BioDrop Duo+ and µLite+ Micro-Volume Spectrophotometers

User's Manual

BioDrop
a division of **Harvard Bioscience, Inc.**

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INTRODUCTION TO BIODROP SPECTROPHOTOMETERS

A spectrophotometer is an optical device that is designed to transmit a beam of light through a sample. Transparent solutions absorb specific wavelengths of light based on their unique molecular composition. Absorbance is proportional to concentration of a sample. Absorbance peaks of a sample can also be used to identify its molecular composition. In kinetic studies, the tracking of absorbance over time can be useful to study chemical reactions and biological processes.

BioDrop spectrophotometers are designed to transmit electromagnetic radiation from the far ultraviolet 190 nm through the visible spectrum to 1100 nm. Many materials, and in particular solutions of materials, will absorb light within this region. This makes BioDrop spectrophotometers useful in a wide range of applications including life sciences, clinical, pharmaceutical, cosmetics, food and drink, agricultural, industrial, environmental, toxicology, water treatment and teaching. There are numerous published methods and assays for these applications.

The BioDrop µLite+ is a split beam spectrophotometer that has a micro-volume sample port for analysis. The built-in sample port has a path length of 0.5 mm.

The BioDrop Duo+ spectrophotometer displays specifications attributed to the µLite+. It contains a micro-volume sample port as well as a standard 10 mm cuvette holder. The cuvette holder may be used in conjunction with the BioDrop micro-volume CUVETTE.

For information about the function of an icon described in this manual, please refer to the Table of Icons section beginning on page 78 of this User's Manual.

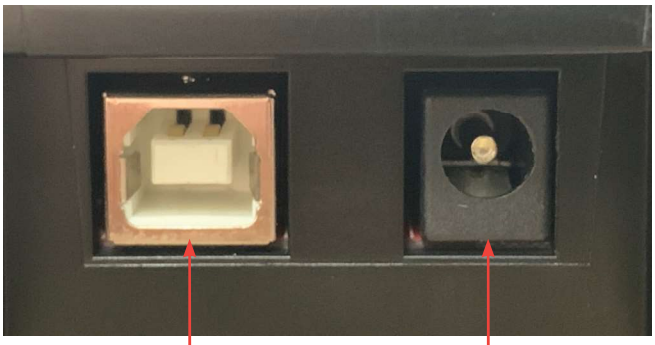
For detailed descriptions of the functions of parameter boxes please refer to the Glossary of Controls section.

Use with BioDrop Resolution PC Software

When connected to a PC the BioDrop spectrophotometer can be controlled using the BioDrop Resolution PC software packages. Operation using BioDrop Resolution PC software is described in the Resolution user manual or Resolution help file.

INTRODUCTION TO BIODROP SPECTROPHOTOMETERS

Instrument Connections



USB connector for PC connection 18V power supply connector

USB connector for USB memory stick

BioDrop µLite+

The BioDrop µLite+ has a sample port for micro-volume analysis.

BioDrop µLite+ sample port

BioDrop Duo+

The BioDrop Duo+ contains a micro-volume sample port and a standard 10 mm cell holder.

Note: *Simultaneous analysis using the micro-volume sample port and the standard cell holder is not possible.*

USER INTERFACE

Frequently Used Icons

	**Forward arrow**	Advances to the next screen in a sequence
	**Back arrow**	Returns to the previous screen in a sequence
	**Accept/Green Check**	Confirms selection/entry. Saves and exits
	**Cancel/Exit**	Exit without saving

Icons on the Sample Measurement Screen

	**Take reference**	Performs a reference measurement
	**Take measurement**	Performs a sample measurement
	**Options screen**	Opens the options menu on the sample measurement screen
	**Method parameters**	Takes the user from the sample measurement screen to the first method parameter screen

Icons on the Options Menu

	**Exit**	Takes the user out of the application and back to the main menu
	**Save data**	Allows the user to manually save sample data to a specified location
	**Save method**	Allows the user to save the current method parameters to the internal memory or a USB stick
	**Print**	Prints the sample data from the specified printer
	**Auto print**	Toggles auto print on and off — green light = on
	**Load Sample**	Accesses Load Sample
	**Trace Manager**	Accesses Trace Manager (wavescan and kinetics only)

USER INTERFACE

First Time Power Up

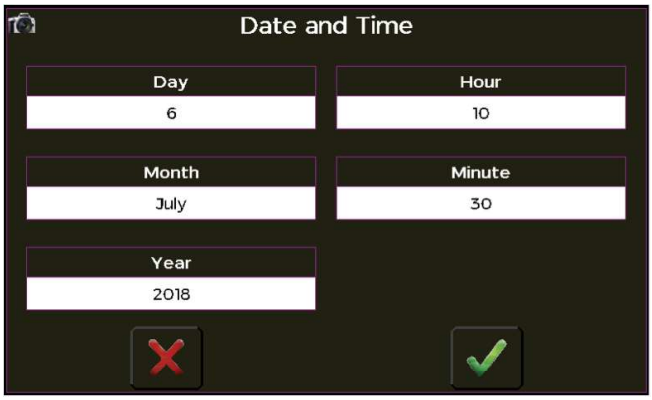
The first time the BioDrop spectrophotometer is powered on, the user will be prompted to set their regional setting preferences for language and local date/time.

Regional

The BioDrop spectrophotometer will arrive with the language set to English. This can be changed by pressing the Language box; the options include English, German, French, Spanish, Italian, Japanese and Chinese.

To save any alterations press the check mark or to exit without saving, press the “X”.

Date & Time



Time and date can be changed on the BioDrop. Press Date and Time to enter the information.

After the desired date and time has been entered, select the check mark to save and exit or the “X” to exit without updating.

Parameter Entry Boxes

The BioDrop spectrophotometer uses different kinds of boxes for parameter selection and entry, these include:

Alphanumeric Text Entry

The alphanumeric text entry box allows the user to enter letters and numbers, symbols, and accents by pressing Ãã, λ!, and Aa respectively. The “shift” icon will change to the Icon displayed below when the Symbol Entry Screen is in use. It is possible to toggle between upper and lower case letters and through a list of symbols by pressing the arrow up button. The green light indicates what keyboard is being used.

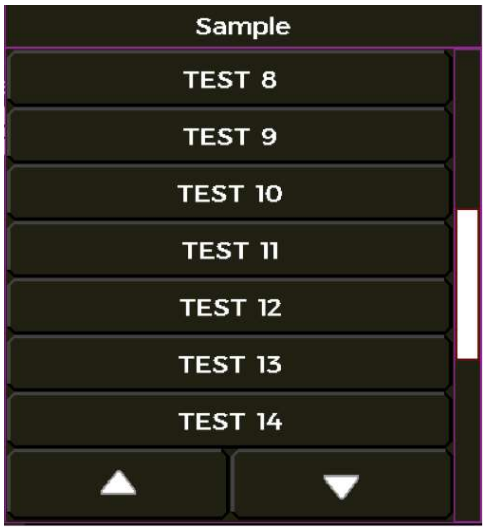
Note: *The layout of the screen is dependent on the text entry mode set under User Interface in Settings.*

USER INTERFACE

Numeric Entry

The numeric entry box allows the user to include numbers in the method parameters. Depending on the numeric box selected it may be possible to add both positive and negative numbers.

Selection List/Combination Box



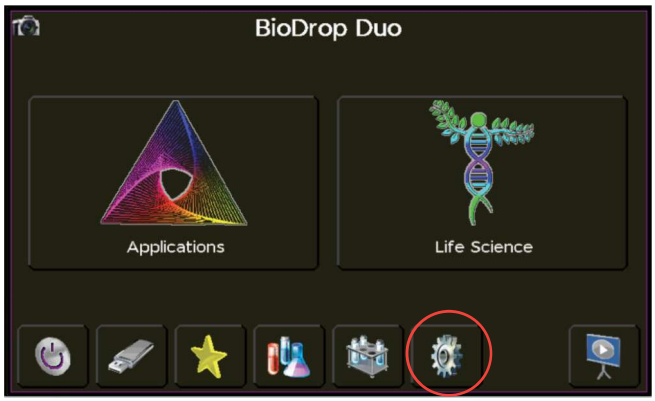
Where there are more than two options, the user will be presented with a list. If there are more than 8 options, the user can scroll through these using either, the page up and page down arrows or by using the scroll bar.

Note: *If a box only contains two options e.g. On or Off, then pressing the box on the screen will toggle between the options and not produce a combination box.*

USER INTERFACE

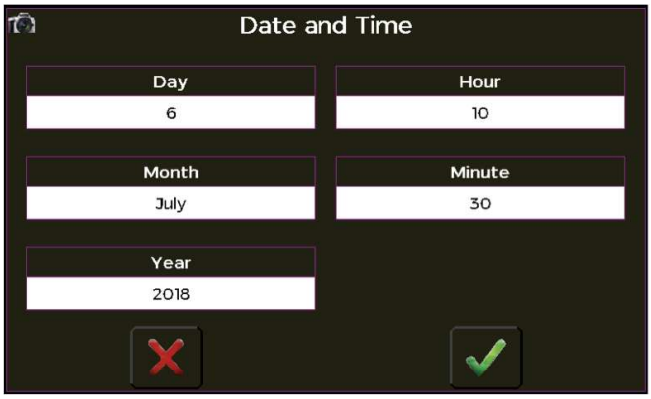
Settings

Settings are accessed via the Settings button on the main screen (see below)



Note: *If User Access has been selected the User Access Icon will only appear for users with Administrator privileges.*

Date & Time



The BioDrop spectrophotometer will arrive with the time and date set. This can be changed by pressing Date and Time.

After the desired date and time have been entered, select the check mark to save and exit or the “X” to exit without saving.

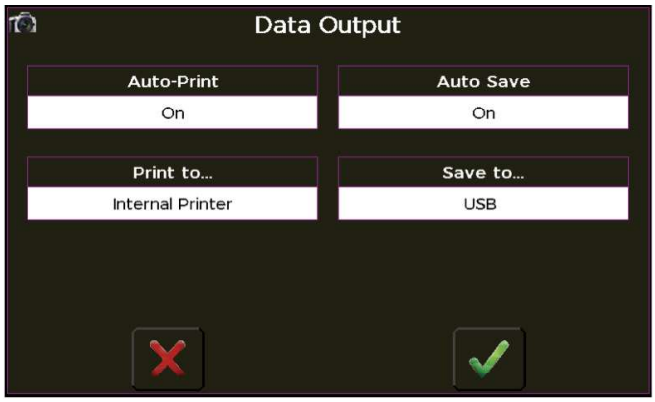
Regional

The BioDrop spectrophotometer will arrive with the language set to English. This can be changed by pressing the Language box; the options are English, German, French, Spanish, Italian, Japanese and Chinese.

To save any alterations press the check mark, to exit without saving press the “X”.

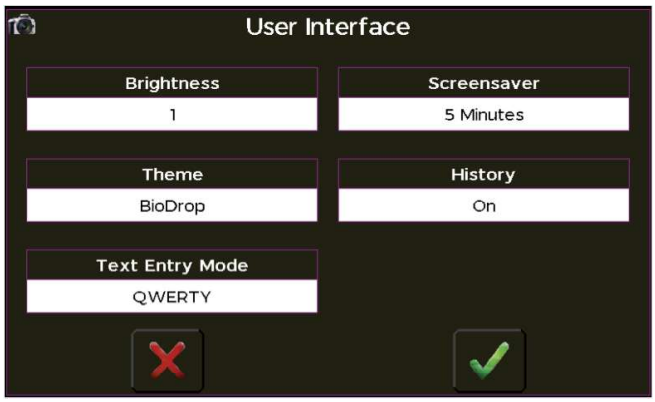
USER INTERFACE

Data Output



This is the default saving and printing settings that will be used in all application method parameters. These can be changed in each applications parameters.

User Interface



The desired brightness level of the screen, the alphanumeric text entry mode and the duration after which the screensaver will be displayed (if required) can be set. Current theme is BioDrop with a black background. The user can also turn the History setting to on, which allows the instrument to store the last settings in the application. If this parameter is Off, all parameters and options will return to their default settings when leaving the application (unless it has been saved as a method).

Service

The Service section is for use only by a trained service engineer or upon recommendation by a member of the technical support team.

USER INTERFACE

Instrument Settings

The following options are included under instrument settings:

Instrument Information

Instrument information displays the product name, product code for service, serial number, user-interface (UI) version and user interface build number.

A Green dot adjacent to an icon (see image) indicates that the icon has been selected.

Instrument Settings

Instrument Settings allows the user to:

1. Collect a new, temporary baseline. This will be stored until the instrument is powered off. 
2. Save the temporary baseline. This will become the permanent baseline and be stored until overwritten. 
3. Restore the original baseline. If measurements show the temporary baseline to be poor quality the permanent baseline can be restored. 
4. View instrument life in days.
5. View service date (set by an engineer).

Lamp Settings

Lamp settings displays the current lamp status.

USER INTERFACE

Instrument Reset

The instrument reset allows users to delete everything that has been saved on the instrument or delete specific sections. This action will delete locked samples and methods, and unlock method folders.

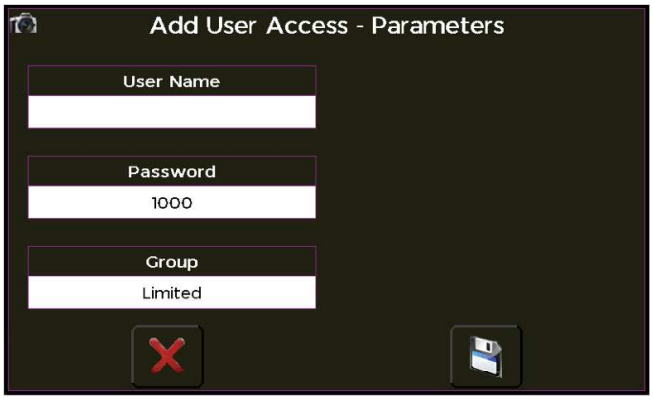
User Access

The BioDrop spectrophotometer has an option to assign users different access rights. These are set via the User Access icon, which is available in Settings.

> ***Note:*** *The User access icon is only available to users who have administrator privileges.*

Adding a user

To add a new user to the instrument, select 'Add User' on the touch screen.  The BioDrop spectrophotometer can store up to 16 individual users.



Each user is given a user name (using alphanumeric entry), a 4-digit password and assigned to one of three user groups depending on the access level they require.

The table below outlines the features each user group can access.

