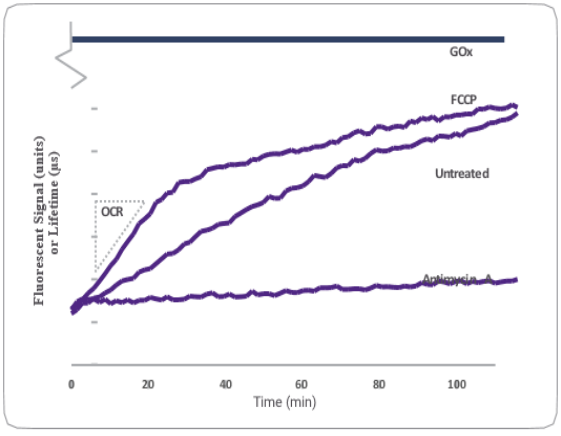


Figure 1. Typical MitoXpress Xtra signal profiles (intensity/TRF [AU] or Lifetime [us]) for adherent cells tested with different ETC compounds, including Antimycin A (recommended as a Negative Control). The effect of glucose oxidase as a positive Signal Control is illustrated schematically.

The **pH-Xtra Glycolysis Assay** measures extracellular acidification, and enables convenient microplate-based interrogations of glycolytic metabolism. It can be used for both metabolic characterization and to provide valuable insights into how specific interventions impact glycolytic activity.

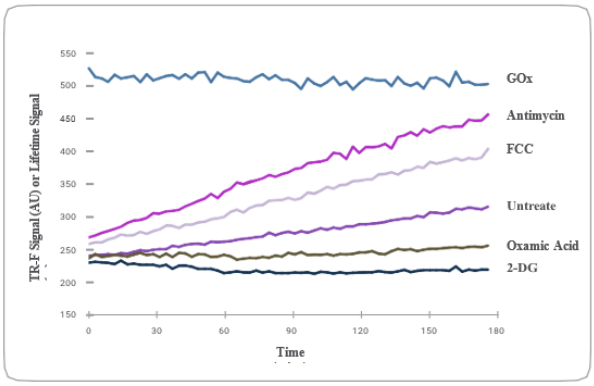


Figure 2. Typical Signal profile (TR-F arbitrary units [AU] or Lifetime [µs]) pH-Xtra for adherent cells, treated with typical control compounds, including 2-Deoxyglucose recommended as a negative control. The effect of glucose oxidase as positive signal control is illustrated schematically.

The Agilent MitoXpress Xtra & pH-Xtra Data Visualization Tool is a Microsoft Excel Macro that automatically transforms experimentally-derived fluorescence data into Kinetic Signal curves (**Figure 3** on page 7) and Tabulated Slope values (**Table 1** on page 8). Figures and data tables in this visualization tool can easily be transferred to other software programs for additional graphing or statistical analysis. The MitoXpress Xtra & pH-Xtra Data Visualization Tool supports analysis of result data generated by a whole host of fluorescence plate reader manufacturers (**Table 3** on page 10) and all recommended assay detection modes.

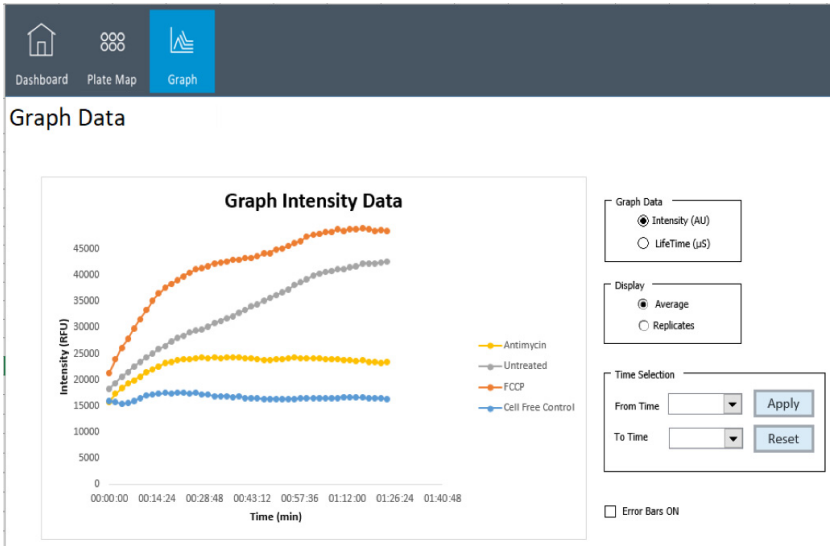


Figure 3. MitoXpress Xtra Kinetic signal curves. Average intensity signal curves of selected samples

		Lifetime (µs/h)		
Group Name	Wells	Slope	Average	Std Dev
	H9	-0.0500		
Cell Free Control	H10	0.1166	0.0333	0.12
	A1	15.60		
	A2	15.76		
FCCP	A3	15.61	15.66	0.09
	A4	10.70		
	A5	11.89		
Untreated	A6	11.23	11.61	0.80
	A10	3.64		
	A11	2.62		
Antimycin	A12	3.45	3.24	0.54

Figure 4. Calculated Slope values for individual sample fluorescence signal curve using the user selected time values (From time and To time). Average slope

Calculations Performed

Table 1 outlines the parameter calculations performed in the MitoXpress & pH-Xtra Data Visualization Tool. Each calculated parameter value represents the 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 MitoXpress Xtra Assay parameters equations (standard assay)

Parameter name	Parameter equation
Blank Subtraction	Sample relative fluorescence units (RFU) Signal - AV Blank RFU signal, at each time point for all individual samples,
Lifetime (LT) Calculation (Advanced Dual Read TR-F detection only)	LT = (70-30)/LN (RFU W1/RFU W2) See the **MitoXpress Xtra Oxygen Consumption Assay User Guide** for further explanation.
Slope	linear equation of a line: $b = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2}$ where the values of x and y are the sample means (the averages) of the known x's and the known y's.

Table 2 pH-Xtra Xtra Assay parameters equations (standard assay)

Parameter name	Parameter equation
Blank Subtraction	Sample RFU Signal - AV Blank RFU signal, at each time point for all individual samples,
Lifetime (LT) Calculation (Advanced Dual Read TR-F detection only)	LT = (300-100)/LN (RFU W1/RFU W2) See the **pH-Xtra Glycolysis Assay User Guide** for further explanation..
Slope	linear equation of a line: $b = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2}$ where the values of x and y are the sample means (the averages) of the known x's and the known y's.

Table 2 pH-Xtra Xtra Assay parameters equations (standard assay) (continued)

Parameter name	Parameter equation
pH Scale	pH Scale = (1687.2 - LT) / 199.12 See the **pH-Xtra Glycolysis Assay User Guide** for further explanation..
$[H^+]$ Scale	[H+] = POWER (10, - pH) See the **pH-Xtra Glycolysis Assay User Guide** for further explanation..

NOTE If Blank wells are not included in the annotation, a blank value of 0 is used.

NOTE Lifetime (LT) Calculation only applicable to data imported correctly form Advanced Dual read TR-F (lifetime) detection mode, where the raw data contains two distinct RFU Intensity data tables.

NOTE See Assay User Guides for further explanation, MitoXpress Xtra Oxygen Consumption Assay User Guide and pH-Xtra Glycolysis Assay User Guide.

Data Visualization Tool Compatibility

Table 3 Compatible fluorescence plate reader By manufacturer

Molecular devices	BMG Labtech	BioTek	TECAN
SpectraMax i3x, i3 SpectraMax Paradigm SpectraMax M series, SpectraMax Flexstation SpectraMax Gemini SpectraMax iD3 or iD5	CLARIOstar FLUOstar Omega POLARstar Omega PHERAstar FS/FSX FLUOstar Optima POLARstar Optima	Cytation 1, 3, or 5 Synergy H1, H4, Synergy Neo or Neo2	Spark (10M/20M) Infinite F Plex Infinite F Nano Infinite F200Pro Infinite M200Pro Infinite M1000Pro Safire or GeniosPro

NOTE

Additional fluorescence plate reader models or manufacturers which are not detailed in this table may allow the export of compatible raw data files, but please be aware they have not been tested.

