EZ Read
Microplate Readers

EZ Read 800 Plus Microplate Reader User Manual

Biochrom Ltd
22 Cambridge Science Park
Cambridge
UK
CB4 0FJ
Tel.: +44/1223 423723

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1.2 Introduction

What is a microplate reader?

A microplate reader is a used to measure the absorbance of liquid samples in a 96-welll plate.

What do microplate readers measure?

Microplate readers measure the amount of specific wavelengths of light that is absorbed by molecules within a solution.

Why is this useful and how do microplate readers measure sample absorbance?

The amount of light absorbed by molecules within a sample is proportional to the sample concentration as described by Beer-Lambert's Law:

$$\mathbf{A = e\,c\,b}$$

A= absorbance
e = molar absorptivity ($Lmol^{-1}\ cm^{-1}$)
c = molar concentration ($mol\ dm^{-3}$)
b = pathlength (cm)

It is important to note that when measuring absorbance in a microplate well, the pathlength is determined by the volume. In a microplate assay, it is very important that all wells have the same volume and that all solutions are dispensed with an accurate liquid handler like an 8-channel pipette.

Common applications of absorbance-based microplate readers are ELISA, total protein (like Bradford or BCA) and cell proliferation assays.

2 Instrument Use

The Biochrom EZ Read 800 Plus Microplate Reader is used for microplate-based applications requiring endpoint or kinetic absorbance measurements from 400-750 nm in optically clear 96-well plates with a standard SBS/ANSI footprint.

Only trained laboratory personnel should operate the Biochrom EZ Read 800 Plus Microplate Reader. The Biochrom EZ Read 800 Plus Microplate Reader is intended for general laboratory and research use only.

3. Instrument Connection and Software Installation and Use

3.1 Instrument connection

1. Connect the USB cable from the PC to the instrument.

2. Connect unit using only the supplied power cords to a power outlet.

   ***Please Note*:** Keep the area around the instrument free from clutter to allow for easy access to the standard mains plug. This is also important to allow an adequate flow of air around the instrument.

3. Switch on main switch (back of the instrument).

4. The power indicator light at the front of the unit will be illuminated when the instrument is switched on.

3.2 Software installation

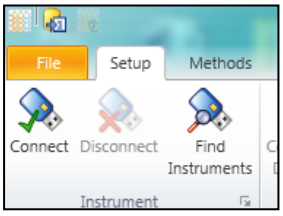
Galapagos is supplied with all EZ Read 800 Plus instruments. The software can be used to control the microplate reader to measure all endpoint, kinetic and multi-wavelength assays.

1. **To connect the instrument to a PC**: Connect to the PC via the supplied USB cable.

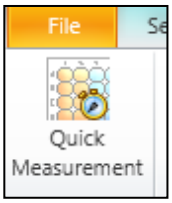
2. **To connect the instrument to Galapagos software**: Insert the CD or USB supplied with the instrument into a PC. Galapagos software includes pre-requisite files (dot.NET Framework 4, Windows Installer and Microsoft SQL Express 2008) which allow installation to occur. Insert the CD or USB and run the setup file. A pop-up menu will appear and guide you through automatic installation.

   Users can save results and templates to a Database. Galapagos uses a database to store data in one place. This is useful for preventing data override and can allow easy data transfer through communication with external laboratory database systems such as LIMS. To create a Database, click 'Setup'>'Select Database'>'Galapagos'. Alternatively users can click on 'Add Database' and enter a new database.

   Connect the instrument to a computer or laptop. Connect by clicking the Galapagos icon and click on the 'Find Instruments' icon which detects the connected instrument. For subsequent use, users can click the 'Connect' icon for recognition:



3. Click ‘Quick Measurement’:

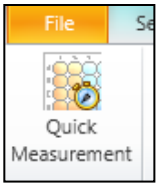


4. The microplate reader is now ready for use.

3.3 Quick Measurement:

Performing a Quick Measurement on Galapagos:

1. Click the ‘Quick Measurement’ icon on the main menu screen:

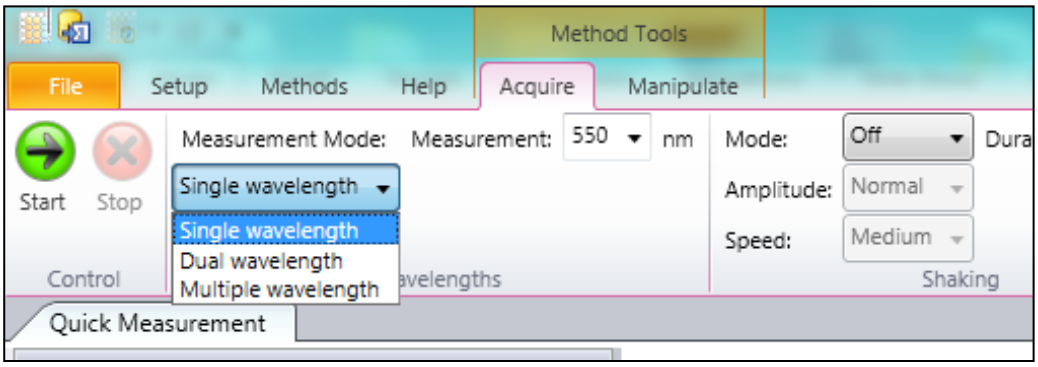


2. Select the appropriate measurement mode (Single, Dual or Multi-wavelength):

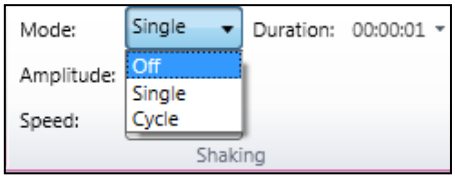
Single wavelength refers to absorbance measurement at one wavelength.

Dual wavelength refers to the measurement of a sample at two wavelengths. The first wavelength is where the sample of interest absorbs. The second wavelength is used as a reference and will be subtracted from the first wavelength. The absorbance measurement is then subtracted from a measurement at a different wavelength.

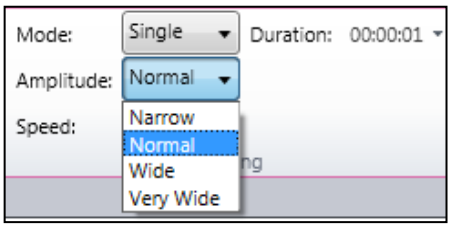
Multiple wavelength refers to a series of absorbance measurements at different wavelengths. In this mode, absorbance measurements are not subtracted from one another.



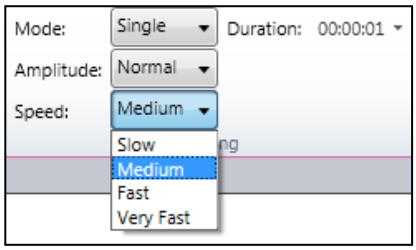
3. Users can also select to shake the microplate. Users can select shaking to be a single event or in timed cycles.



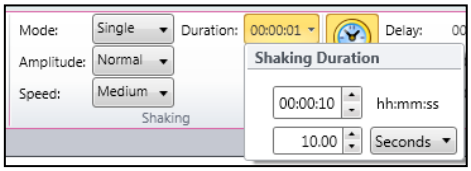
4. Select the amplitude (degree of linear shaking).



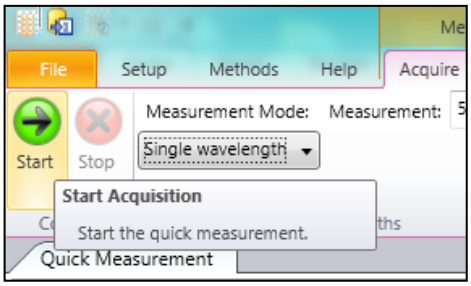
5. Select the speed for linear shaking.



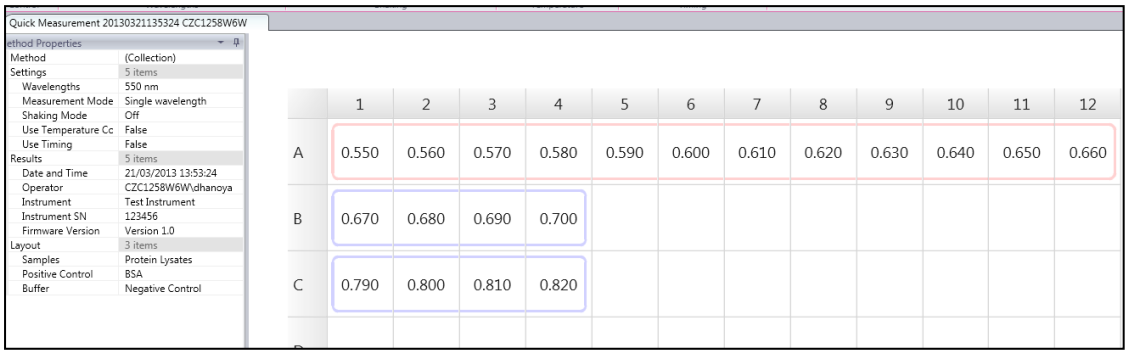
6. Users can select the duration of shaking.