User Group	Run Applications & Saved Methods	Save Sample Data	Delete Sample Data from the instrument's memory	Save Methods	Access Settings Menu	Access User Settings
Limited	✓	✓	×	×	×	×
Supervisor	✓	✓	✓	✓	×	×
Admin	✓	✓	✓	✓	✓	✓

Editing a User

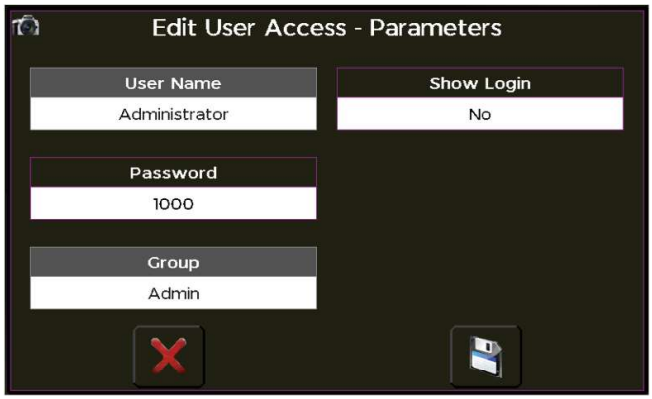
To edit a user's details, highlight the desired user and select 'Edit user' on the touch screen.  This allows the username, password and user group to be edited/updated as above.

Deleting a User

To delete a user from the instrument, highlight the desired user and press the 'Delete User' icon. 
Any methods or data created by this user will not be deleted.

> ***Note:*** *It is not possible to delete the default administrator account.*

Editing User Access



To disable user logins and user access, highlight the default administrator account and press 'Edit User' to display the screen left.

> ***Note:*** *With the Show Login set to "No," the instrument will not prompt for User Login at start up, The 'Switch User' icon will not be displayed on the main screen and the instrument will always be in Administrator mode.*

PERFORMING A MEASUREMENT

BioDrop Spectrophotometers

BioDrop spectrophotometers are split-beam UV visible spectrophotometers that contains a single cuvette holder for both reference and sample measurements Therefore, before performing sample measurements, it is necessary to perform a reference measurement to correct for solvent and/or cuvette effects.

To perform measurements using the cuvette holder:

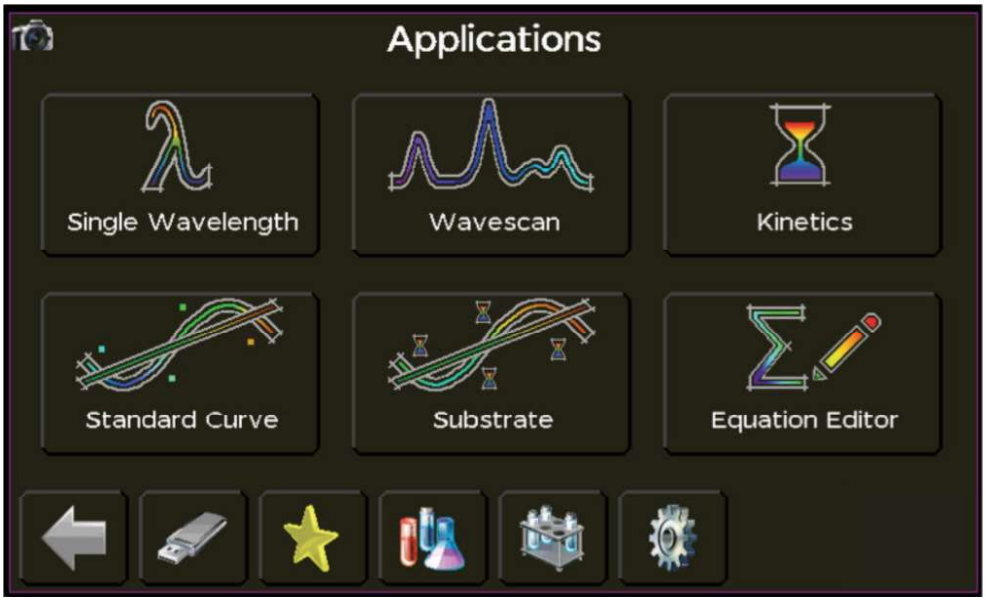
1. Insert a cuvette containing the solvent/buffer in the cuvette holder.
2. Press Take Reference.
3. When the reference is complete, remove the cuvette containing solvent/buffer from the cuvette holder, and insert a cuvette containing the sample solution.
4. Select Take Measurement.
5. Repeat steps 3 & 4 until all sample data has been collected. See the section Saving and Printing for post measurement options.

To perform measurements using the built in sample port:

1. Load minimum volume of 0.5 µl of reference in the sample port.
2. Press Take Reference.
3. When the reference is complete, remove the reference material by wiping with a lint free cloth from the sample port, and load sample of interest.
4. Select Take Measurement.
5. Repeat steps 3 & 4 until all sample data has been collected. See the section Saving and Printing for post measurement options.

Note: *A single reference will suffice for subsequent analysis for samples with the same solvent.*

APPLICATIONS



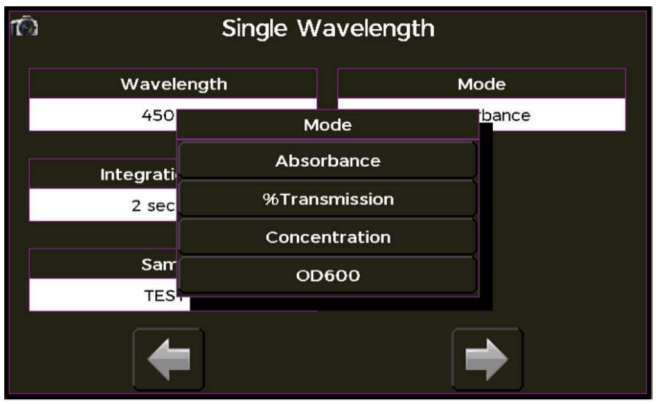
Single Wavelength	Absorbance, % transmission, concentration or OD600 measurements at a single, specified wavelength.
Wavescan	Wavelength scan between two, user-defined wavelengths in the range 190 to 1100 nm. The BioDrop allows data overlay, post-scan data manipulation and user configurable peak and valley functions, and multiwave function.
Kinetics	Measurements of Absorbance versus time to determine rate or end points. The BioDrop spectrophotometers allow data overlay, post-scan data manipulation and user defined sectors.
Standard Curve	Concentration measurement at a single wavelength determined by the generation of a calibration curve of known standards.
Substrate	Enzymatic determination of compounds with reagent test kits.
Equation Editor	Allows users to create their own unique methods including calculations and thresholds.

APPLICATIONS

Single Wavelength

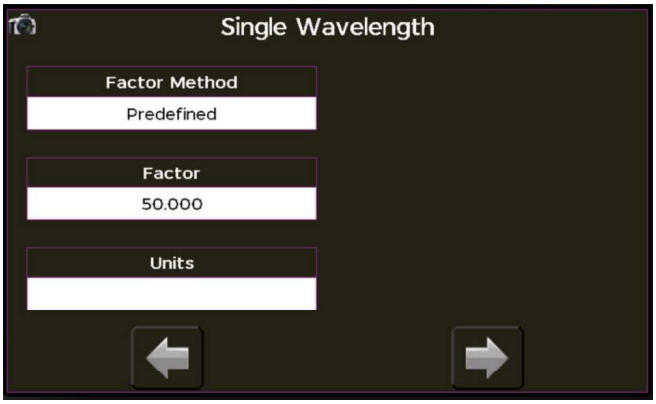
The Single Wavelength application performs simple absorbance (A) and % transmission (%T) measurements on samples, measuring the amount of light that has passed through a sample relative to a reference (this can be air).

Measurement Parameters

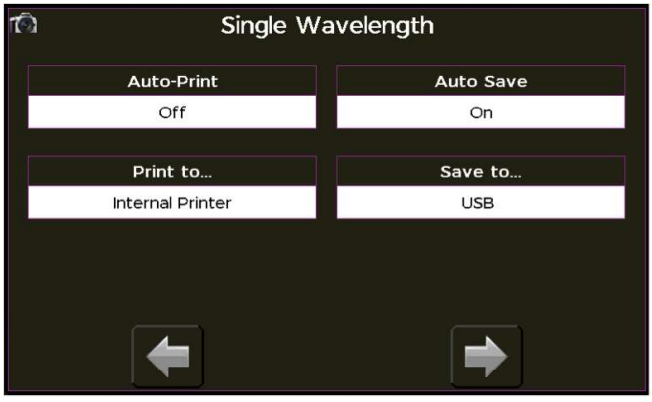


Set Mode to Absorbance, %Transmission, Concentration, or OD600. The Sample Seed entered under Sample will be the filename used for any data file saved automatically.

You may advance to the next screen at any time by selecting the forward arrow or return to the previous screen by selecting the back arrow.



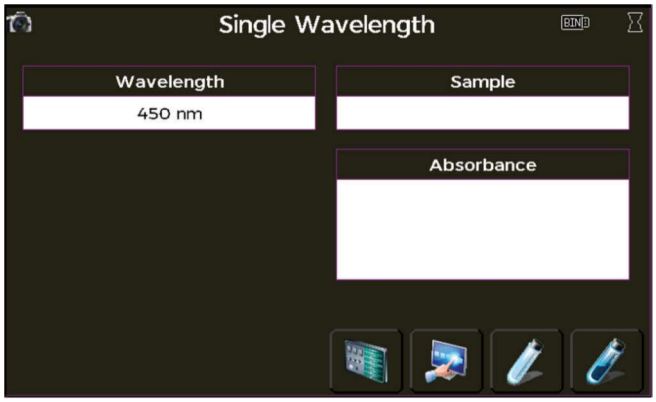
If Concentration Mode is selected, set Factor Method as required, enter Factor using numeric entry and Units using alphanumeric entry.



Set the outputs required in your method. For more information see the section Saving and Printing.

APPLICATIONS

Taking a Measurement



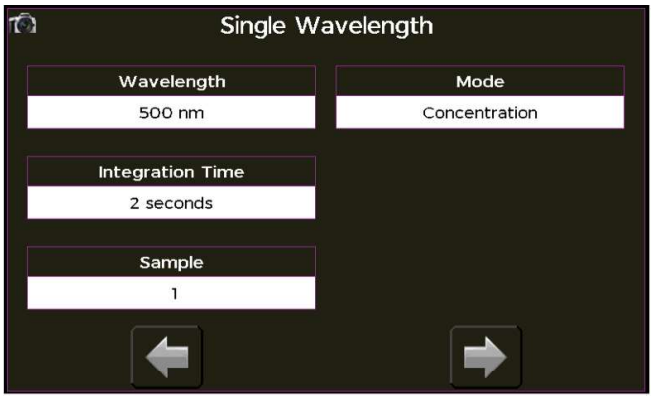
To perform a measurement, insert a cuvette containing the reference solution in the cuvette holder (or load reference directly on the µLite+ sample port) and press the reference button. Remove and replace with a cuvette containing the sample. For the µLite+ sample port, wipe away the reference with a lint free cloth and load the sample directly on the port. When the sample is loaded, press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

Concentration via Factor

This mode is within the Single Wavelength application for simple concentration measurements on samples. Concentration is obtained by multiplying the measured absorbance at a specific wavelength by a factor. The factor may be known in advance or calculated by the instrument by measuring a standard of known concentration. Examples of concentration measurements include DNA or protein.

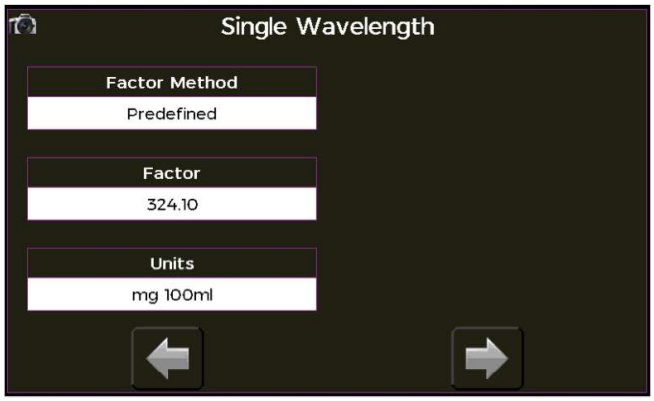
Measurement Parameters

From the main screen of the BioDrop spectrophotometer, select Applications followed by Single Wavelength to display the screen below.



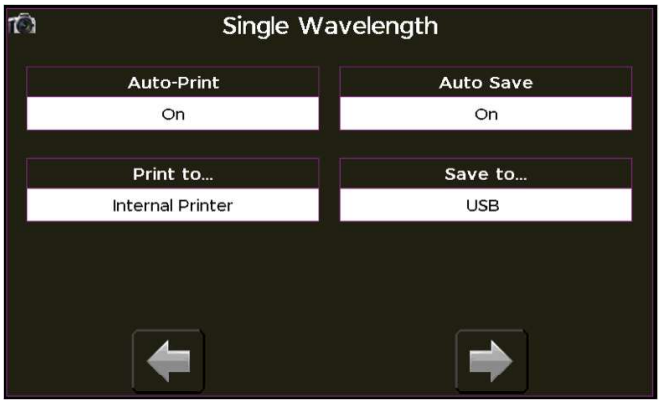
Set Mode to Concentration. Wavelength and Integration Time can be set as required. The Sample Seed entered under Sample will be the filename used for any data file saved automatically.

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.



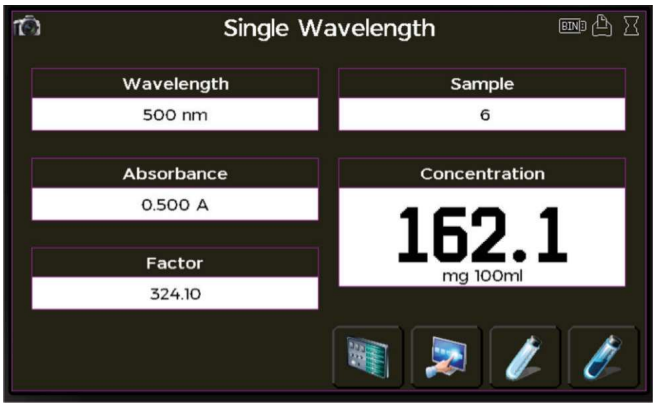
Set Factor Method as required. Based on Factor Method selection, enter either Factor (shown in example) or Concentration using the numeric entry. Enter Units using the alphanumeric entry.