The Data Visualization Tool is compatible with data file types exported from the versions of plate reader software found in **Table 4**.

Table 4 Compatible fluorescence plate reader software

BMG Labtech	BioTek	Molecular devices	TECAN
MARS version 3.10 R5 or higher required	Gen5 version 3.00 or higher required	SoftMaxPro V4 or 5 use .txt in time format	iControl version 1.10.4 or higher required
MARS version 3.31 upwards requires additional table settings		SoftMaxPro V6 and 7 or higher use .txt or .xls in column format	SPARKControl version 2.1 or higher required

NOTE

Older versions of the plate reader software may also be compatible, exporting suitable data files for import. However, these have not been verified and tested and are not currently supported. We recommend using the software versions described in the "Export Data from Plate Reader Software" on page 17.

NOTE

See "Export Data from Plate Reader Software" on page 17 for specific plate reader software directions.

Compatible Detection Modes

- Basic: Intensity measurement, (MitoXpress Xtra assay applicable only)
- Standard: Time-resolved fluorescence measurement (TR-F), and
- Advanced: Dual-read Ratiometric TR-F measurement (Lifetime calculation)

In Basic and Standard detection modes, the exported data file from the plate reader will contain a single table of Intensity signal data in Relative Fluorescence Units (RFU) versus time.

In advanced detection modes, the exported data file from the plate reader will contain two separate tables of RFU Intensity signal data versus time. (for example, RFU signal detected at two distinct time-resolved (TR-F) delay times). It is critically important to export both RFU data tables when using the Data Visualization Tool for use with the Advanced: Dual-Read Ratiometric TR-F detection mode.

NOTE

See Assay User Guides for further detail on detection modes, MitoXpress Xtra Oxygen Consumption Assay User Guide and pH-Xtra Glycolysis Assay User Guide.

MitoXpress Xtra & pH-Xtra Data Visualization Tool Overview

For the standard MitoXpress Oxygen Consumption assay and the pH-Xtra Glycolysis Assay, the Data Visualization Tool displays results data and other assay-related information on the tabs described below:

- **Dashboard:** Used to import and load new data files. Contains user license agreement information.
- **Plate map:** Used to annotate designated replicate wells of 96-well plate for Samples, Controls, and Blank wells of the imported experiment data. This is an important step for correct blank correction and statistical analysis performed later.
- **Graph:** Graphical summary of the imported MitoXpress Xtra data per group plotted as Fluorescence signal curves versus time. Intensity or lifetime data is graphed depending on the detection mode of import data. A subsequent Table of calculated Slopes for the annotated samples is generated based user defined Time Selection values (start time/ stop time)

  In pH-Xtra assay only, an additional pH/ [H+] Graph is presented in the Graph tab illustrating signal curves in pH /[H+] scale for the same selected groups/samples, and subsequent Calculated [H+] slopes are presented in the Slope table.
- **Common Format Data:** Raw Intensity Data sorted in individual columns for each sample well versus time, orientated from A1-A12, B1-B12 etc., until H12. This data is used as the input data for subsequent macro calculations. It can be exported to other software by the end user.
- **Summary Intensity Data:** Table Data of blank subtracted Intensity values of individual annotated samples and sorted using the plate map information. Samples sorted in columns versus time and are orientated Blanks, Controls and Samples. It contains the calculated Average, Standard Deviation, and %CV for the replicate Intensity values at each timepoint. This data is used as the input data for subsequent graphing and slope calculation.

- **Summary Lifetime Data:** Table Data of calculated Lifetime values of individual samples which has been annotated using the plate map information sorted in columns versus time and are orientated Blanks, Controls, and Samples. (only applicable to Advanced Dual-read detection mode). It contains the calculated Average, Standard Deviation, and %CV for the replicate Lifetime values at each timepoint. This data is used as the input data for subsequent graphing and slope calculation. It can be exported to other software by the end user.
- **pH Calculation:** In pH-Xtra assay only, it contains summarized pH values of the same samples described in the summary lifetime data tab.
- **Graph Intensity/Lifetime tabs:** The content depends on the chosen groups selected for inclusion in the Graph tab.
- **pH Graph Data:** In pH-Xtra assay only, content depends on the chosen groups selected for inclusion in the Graph tab

NOTE **Data in these tabs can be exported to other software by the end user.**

1 Introduction

MitoXpress Xtra & pH-Xtra Data Visualization Tool Overview

2 How To

Configure Microsoft Excel to Enable Macros

The **MitoXpress & pH-Xtra Data Visualization Tool** is a Microsoft Excel Macro-Enabled Template compatible with Microsoft Excel versions 2013, and 2016 for Windows PCs. To use this Data Visualization Tool, Excel must be configured to allow macros to run. To enable macros once, double-click the **Agilent MitoXpress & pH-Xtra Data Visualization Tool.xlsm** file, then click **Enable Editing** and **Enable Content** (yellow information bar) if prompted (**Figure 5**).

Figure 5. Enable macros using the Enable Editing button as seen on the yellow information bar. This needs to be performed once upon opening the MitoXpress & pH-Xtra Data Visualization Tool for the first time after download.

To always enable macros (recommended for the best experience using this software tool):

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**.

Export Data from Plate Reader Software

The specific steps and settings required for data file export are specific to your chosen plate reader and software version.

The following are described below:

- BMG Labtech
- Molecular Devices
- BioTek
- Tecan

BMG Labtech Software

Applicable to MARS version 3.0 to 3.29

1 Open the data file in MARS data analysis software.

2 Click on **Format and Settings** tab then click the **Number Format Settings** button to open a new window. Click **Number Formats** on the left side. Under the **Global time format options**, click **User defined format:** and select **hh:mm:ss** with **Time Separator Character** as **:**.

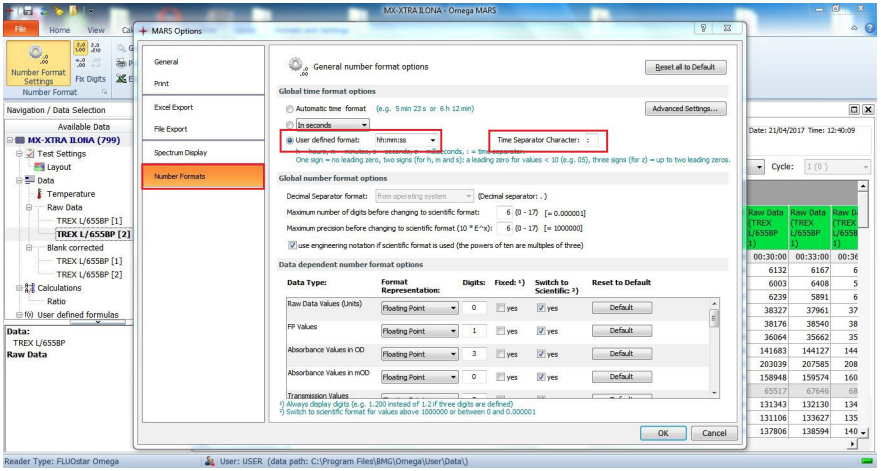


Figure 6. Number format setting options

3 Select **File Export**. The **File Extension** can either be **CSV** or **txt**, and select the **separator** as **,**. In the **Auto mode options**, select the **Export table view** option.

The Output directory for the created text files and the file name can be as per User discretion. Click **OK**.