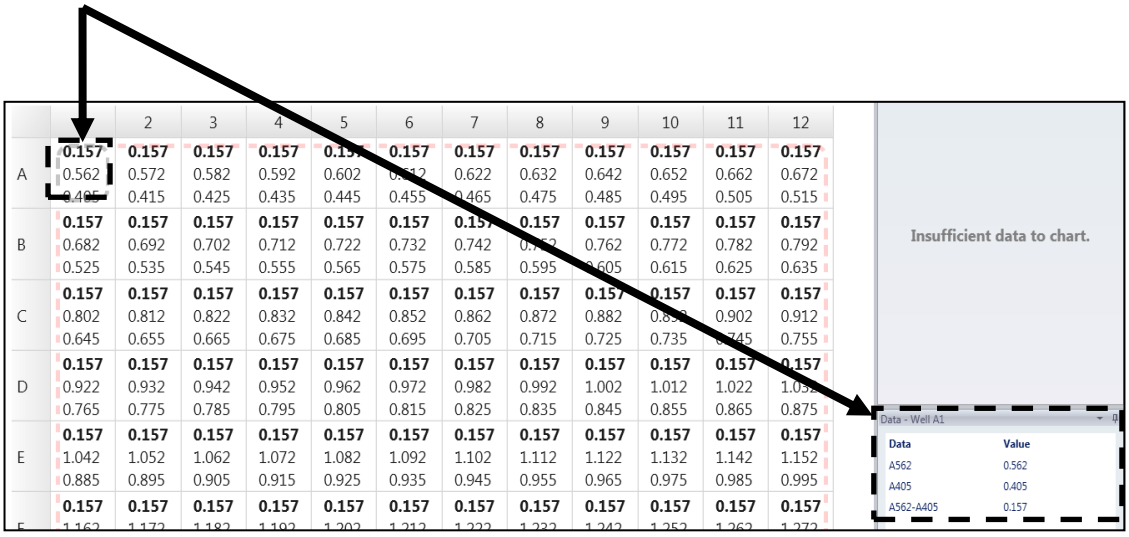


7. Click ‘Start’ to begin acquisition.

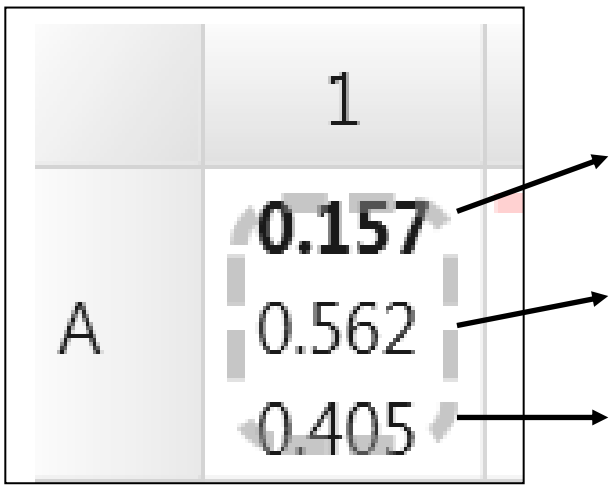


8. Raw absorbance values for dual wavelengths measurements are displayed in the wells. Details of corresponding wavelengths can be displayed when clicking on the individual well.





9. When users click onto a well, it is highlighted (dashed lines). In regards to dual wavelength measurements, three absorbance values are displayed.

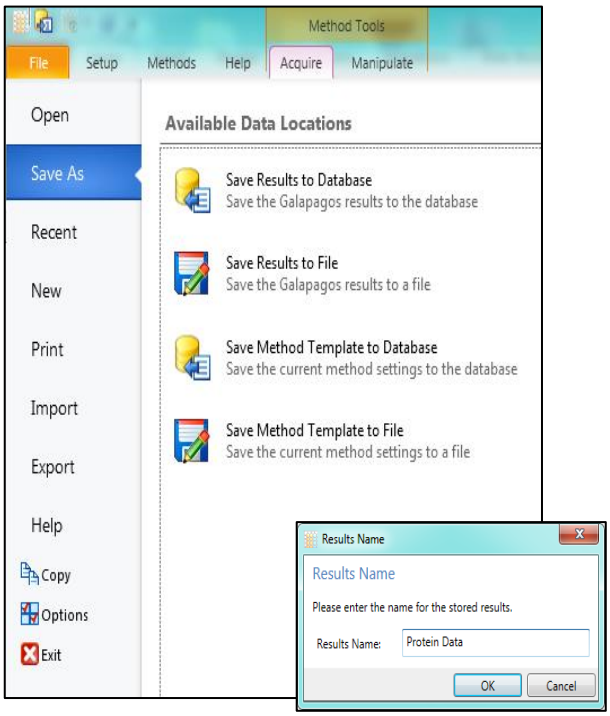


The bold value refers to the absorbance measurement after wavelength subtraction of the absorbance at the measurement wavelength from the absorbance at the reference wavelength.

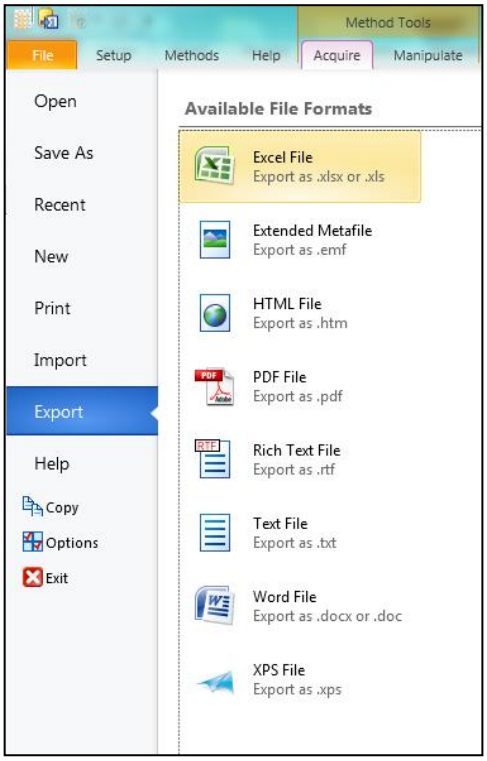
The second value is the absorbance at the measurement wavelength

The third value is the absorbance at the reference wavelength.

10. Results can be saved to ‘Database’. A database is useful for saving, sharing and extracting data via communication with an external database system such as LIMS. Users can save files and templates onto the database, ‘File’ (save to own personal file), or ‘Save Method Template’ (saves the method protocol for future use).



11. Results can also be exported as Excel, Extended Metafile, results exported as a HTML file, PDF, Rich Text File, Text File, Word File and XPS File.

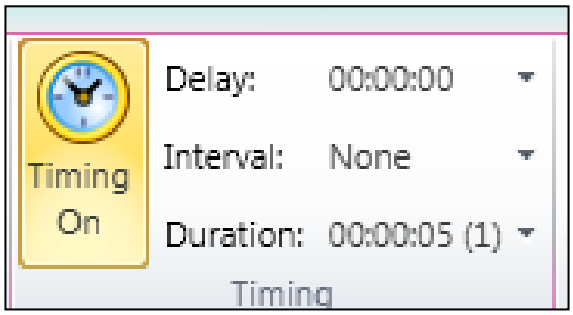


3.4 Kinetic Measurements

Performing a kinetic measurement on Galapagos:

Kinetic measurements refer to recorded measurements over the course of a set time period. To perform a kinetic measurement, open Galapagos as mentioned in section 3.2.

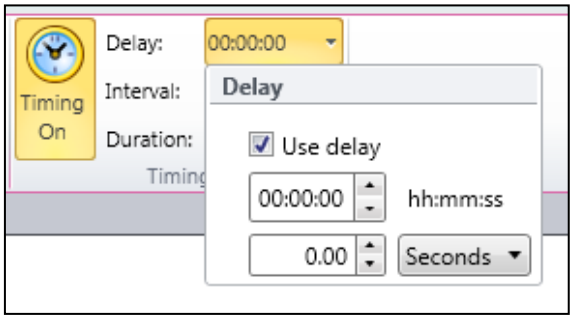
1. For kinetics measurements, click on the timer icon for timed measurements. This is indicated by 'Timing On'



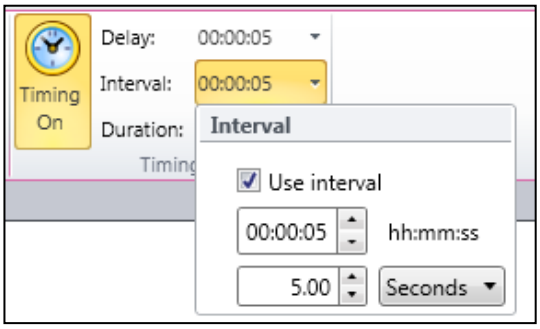
Users can then input the required kinetic parameters for a measurement cycle. The measurement cycle refers to the point of absorbance measurement including any timed delay and/or interval until the next reading.

Note: the use of a delay is optional (tick or un-tick), whereas the interval and the number of cycles is not.

