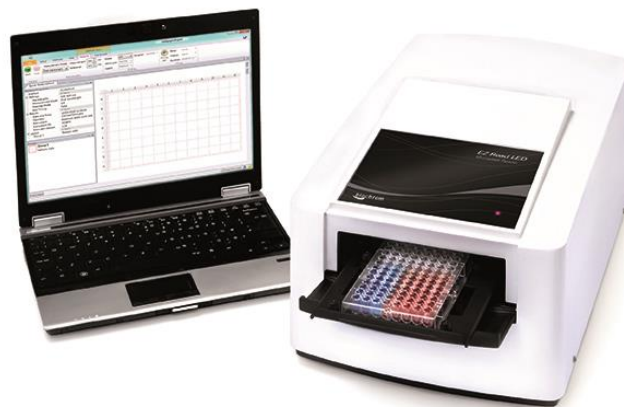




EZ Read LED Microplate Reader

User Manual



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1 Introduction and Intended Use

1.1 What to expect from the user's manual

- ✓ Instrument use
- ✓ Software installation
- ✓ Quick start guides for operational use (to perform a quick measurement, kinetics and multi-wavelength measurements).
- ✓ A technical explanation of how the instrument operates
- ✓ General features
- ✓ Helpful hints for obtaining the best measurements using the instrument.
- ✓ Maintenance

1.2 Introduction

What is a microplate reader?

A microplate reader is used to measure the absorbance of liquid samples in a 96-well 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:

$$A = e c b$$

A = absorbance

e = molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)

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 LED Microplate Reader is used for microplate-based applications requiring endpoint or kinetic absorbance measurements in optically clear 96-well plates with a standard SBS/ANSI footprint.

Only trained laboratory personnel should operate the Biochrom EZ Read LED Microplate Reader. The Biochrom EZ Read LED 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.

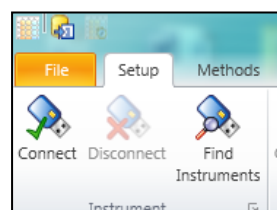
3.2 Software Installation

Galapagos is supplied with all EZ LED 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.

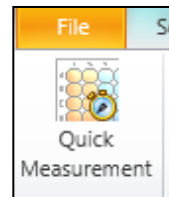
Users can save results and templates to a Database Galapagos uses a database to store data in one place. This is useful for preventing data override and can allow easy data transfer through communication with external laboratory database systems such as LIMS. To create a Database, click 'Setup' > 'Select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

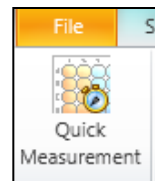


4. The microplate reader is now ready for use.

3.3 Quick Measurement:

Performing a Quick Measurement on Galapagos:

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

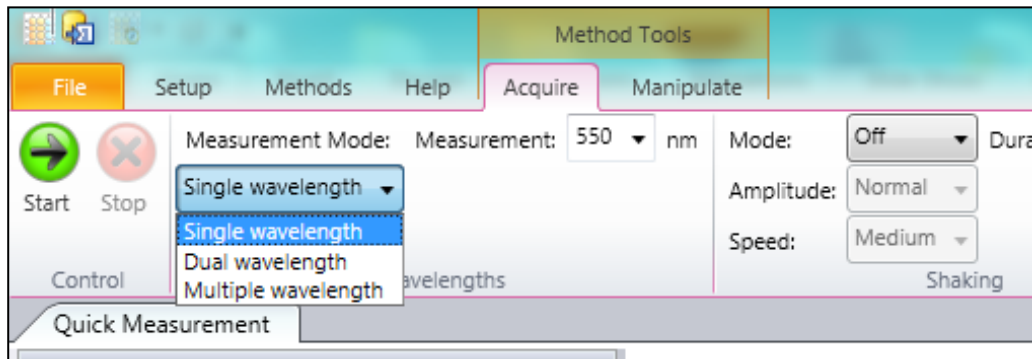


2. Select the appropriate measurement mode (Single, Dual or Multi-wavelength):

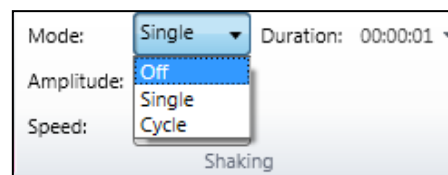
Single wavelength refers to absorbance measurement at one wavelength.

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.