APPLICATIONS

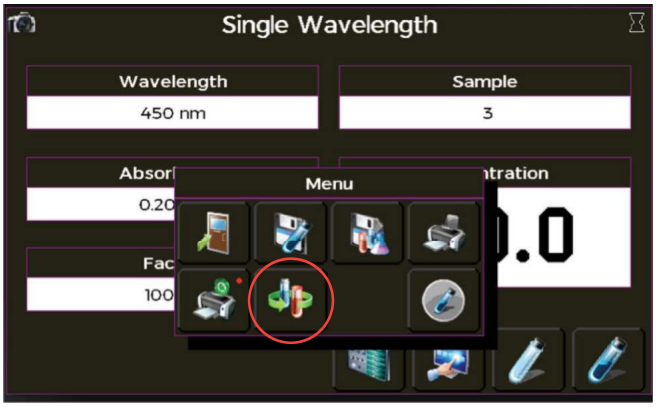


Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



To perform a measurement, insert a cuvette containing the reference solution in the cuvette holder (or load reference directly on the µLite+ sample port) and press the reference button. Remove and replace with a cuvette containing the sample. For the µLite+ sample port, wipe away the reference with a lint free cloth and load the sample directly on the port. When the sample is loaded, press the Take Measurement button.



When performing a measurement in Standard Concentration mode, a Run Standard Dialog box will display to run the standard. The Run Standard dialog box can be displayed again when the application status is Sample or Standard by entering the options menu, and selecting the Rerun Standard button as shown in the example.

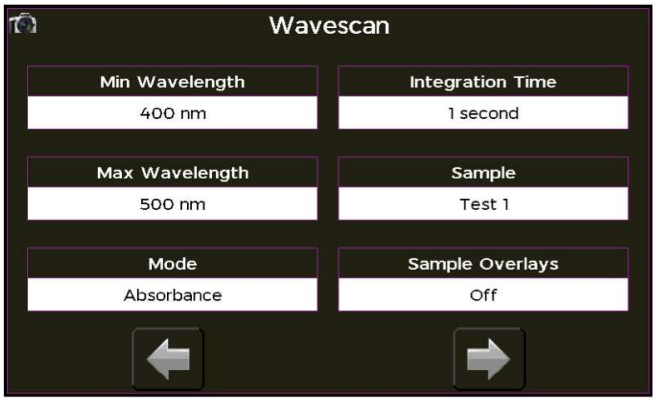
Note: *See Standard Curve section for details on how to perform calibration using prepared standards.*

Wavescan

A measurement of absorbance or % transmission of a sample over a specified wavelength range is one of the most useful physical characteristics of a compound, both as means of identification (qualitative analysis) and of estimation (quantitative analysis). The observed features arise due to the various electronic transitions that are possible within a molecule. The BioDrop spectrophotometers offer a range of post-scan data manipulation options including: 1st order derivative, enabling identification of multiple, unresolved peaks; 2nd order derivative, enabling identification of peak shoulders (inflections); 4th order derivative, which identifies both multiple peaks and inflections at the same time; Smoothing, utilizes the Savitzky-Golay algorithm to smooth data and improve the signal to noise ratio; Enhanced, which enhances features, sharpening peaks and valleys.

APPLICATIONS

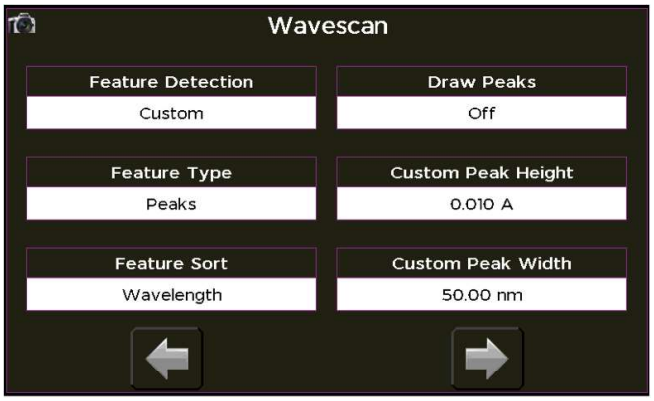
Measurement Parameters

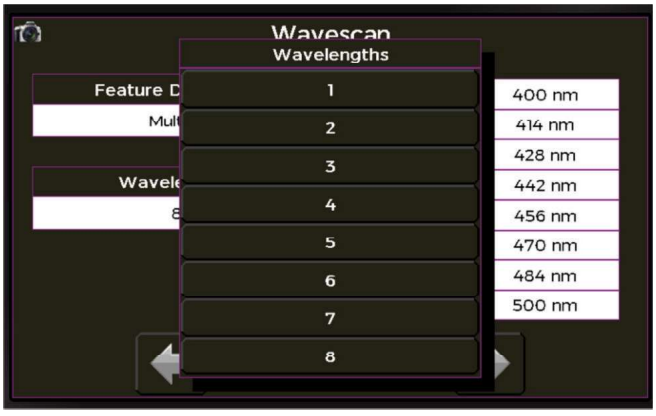


Use Min Wavelength and Max Wavelength to set the required wavelength range. Set Mode and Integration time as required. The Sample Seed entered under Sample will be the filename of any automatically saved file. Sample overlays are described below.

Note: *With Sample Overlays set to ≥2, all wavelength scans will be automatically saved to the instrument's internal memory and will be displayed in Trace Manager.*

You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.





This measurement parameters screen allows the user to set the following parameters:

Feature Detection: Determines the number of peaks or valleys that will be automatically detected. Options include: Off, Coarse, Sensitive, Custom and Multi λ.

Feature Type: The feature types that will be detected by the software. Options are Peaks or Valleys.

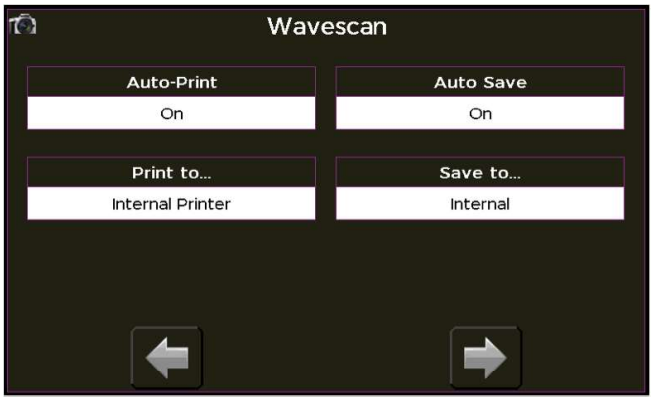
Feature Sort: Determines how the features will be displayed in the data table. Wavelength shows the peaks in ascending wavelength whilst Magnitude displays then in descending size.

Draw Peaks: When set to ON, peak is drawn by highlighting the section of the graph determined to be a peak in a different color.

Custom Peak Height: Only displayed if Feature Detection is set to Custom. This is the minimum height the peak has to be above the higher of the two adjacent minima for the peak to be detected.

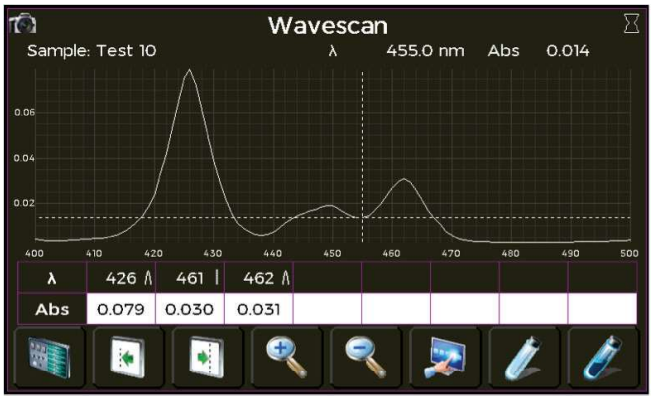
Custom Peak Width: Only displayed if Feature Detection is set to Custom. This is the minimum width of the peak as determined by the difference in wavelength between the higher of the two adjacent minima and the opposing intersection of that higher minimum level and the peak profile.

Wavelengths: Only displayed if Feature Detection is set to Multi λ. Input up to 8 wavelengths via a numeric entry box. Example shown to left.



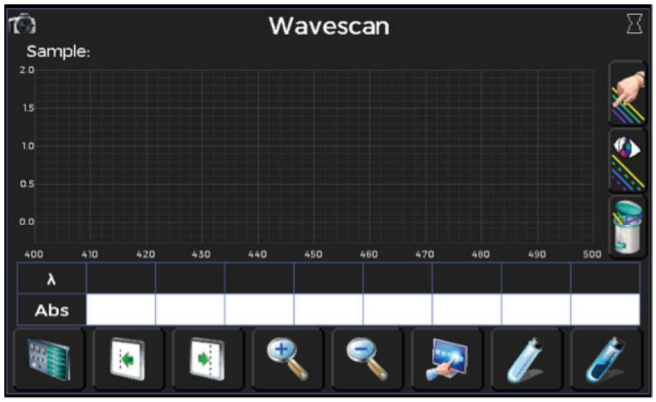
Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



To perform a measurement, insert a cuvette containing the reference solution in the cuvette holder or load reference directly on the µLite+ sample port and press the reference button. Remove and replace with a cuvette containing the sample. For the µLite+ sample port, wipe away the reference with a lint free cloth and load the sample directly on the port. When the sample is loaded, press the Take Measurement button. Selecting the Options Menu icon provides access to additional options, including toggling of cursors on/off, auto-print, and Trace Manager.

With Feature Detection set to Coarse, Sensitive, Multi λ, or Custom the sample measurement screen will display a table below the scan. This table will display the Feature Type selected in the method parameters. To manually add a peak or valley to the table, position the cursor over the desired feature by either touching the feature or using the left and right cursors and press an empty cell in the table.



When the number of samples in the application parameters is greater than zero, the Trace Panel controls are added to the right-hand side of the graph. See table on the next page.

APPLICATIONS

OPEN CONTROL	CONTROL	PURPOSE
Opens the trace panel selector control pane 		• Traces are identified by a line of the same color. • Selected trace is shown in the down or checked position. • Only one trace can be selected at any given time. • Where there is no trace available, or the trace is hidden, the button is greyed out.
Opens the trace hide panel 		• Traces are identified by an eye icon of the same color. • Hidden traces are identified as a button in the down or checked position. • Multiple traces can be hidden at the same time, hiding a selected trace causes the nearest visible trace to be selected. • Where there is no trace available the button is greyed out.
Opens the trace delete panel 		• Traces are identified by a bin icon of the same color. • Where there is no trace available the button is greyed out.

Note: *If the application is started with sample overlays off, the user can run samples then load in previously saved samples to compare. In this case the selection panel can be used to select different traces — or de-select all of them to have the live trace selected and its data presented.*

Details of how to perform overlays, data manipulation and selecting saved files can be found in the Trace Manager section.

APPLICATIONS

Kinetics

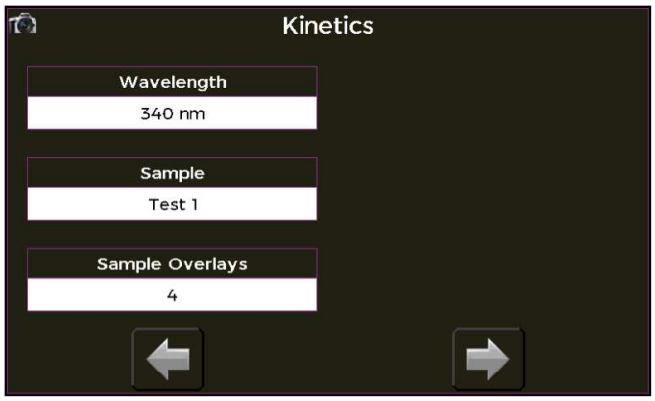
Kinetics measurements made using a UV/visible spectrophotometer measure the change in absorbance at a single, fixed wavelength over a specified period. This can be used to provide useful information when an appropriate factor, defined in a reagent kit protocol, is applied. Reagent test kits are routinely used for the enzymatic determination of compounds in food, beverage and clinical laboratories.

UV/visible spectrophotometric kinetic assays are considered one of the most convenient measurements for enzymatic assays since they allow the rate of the reaction to be measured continuously.

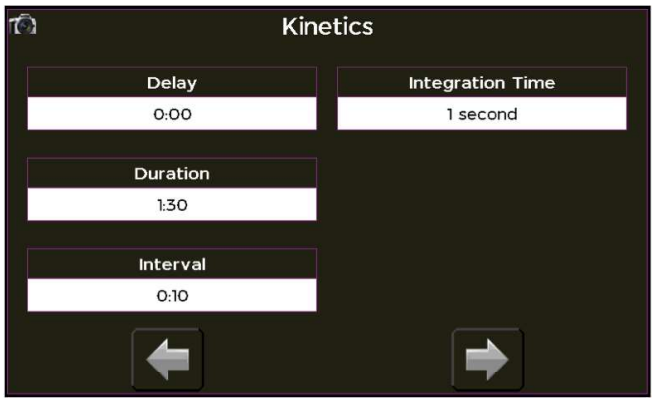
Serial Kinetics Measurements

Serial kinetics is the measurement of the absorbance of a single sample over a specified duration at a specified interval.

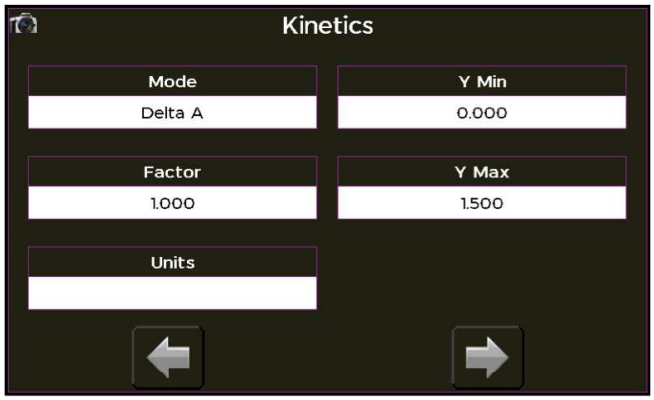
Measurement Parameters



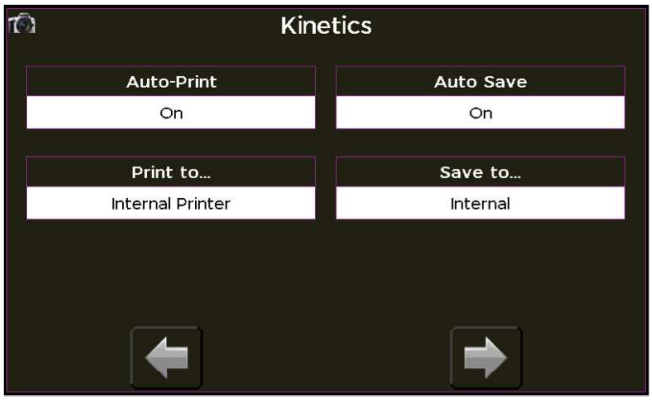
Wavelength can be set as you require. The Sample Seed entered under Sample will be the filename of any automatically saved file. Sample Overlays is described below.