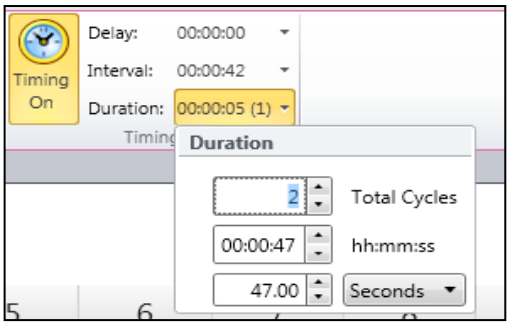
2. Users can input delays between measurements.



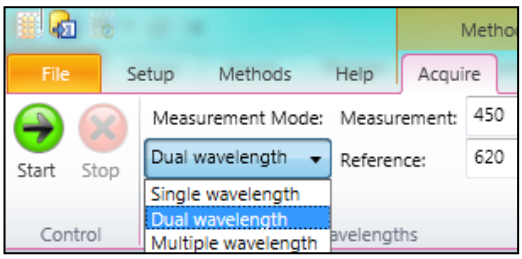
3. Users can input timed intervals between readings.



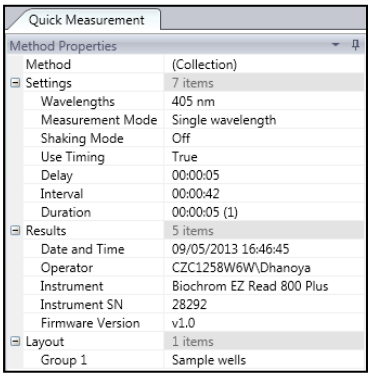
4. The measurement cycle (delay and interval) can be inputted and multiple cycles can be selected.



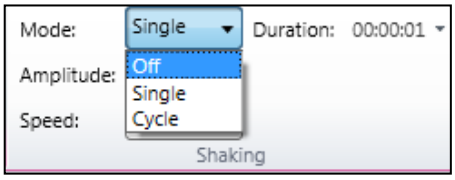
5. Select either: Single, Dual or Multiple wavelength modes. Note: measuring at more than one wavelength will affect the interval that can be used. The interval time must take into account the measurement time at a single wavelength (e.g. this is 5 seconds at a single wavelength for the EZ Read 800 Plus).



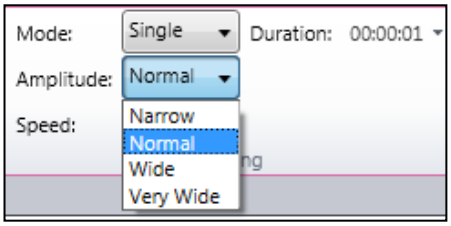
Note: Method details of the kinetic experiment are listed on the left-hand panel of the screen.



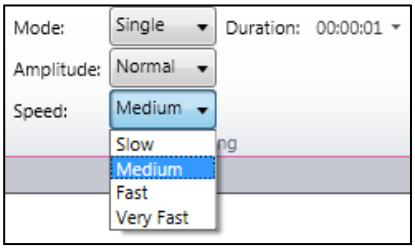
6. Users can also select to shake the microplate. Users can select shaking to be a single event or in timed cycles.



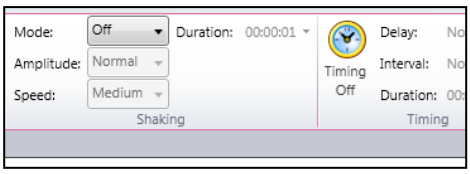
7. Select the amplitude (degree of linear shaking).

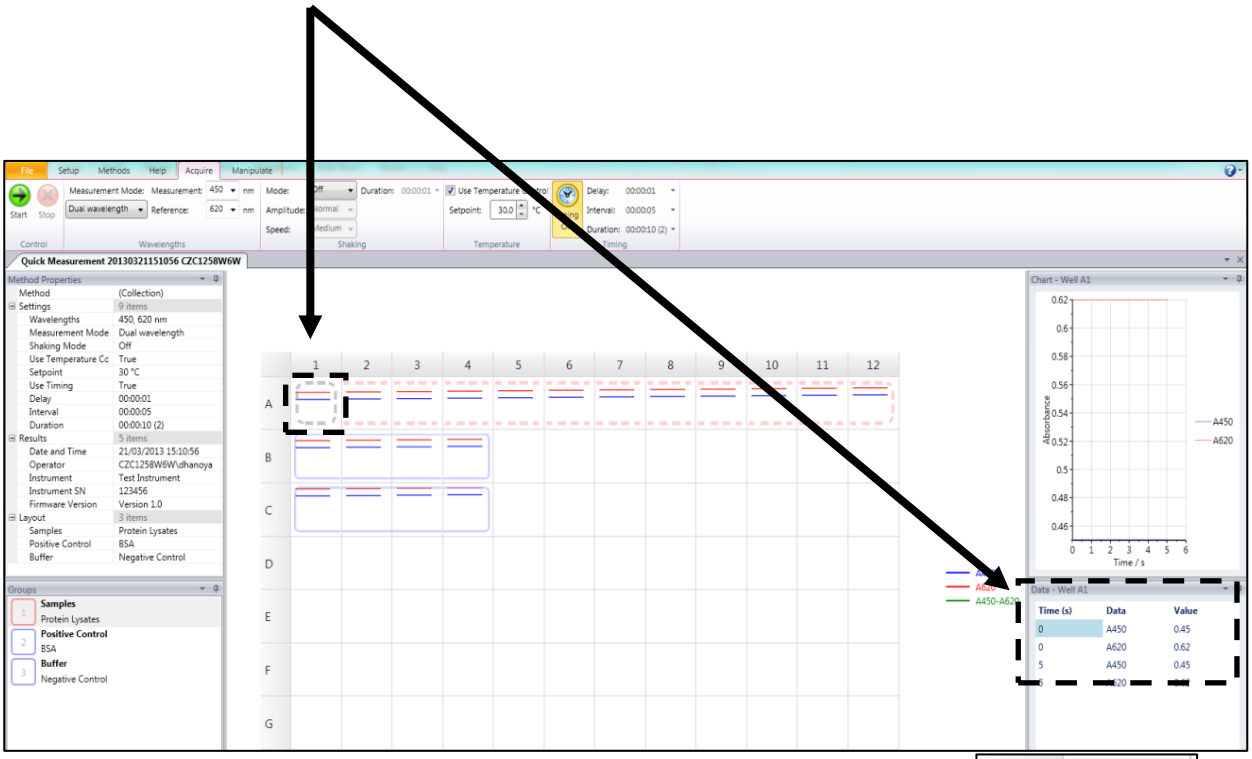


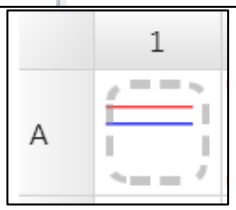
8. Select the speed for linear shaking.



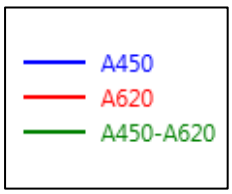
9. Users can select the duration of shaking.



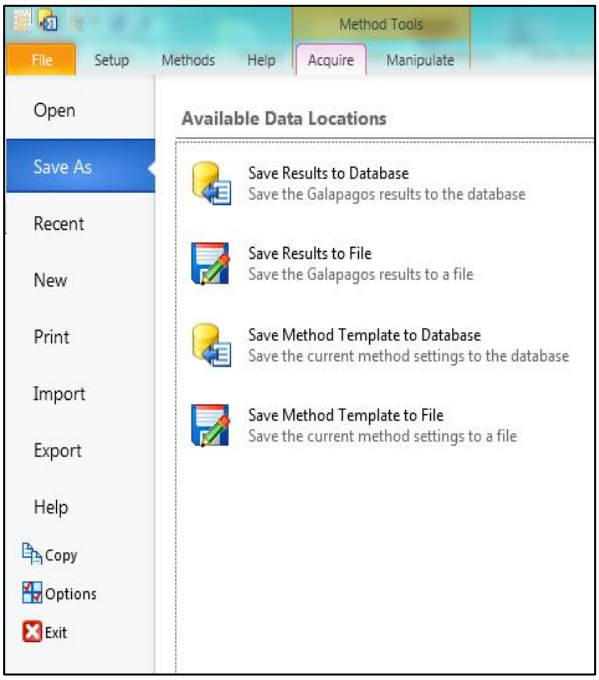




The lines shown in each well refer to the measurement at one wavelength over time. Different wavelengths used for absorbance measurements are assigned a specific colour and identified by a legend.



10. Results can be saved to ‘Database’. A database is useful for saving, sharing and extracting data via communication with an external database system such as LIMS. Users can save files and templates onto the database, ‘File’ (save to own personal file), or ‘Save Method Template’ (saves the method protocol for future use).