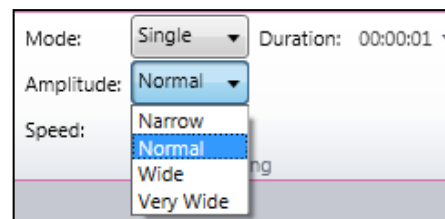
Multiple wavelength refers to a series of absorbance measurements at different wavelengths. In this mode, absorbance measurements are not subtracted from one another.



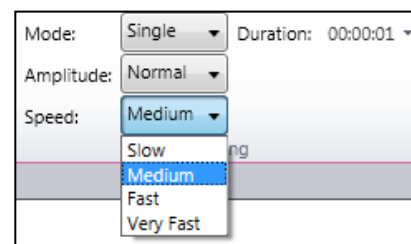
- Users can also select to shake the microplate. Users can select shaking to be a single event or in timed cycles.



- Select the amplitude (degree of linear shaking).

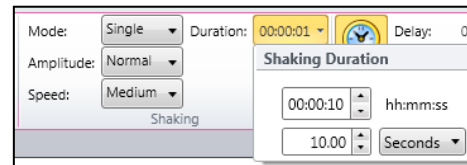


- Select the speed for linear shaking.

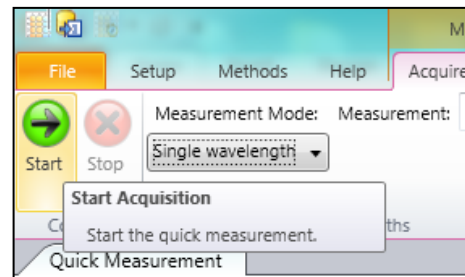




- Users can select the duration of shaking.



- Click 'Start' to begin acquisition.



- 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.

Quick Measurement 20130321135324 CZC1258W6W												
Method Properties												
Method (Collection)												
Settings 5 items												
Wavelengths 550 nm												
Measurement Mode Single wavelength												
Shaking Mode Off												
Use Temperature Cc False												
Use Timing False												
Results 5 items												
Date and Time 21/03/2013 13:53:24												
Operator CZC1258W6Wdhanaya												
Instrument Test Instrument												
Instrument SN 123456												
Firmware Version Version 1.0												
Layout 3 items												
Samples Protein Lysates												
Positive Control BSA												
Buffer Negative Control												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.550	0.560	0.570	0.580	0.590	0.600	0.610	0.620	0.630	0.640	0.650	0.660
B	0.670	0.680	0.690	0.700								
C	0.790	0.800	0.810	0.820								



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.157 0.562 0.405	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157
B	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157
C	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157
D	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157
E	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157
F	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157	0.157

Data - Well A1

Data	Value
A562	0.562
A405	0.405
A562-A405	0.157

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

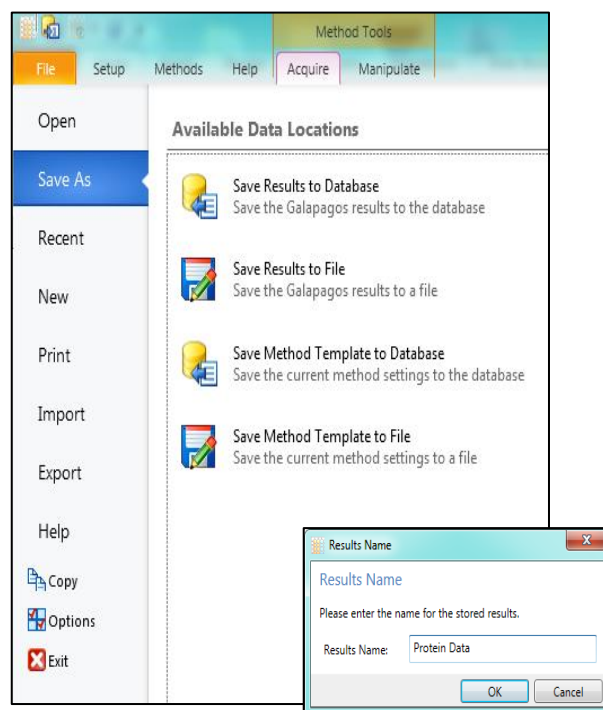
	1
A	0.157 0.562 0.405

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.