Set the Delay (time before first measurement), Duration (total measurement time, up to 180 minutes), Interval (duration between readings, from 1 second to duration) and Integration Time that you require.

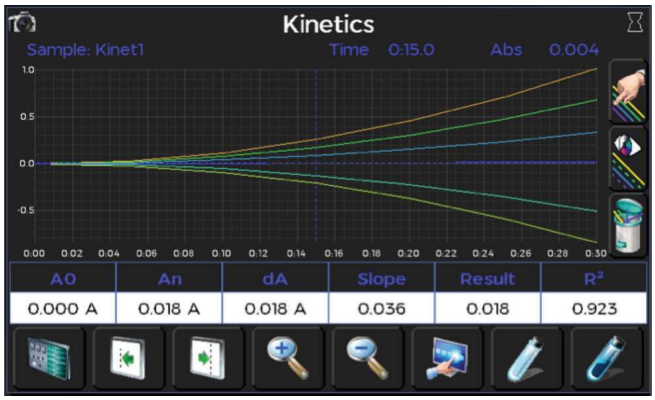


Mode has options for Delta A, Final A and Slope and is the value that will be multiplied by the Factor to give the Result on the sample measurement screen. Units are entered using alphanumeric text entry and will appear on any printed or exported data. Y min and Y max are displayed during the measurement, the y axis auto-scales upon completion.

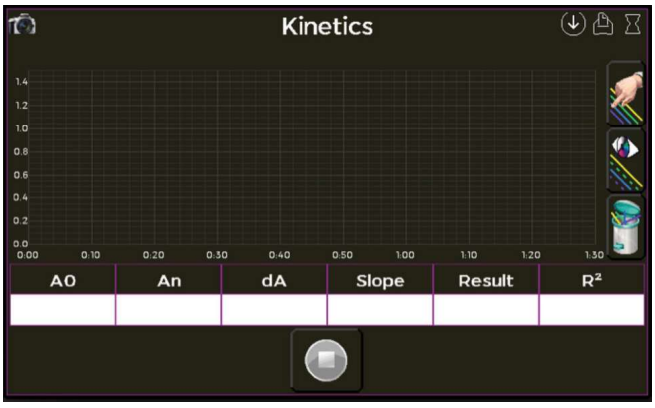


Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



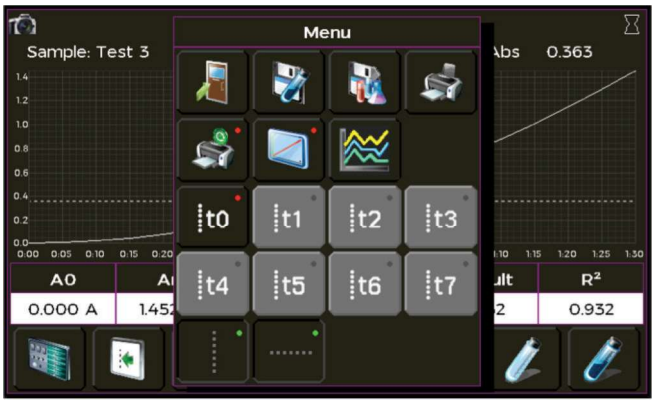
To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series. With overlays on, or with loaded samples, the trace management icons appear to the right hand side of the graph.



Note: *Measurements will commence after the specified Delay period (if applicable). Delay can be skipped using fast forward button.*

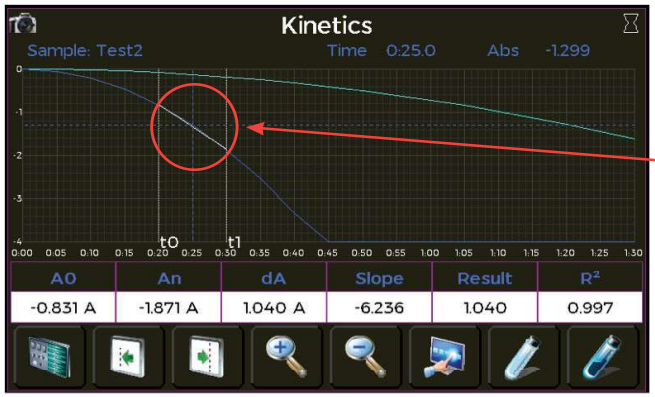
A measurement can be stopped at any time by pressing the Stop button at the bottom of the screen. All data collected to this point will be displayed on screen and can be saved.

APPLICATIONS



The data displayed in the table below the graph refers to the full measurement range. To obtain data for a specific section it is necessary to add sections, this is done as follows: Set the cursor to the desired start position by either pressing on the scan or using the cursors, select t0 from the options menu, set the cursor to the desired end position and select t1 from the options menu. This can be repeated to add up to 4 discrete sections. Cursors can be toggled on / off using the cursor toggle buttons.

Note: *Sections must be added in numerical order i.e. t1 must be added after t0, t2 after t1 etc.*



With sections defined, the data displayed in the table below the scan is determined by the position of the cursor. Only when the cursor is positioned in the section of interest will this data be displayed.

Line of best fit. When the draw slope function is turned on from the options menu, the line of best fit will be drawn for the section of data the cursor is currently in.

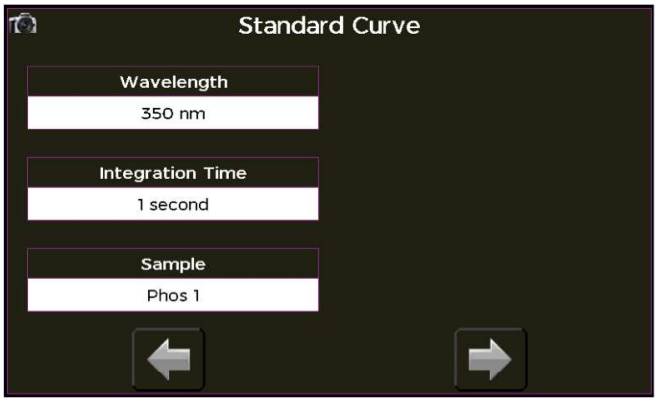
Note: *Details of how to perform overlays, data manipulation and selecting saved files can be found in the Trace Manager section.*

APPLICATIONS

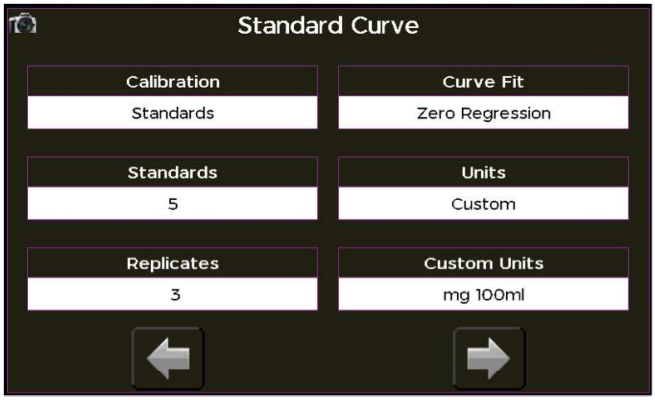
Standard Curve

The construction of a multi-point calibration curve from standards of known concentration to quantify unknown samples is a fundamental use of a spectrophotometer. The BioDrop spectrophotometers have the advantage of being able to store calibration curves with a method. Each calibration curve can be created using up to 9 standards, with each standard measurement being made of up to 3 replicates.

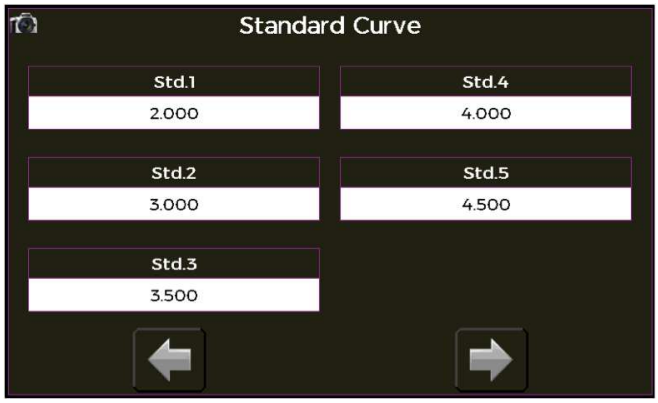
Creating a Standard Curve



Set Wavelength and Integration Time as required. The Sample Seed entered under Sample will be the file name of any file saved automatically.

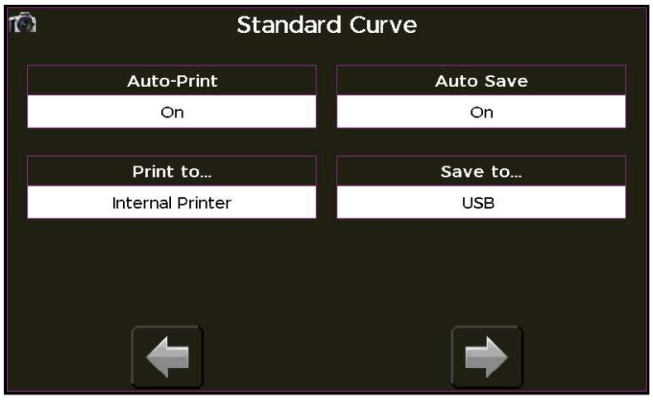


Calibration can be set to Standards (the user is required to prepare and measure standards) or Manual (the user inputs both the standard concentrations and standard absorbencies). Set Standards, Replicates, Curve Fit and Units as required in your application.

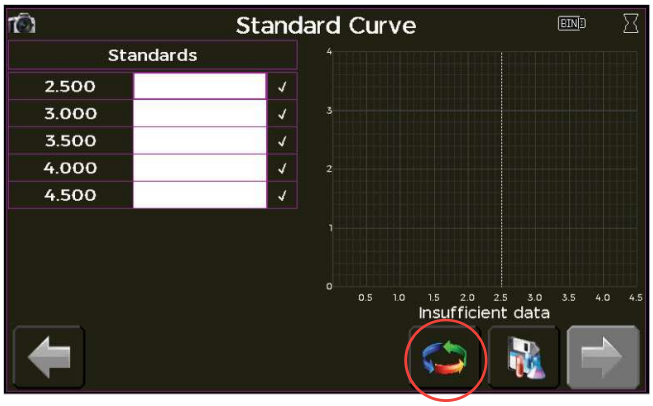


Set the concentration values for each of the standards using the numeric entry box.

APPLICATIONS

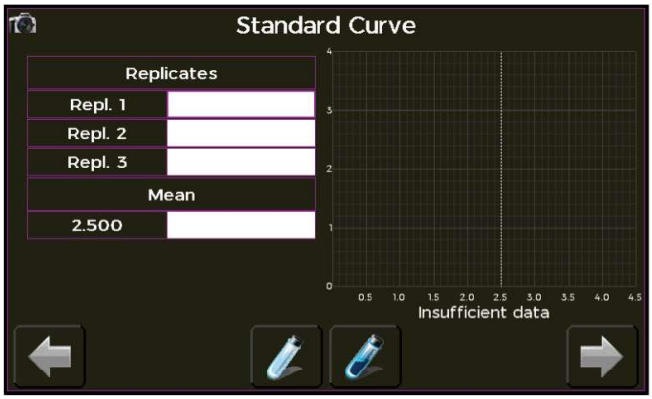


Set the outputs required in your method. For more information see the section Saving and Printing.



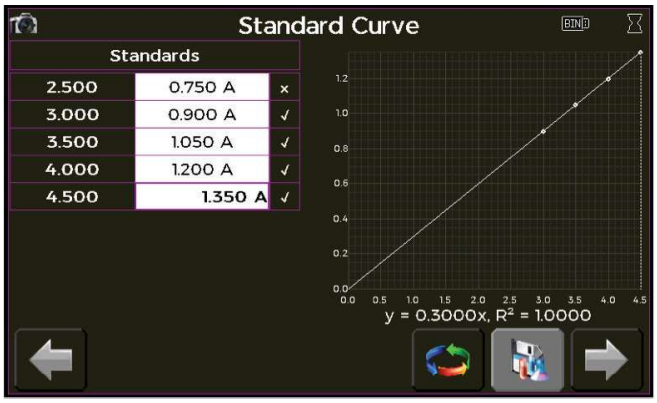
To create the standard curve when using replicates, press the Replicates button to take you to the screen shown below. With Replicates off, standards can be measured directly as described below.

> ***Note:*** *Pressing the save method icon before any standards have been measured will save the method parameters only. Recalling a method containing method parameters only requires the user to construct a standard curve before measuring samples.*



To create a standard curve, insert the cuvette containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the first standard/replicate in the series and press the Take Measurement button. A single reference suffices for standard curve creation.

Once all replicates have been measured for the standard, press the arrow forward button to continue recording all standards/replicates until the standard curve has been completed. To repeat any standard measurements, simply press the desired result, insert the correct standard and press Take Measurement.



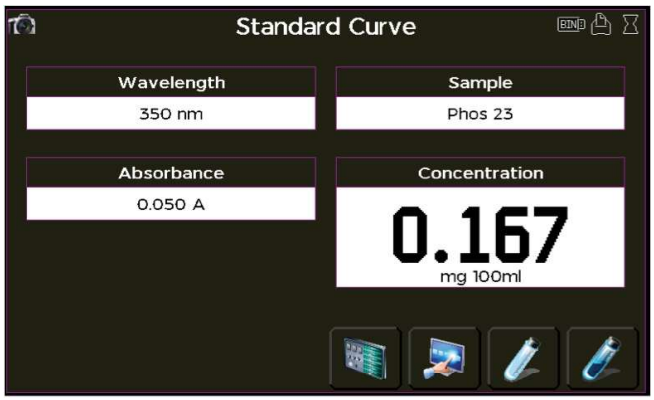
After all replicates have been measured, press the back arrow button to take you to the standards screen, as shown below.

To ignore any outlying standard measurements, press the check mark in the appropriate row to toggle it to an “X”. Any ignored measurement will be automatically removed from the standard curve. These can be reinstated by pressing the “X”.

APPLICATIONS

Taking a Measurement

After the standard curve has been collected, press the forward arrow to proceed to the sample measurement screen.



To perform a measurement, insert the cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the sample and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

To view the standard curve whilst on the sample measurement screen, simply press the 'View Curve' icon that appears under the options menu.