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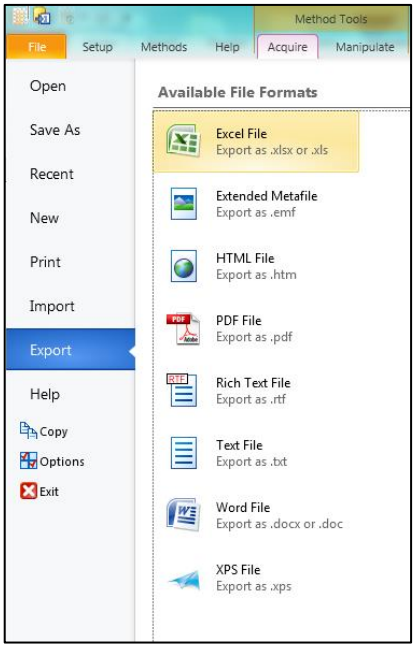
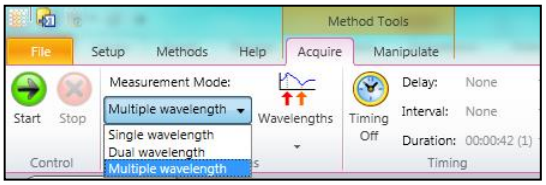


3.5 Multi-wavelength Measurements

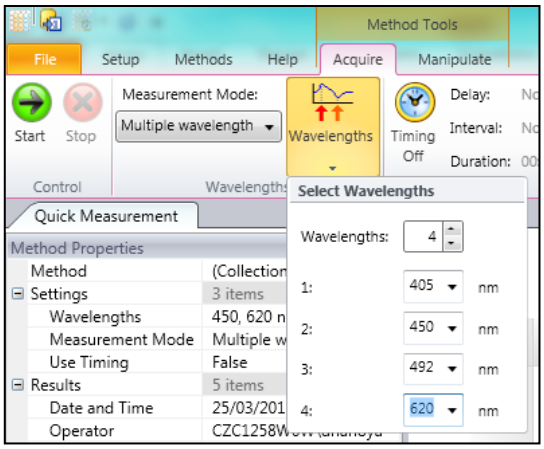
Performing a multi-wavelength measurement on Galapagos:

The Multiple wavelength application allows users to measure absorbance at different wavelengths. Mathematical manipulations of this data can often reveal information about the sample's composition or purity.

1. To perform a multi-wavelength measurements click 'Multiple Wavelength' from the drop down menu.

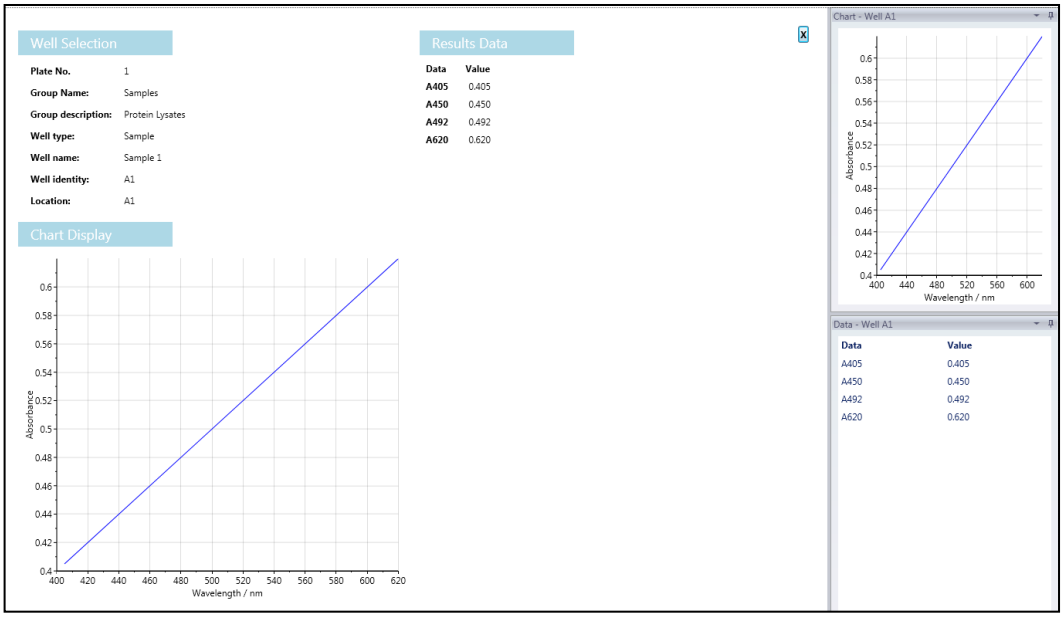


2. The menu is adjusted to allow users to select up to 4 wavelengths for measurement. Each wavelength must be different. For the EZ Read 800 Plus ELISA, users can input wavelengths of 405, 450, 492 and 620nm. For the EZ Read Research model, users can choose four wavelengths from 405, 450, 492, 562, 595 and 620nm. Click 'Start' to begin the measurement.

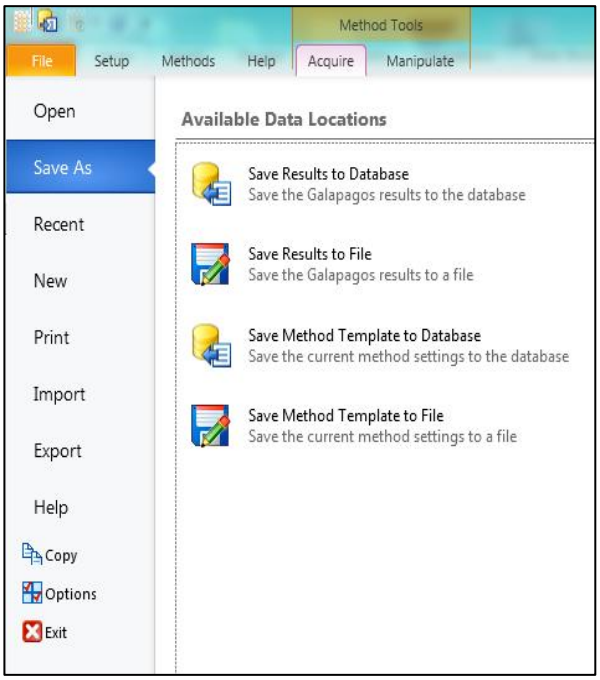


3. Individual multi-wavelength measurements are displayed in each well, with raw absorbance displayed on the right hand panel as a graph of absorbance vs. wavelength.

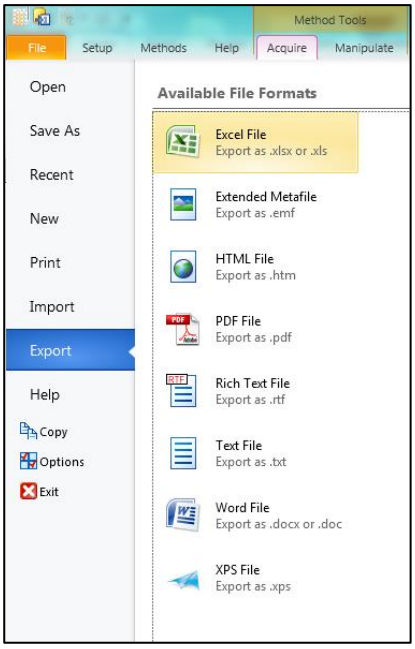
4. Alternatively users can click on an individual well to view the scan in more detail and raw results. Both tabulated and graphical chart data are displayed.



5. Results can be saved to 'Database'. A database is useful for saving, sharing and extracting data via communication with an external database system such as LIMS. Users can save files and templates onto the database, 'File' (save to own personal file), or 'Save Method Template' (saves the method protocol for future use).



6. Results can also be may be exported as file types such as Excel, Extended Metafile, results exported as a HTML file, PDF, Rich Text File, Text File, Word File and XPS File.



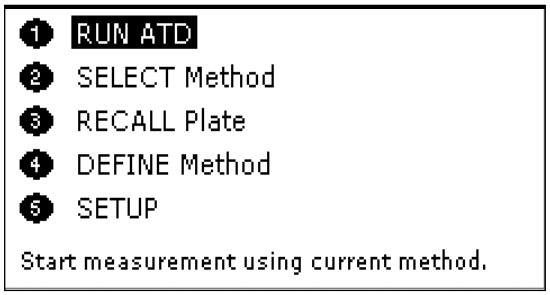
3.6 Calibrating the instrument

The EZ Read 800 Plus performs an automatic calibration of the lamp energy before it measures a microplate; however, the user may use a calibration plate containing a series of neutral density filters with known optical density to verify reader performance. See Section 8: Ordering for order details.

3.7 Using the user interface on the EZ Read 800 Plus

The 240 x 128 pixel high resolution, graphical liquid crystal display provides the user with set up parameters and experimental results. The keypad is used for navigation and has a spill-proof membrane. Navigation using the on-board menu is very simple:

1. Press the corresponding number on the keypad to enter the user mode choices or numeric data.



2. Press keypad directly below the corresponding option on the display (F1, F2, F3 and F4) to select the appropriate option.

3. Use the four ◄ ► ▲ ▼ cursor keys to navigate around the options when the prompts appear.

4. Use the enter key to move forward to the next display page.

5. Use the ESC key to go back to the previous screen.

6. Incorrect entries can be removed using the ← key.

7. The keypad can be locked to prevent unauthorized access. (default PIN is 1505). Service engineers can access service functions from here by entry of a specific PIN.