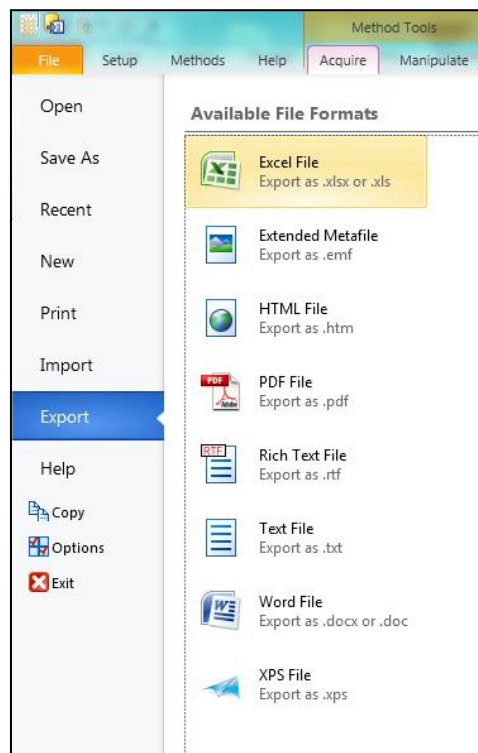
The second value is the absorbance at the measurement wavelength

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.



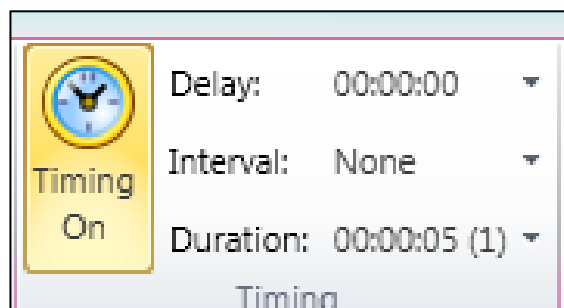


3.4 Kinetic Measurements

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.

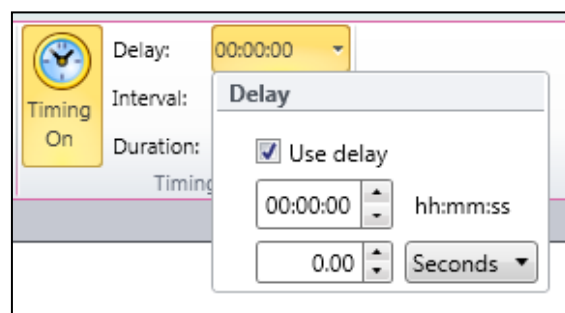
1. For kinetics measurements, click on the timer icon for timed measurements. This is indicated by 'Timing On'



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.

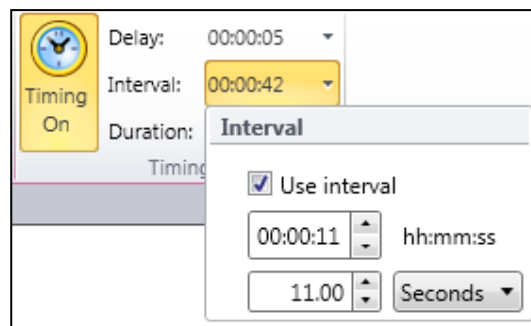
Note: the use of a delay is optional (tick or un-tick), whereas the interval and the number of cycles is not.

2. Users can input delays between measurements.

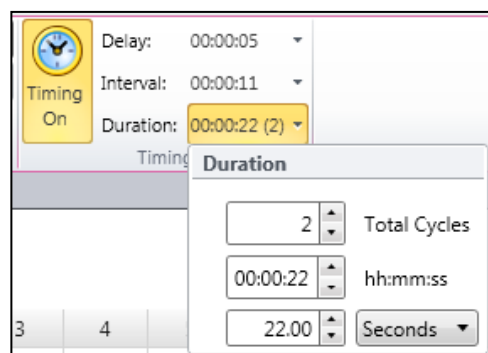




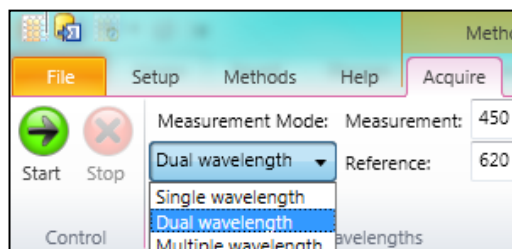
- Users can input timed intervals between readings.