Note: *Saving the method using the Save Method icon that appears on the options menu will save both the method parameters and the standard curve. Recalling a method containing method parameters and standard curve allows the user to measure samples directly.*

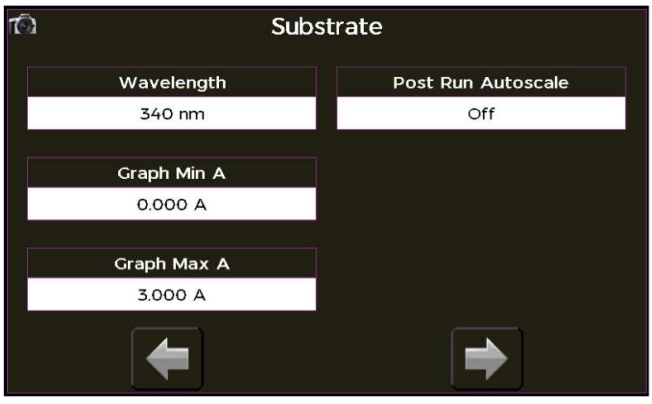
Substrate

Reagent test kits are routinely used for enzymatic determination of compounds in food, beverage and clinical laboratories. The change in absorbance over a specified time period can be used to provide useful information when an appropriate factor is applied; the start and end times as well as the factor are defined in the reagent kit protocol. The curve fit usually used is linear regression.

The reaction rate and enzyme activity can be calculated if the factor used takes account of the absorbance difference per unit time as opposed to the absorbance per se.

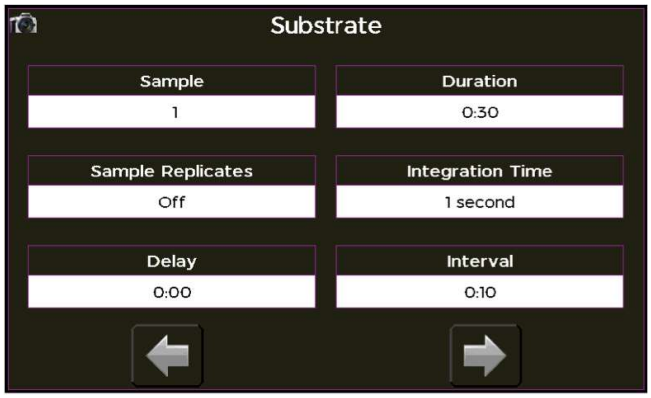
If a factor needs to be applied to a change in absorbance with time, use the kinetics application.

Measurement Parameters

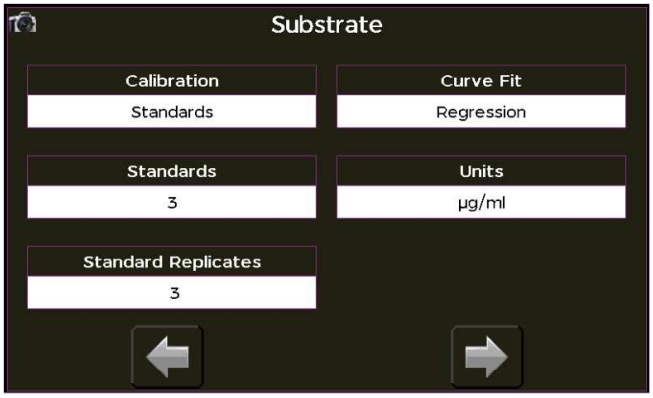


Set wavelength, minimum and maximum absorbance for the graph to display. Select if automatic scaling of the results to fit the display is required post run.

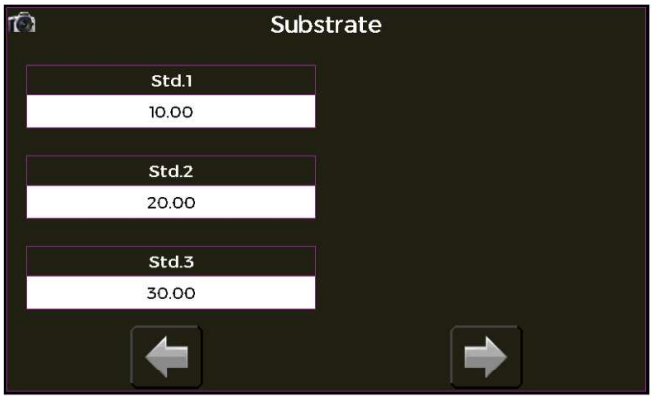
APPLICATIONS



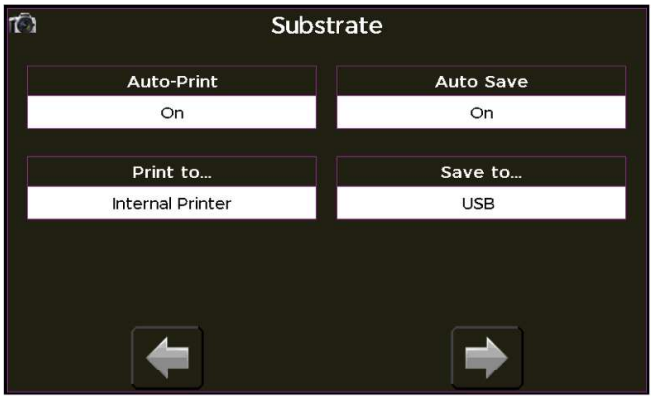
The Sample Seed entered under Sample will be the filename used for any data file saved automatically. Set the Duration (total measurement time), Replicates (up to 3) for each sample, Integration time (the duration in which the instrument will take the reading), Delay (time before the first measurement), and Interval (duration between readings).



Set the Calibration (Manual / Standards), number of Standards (up to 9), Replicates (up to 3) for each standard, Curve Fit, and Units (custom option available).

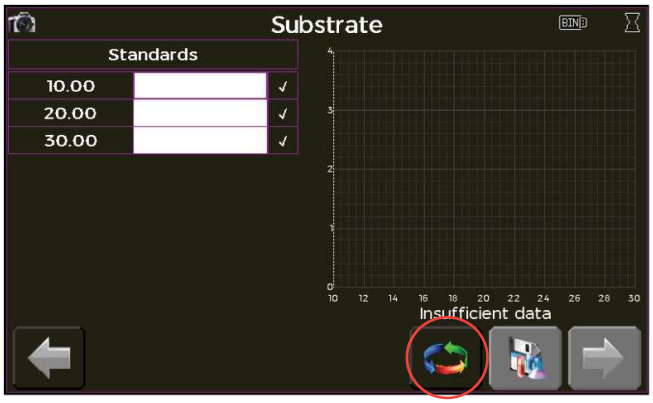


Set the concentration values for each of the standards using the numeric entry box.



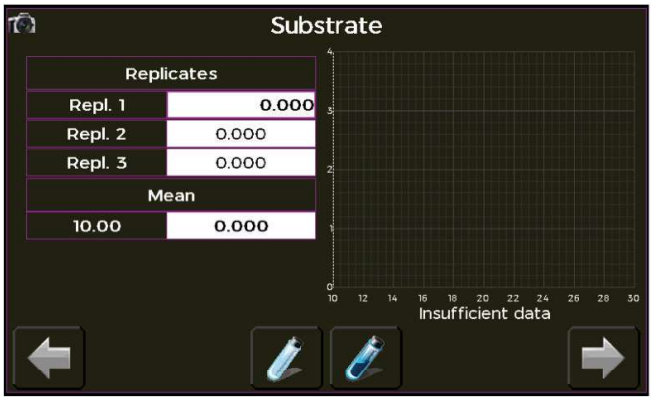
Set the outputs required for your methods. For more information, see the section Saving and Printing.

APPLICATIONS



To create a standard curve when using replicates, press the Replicates button to take you to the screen shown below. With Replicates off, standards can be measured directly as described below

Note: *Pressing the save method icon before any standards have been measured will save the method parameters only. Recalling a method containing method parameters only requires the user to construct a standard curve before measuring samples.*

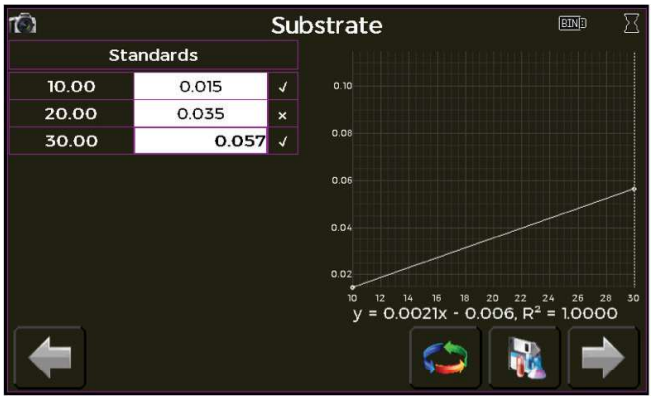


To create a standard curve, insert the cuvette containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the first standard/replicate in the series and press the Take Measurement button. A single reference suffices for standard curve creation.

Once all replicates have been measured for the standard, press the arrow forward button to continue recording all standards/replicates until the standard curve has been completed. To repeat any standard measurements, simply press the desired result, insert the correct standard and press Take Measurement.

Note: *A measurement can be stopped at any time by pressing the Stop button at the bottom of the screen. All data collected to this point will be displayed on screen and can be saved.*

After all the replicates have been measured, press the back arrow button to take you the standards screen, below.

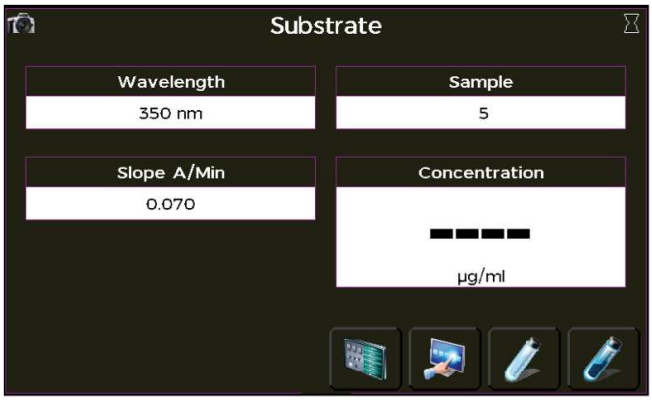


To ignore any outlying standard measurements, press the check mark in the appropriate row to toggle it to an “X”. Any ignored measurement will be automatically removed from the standard curve. These can be reinstated by pressing the “X”.

APPLICATIONS

Taking a Measurement

After the standard curve has been collected, press the forward arrow to proceed to the sample measurement screen.



To perform a measurement, insert the cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the sample and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series. View graph is available from the Options Menu.

Note: *A measurement can be stopped at any time by pressing the Stop button at the bottom of the screen. All data collected to this point will be displayed on screen and can be saved.*

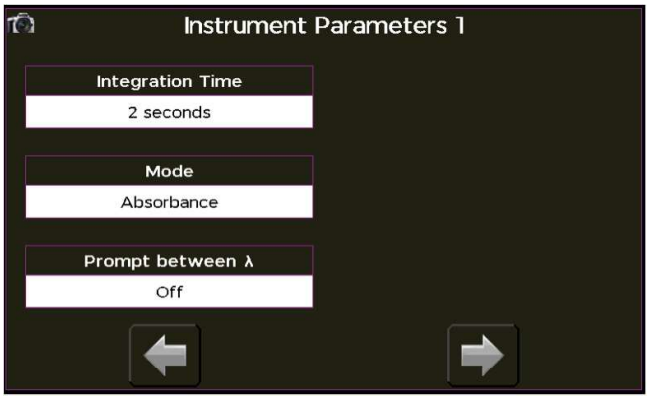
To view the graph whilst on the sample measurement screen, simply press the 'View Curve' icon that appears under the options menu.

Note: *Saving the method using the Save Method icon that appears on the options menu will save both the method parameters and the standard curve. Recalling a method containing method parameters and standard curve allows the user to measure samples directly.*

Equation Editor

The Equation Editor application allows users to create their own unique methods that include calculations and thresholds. Examples of methods that can be created using Equation Editor include percentage strength calculations and olive oil and chlorophyll analysis.

Measurement Parameters



Set the measurement parameters that will be used for all subsequent measurements.

Prompt between λ off

The instrument will measure wavelength 1, measure wavelength 2, measure wavelength 3 etc and then perform any calculations.

Prompt between λ on

The instrument will measure wavelength 1, prompt, measure wavelength 2, prompt, measure wavelength 3, etc., and then perform any calculations. This is used for equations that require wavelength measurements of different samples e.g. chlorophyll analysis.

You can advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

APPLICATIONS

Using the Sample Measurement table, the user inputs all of the measurements that are required in the method i.e. the screen left shows a fixed wavelength measurement at 430 nm that is named 430. Data is entered as described below. Before any data is entered, Name will read 'Not set' and will not appear in the Sample Data list on the Equation Builder.

Name — Enter an alphanumeric name for the measurement. The name entered here will be used as the Sample Data in the Equation Builder (entered data only appears in the Equation Builder if the user has defined a name).

Wavelength — Enter the (λ)wavelength for measurement.

Function — The combination box will appear with the following options.

Abs / %T at λ — measurement will be at the wavelength inputted by the user only.

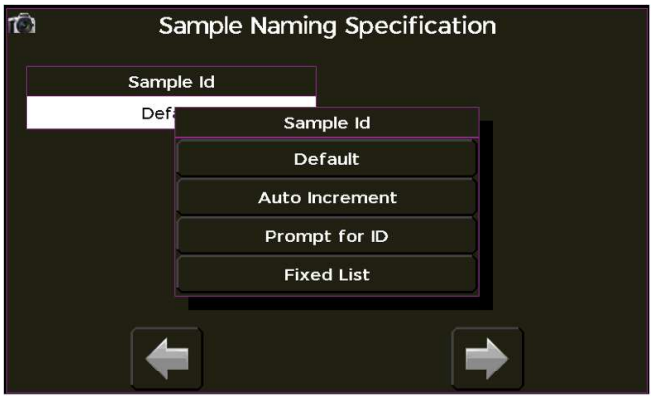
Peak closest to λ — the instrument automatically finds the peak closest to the inputted wavelength.

Valley closest to λ — the instrument automatically finds the valley closest to the inputted wavelength.

+/- — Used with Peak closest to λ and Valley closest to λ only. This is the range over which the instrument will scan for a peak or valley from the wavelength entered.

Del — Pressing the "X" deletes the row. If the data is used in an equation this will also be deleted.