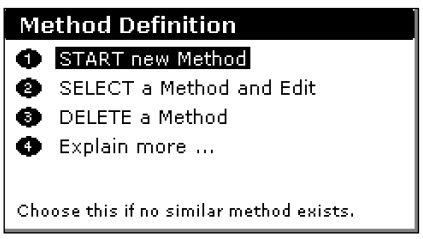
3.7.1 Description of the main menu options

1. Run	This option starts a measurement with the last used or defined method.
2. Select Method	This option selects a pre-defined or previously stored method to use.
3. Define Method	This option enables a new method to be defined (**Method Definition**), either from the beginning or by modification of a previously stored or default method.
4. Recall Plate Data	This option recalls previously stored measurement data.
5. Setup	This option enables the basic plate reader parameters, such as printer driver and filter set up/calibration, to be set up.

3.7.2 Method Definition

The method definition submenu offers three choices. These include:

- Define a new method.
- Edit an existing method which can replace an original method or stored using a new name.
- Delete an existing method.



3.7.2.1 Method Definition: Filters

1. Measurement	Use the ◄ ► keys to select the filter to be used for measurement.
2. Reference	Use the ◄ ► keys to select the filter to be used as a reference. Select "none" for no filter. The reference value (usually 620nm), is subtracted from the measured value to correct for imperfections etc. on the plastic microplate; these can have a great effect on low OD measurements.
3. Shake	Press F2 if plate shaking is required before measurement. This is often used when working with cells in order to prevent samples from settling on the bottom of the well. Enter the shaking time required in seconds and the degree of shaking. The options are: slow / medium / fast / very fast and a combination of speed and travel distance. Press F4 to accept the entries.
4. Kinetics	Press F4 if kinetics is required. Enter the interval time (time between readings) and the number of measurements. The total process time is calculated and indicated on the screen. The minimum interval time is 10 seconds and the maximum number of measurements is 100.

NOTE: Up to 100 plate data results can be stored in the instruments memory. If during the kinetic measurement the maximum capacity is reached the new results will overwrite the results stored first. When previous measured data should be kept, save these data with the Connect Plus program. Press F4 to accept the entries.

Press enter to move to the next method definition box.

3.7.2.2 Method Definition: Layout (for controls and standards)

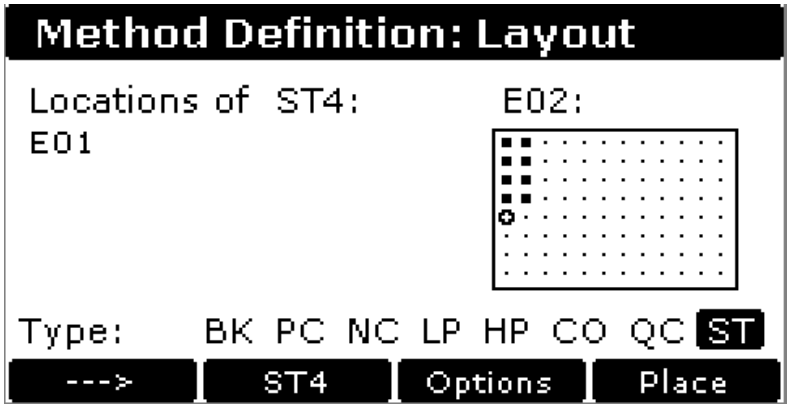
1. Options	Press F3 to define default directions (horizontal or vertical) for replicates of controls and for samples. Move the ◄ ► symbol to the desired option with the up / down arrow keys. Select the option by pressing either the left or right arrow

key. Press F4 to accept the entries.

2. Choose well definition

Press F1 '--->' to select the well type required from the options listed at the bottom of the display box. With the exception of BK and ST the terms are names and whatever you want them to be. They are not "tied".

For example, LP and HP can be used to substitute for terms T1 and T2 defined in a specific test kit. The terms can be used in calculations defined later in the method definition.



BK	Blank	The absorbance of the blank value(s) is **automatically** subtracted from the absorbance of the other wells.
NC	Negative control	Used for qualitative / screening tests.
PC	Positive control	Used for qualitative / screening tests.

LP	Low positive	Used to see if results are outside pre-defined limits
HP	High positive	Used to see if results are outside pre-defined limits
CO	Control	Use as required.
QC	Quality control	Use as required.
ST	Standards	Use for quantitative determinations; a maximum of 16 standards can be used.

3. Placement

Press F4 to place the required well type at the location shown on the display. Enter replicates by repeat pressing of F4 as appropriate. By alternating between F1 and F4 it is possible to build up the plate as required. You can come back to edit the layout if required. Use the arrow keys to move around the plate layout. Above the plate illustration the current position and well type is shown.

4. Standards

When ST (standards) is selected, 'ST1' option appears as F2. After placing these on the layout, press F2 to get ST2. A total of 16 standards, with replicates, can be built up in this way. When doing standards, note that the concentrations are entered later on.

5. Press enter to move to the next method definition box.

3.7.2.3 Method Definition: Samples

Samples can be placed either individually by moving the cursor to the desired location. Alternatively all wells not already used can be filled with samples. Replicate samples can be used in combination with single samples when desired. There is no limit on the replicate number.

1. Options — Press F3 to define default directions (horizontal or vertical) for replicates of samples and for different samples. Move the ◂ ▸ symbol to the desired option with the up / down arrow keys and select the option by pressing either the left or right arrow key. Press F4 to accept the entries.

2. Place samples — Place the first sample (SM1) and replicates using F4. Press F1 to get SM2. Use F4 to place the second sample and repeat as necessary. Replicates of samples can be placed in any free location; it is not necessary that the wells are adjacent.

3. Fill all — To reproduce the first sample pattern for all samples: Sample 1 has to be defined. The Function 'Fill All' (F2) will fill the plate accordingly as set in 'Options'.

4. To erase all already defined sample positions press the Backspace (🡐) for a few seconds.

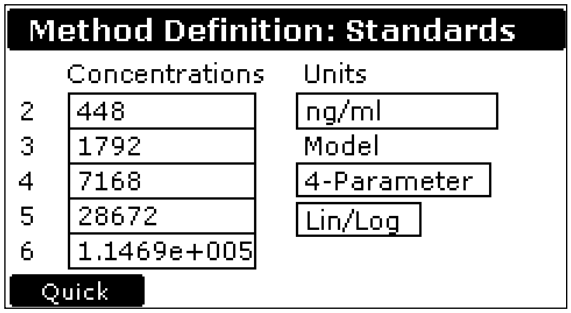
5. Press enter to move to the next method definition box.

3.7.2.4 Method Definition: Standards

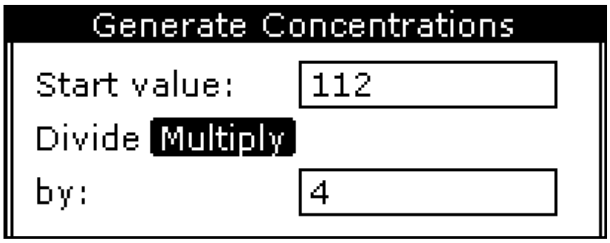
In case standards have been defined in the layout section, the calibration screen appears.

Use this option to enter the values of the standards in addition to the units for the concentration and to choose the curve-fit method (model).

1. The concentrations for the standards should be entered first. The screen offers as many entry fields as standards have been defined.



2. In case the standards are in linear serial dilution, the Quick key (F1) offers a quick way of entering the concentrations.



3. Units can be defined using the alphanumeric routine.

4. Select the analysis model for the standard curve. The options are Linear regression, Parabolic, Cubic, Point to Point (linear interpolation), Spline and 4-parameter.

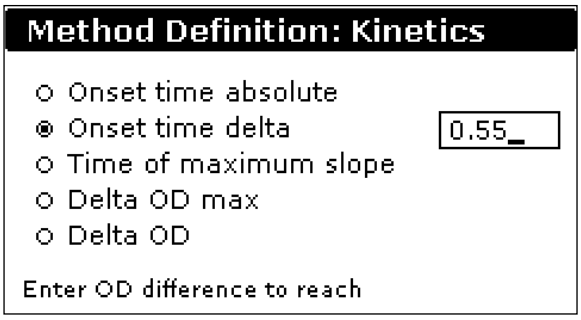
5. Select the y- and x-axis format required for presentation of the results; linear/linear, log/linear, linear/log, log/log.

6. Press enter to move to the next method definition box.

3.7.2.5 Method Definition: Kinetics

If kinetic measurements are to be defined, the Kinetics calculation definition window will appear after the layout has been set up. The calculated results for these kinetics options are normally used as the basis for quantitative, qualitative or combined sample evaluation.

1. Five kinetic calculation options are available:



1. Onset time absolute

This option calculates the time required to reach the absorbance value specified in the input field, for each well. A linear regression is used to interpolate the time between the kinetic measurement points.

2. Onset time delta

The option calculates the time required for the Absorbance difference between that of the first measurement and that specified in the input field to be reached, for each well. A linear regression is used to interpolate the time between the kinetic measurement points.

3. Time of maximum slope

This option calculates the time at which the maximum slope is reached. A linear regression is performed for every three consecutive readings over the whole measurement time. The center point in time of the regression with the steepest slope is the time the maximum slope.

4. Delta OD max

This function calculates the absorbance change, normalized to a minute; for the three readings at the maximum slope.