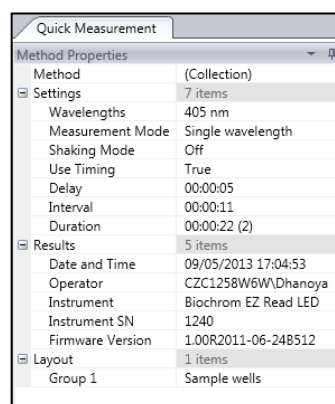
- The measurement cycle (delay and interval) can be inputted and multiple cycles can be selected.



- 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 11 seconds at a single wavelength for the EZ Read LED).



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.

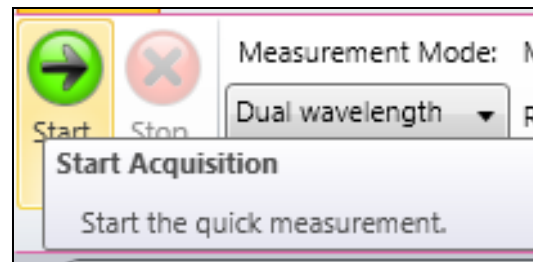
7. Select the amplitude (degree of linear shaking).

8. Select the speed for linear shaking.

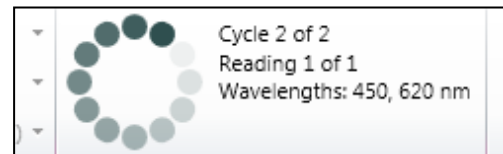
9. Users can select the duration of shaking.



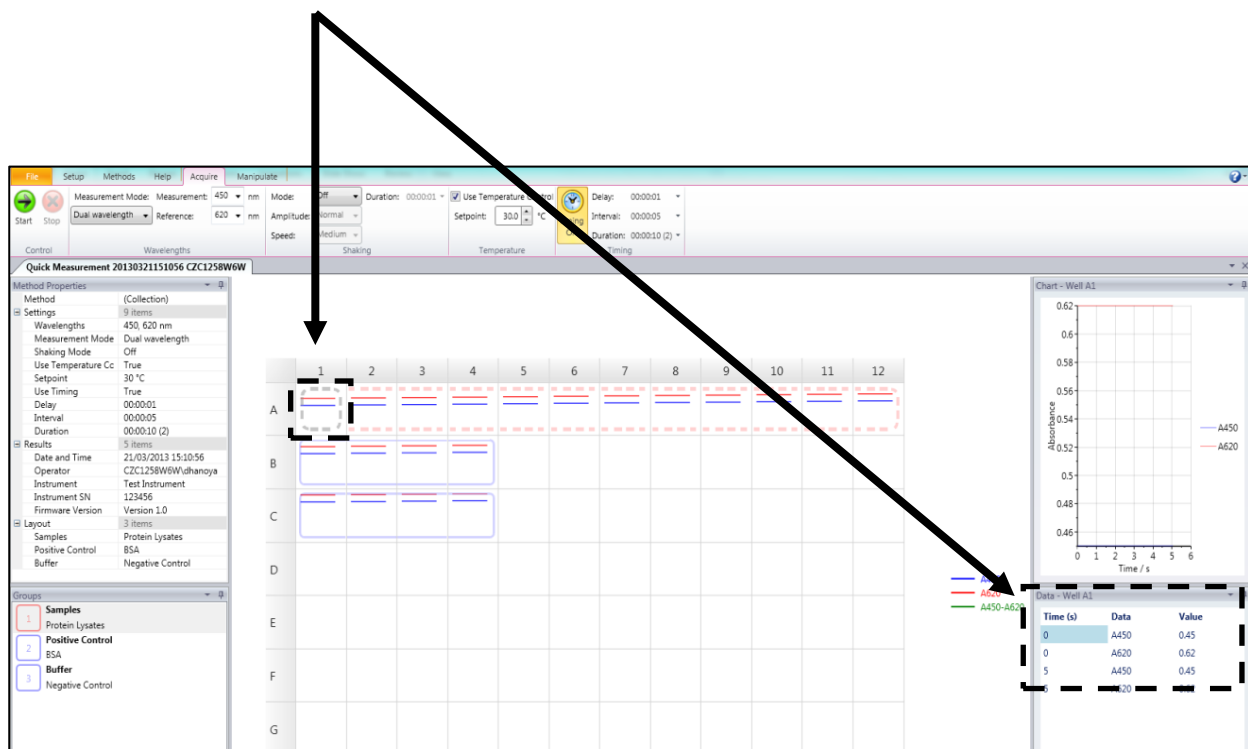
10. Click 'Start' to begin the measurement.