APPLICATIONS



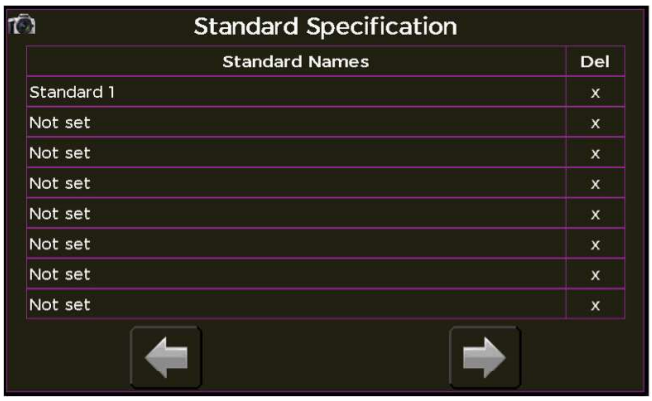
Equation Editor has four options for sample naming conventions. These are shown below:

Default The sample name consists of Sample and an incrementing number.

Auto Increment The sample name is a combination of sample seed and an incrementing sample number. The user will be prompted to enter the sample seed for each new batch of samples.

Prompt for ID The user is prompted to enter the sample name before running each sample.

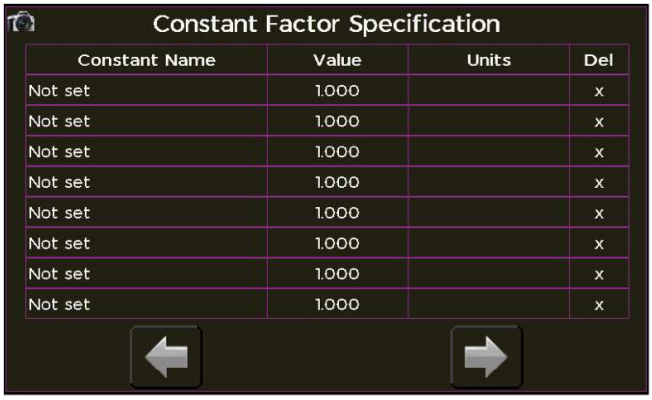
Fixed List The user will be prompted to enter the number of samples required. Sample names for each sample are then entered on the subsequent screens. The sample names that are assigned are saved for each method.



The Standard Specification screen is used to declare a list of all of the standard solutions which will be referenced when creating an equation.

E.g. the equation for percentage strength at 500 nm compares the absorbance of a sample to the absorbance of a control standard. This is where the control standard would be defined.

Standard names are entered by pressing on the desired row and entering the standard name using alphanumeric text entry. Standard measurements can be made for any measurements specified in the Samples table.



The Constant Factor Specification is used to declare any constants used in the equation. Data is entered as described below. Before any data is entered, Constant Name will read 'Not set' and will not appear in the Constants list on the Equation Builder.

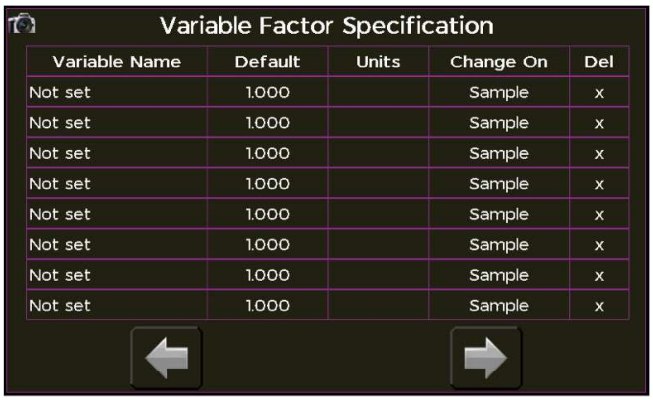
APPLICATIONS

Constant Name — Enter a alphanumeric name for the constant.

Value — Input the value of the constant.

Units — Enter the units for the constant using alphanumeric text entry. If this column is left blank, no units will be displayed on exported or printed data.

Del — Pressing the "X" deletes the row. If the constant is used in an equation this will also be deleted.



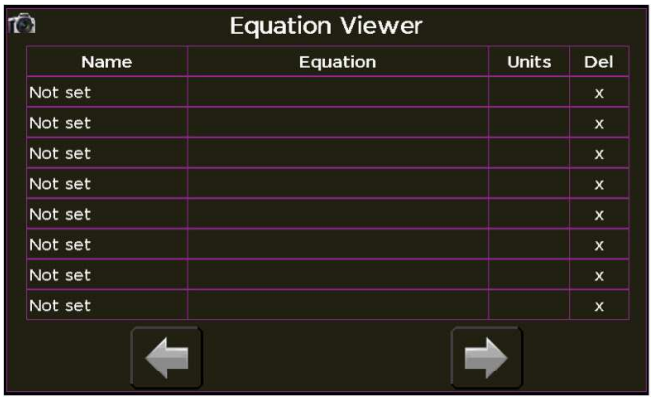
The Variable Factor Specification is used to declare any variables used in the equation. Data is entered as described below. Before any data is entered, Variable Name will read 'Not set' and will not appear in the Variables list on the Equation Builder.

Variable Name — Enter an alphanumeric name for the variable factor.

Default — Enter the default variable factor. Default values can be edited during a measurement.

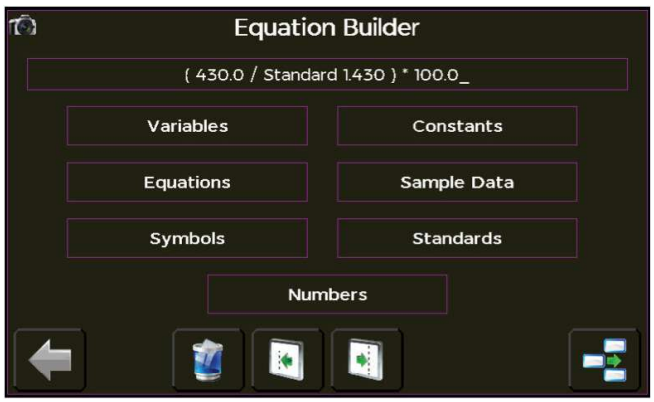
Units — Enter the units for the variable factor using alphanumeric text entry. If this column is left blank, no units will be displayed on exported or printed data.

Change On — This allows you to toggle between Sample and Batch. When set to Sample, the method will prompt for a variable to be entered before each sample measurement. When set to Batch, the method will prompt for a variable to be entered at the start of each sample batch.



The Equation Viewer provides an overview of any equations created, as well as allowing the user to create new or edit existing equations. Data is entered as described below. Before any data is entered, Name will read 'Not set' and will not appear on the results screen.

Name	Enter a unique, alphanumeric name for the equation. The names entered here will be displayed in the 'Equations' combo box on the Equation Builder screen as well the results screens. Therefore, any equation can be easily identified when using it in other equations.
Equation	This takes the user to the 'Equation Builder' (see below). Any equation constructed in the Equation Builder will be displayed in this box.
Units	Enter the units for the result of the equation using alphanumeric text entry. If this column is left blank no units will be displayed alongside the result.
Del	Pressing the "X" deletes the row and removes the equation.

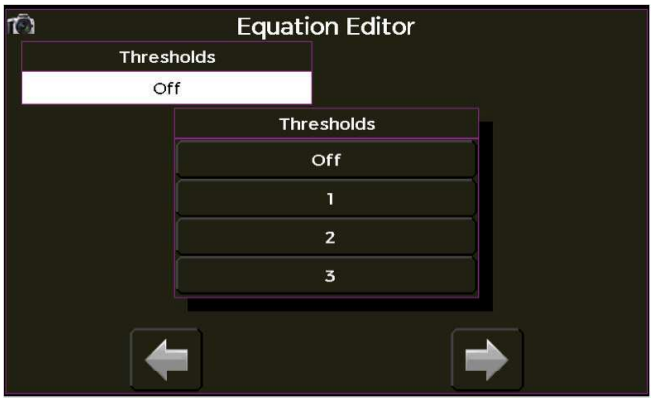


The Equation Builder allows the user to create any equations required in the method. Data is entered as described below. To allow data to be inserted or deleted, the cursor can be moved left and right using the left and right arrows. Data can be deleted using the delete icon and thresholds added using the thresholds icon (see below).

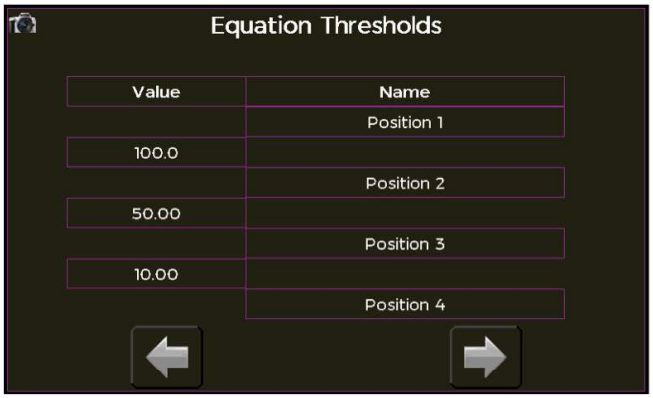
Variables	Contains any variables added to the 'Variables' table by the user. Selecting the desired variable enters it into the equation.
Constants	Contains any constants added to the 'Constants' table by the user. Selecting the desired constant enters it into the equation.
Equations	Contains any equations that have been created in this method. Selecting the desired equation enters it into the equation.
Sample Data	Contains all of the readings specified by the user in the Sample Measurement table. Selecting the desired sample data enters it into the equation.
Symbols	Contains mathematical symbols and the logic gates AND and OR. Selecting the desired symbol enters it into the equation.
Numbers	Allows the user to directly enter numbers into the equation.

Note: *All of the data above appears in the lists as it was entered in the appropriate table.*

After the equation has been entered, the user has two options. If the result is to be viewed as a number, press the back arrow to return to the Equation Viewer screen. If the result is to be viewed with a user specified pass/fail limit, press Thresholds to set appropriate thresholds for the measurement.



The first thresholds screen allows the user to input how many thresholds are required for a result.



With Thresholds set to 3, the screen will display the table layout shown left. Setting Thresholds to 2 and 1 will reduce the number of values you can input to 2 and 1, respectively.

Value

Allows the user to directly input numbers for threshold values.

Name

Enter the text that will be displayed for each result using alphanumeric entry.

Using the example above: A result of ≥100 will return the answer Position 1, a result of <100 and ≥50 will return the answer Position 2, a result of <50 and ≥10 will return the answer Position 3 and a result of <10 will return the answer Position 4.

Note: *When using AND or OR logic in an equation, the result will be returned as a binary (1 = true and 0 = false). These can be incorporated into the thresholds by setting the Thresholds to 1, Value to 1 and setting the appropriate names (as the name above value corresponds to ≥1 this is the response that will be displayed for true results).*

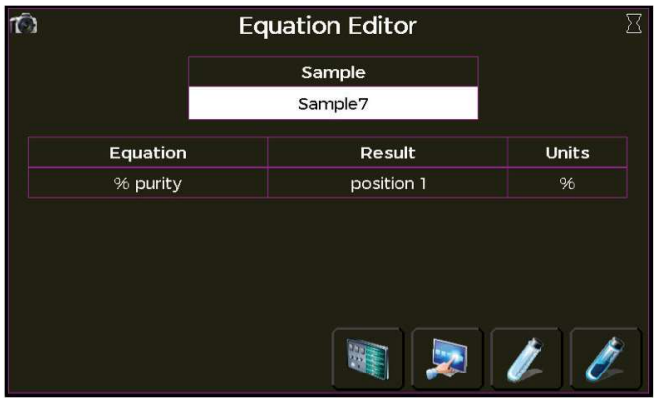
After all of the thresholds have been set, pressing the forward arrow will return the user to the Equation Builder screen. The Equation Viewer screen can be accessed using the back arrow.

Continue as described above until all equations have been created. Once complete, press the forward arrow to the output options screen below.

Set the outputs required in your method. For more information see the section Saving and Printing.

Note: *Automatically saving sample data with sample naming set to 'Prompt for ID' or 'Fixed list' will save the data using the first entered sample name as the filename.*

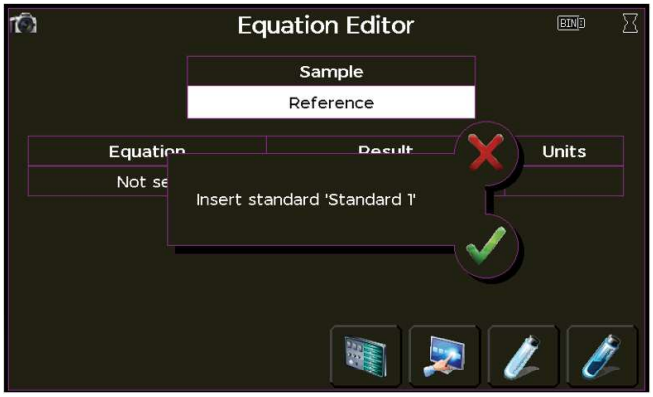
Performing a Measurement — No standards



To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

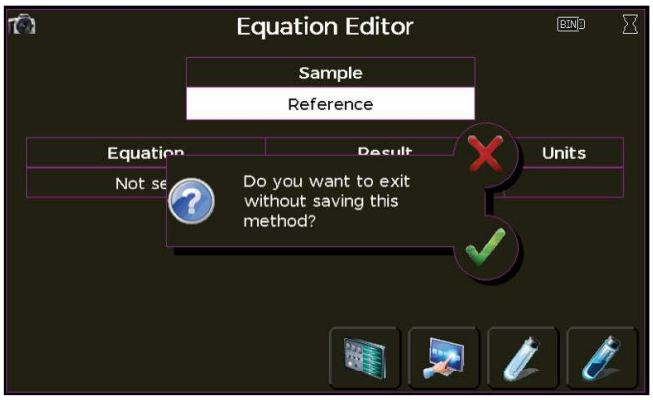
Note: *User can click on the equation name to show the calculation and values used.*

Using Standards



When performing a measurement using a method that includes standards, the first press of the Take Measurement button will produce a message box that prompts the user to insert a specific standard. After all standards have been measured, subsequent presses of the Take Measurement button will perform sample measurements.

Saving Methods and Exiting



As methods developed using Equation Editor may have taken time to input, the software will prompt the user to save the method before exiting. Pressing the "X" will return to the user to the results screen, where they can save the method, pressing the check mark will exit without saving. Details of method saving can be found in the Saving Methods section.