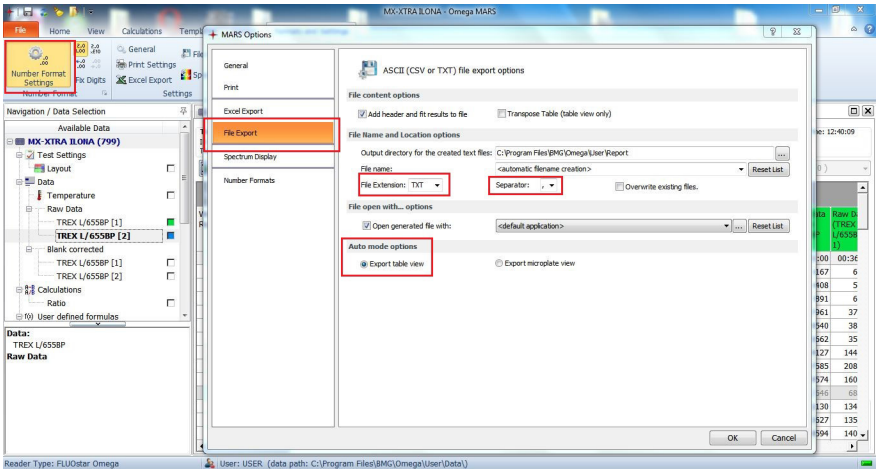


Figure 7. File Export options

4 Navigate to the data, as shown in **Figure 8** on page 19. Select the **Table View** tab. Select **All** cycles. Select **Raw Data** for Window 1 and Window 2 (if measured) in the **Navigation / Data Selection** section.

In Advanced TR-F, lifetime measurement 2 TRF intensity data sets should be present. In single TR-F measurement only one data sets is present.

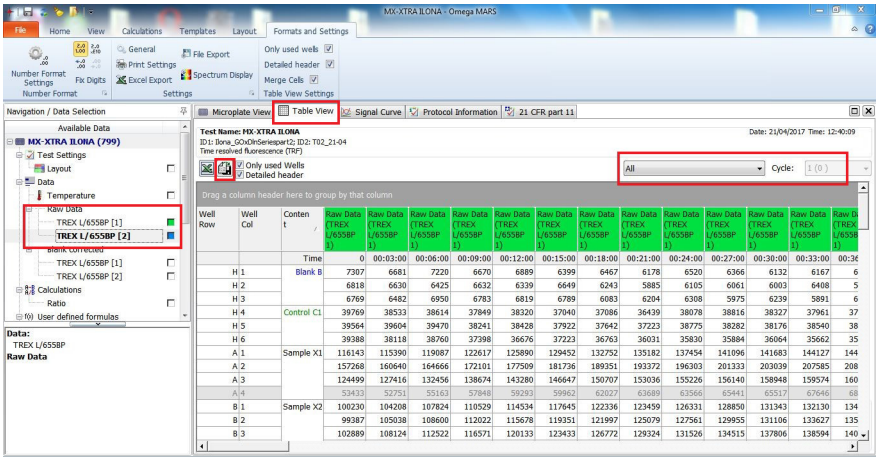


Figure 8. Table View options

5 Click the **ASCII Export** button to export the file. Alternatively, the data file can be exported using the **Export Table to ASCII** in the **Home** tab.

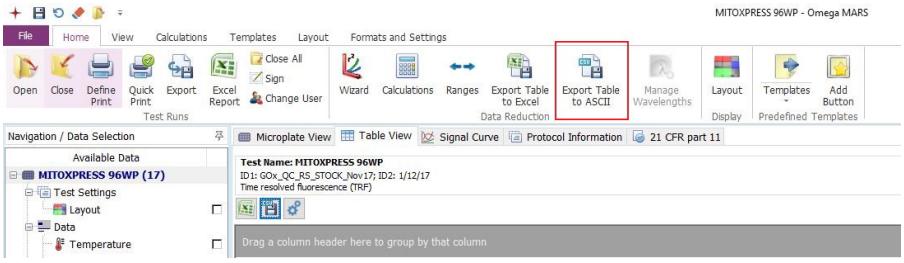


Figure 9. Alternative Way to export the Data File in ASCII Format

Applicable to MARS version 3.30 and above

1 Use steps 1-3 from **"Applicable to MARS version 3.0 to 3.29"** on page 17.

2 Click **Table View** (SEPARATE TAB IN Ver. 3.31 and above), and deselect the **Display well name (row letter and column index) in one column** option, if it is selected by default.

3 Select the option, **A..f/1..48 (use leading space)** from the **Well name format** dropdown menu. See **Figure 10**.

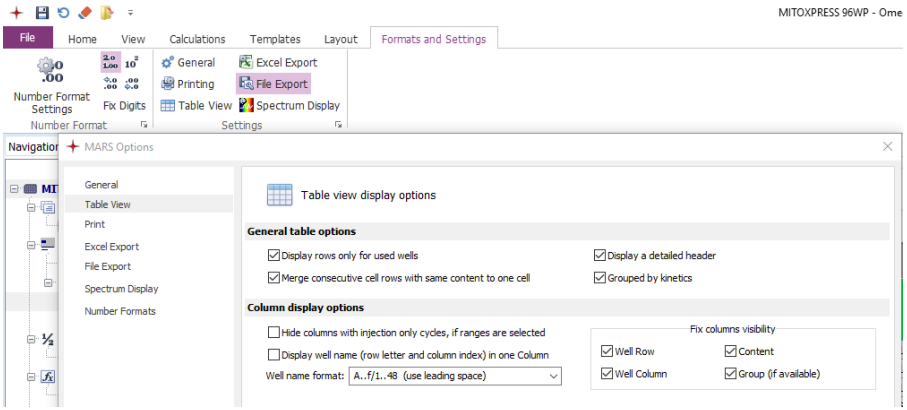


Figure 10. Well name format option

Molecular Devices Software

Applicable to SoftMaxPro Version 4 and 5:

1 Click on **Plate Reader Name** to open a window titled **Preferences**. Ensure that, in the **Manual Export Format**, **Time** is selected, and all other tick boxes are deselected. In the **Template Export Format** section make sure **Tab-delimited Text** is selected, Click **Ok**. See **Figure 11**.

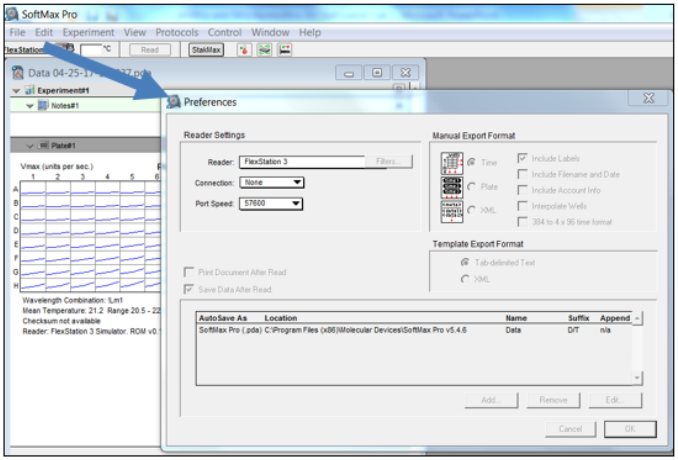


Figure 11. Preferences section for Exporting a Data File from Softmax 4/Softmax 5. Ensuring the data is exported in Time format.

2 Return to the open Experiment data file. Select **File**, click on **Import/Export**, select **Export**, the **Export Data to** window appears. See **Figure 12** on page 22.

3 Select **Plate Sections**, and click on **All**. Ensure **Group Sections** are deselected, save the files as .txt file type, and click **Save**. See **Figure 12** on page 22.

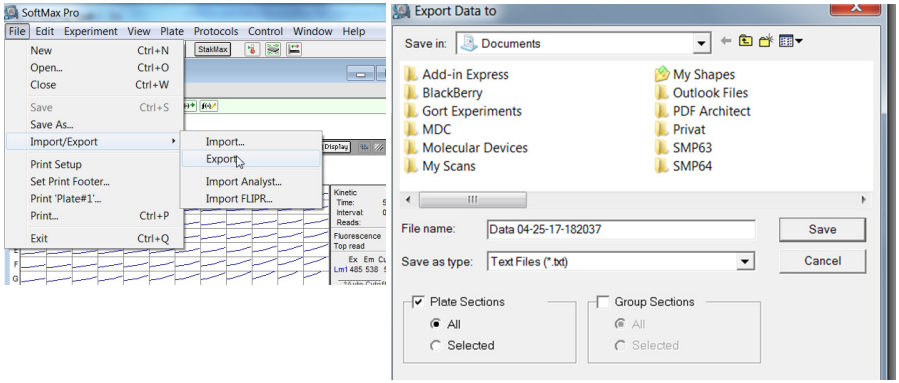


Figure 12. Exporting a data file from Softmax 4/Softmax 5.