11. As the measurement is recorded, the status of the experiment is displayed showing both readings and cycle status at the measured wavelengths.

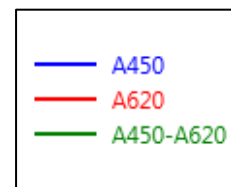
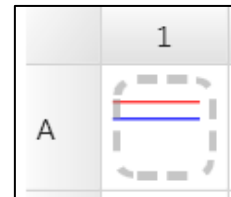


12. Results are displayed in each well. If dual wavelength measurements are carried out, absorbance measurements at both wavelengths are displayed. Absorbance measurements are also displayed on the bottom right hand panel if a well is selected.

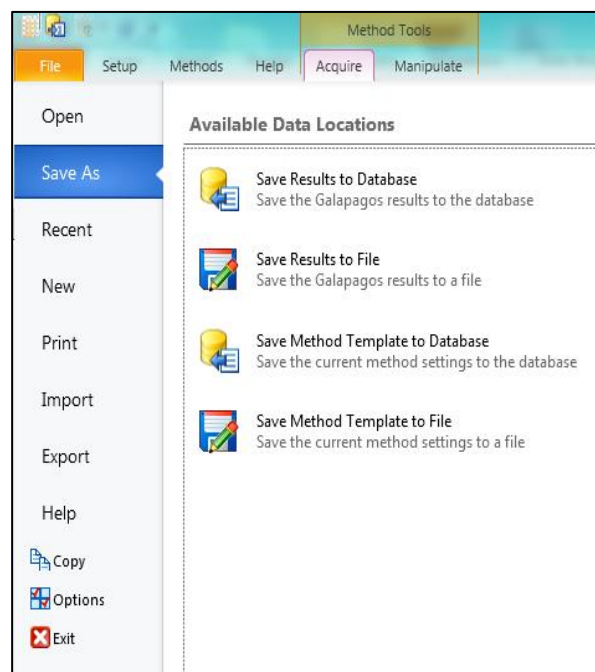




The lines shown in each well refer to the measurement at one wavelength over time. Different wavelengths used for absorbance measurements are assigned a specific colour and identified by a legend.

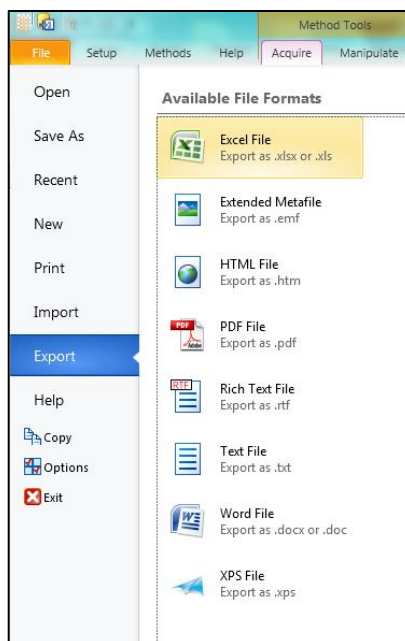


13. Results can be saved to 'Database'. A database is useful for saving, sharing and extracting data via communication with an external database system such as LIMS. Users can save files and templates onto the database, 'File' (save to own personal file), or 'Save Method Template' (saves the method protocol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





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

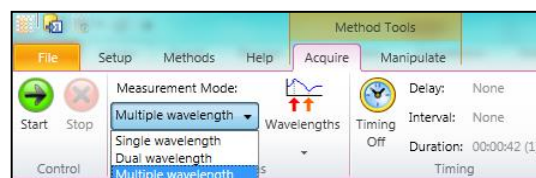


3.5 Multi-wavelength Measurements

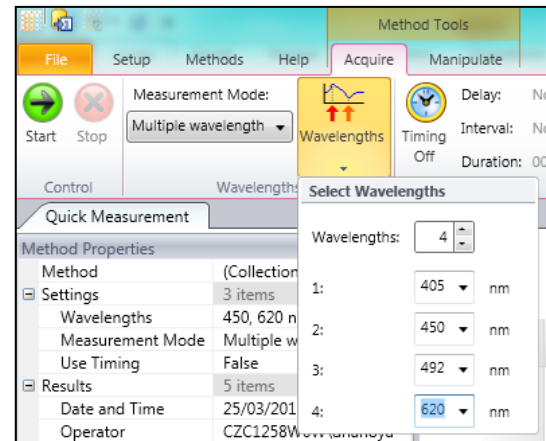
Performing a multi-wavelength measurement on Galapagos:

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.