Trace Manager — Overlaying & Manipulating Wavescan & Kinetics Files

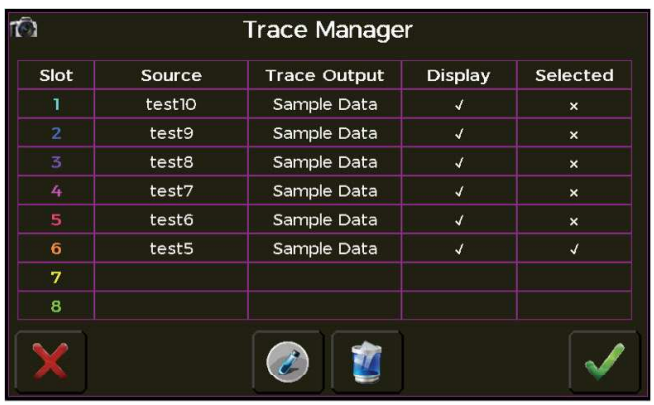
Trace Manager is the application used by the BioDrop spectrophotometers to overlay and manipulate wavescan and kinetics files. Samples are loaded into Trace Manager as described below:

Using Sample Manager

Wavescan and kinetics files can be loaded directly into Trace Manager by selecting the required files from Sample Manager on the main screen located at the bottom toolbar.

Highlight the required files by pressing on the appropriate row and load these into Trace Manager by pressing the Load Sample Icon (bottom right corner). If the files you require are not displayed on the screen you can scroll through the pages using the up and down arrows. Pressing on the Filename and Date column headers will sort the files alphabetically and chronologically, respectively.

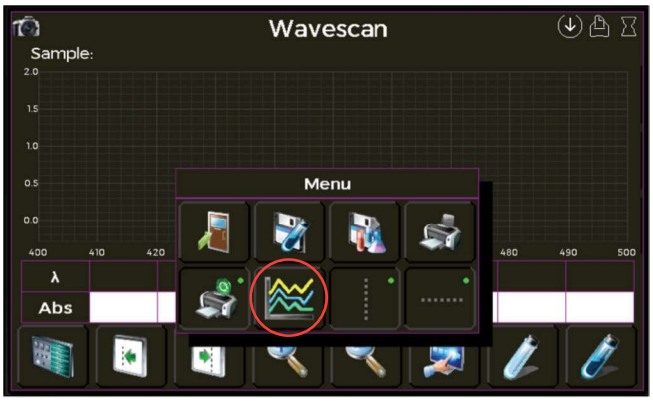
Note: *If a USB memory stick is inserted, it is possible to toggle between files stored on the internal and USB memories using the icon towards the left hand corner. The icon in the top right corner of the screen will display the memory that is currently in use – instrument or USB.*



All recalled files will be displayed on the Trace Manager screen (up to 8). The color of the Slot number will be the color of the trace when it is displayed on screen.
To toggle which files are displayed on the measurement screen, press in the appropriate Display box (up to 8 files can be displayed). To select which files data will be displayed on the measurement screen press in the appropriate selected box (only one file may be selected). Press the check mark to view the overlaid data.

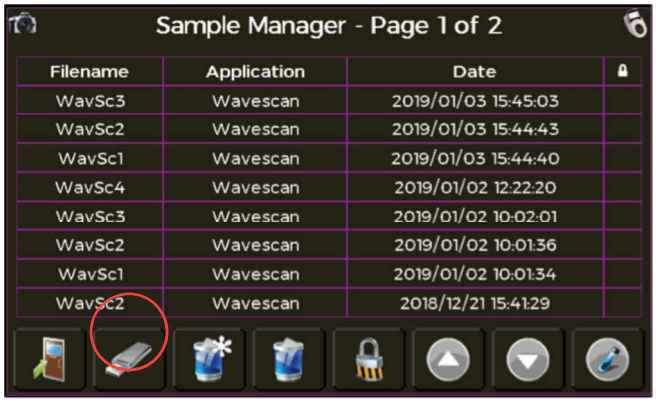
From Within an Application

To overlay saved files with a live trace displayed, Trace Manager can be accessed from within the application. This procedure is required for Limited users as they do not have the ability to access Sample Manager on the main screen.



Trace Manager can be accessed from the Wavescan and Kinetics measurement screens using the Trace Manager icon on the options menu.

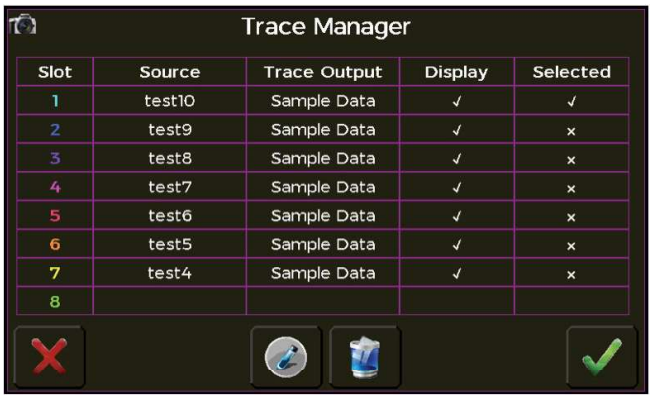
When accessed with no overlays displayed, Trace Manager will be empty. Files are added to this screen by selecting the Load Sample Icon in the bottom centre of the screen and loading saved files.



Highlight the required files by pressing on the appropriate row and load these into Trace Manager by pressing the Load sample icon. If the files you require are not displayed on the screen you can scroll through the pages using the up and down arrows. Pressing on the Filename and Date column headers will sort the files alphabetically and chronologically, respectively.

Note: *If a USB memory stick is inserted, it is possible to toggle between files stored on the internal and USB memories using the icon towards the left hand corner. The icon in the top right corner will display the memory that is currently in use.*

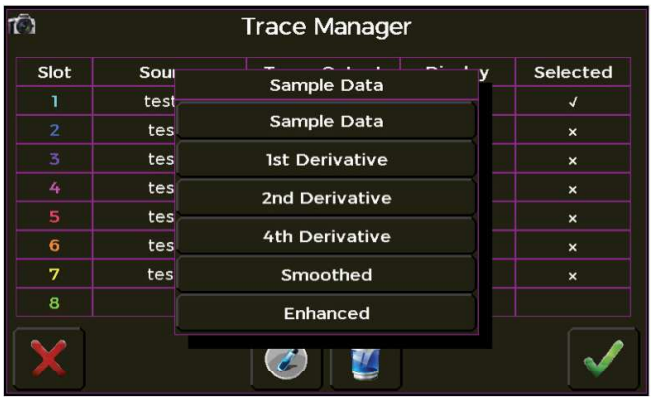
APPLICATIONS



All recalled files will be displayed on the Trace Manager screen (up to 8). The color of the Slot number will be the color of the trace when it is displayed on screen. To toggle which files are displayed on the measurement screen, press in the appropriate Display box (up to 8 files can be displayed). To select which files data will be displayed on the measurement screen press in the appropriate selected box (only one file may be selected). Press the check mark to view the overlaid data.

Post Scan Manipulation

Trace Manager allows the user to manipulate recalled wavescan and kinetics data using the procedure outlined below.



With the required files loaded into Trace Manager press the appropriate Trace Output box to display the manipulation options.

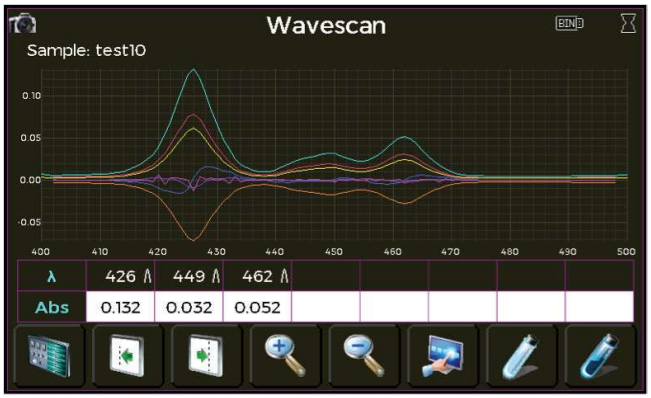
Wavescan post scan manipulations are:

Sample Data	Displays the raw wavescan data (this is the default option).
1st – 4th Derivative	Displays the derivative data to the desired order.
Smoothed	Uses the Savitzky-Golay algorithm to reduce noise and smooth the data.
Enhanced	Enhances features, sharpening peaks and valleys.

Kinetics post scan manipulations are:

Sample Data	Displays the raw kinetics data (this is the default option).
Low	Applies a low level of smoothing to the data.
Medium	Applies a medium level of smoothing to the data.
High	Applies a high level of smoothing to the data.

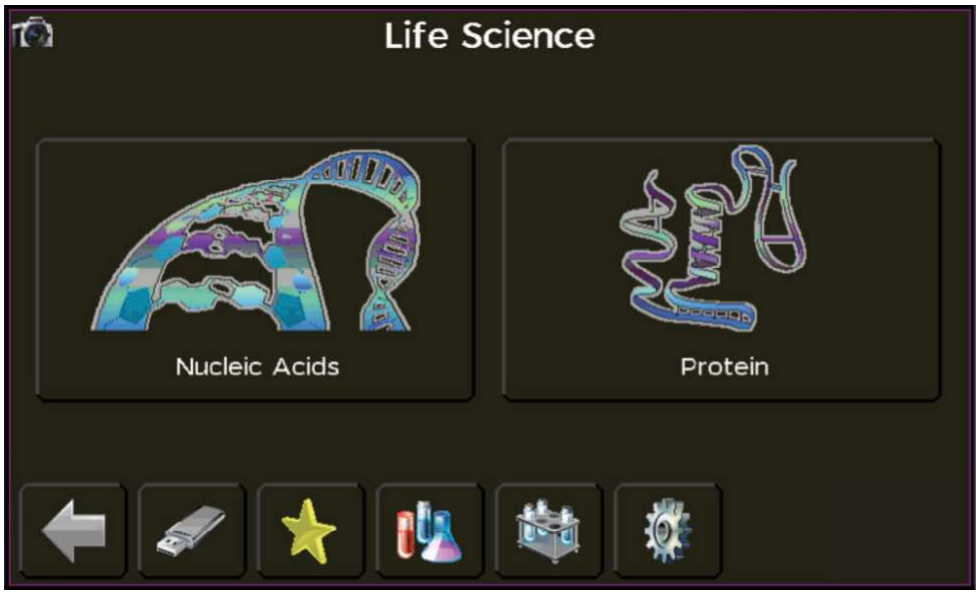
APPLICATIONS



After the manipulations and display options have been set as required, press the check mark button to display the recalled data on the sample measurement screen.

Note: *For wavescan and serial kinetic measurements with overlays set to >1, data will be automatically saved into the instrument's internal memory and displayed in Trace Manager. To ensure the optimum performance of the instrument, it is recommended that unwanted files are deleted from the internal memory at regular intervals (see Sample Manager — deleting files from the internal memory).*

LIFE SCIENCE APPLICATIONS



Nucleic Acids

DNA	Utilizes the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for DNA samples.
RNA	Utilizes the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for RNA samples.
Oligo	Utilizes the absorbance measurements at 230, 260 & 280 nm with optional background correction to perform a concentration and purity check for oligo samples.
Fluorescent Dye	Measures the labelling efficiency of fluorescently labelled DNA probes to ensure that there is sufficient amount of each probe to give satisfactory signals. The DNA yield is measured at 260 nm whilst the incorporation of the dyes is measured at the absorption maxima. This method is also useful for measuring the yields and brightness of fluorescently labelled in-situ hybridization probes.
TM Calc	The Tm Calculation application calculates the theoretical melting point from the base sequence of a primer. It is done using nearest neighbor thermodynamic data for each base in the nucleotide chain in relation to its neighbor.

LIFE SCIENCE APPLICATIONS

Protein

Protein UV	Direct UV determination of protein concentration at 280 nm using the Christian Warburg calculation, BSA, IgG, Lysozyme, Molar Extinction. Mass Extinction or E1% calculations.
Colorimetric Protein:	
BCA	Quantitative determination of protein concentration utilizing the absorbance measurement at 562 nm.
Bradford	Quantitative determination of protein concentration utilizing the absorbance measurement at 595 nm.
Lowry	Quantitative determination of protein concentration utilizing the absorbance measurement at 750 nm.
Biuret	Quantitative determination of protein concentration utilizing the absorbance measurement at 546 nm.
Pierce	Quantitative determination of protein concentration utilizing the absorbance measurement at 660 nm.
Protein Dye	Measures the labelling efficiency of fluorescently labelled protein probes to ensure that there is sufficient amount of each probe to give satisfactory signals.

NUCLEIC ACID APPLICATIONS

DNA, RNA & Oligo

Nucleic acids can be quantified at 260 nm because it is well established that solutions of DNA and RNA in 10 mm pathlength cuvettes with an optical density (absorbance) of 1.0 have concentrations of 50 µg/ml and 40 µg/ml, respectively. Oligonucleotides typically have a factor of 33 µg/ml, although this does vary with base composition and can be calculated if the base sequence is known.

$$\text{Concentration} = \text{A260} \times \text{Factor}$$

BioDrop spectrophotometers use the default factors 50, 40 and 33 for DNA, RNA and oligonucleotides, respectively. Compensation for dilution and pathlength can also be entered.

The BioDrop Duo+ can be used with the BioDrop CUVETTES. The BioDrop CUVETTE is available in two pathlength configurations, the BioDrop 125 has a pathlength of 0.125 mm and the BioDrop 500 has a pathlength of 0.5 mm. The BioDrop µLite+ and Duo+ contain a direct micro-volume sample port with a pathlength of 0.5 mm which can be selected from the drop-down menu of the Pathlength list. We recommended the use of the BioDrop 500 for the highest sensitivity and BioDrop 125 and µLite+ sample port analysis (0.5 mm pathlength) for low volume samples. Pathlength factors are pre-programmed in the software for quick calculations. For example, if measuring dsDNA in a BioDrop 125, the calculation would be as follows:

$$\text{Concentration} = \text{A260} \times 50\ \mu\text{g/ml} \times 80$$

If measuring dsDNA in the BioDrop 500, the calculation would be:

$$\text{Concentration} = \text{A260} \times 50\ \mu\text{g/ml} \times 20$$

Nucleic Acid Purity Checks

Nucleic acids extracted from cells are accompanied by proteins and extensive purification is required to separate these protein impurities. The ratio of A260/A280 gives an indication of a sample's purity, with pure DNA and RNA preparations typically having ratios of ≥1.8 and ≥2.0, respectively. Deviations from these values indicate the presence of impurities, but care must be taken when interpreting results.

Concentration also affects both the A260 and A280 readings. If a solution is too dilute, the readings may be at the instrument's detection limit and results may vary as there is less distinction of the A260 peak and A280 slope from the background absorbance. For accurate measurements A260 should always be greater than 0.1.