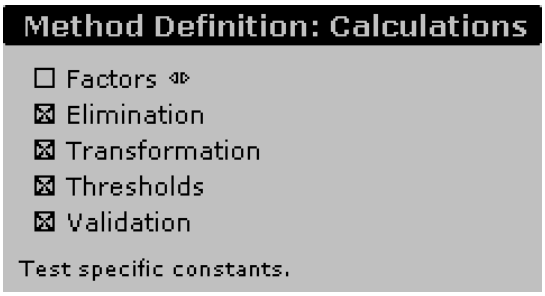
5. Delta OD

The absorbance change, normalized to a minute, over the whole measurement time is calculated.

NOTE: If none of these kinetic options is chosen, the raw absorbance data for each well at the specified time intervals can be printed out.

3.7.2.6 Method Definition: Calculations

1. If kinetics is not selected, the Calculation window will appear after the layout has been set up.



2. Use the left / right keys to select the option required, and up / down keys to move onto the next.

3. Press enter to move to the next method definition screen. Depending on the selected options different screens will be shown.

NOTE: In case controls are used in replicates they must be referred as avg(xx), otherwise they are not recognized in a formula (xx should be replaced by the name of the control).

3.7.2.7 Method Definition: Factors

1. Use this option to enter a constant specific to a particular test, for example to compensate for batch variations if biological materials are being used as part of the assay kit. Thus if the material is less biologically active than it should be, the manufacturer will specify the use of a factor or factors to normalize results to what they should be.

2. These factors can be used in any further calculation step, e.g. in the transformation formulas.

3. Press enter to move to the next method definition screen.

3.7.2.8 Method Definition: Elimination

This option allows users to define conditions for the automatic elimination of individual controls which do not meet the set criteria. Two different controls can be evaluated.

1. The selection field *Ctrl:* shows the selected control types, the left / right cursor keys are used to scroll through the available ones. Only control types which are defined in the layout are shown.

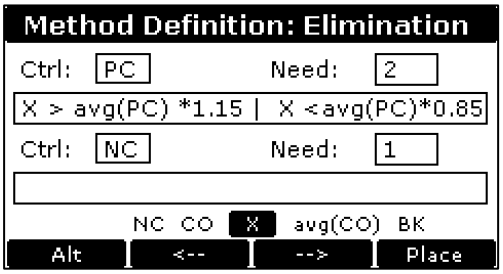
2. The field *Need* is used to enter the number of controls which should be left after the elimination process. When this condition is not met, the test is declared invalid.

3. The next entry field is used to enter the condition for the elimination. For example individual positive controls should be eliminated which are deviating more than 15% from the mean value. The condition is entered as following:

   X > avg(PC) *1.15 | X < avg(PC) *0.85

   X represents any single well of the positive control well, the '|' stands for a logical or.

4. The formula can be read as: If as single positive control is higher than 115 % of the mean value of the positive control or if a single positive control is lower than 85 % of the mean value then eliminate the respective single positive control.



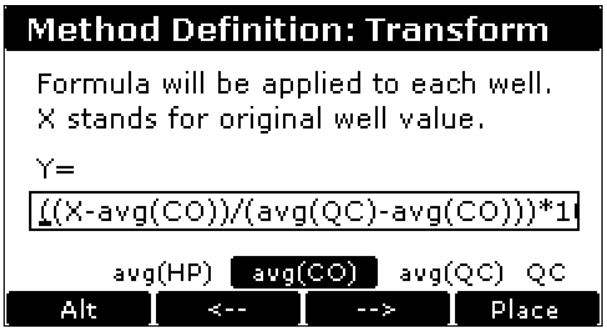
5. When the cursor is in the entry field the function keys are active and users can select different options. There are three groups of elements that can be selected by pressing the F1 (Alt) key:

- Controls (single and average) and the factors.
- Operates +, -, /, etc.
- All available mathematical and logical functions.

6. Use F2 and F3 to select the desired element and F4 to place it.

3.7.2.9 Method Definition: Transformation

Use this option to define a calculation equation to be applied to each well. All typical mathematical functions are available (+ /- , sqr, abs, log, log10, x^2, etc.) and Pow (power of x).



1. There are three groups of elements that can be selected by pressing the F1 (Alt) key:

- Controls (single and average) and the factors.
- Operates +, -, /, etc.
- All available mathematical and logical functions.

2. Use F2 and F3 to select the desired element and F4 to place it.

NOTE: If a Blank (BK) is defined it will be automatically subtracted from the absorbencies of all other wells.

3. Example: An index (in per cent), based on the absorbance of the CO control should be calculated for each well (Absorbance of the CO control = 100 %).

   The formula should be entered as follows: (X*100) / avg(CO)

4. Press enter to move to the next method definition screen.

3.7.2.10 Method Definition: Thresholds (Cut-off)

Use this option to define the threshold limits for a qualitative result. Up to three different classes (ranges) are possible. The label for the classes can be user defined. For **n** thresholds you have n+ 1 quantitative label.

Cut-off limits can be defined for the measured absorbance values (OD.), kinetic values, transformed values or concentration values, depending on the test protocol.

1. To define the threshold(s) proceed as follows:

   Select the data source first. The options are:

- 'Val' (absorbance or kinetic data),
- 'Trf' (transformed data)
- 'Conc' (concentration)

   Next enter the label for the upper limit in the top left edit field. This can be a text like 'Positive' or a symbol like '+'. Now enter the threshold condition for the upper limit. This can be a value or an equation. After the condition for the upper limit has been entered, labels for other classes and corresponding conditions can be entered.

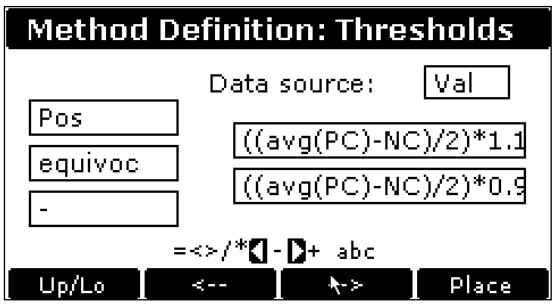
2. The threshold limits work as follows:

Label 1	If X > A → Label 1
Threshold A	
Label 2	If X <=A and X >B → Label 2
Threshold B	
Label 3	If X <= B → Label 3

(X ... Value of well)

3. The example below shows the setup for a typical ELISA assay. Here a sample is considered to be positive if the absorbance value is above (mean Positive control - Negative control) / 2.

   In addition it is specified that samples that are +/- 10% of the cut-off are considered as equivocal.



4. Press enter to move to the next method definition box.

3.7.2.11 Method Definition: Validation

Use this option to apply parameters to ensure that the test is valid within the requirements stated by the assay test protocol. If these conditions are not met, the result of the test is considered not valid. This is indicated on the printout.

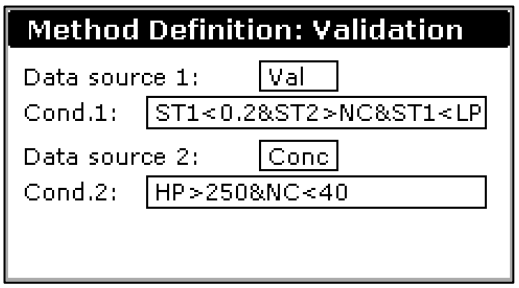
Validation criteria can be defined for the measured absorbance values or kinetic values (Val), transformed values (Trf) as defined in Transformation or concentration values (Conc), depending on the test protocol.

1. To define a validation condition chose the data source first. Then enter the first condition. At the bottom of the screen you can see various options like avg(PC), Fct1, etc. Use the function keys F2 and F3 to select the desired option and press F4 to place the selected option. 'Alt' (F1-key) toggles between well types, mathematical operators and mathematical functions.

2. To connect 2 or more conditions of the same data source use the '&' sign. If you use '&' between two conditions BOTH must be fulfilled in order for the measurement to be valid.

   Use >= for ≥ and <= for ≤.

3. For example for an ELISA assay the following criteria must be fulfilled:

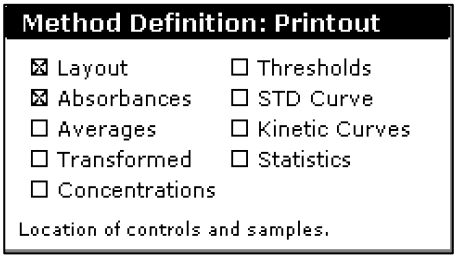


- The absorbance of the Standard 1 must be < 0.2.
- The absorbance of the Standard 2 must be higher than that of the Negative Control.
- The absorbance of the Standard 2 must be lower than that of the Low Positive Control.
- The High Positive Control must be > 250 IU/ml.
- The Negative Control must be < 40 IU/ml.

4. Press enter to move to the next method definition box.

3.7.2.10 Method Definition: Printout

Use this option to select the content of the printout. You can select one, several or all options. Only options relevant to the assay that has been defined / selected are displayed. The image below shows all options that are possible (if kinetics has been selected, the option for absorbances is replaced by Kinetic values).



1. If replicates have been used, the average value can be printed out.

2. Press enter to move to the next method definition box.

3.7.2.10 Method Definition: Saving Method Definition

The complete method has now been defined and should be saved in the non-volatile memory. There are three options how the new methods should be stored.