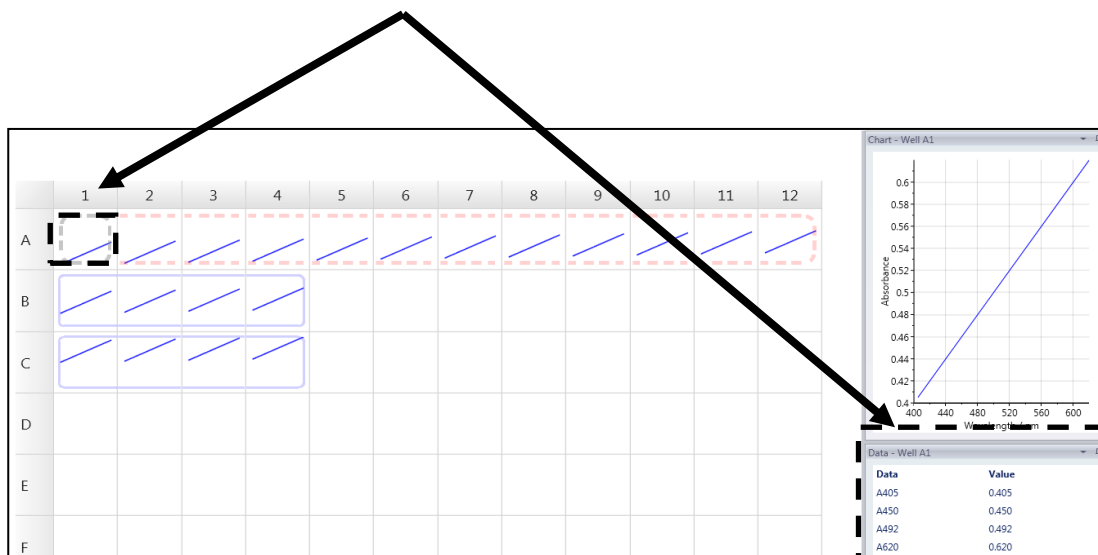
1. To perform a multi-wavelength measurements click 'Multiple Wavelength' from the drop down menu.



- The menu is adjusted to allow users to select up to 4 wavelengths for measurement. Each wavelength must be different. For the EZ Read LED, users can input wavelengths of 405, 450, 492 and 620nm. Users can also measure at 340nm when using the EZ Read LED UV model. Click 'Start' to begin the measurement.

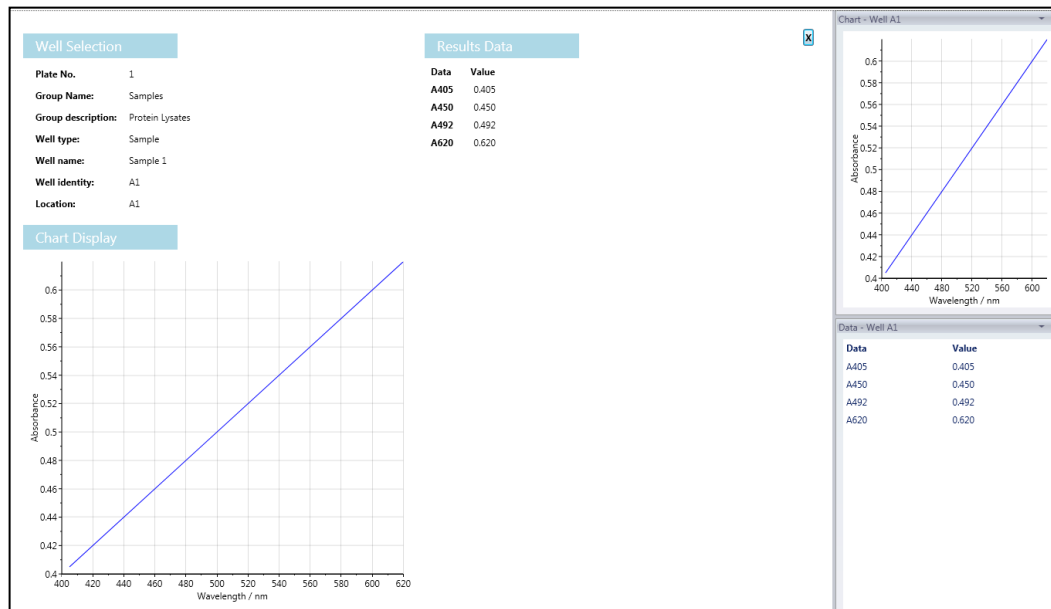


- Individual multi-wavelength measurements are displayed in each well, with raw absorbance displayed on the right hand panel as a graph of absorbance vs. wavelength.