Applicable to SoftMaxPro Version 6 and 7

1 Open the Data File using the relevant software version of SoftMax Pro.

2 Select the microplate icon on the top left of the window, and select **Export**. See **Figure 13**.

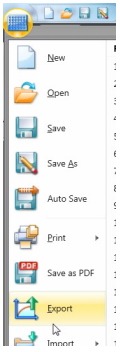


Figure 13. Exporting a Data File from SoftMaxPro 6 and 7

3 Select the correct plate and the correct mode to export. In the **Plate Export Options**, select **Raw** as the **Plate Data Options** and **Columns (.txt or .xls)** as the **Output format**. See **Figure 14** on page 23.

4 Click **OK**.

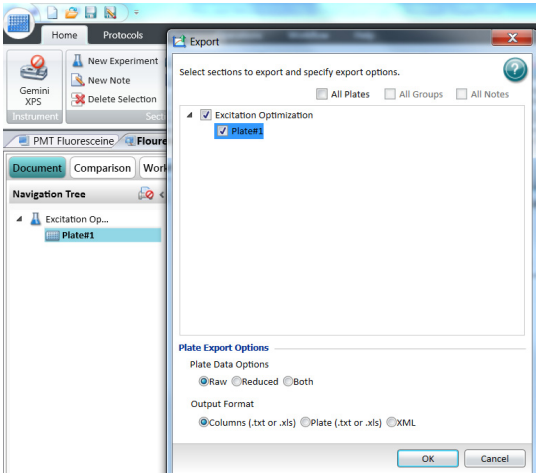


Figure 14. Plate Export Options defined for SoftMaxPro 6 and 7.

BioTek

Applicable to Gen5 software version 3.00 upwards required

1 Open the Data file in Gen5 software. Click the **Report/Export** button. See **Figure 15**.

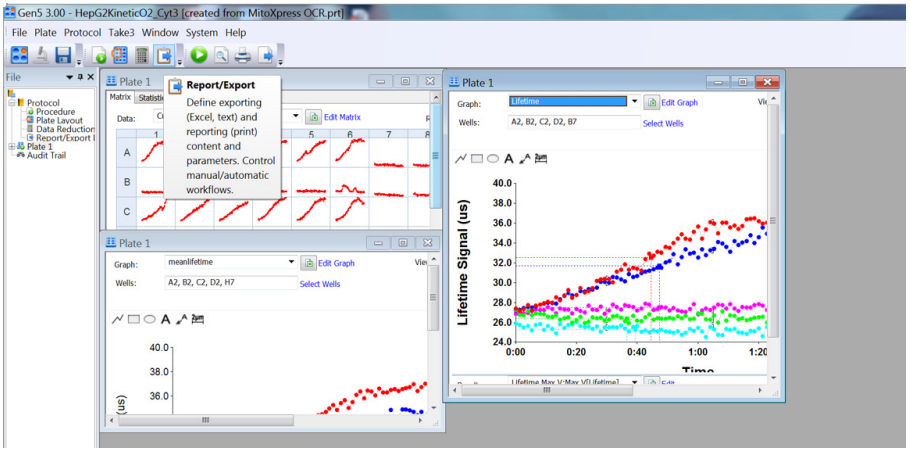


Figure 15. Exporting a Data File in Gen5 Software, Creating a new Report/Export

2 Click on **New export to text...** when the Report/Export Builder Window opens. See **Figure 16**.

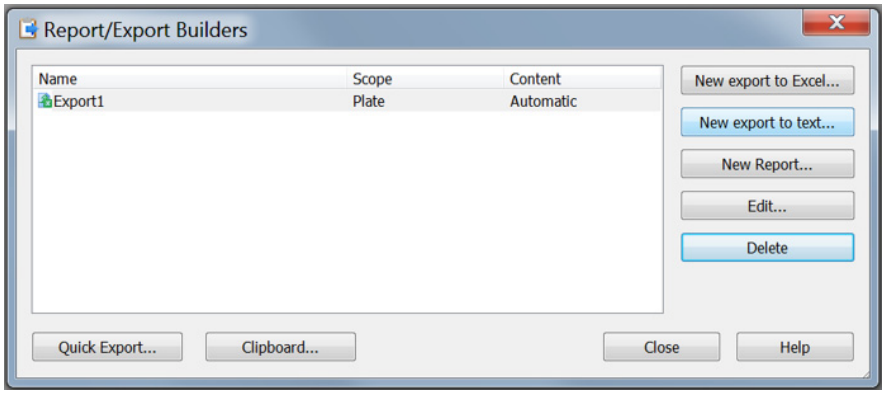


Figure 16. Exporting a Data File in Gen5 Software, Creating 'a text file exporter'.

3 Select **Properties** when the **New Export to text...** window opens.

a Select **Plate** for the **Scope**. See **Figure 17**.

b Select **Automatic** for the **Content**. See **Figure 17**.

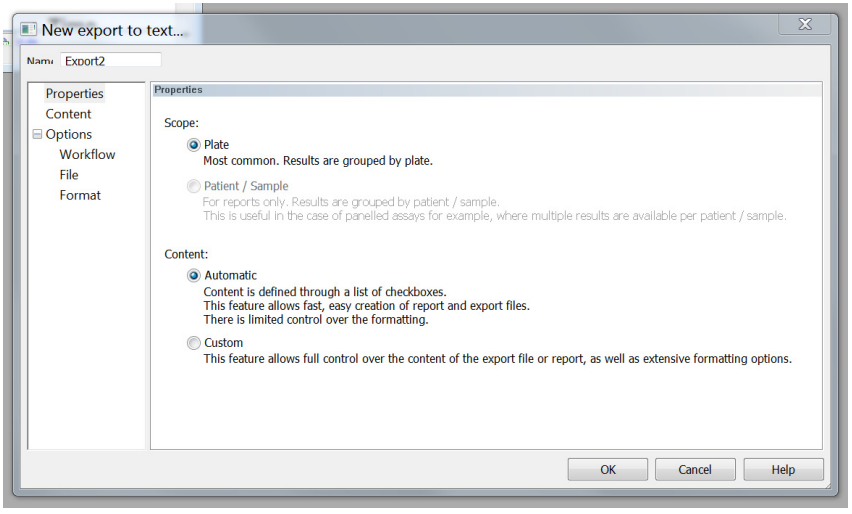


Figure 17. Exporting a Data File in Gen 5 Software, Settings for Properties Tab.

4 Select **Content**. See **Figure 18**.

a Deselect all four options in the **General Information**.

b Select **Raw Data** from the **Data/Include** section.

c Select **Row-wise table** as the **General Format**.

d Deselect **Regroup data in one matrix/table when possible** and **Include curve/scan/image pictures**.

e Select **Row-wise table** from the **Kinetic/spectrum/scanning data format**.

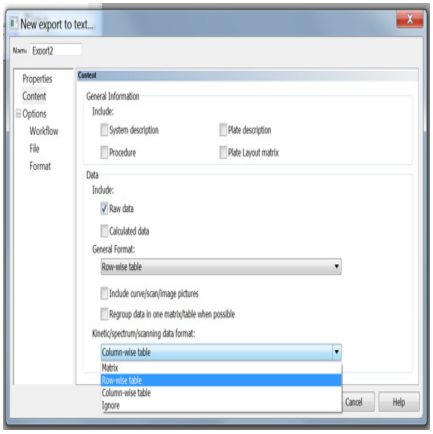


Figure 18. Exporting a Data File in Gen 5 Software, Settings for Content Tab.

5 Select **Workflow**. See **Figure 19**.

a Select **All plates in the same file**.

b Deselect every other option.