1. The new method can be appended to the list of already existing methods by scrolling down the list and pressing the **Append** (F4) key.

2. It is possible to store the new method at any position in the list by scrolling to the desired position for the new method. Press now the **Insert** (F4) key, the new method will be inserted before the current selected position.

3. In case an existing method should be replaced by the new one, scroll to the method to be replaced and press the **Replace** (F3) key.

4. Enter the method name using the alphanumeric function and press enter. This new method is then placed as the default method for the next measurement.

3.7.3 Running a Method

This can either be done immediately after defining a method (press 1) or by recalling an existing method (press 2, select using ▲ ▼ and F4). The plate carrier will move out now, allowing loading the microplate.

1. Each plate requires a unique ID. This ID is used to identify the plate on the printout and the measured data for later recall.

2. In case an ID already used is entered, the screen will show a warning, asking if the previous measured data with the same ID should be overwritten.

3. The maximum number of samples for the chosen method is displayed below the plate ID. When less than the maximum number of samples should be processed, the number of existing samples can be entered. This will avoid that empty well positions show unwanted results.

4. Press the F3 key to select the post run option from three choices:

Printer	Use if a printer is connected (results are sent out via both the parallel and serial data ports)

Serial	Use if PC connected to send results through the serial port.
None	Use if neither printer nor PC are connected. Results are stored in the memory and can be recalled later using the Recall Plate Data option. This can speed up the measurement of a batch of plates significantly.

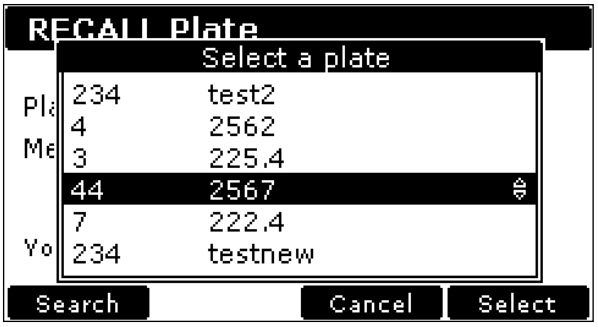
5. When the plate ID has been entered press the RUN or the ENTER key to start the measurement. During reading the screen shows a status message. A soft-key labeled 'STOP' is the only key active during reading and subsequent printing. Up to 100 plate data results can be stored in memory. After this is full the new results overwrite the previous results. To avoid this, results can be downloaded to PC for archiving.

3.7.4 Recalling a plate

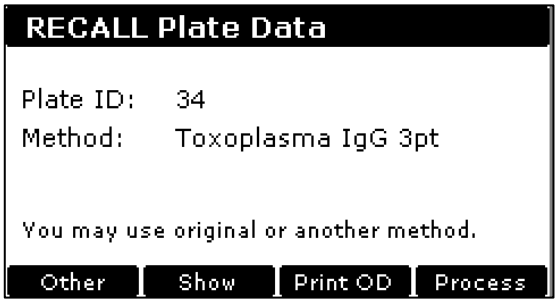
Previously run assays are stored using the unique plate ID. The Recall menu can be called from the main menu by pressing key 3 and the ID.

1. The screen below shows a list of stored plates. The left column indicates the plate ID, the center column shows the method used for the measurement.

   A plate can be chosen using the up / down arrow keys or with the help of the search function. The search function is especially useful in case of many stored plates.



2. After a plate has been chosen press either F4 or the Enter key to load the data from the memory.



3. Further options include:

Other	Use a different method. Available methods are displayed (see earlier).
Show	Show the plate data
Print OD	Print the OD values obtained for the plate. No calculations are carried out

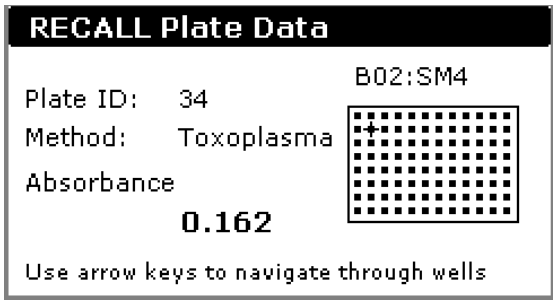
NOTE: It is the sole responsibility of the user to make sure that the chosen method gives useful results. The program cannot determine if the selected method makes sense with the existing data.

4. When the measured OD values should be printed press the key F3 (Raw). No calculations will be performed with this option.

5. To show the measured OD values on the screen of the reader press F2 (Show).

6. The new screen shows the plate ID, the original method and the absorbance (OD. value) of the selected position.

   On the right side of the screen there is a representation of a microplate. You can move across the plate with the cursor keys to select a particular well. At top of the plate representation the actual well location and the well designation is indicated.



3.7.5 Setup

3.7.5.1 Setup: Printer

Use this option to define which printer is connected to the plate reader. You can chose from a wide list of printer models.

Many different printers can be used with generic type drivers. Consult the printer manual in case your printer is not listed, to find out the nearest compatible type.

Use the up / down arrow keys to highlight the printer required and press enter to confirm your selection. Depending on the printer type some options like paper size may be shown. Use the function keys to select the required option and press the OK key to confirm the selection.

3.7.5.2 Setup: Filters

The Filter submenu serves to review the installed filters, inform the system about new or removed filters and to perform a calibration. To remove a filter from the list enter 0 (zero) for the wavelength and press F3 (Curr). To add a filter, enter the wavelength of the newly installed filter in the respective edit field corresponding to the position on the filterwheel and press F3.

Any new installed filter must be calibrated to save the wavelength information.

It is strongly recommended that all filters be calibrated before the instrument is used the first time.

For a technical description of the calibration process please refer to chapter 3.

3.7.5.3 Setup: Date/Time

Use this menu option to change the date and time with ▲ ▼ and the keypad. A choice of AM, PM or 24 hour clock is available.

3.7.5.4 Setup: Contrast

Use this option to change the contrast of the liquid crystal display using the F1 and F2 keys.

3.7.5.5 Setup: Language

Use ▲ ▼ to select the language that is used on the displays and printouts.

4. Technical Information

Please see Biochrom's website: www.biochrom.co.uk for complete technical specifications.

5. General Features

5.1 Measurement modes

Single, dual and multi-wavelength and kinetics measurements with absorbance data output in OD on display.

5.2 Integrated quality control

- ✓ Post-sample filtering to eliminate ambient light.
- ✓ Automatic calibration prior to each measurement.

- ✓ Plate centering system positions the well accurately and reproducibly under the optical path only if it is a 96-well microplate that conforms to the ANSI SBS 1_2004 standards.

5.3 Scope of supply

- ✓ Instrument
- ✓ CD containing user's manual and Galapagos Software.
- ✓ USB PC cable
- ✓ Dust cover
- ✓ Power supply
- ✓ Mains power cord

Please Note: Only use the power supply and mains power cord supplied with the instrument.

5.4 Liability

In its original condition the instrument meets all safety regulations for safe operation. If the instrument is used in a manner not specified in this user's manual, safety and performance may be affected. Biochrom is not liable for damages or costs caused by unauthorized alterations, repairs or modifications of the equipment.

Maintenance

6.1 Approved Parts

Except for the parts shown in the following list, only parts supplied by Biochrom or an authorized Biochrom Distributor may be installed in or used with the EZ Read 800 Plus.

6.2 Cleaning and Disinfection

All parts of the reader that come into contact with potentially infectious material must be treated as potentially infectious and should be periodically clean and disinfected. Only authorized and trained personnel in a well-ventilated room while wearing disposable gloves, protective glasses and clothing should perform the cleaning and disinfection procedures

The following materials are recommended for cleaning and disinfection of the reader:

- ✓ Lint-free tissues.
- ✓ 70% ethanol or a 0.5% bleach solution

6.3 Instrument cleaning

The following cleaning procedure should be regularly performed and after any spillages.

1. Disconnect the instrument from the power supply and the PC.
2. Carefully wipe off the entire reader with lint-free tissues that have been moistened in a 70% ethanol or a 0.5% bleach solution.
3. Put lint-free tissues that have been moistened in 70% ethanol or a 0.5% bleach solution onto the plate transport mechanism and let it soak for ± 30 minutes.
4. When a bleach solution has been used, carefully wipe off the entire reader with lint-free tissues that have been moistened in water.
5. Dry the reader by wiping it off with lint-free tissues.

6.4 Additional Maintenance Tips

- ✓ Never spray liquids directly into the interior of the reader.
- ✓ Do not use force when cleaning the reader.
- ✓ Wipe up spills immediately.
- ✓ Avoid buildup of dust on the instrument and wipe off visible dust.
- ✓ Cover the instrument with the dust cover if not in use.

Warranty and Return to Base

7.1 Warranty Terms and Conditions

This warranty refers to the obligations of Biochrom and can only be amended upon the written consent of Biochrom Ltd.

A warranty period of 24 months shall be granted to the original buyer of the Biochrom EZ Read 800 Plus Microplate Reader.