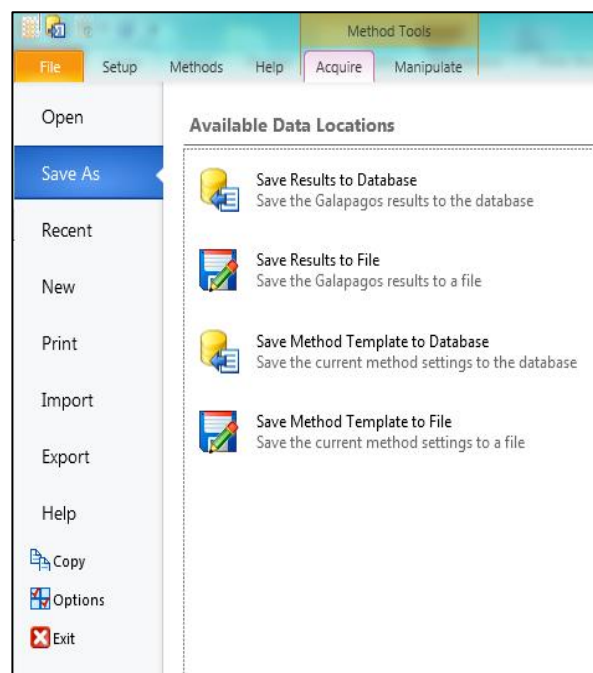




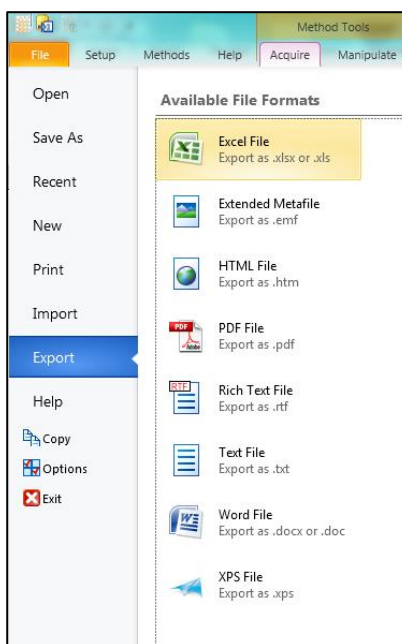
- Alternatively users can click on an individual well to view the scan in more detail and raw results. Both tabulated and graphical chart data are displayed.



- 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).



- 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 LED performs an automatic calibration prior to each measurement. Alternatively, 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.

The EZ Read LED contains light emitting diodes (LED) coupled to high quality interference filters for light source and wavelength selection. The advantages of LEDs over conventional lamp sources is the high energy light source and increased life span (therefore avoiding the need to constantly replace lamps).



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

- ✓ 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 LED.

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 \pm 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 LED Microplate Reader.

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

- ✓ Biochrom EZ Read LED Microplate Reader is not used in the defined scope of application.
- ✓ Biochrom EZ Read LED 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 LED 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 LED Microplate Reader serial number is no longer legible, because it has been removed or altered.
- ✓ Biochrom EZ Read LED Microplate Reader has not been installed in accordance with supplied instructions.
- ✓ Biochrom EZ Read LED Microplate Reader has been damaged during return transport due to wrong packing (e.g. not in original packing material).



- ✓ Biochrom EZ Read LED 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	
80-4002-10	Biochrom EZ Read LED Microplate Reader
80-4002-11	Biochrom EZ Read LED UV Microplate Reader
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
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 menisci, 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 LED 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 LED and how does this specification differ from the linear range?

The Biochrom EZ Read LED has an indication range of 0.000-4.000 OD and a linear range of 0.005-3.500 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 LED 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 LED, can specific wells be designated as blanks and can those values be automatically subtracted from the remaining wells?

The Biochrom EZ Read LED comes with standard software, Galapagos Software, which does allow 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 LED (80-4002-10)

EZ Read LED UV (80-4002-11)

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