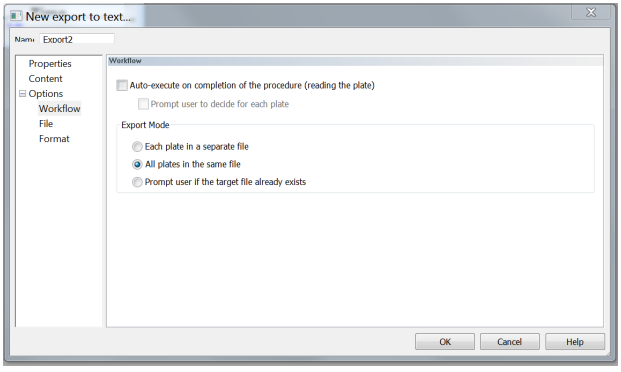


Figure 19. Exporting a Data File in Gen 5 Software, Settings for Workflow Tab.

6 Select **File**. See **Figure 20**.

a Select **Extension** to **txt**.

b The remaining settings are user preferred.

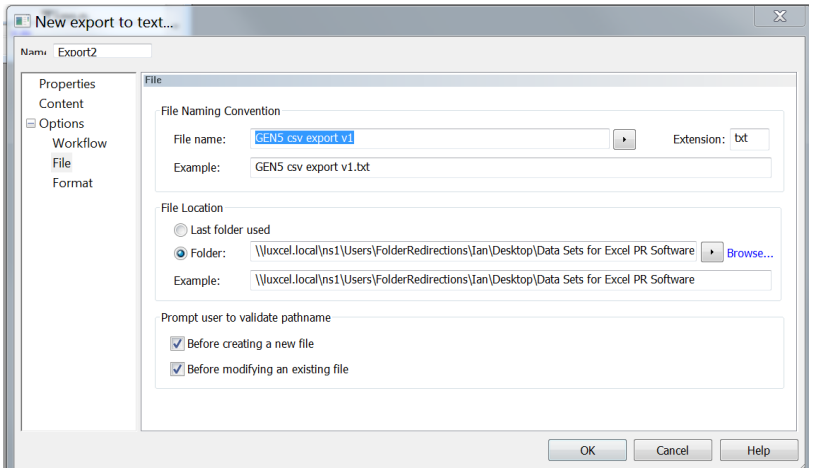


Figure 20. Exporting a Data File in Gen 5 Software, Settings for File Tab.

7 Select **Format**. See **Figure 21**.

a Select **Statistic column labels** in the **Include** section.

b Select **TAB** as the **Separator**.

8 Click **OK**.

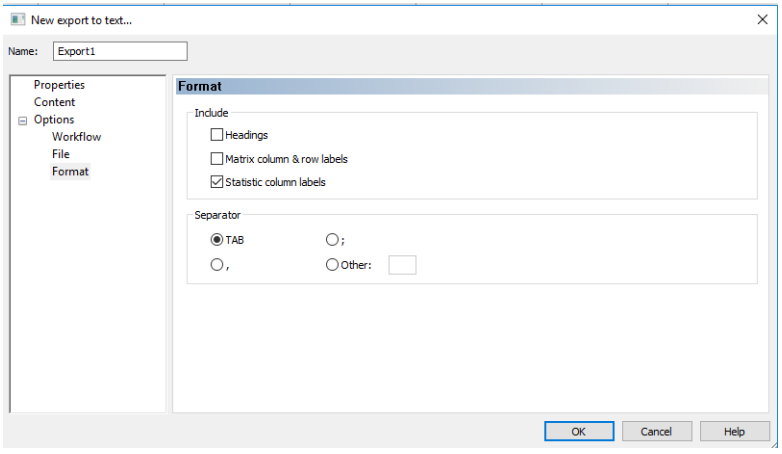


Figure 21. Exporting a Data File in Gen 5 Software, Settings for Format Tab.

9 Return to the experiment data file. Click the **Export** button.

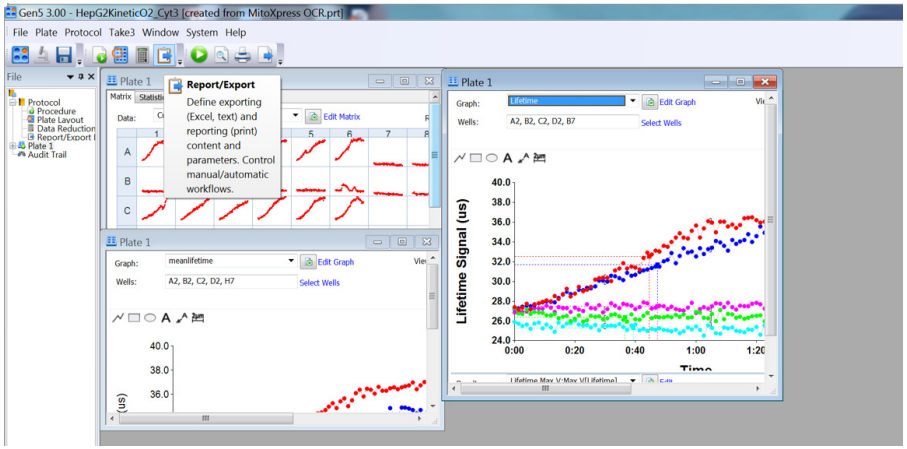


Figure 22. Exporting a Data File in Gen5 Software, clicking on Export button to export the file.

10 Select the recently created Export template. (**Export1**, in this instance, see **Figure 23**.). Save the .txt file with appropriate name and location.

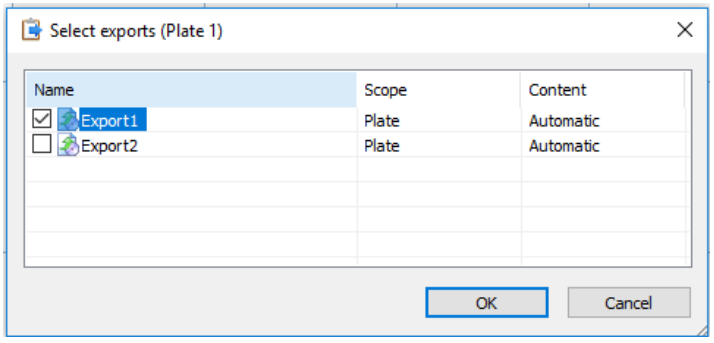


Figure 23. Exporting a Data File in Gen5 Software, Selecting the export for the final export.

TECAN

Applicable to iControl software version 1.10.4.0 or above required

The export settings need to be defined prior to performing the measurement.

1 Click on the **Settings** Menu, and select **User Settings**.

2 To edit the options for the excel export, click on **Result Presentation Settings**. See **Figure 24**.

3 Set the **Destination** as **New Worksheet**.

4 Select the **View Mode** as **List**. Once **List** mode is chosen, please set the following settings:

- **Show** = **Measured** (Displays only Measured Values)
- **Align** = **A1A2** (Arranged in Rows)
- **Rotation** = **Row-wise**
- **Display Times** = **Time per well** (Only values are displayed)

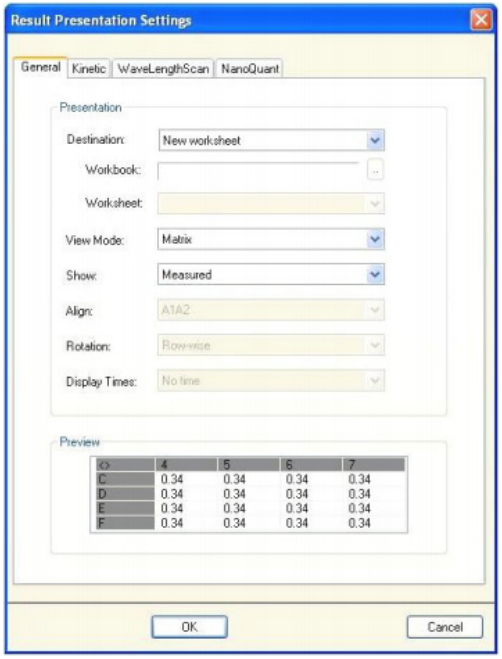


Figure 24. Result Presentation Settings; Setting the Excel export conditions

5 Click on the **Kinetic** Tab and set the following settings:

- **Rotation** = **Row-wise**
- **Align** = **A1A2**
- **Display Times** = **Time per cycle** (displays Time span per cycle)
- **Range** = **ALL**

6 Click **OK.**

The Default settings for kinetic measurement are now correct.