The warranty shall lose effect if any of the below conditions occur:

- ✓ Biochrom EZ Read 800 Plus Microplate Reader is not used in the defined scope of application.
- ✓ Biochrom EZ Read 800 Plus Microplate Reader has obviously been damaged by external influences which are not in accordance with the provisions for the nominal range of use.
- ✓ Biochrom EZ Read 800 Plus Microplate Reader has been modified or parts exchanged by a person other than Biochrom personnel or an authorized servicing agent.
- ✓ The warranty seals on the housing of the instrument are broken.
- ✓ The parts and subassemblies have been installed, which are not original from Biochrom.
- ✓ Biochrom EZ Read 800 Plus Microplate Reader serial number is no longer legible, because it has been removed or altered.
- ✓ Biochrom EZ Read 800 Plus Microplate Reader has not been installed in accordance with supplied instructions.
- ✓ Biochrom EZ Read 800 Plus Microplate Reader has been damaged during return transport due to wrong packing (e.g. not in original packing material).
- ✓ Biochrom EZ Read 800 Plus Microplate Reader was damaged due to improper operation and outside of the use described in this manual.

If an instrument is returned, Biochrom will repair or replace any defects, which have resulted from faulty material or during production as it sees fit. No costs shall arise for the client (except the cost of shipping the instrument).

All components found in the original equipment, or an adequate and full compatible alternative shall be available for a period of 7 years after manufacture. Instrument parts are available for 7 years after the instrument is no longer manufactured.

7.2 Returns Terms and Conditions

In the event that you need to return your instrument to us for repair or to use our in house recalibration service, the following instructions which should be carried out in full:

If your instrument is in warranty contact technical support: **support@biochrom.co.uk**

If your instrument is out the warranty period, you must provide a purchase order number for the repair on your returns form.

If you need to know that cost of repair/recalibration, please send an e-mail to **support@biochrom.co.uk.**

All policies and procedures, forms and labels can be found in returns in the support section of the Biochrom website: www.biochrom.co.uk.

7.3 Returns Procedure

1. On the Biochrom website, complete the **returns authorisation form** (http://www.biochrom.co.uk/content/1/65/returns.html) and send this to Biochrom to obtain a returns authorisation number. This number needs to be written clearly on the outside of your returns package.

2. Please do not send your package to us until you have received a returns authorisation number.

3. Download a decontamination certificate from and include this certificate with your package. Please put a copy on the outside and inside of your package.

   Please Note: Instruments which are returned without a decontamination certificate will not be processed. This would result in significant delays and additional administration charges for your repair.

   IMPORTANT: For returns outside the EU, please complete your commercial invoices, making sure that you indicate the true commercial value of the return. Please also state the following on your invoice:
   "Temporary Import – Item Returning to Supplier for Repair"

4. Send the package to Biochrom using the address label (found on the Biochrom website: (http://www.biochrom.co.uk/content/1/65/returns.html) and your own carrier. The goods being returned must be securely packaged and in a manner to prevent transit damage.

5. Below is a checklist to make sure that you have completed all steps:

   - ✓ Purchase order number for repair
   - ✓ Return number on outside of package
   - ✓ Decontamination certificate included on outside and inside of package

- ✓ Customs declaration of true value
- ✓ Customs declaration: Temporary Import etc.
- ✓ Have read and understood the returns terms and conditions (http://www.biochrom.co.uk/returns_terms.html)
- ✓ For all returns enquires please contact returns@biochrom.co.uk or +44(0)1223 427861

8. Ordering Information and Accessories

Table 1 Ordering Information

ORDERING INFORMATION	
80-4002-02	Biochrom EZ Read 800 Plus ELISA
80-4002-03	Biochrom EZ Read 800 Plus Research
SS01751	EZ Read Microplate Reader Check Plate

See www.biochrom.co.uk for a list of filters that are available for purchase.

9. Contact Information

Table 2 Contact Information

Department	Email contact	Telephone contact
Sales	enquiries@biochrom.co.uk	+44 (0) 1223 423723
Technical Support	support@biochrom.co.uk	+44 (0) 1223 427890
Orders	orders@biochrom.co.uk	+44 (0) 1223 427861 +44 (0) 1223 420 164 (Fax)
Returns	returns@biochrom.co.uk	+44 (0) 1223 427861
Human Resources	hr@biochrom.co.uk	
Accounts	accounts@biochrom.co.uk	+44 (0) 1223 427816

Appendix:

10.1 Helpful Hints for Successful Microplate Assays

- ✓ Always insert the plate with well A1 on the upper left position.
- ✓ Keep plates clean: measurements in microplates are made through the surface of the plate and thus the surface should be as free as possible of debris, scratches and smudges. Wipe the plate clean with a lint-free tissue if necessary.
- ✓ Measurements may be affected by uneven or heterogeneous well contents. Visually inspect the plate for foaming, uneven meniscuses, bubbles or particles in the well, all of which can interfere with absorption. Centrifuge the plate if necessary to obtain a homogeneous level in the wells.
- ✓ Use a reference measurement for reducing measurement error caused by interference from the plate material or contamination. (Use a reference wavelength at which your molecule of interest does not absorb. Many assays recommend a reference measurement at 620 nm).
- ✓ When using plate frames for stripwells or single wells (breakable strips) check that all strips and wells are pushed down completely and are level with the frame.
- ✓ Keep the bottom of the plate dry. If liquid has contaminated the well bottoms or if condensation is present, dry the surface with a lint-free tissue.
- ✓ Use high quality optically clear 96-well microplates (such as virgin polystyrene) with standard ANSI SBS footprint for the best results.

10.2 Troubleshooting and Frequently Asked Questions

Biochrom's technical support team is available to answer all of your questions regarding your EZ Read 800 Plus microplate reader:

E-mail: support@biochrom.co.uk
Telephone: +44 (0)1223 427890
Fax: +44 (0)1223 427857

Please also see the support section of our website which is frequently updated with technical and applications support information.

Below is a list of frequently asked questions and answers:

1. What is the indication range of the EZ Read 800 Plus and how does this specification differ from the linear range?

   The Biochrom EZ Read 800 Plus has an indication range of 0.000-3.300 OD and a linear range of 0.100-2.500 OD. We recommend that the broader indication range should be used only for relative determinations but not for determining the quantity of the absorbing material. Only measurements made within the linear range should be used for quantitative calculations.

2. If a measurement is made by the Biochrom EZ Read 800 Plus that exceeds the maximum indication range, how is that measurement represented?

   If a well measurement at a particular wavelength exceeds the maximum indication range, then the well will be represented with ''O/R''.

3. When using Galapagos software to control the Biochrom EZ Read 800 Plus, can specific wells be designated as blanks and can those values be automatically subtracted from the remaining wells?

   The Biochrom EZ Read 800 Plus comes with standard software, Galapagos Software, which does allows the user to define specific wells. Specific wells can be allocated to certain controls (such as positive or negative controls). Users can input details of the controls such as the name and type of control. Users can use threshold calculations to subtract readings from other wells.

4. Which Microsoft Windows operating systems are Galapagos compatible with?

   Galapagos is compatible with Windows XP with SP3 and higher versions.

5. Why do I get different readings from the same sample in different wells in the microplate?

   Check correct sample volume has been dispensed. This is important as pathlength (which is used to calculate sample concentration using Beer's Law) is not standardised and is dependent on sample volume. Therefore the volume must be equal throughout the plate.

   The quality of the microplate is paramount to the quality of the measurements because a microplate reader sends light through a sample vertically. Use high quality, optically clear microplate for best results.

   In addition, it is important to use a reference wavelength when measuring a microplate. Microplates may have smudges, scratches, lids and other forms of optical interference which may obstruct the light beam. A reference wavelength allows for a well by well control of imperfections in the plate.

   When choosing a reference wavelength, use a wavelength at which the sample of interest

does not absorb. 620nm is a frequently used reference wavelength in many absorbance applications in the visible range. Check the product data sheet for recommended reference wavelength for your specific assay.

6. How can I connect the instrument to Galapagos?

   Refer to section 3.2.

Declaration of Conformity for Biochrom Manufactured Products

This is to certify that the following Biochrom manufactured products:-

EZ Read 800 ELISA Plus (80-4002-02) and EZ Read 800 Research Plus (80-4002-03)

Conform to the requirements of the following Directives:-

2006/95/EC	Low voltage directive (LVD)
2004/108/EC	Electromagnetic Compatibility (EMC) directive
2012/19/EU	Waste Electrical and Electronic Equipment directive recast (WEEE Recast)
2011/65/EU	Restriction on the use of certain hazardous substances (ROHS) directive
2006/42/EC	Machinery directive
98/79/EC	In Vitro medical devices directive (Annex I & III)

Standards, to which conformity is declared, are as follows:-

EN61010-1:2010	Safety requirements for electrical equipment for measurement, control and laboratory use. General requirements
EN61010-2-101:2002	Safety requirements for electrical equipment for measurement, control and laboratory use. Particular requirements for in vitro diagnostic (IVD) medical equipment
EN61326-1:2006	Electrical equipment for measurement, control and laboratory use -EMC Requirements*
EN ISO 12100:2010	Safety of machinery-General principles for design, risk assessment and risk reduction

*This equipment has been tested and found to comply with the limits for a CLASS A digital device, pursuant to part 15 of the FCC Rules.

For further information, including unpacking, positioning and installation of the products please refer to the user manual.

Signed: Dated: 07th May 2013

Sam Luke

Group Managing Director, Biochrom Ltd