Applicable to SPARK Control version 2.1

1 Prior to making a measurement, ensure to set the correct Export settings.

2 Open the SparkControl software. Click on the **Data Handling icon** in the **Settings** tab of the SparkControl DashBoard. See **Figure 25**.

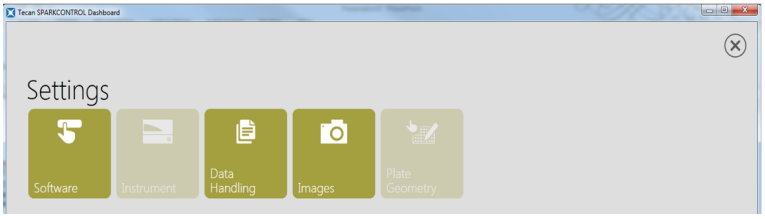


Figure 25. Exporting a Data File in SparkControl Software. Data Handling in Settings Tab.

3 Deselect the **Include plate layout**, if selected, and choose the export path if needed. See **Figure 26**.

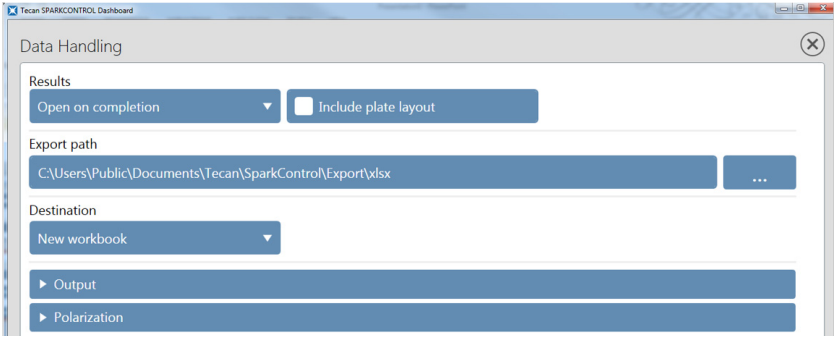


Figure 26. Exporting a Data File in SparkControl Software; Choosing export path and deselecting plate layout.

4 Click on the **Output** Drop-down menu.

5 Click on **Kinetic** to expand the options. See **Figure 27**.

6 Select the following Settings:

- **Show**: **All**.
- **List Orientation**: **Vertical**
- **Export Mode**: **List**
- **Time**: **Time per cycle**
- **Well Order**: **A1A2**

7 Click **OK**.

The Default settings for kinetic measurement are now correct.

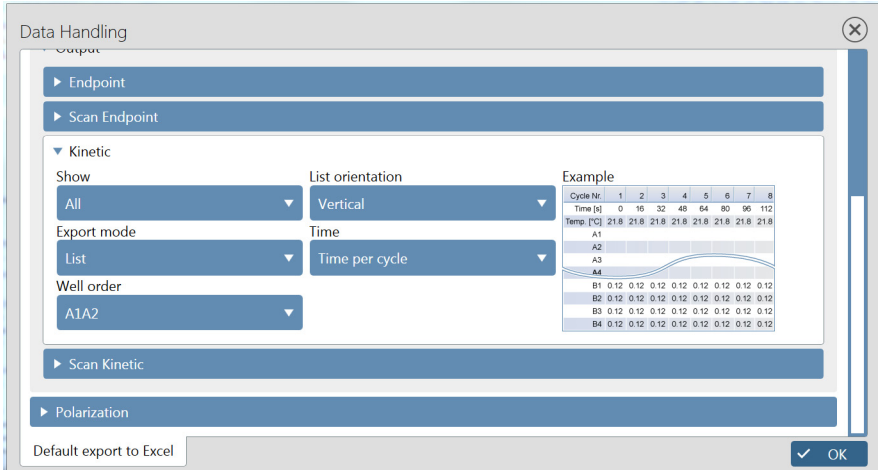


Figure 27. Exporting a Data File from SparkControl Software. Choosing the Kinetic Settings.

Analyze Data in the Data Visualization Tool

Import Excel/CSV/TXT file

1 Open the MitoXpress Xtra & pH-Xtra Data Visualization Tool.

2 Click **Load New Data File** in the Dashboard.

3 Locate the Microsoft Excel/CSV/TXT file (exported from plate reader software), and click **Next**.

Plate Map annotation

After assay result data has been imported, use the **Plate Map Tab** to select groups from the assay to display, and click **Data Reduction** (X). The Data Visualization Tool automatically calculates the parameters for each group selected, and displays kinetic signal curves on the **Graph** tab.

How to annoate:

1 Enter the number of replicate wells for the entry.

2 Choose the wells for entry in the 96-well plate layout.

3 Click the group Button; **Blank**, **Control or Sample** depending on what the sample is. The entry will appear in the table on the right.

4 Enter additional details in the **Description** section, if needed.

5 Repeat steps 1 through 4 to make the next entry.

6 Use the **Clear well / All** button to undo mistakes are make edits.

7 Once all required wells are annotated, Click **Data Reduction** button.

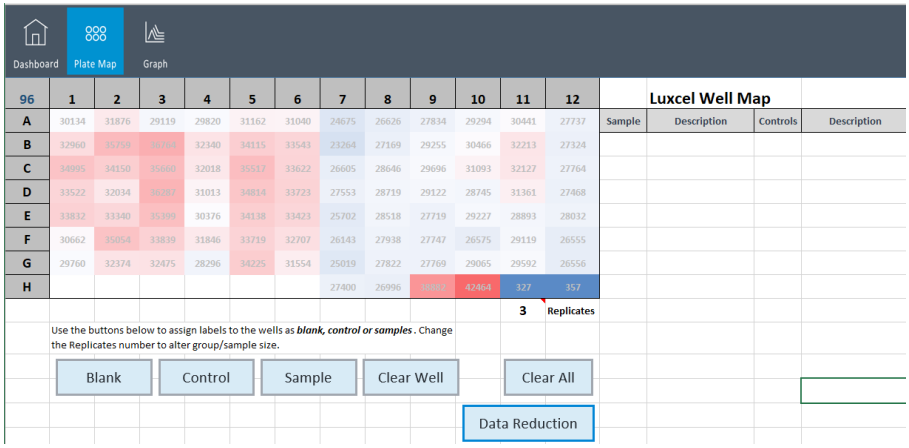


Figure 28. Plate Map Annotation; the values shown in plate map orientation are the RFU (W1) at cycle 1 of the measurement. These values are color coded based on their intensity (RFU) value, with red identifying lower AV RFU values compared to blue representing higher AV RFU value (i.e. Blank wells).

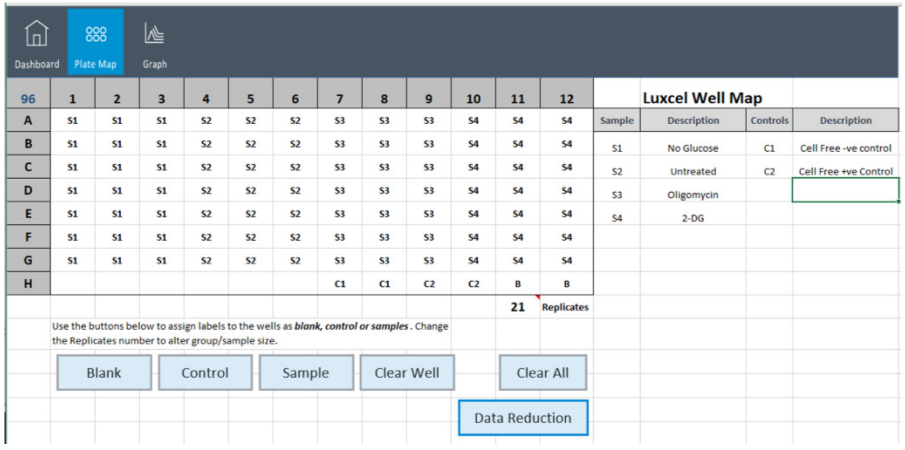


Figure 29. A completed Plate Map annotation in the Data Visualization Tool showing selected groups of Samples, Controls, and a Blank. Description information was added in the Table.

Graph

After the **Plate Map** has been annotated and **Data Reduction** performed, the visualization tool automatically calculates the parameters for each group and displays kinetic signal curves and calculated slopes on the **Graph** tab. The tab can be used to visualize and process the chosen wells as preferred by the user.

Selection options:

- Select between Intensity or Lifetime and pH/H+ scale data in the graph data selection box depending on the input data detection mode
- Select between Average or Replicate data in the Display box
- Select/deselect to include error bars in the graph
- Select/deselect a Group or Wells by selecting them in the Summary Group Table. See **Figure 33** on page 35. Greyed cells are deselected from the graph and analysis.

These Graphs and Tables can be easily exported to another Excel file or another software for further processing and interpretation as required.

MitoXpress Xtra Sample Signal Curves

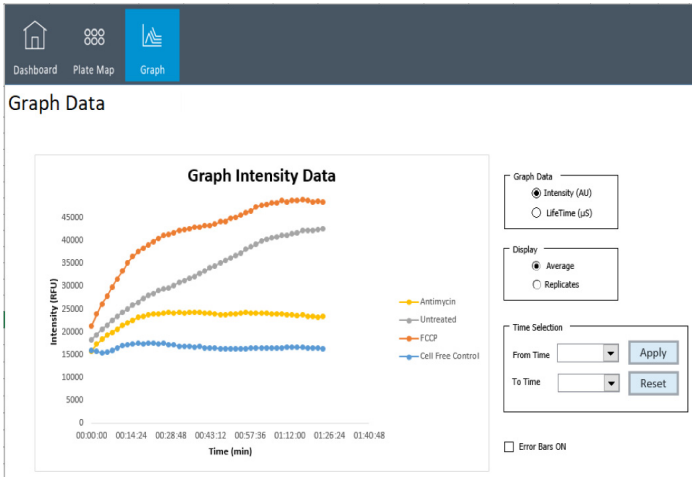


Figure 30. Graph Example: Intensity Scale Data. Average intensity signal curves of the selected samples.

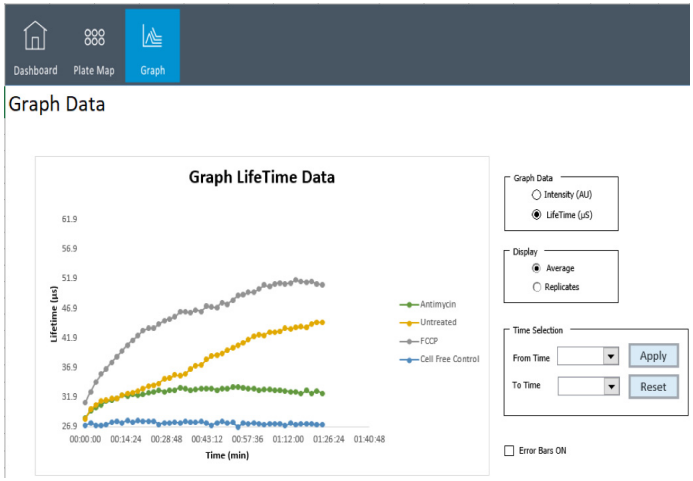


Figure 31. Graph Example: Lifetime Scale Data. Average Lifetime signal curves of selected samples.

pH Xtra Sample Signal Curves

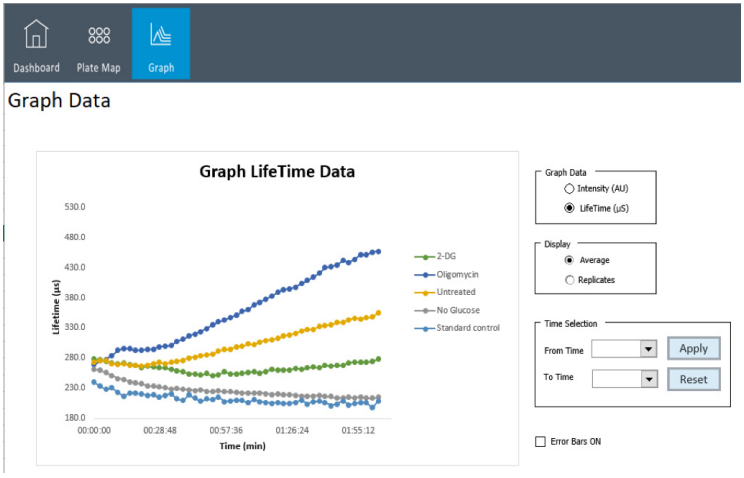


Figure 32. Graph example: pH-Xtra lifetime scale data. Average lifetime signal curves of selected samples.

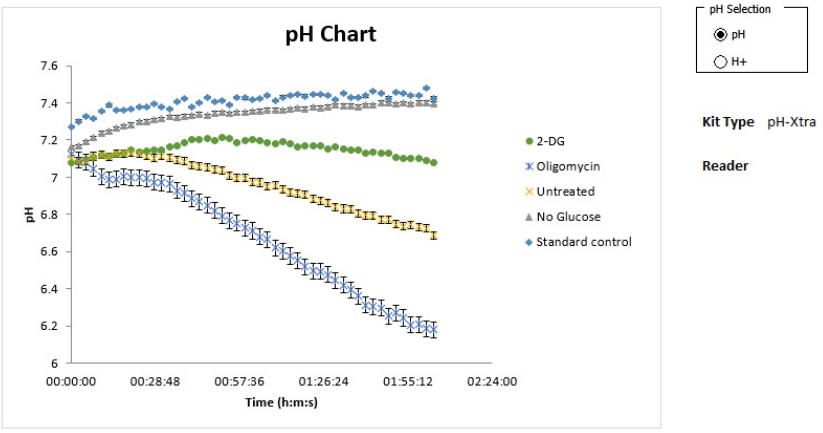


Figure 33. Graph Example: pH-Xtra pH Scale Data. Average pH scale signal curves of selected samples.

Group Name		Wells			
Cell Free Control		H9	H10		
Cell Free Gox Control		H7	H8		
FCCP		A1	A2	A3	
Untreated		A4	A5	A6	
Comp. E		A7	A8	A9	
Antimycin		A10	A11	A12	
Comp. A		B1	B2	B3	
Comp. B		B4	B5	B6	
Comp. C		B7	B8	B9	
Comp. D		B10	B11	B12	

Figure 34. Summary Group Table

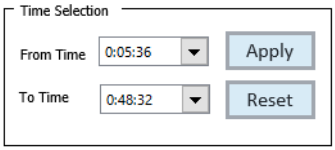
The table contains all annotated samples by group name and well number. Greyed cells are deselected from the graph and analysis. The user simply selects or unselects Groups or Wells for inclusion in the analysis. See **"Exclude Outliers/Groups from Analysis"** on page 42 for more information. Data selected for analysis are illustrated in the graph and subsequent slope calculated are reported in the Slope table.

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 visualization tool.

Adjust Time for Slope Calculation

Input values in the drop-down boxes under Time Selection and click **Apply**. This allows the user to select the linear portion of the signal curves which are used for slope calculation, as recommended in the Assay User guide. The calculated slopes are reported in the Slope Summary Table.



The Input data used for the Graph Data box is used as the input data for the slope calculation. If the data is switched from Intensity to Lifetime, then the Slope values presented in the Slope table will update and switch to Lifetime based slope (for example, µs/h).

In this example, the time selection is set to use signal between 5 minutes 36 seconds, and 48 minutes 32 seconds. Data between these two times is presented in the graph and used for the Slope calculation with values presented in the slope summary table. See **Figure 35** on page 37.

The time selection can be adjusted until the R^2 value is suitable for the given set of samples.

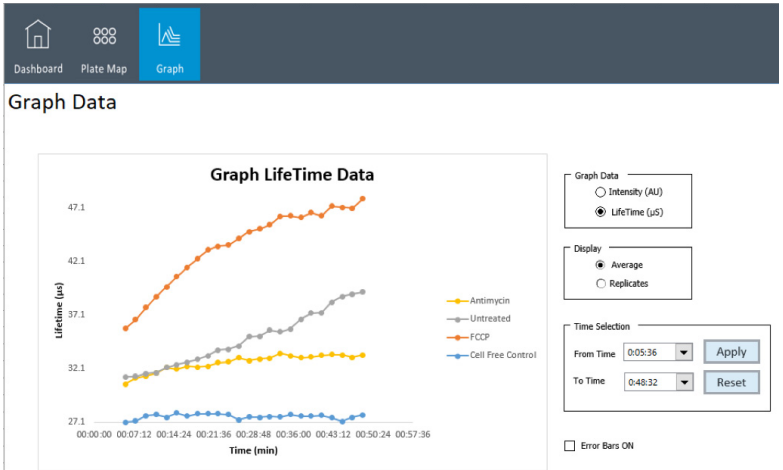


Figure 35. Average lifetime signal curve with time section set between 5 minutes 36 seconds, and 48 minutes 32 seconds.

	Lifetime (µs/h)			
Group Name	Wells	Slope	Average	Std Dev
Cell Free Control	H9	-0.0500	0.0333	0.12
	H10	0.1166		
FCCP	A1	15.60	15.66	0.09
	A2	15.76		
	A3	15.61		
Untreated	A4	10.70	11.61	0.80
	A5	11.89		
	A6	11.23		
Antimycin	A10	3.64	3.24	0.54
	A11	2.62		
	A12	3.45		

Figure 36. Example Slope Summary Table, shoeing calculated Slope values (µs/h) for replicate and average samples.

Save a Summary

Save/Save as

Use the **Save/Save as** function to save the customized Summary Report as an Excel Macro file format (*.xlsm).

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.

When saved as an Excel Macro file format, the Summary Report can be re-opened to view the calculated parameters for the selected groups, format/customize the appearance of graphs and figures, or import another data file for analysis with the Data Visualization Tool.

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

The Data Visualization Tool default file type is a Microsoft Excel Macro-Enabled Template (*.xltm) - this file cannot be overwritten. Saving the Data Visualization Tool as an Excel workbook or any other file type than the default file type (Excel Macro: *.xlsm) will render the macro inoperable - modifying the groups selected or importing additional assay result data is not supported as an Excel Workbook file type.

Suggested additional processing

Bar Charts

Create Bar Chart(s) using the calculated average slope and standard deviation values. Copy the Summary Slope Table to a New worksheet or new Excel file and plot Average Slope values using the Bar Chart function in Excel of the chosen samples. Add errors bars using the calculated Standard deviation. Additional data processing software applications can be used.

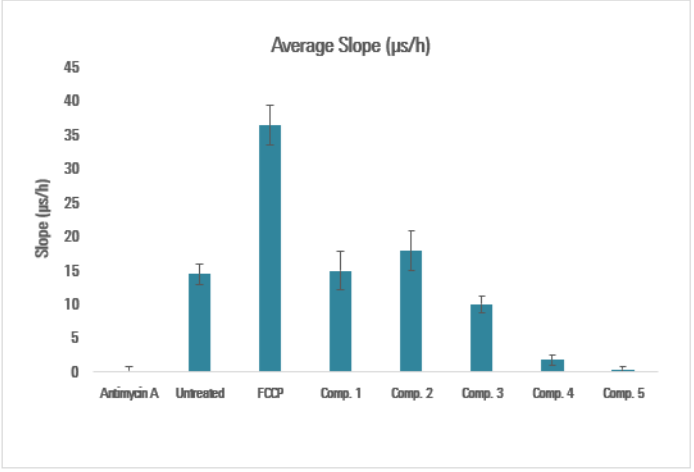


Figure 37. Example Bar Chart of Average Lifetime Slopes, including Untreated, Antimycin A and FCCP controls and other unknown compound samples.

Normalize Sample Slopes as % Untreated Control Slope

Normalize the slope values of all samples to the Slope Untreated Control Sample in the experiment, where the untreated control is 100%.

Use the follow formula: % of Untreated Control = (X/m)*100

Where X is the calculated slope of a given sample (µs/h) and m is the calculated slope (µs/h) for the untreated Control Sample. Value are express as %.

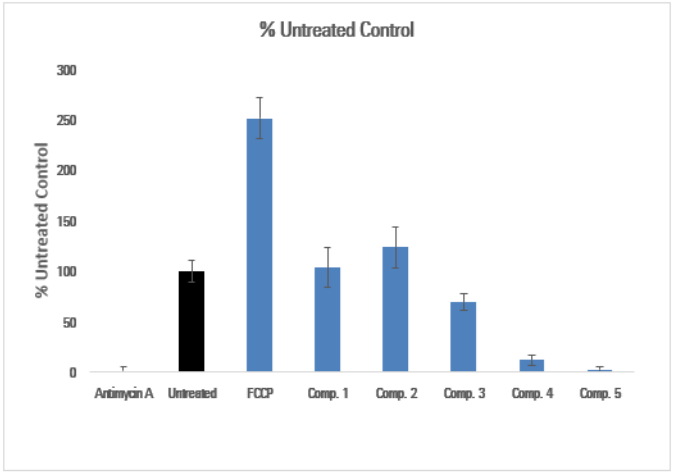


Figure 38. Example Bar Chart of % Untreated Control, where Sample slopes have been normalized to AV Slope of the Untreated Control.

Error Bar Calculations

Error Bar Type is a universal setting and applies to ALL graphs and charts in the Data Visualization Tool. **Standard Deviation** is the default error bar type and cannot be changed to an alternate error bar calculation within the Data Visualization Tool.

- Error bars are calculated from each replicate of the rate measurement used to determine the assay parameter (**Table 1** on page 8).
- Standard deviation is calculated using the Microsoft Excel function: =STDEV.P

Exclude Outliers/Groups from Analysis

Individual assay wells or entire groups/conditions can be excluded from parameter calculations in the visualization tool. Greyed cells are deselected from the graph and analysis. Simply select Groups or Wells to exclude in the analysis. Data excluded for analysis in the Graph and in the Slope calculations are shown as greyed in the table. Only Groups which are not greyed out, are illustrated in the graph and reported in the Slope table.

Group Name		Wells			
Cell Free Control		H9	H10		
Cell Free Gox Control		H7	H8		
FCCP		A1	A2	A3	
Untreated		A4	A5	A6	
Comp. E		A7	A8	A9	
Antimycin		A10	A11	A12	
Comp. A		B1	B2	B3	
Comp. B		B4	B5	B6	
Comp. C		B7	B8	B9	
Comp. D		B10	B11	B12	

Figure 39. Group table: Assay Groups Cell Free GOx Contorl. A, B, and E have been deselected in table (grey cells), therefore they are not used for the graph or calculations.

3 Frequently Asked Questions

What calculations are performed on the data in this visualization tool?

Parameter equations are described in **Table 1** on page 8 of this User Guide, in the **"Calculations Performed"** on page 8 section.

How do I remove outlier wells in the Data Visualization Tool?

See the **"Exclude Outliers/Groups from Analysis"** on page 42 for more information.

Can I use any file format (.xml/ csv/ .txt) exported from the plate reader for importing into this Tool?

No, only the file formats described in the **"Export Data from Plate Reader Software"** on page 17 section are compatible with this Data Visualization Tool. If you are having difficulties importing the recommended file format for import, please contact Agilent Cell Analysis Technical Support.

There is a large drop in fluorescence signal in the beginning of my results what do I do?

This decrease in signal is due to temperature equilibration of the plate, and data illustrating this effect should not be used for slope analysis purposes.

We recommend moving the time selection to calculate slope after temperature equilibration effect has plateaued.

Please refer to the user guide for more information on plate Temperature equilibration, and steps to reduce it.

How do I choose the linear portion of the signal curves?

Use the time selection values to select for the portion of the signal curves you wish to be included in the analysis. Once chosen, review the R^2 values recorded in the Summary Slope table to give you an indication of the linearity of the Slope calculation. Based on these values, the time selection can be adjust to further improve the R^2 value for a given data set as required.

Feedback

Feedback for the Data Visualization Tool or other products is always encouraged. Please direct any questions, concerns or suggestions to Agilent Technical Support at: cellanalysis.support@agilent.com

www.agilent.com

For Research Use Only.
Not for use in diagnostic procedures.

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