Elevated A230 values can also indicate the presence of impurities (230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since EDTA and other buffer salts absorb at this wavelength). When measuring RNA samples, the A260/A230 ratio should be >2.0. Ratios lower than 2.0 generally indicate contamination with guanidinium thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230 – 260 nm range. A wavelength scan of the nucleic acid is particularly useful for RNA samples.

For DNA, RNA and Oligo applications, if the ratio is less than or greater than the suggested values shown below, a yellow warning symbol is displayed in the control to inform the user there may be an issue with the sample.

Ratio values that trigger a warning.

DNA	A260/A230	<2.0 or >2.2
	A260/A280	<1.7 or >2.0
RNA	A260/A230	<2.0 or >2.2
	A260/A280	<1.9 or >2.2
Oligo	A260/A230	<2.0 or >2.2
	A260/A280	<1.9 or >2.2

Ratio Warning Symbol

NUCLEIC ACID APPLICATIONS

Background Correction

- To compensate for the effects of background absorbance caused by turbidity, high absorbance buffer solutions and the use of reduced aperture cuvettes the BioDrop spectrophotometers have the option of background correction at a 320 nm.
- When used, A320 is subtracted from A260 and A280 prior to use so that:

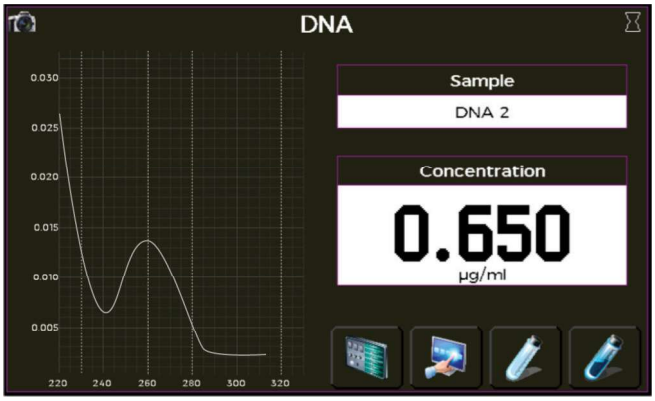
$$\text{Concentration} = (A260 - A320) \times \text{Factor}$$

$$\text{Abs ratio} = (A260 - A320) / (A280 - A320)$$

$$\text{Abs ratio} = (A260 - A320) / (A230 - A320)$$

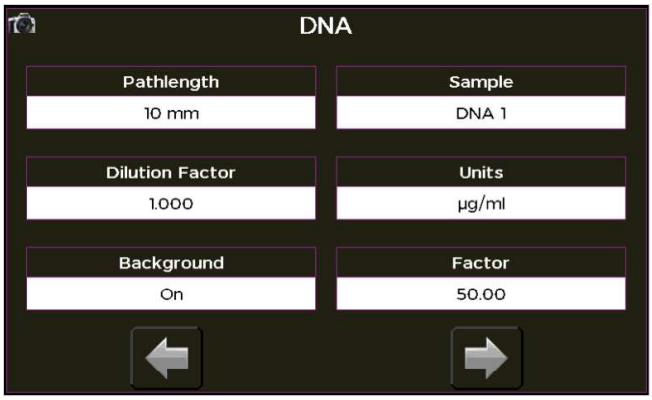
- The use of background correction can remove variability due to handling effects of low volume disposable cuvettes.

Spectral Scan of Nucleic Acid

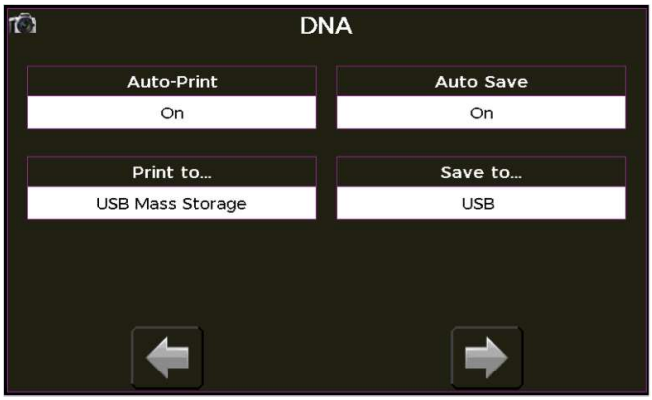


Note: *An absorbance maximum near 260 nm and absorbance minimum near 230 nm, a flat peak near 260 nm and steep slope at 280 nm and very little absorbance at 320 nm*

Measurement Parameters



Set Pathlength to match the cuvette being used and Dilution Factor if required. If using low volume cuvettes, set Background correction to On. Set Units to encompass the expected concentration of your samples, the default Factor will update automatically depending on the units selected i.e. for units of µg/ml the default factor will be 50.00. If the Factor required differs from the default value this can be edited using numeric entry. The Sample Seed entered under Sample will be the filename of any saved file.

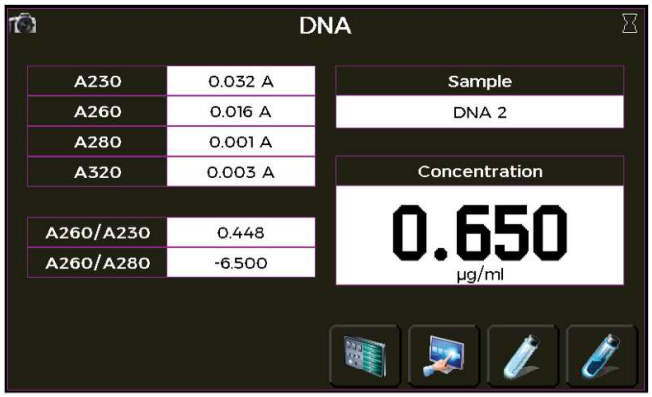


You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

Set the outputs required in your method. For more information see the section Saving and Printing.

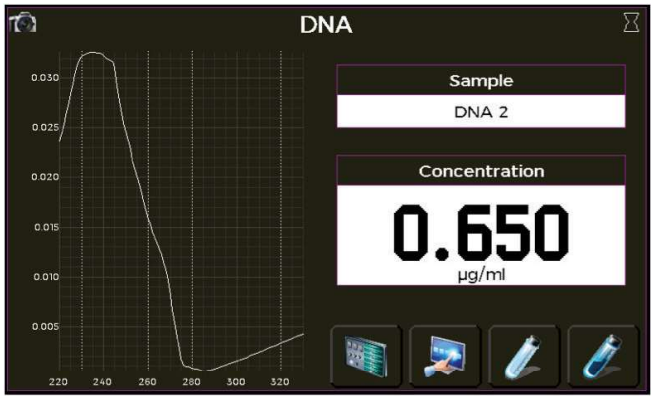
NUCLEIC ACID APPLICATIONS

Taking a Measurement



To perform a measurement, insert a cuvette (or load directly onto the µLite sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

If *Background* is set to *On*, the A320 result will be included in the left hand column and automatically subtracted from the displayed A230, A260, A280, A260/A230, A260/A280 results.



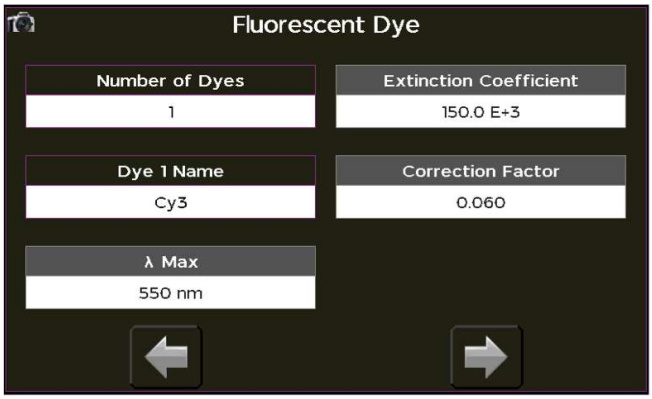
A scan of the most recently run sample can be viewed by pressing the View Scan icon in the options menu.

> ***Note:*** *When saving sample data, scan files will not be saved. The Wavescan application should be used to save scans of nucleic acid samples.*

Fluorescent Dye

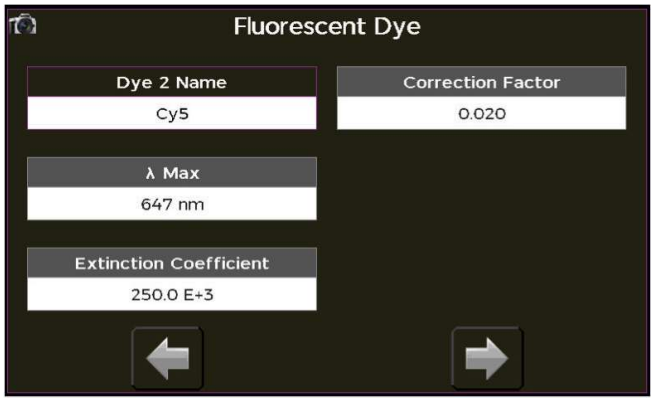
The measurement of the labelling efficiency of fluorescently labelled DNA probes before 2-color micro-array hybridization ensures that there is sufficient amount of each probe to give satisfactory signals. The data also provides an opportunity to balance the relative intensities of each fluorescent dye by adjusting the concentration of each probe before hybridization. The DNA yield is measured at 260 nm whilst the incorporation of the dyes is measured at the absorption maxima. This method is also useful for measuring the yields and brightness of fluorescently labelled in-situ hybridization probes.

Measurement Parameters

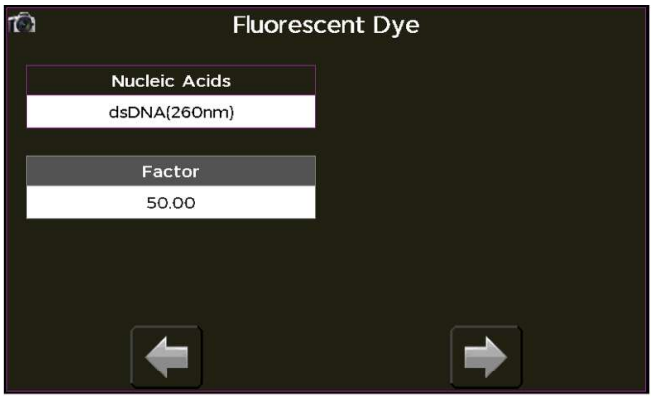


Number of Dyes, this can be set to 1 or 2. Dye 1 Name allows the user to select the dye used in the measurement, the BioDrop spectrophotometers have 19 dyes pre-programmed and the option for user entry using the Custom Dye option. λ Max, Extinction Coefficient and Correction Factor are only editable when using the Custom Dye option.

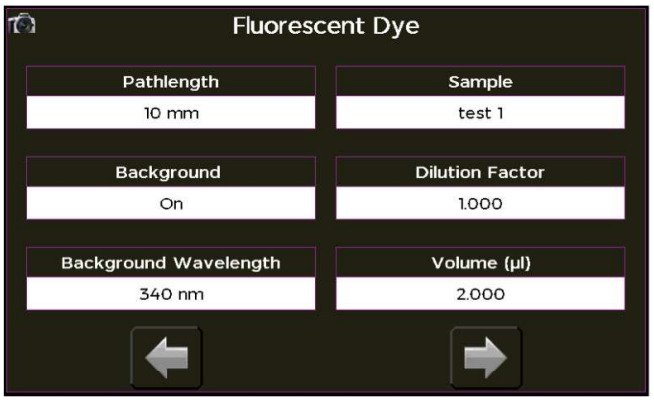
You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.



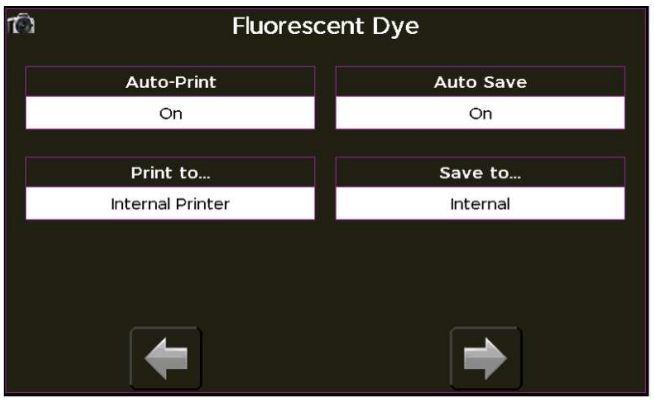
With Number of Dyes set to 2, the next method parameter screen allows the user to specify the second dye used in the measurement.



If the measurement requires the calculation of DNA Concentration and/or DNA Quantity the relevant nucleic acid can be entered. A custom factor can be entered by selecting Custom in Nucleic Acids.

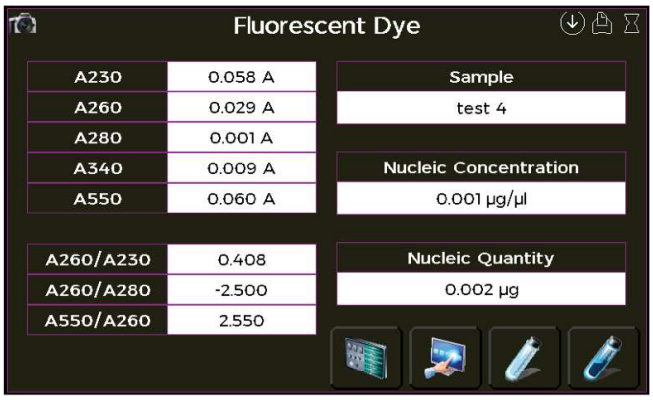


This parameters screen allows the user to set the pathlength of the cuvette being used, whether background correction is required and at what wavelength, the dilution factor and volume of the sample in µl. All of these will be used in the calculations. The Sample Seed entered under Sample will the filename of any saved file.



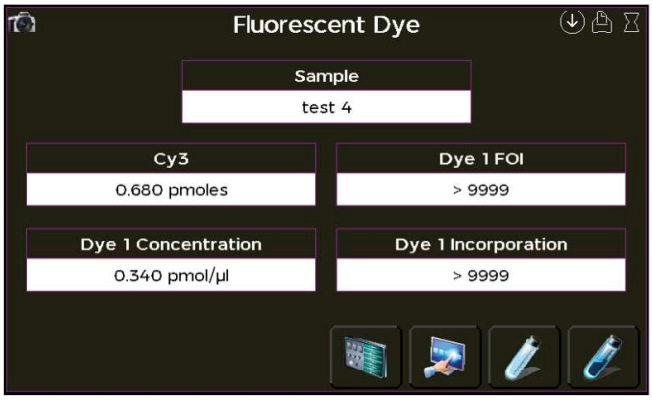
Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

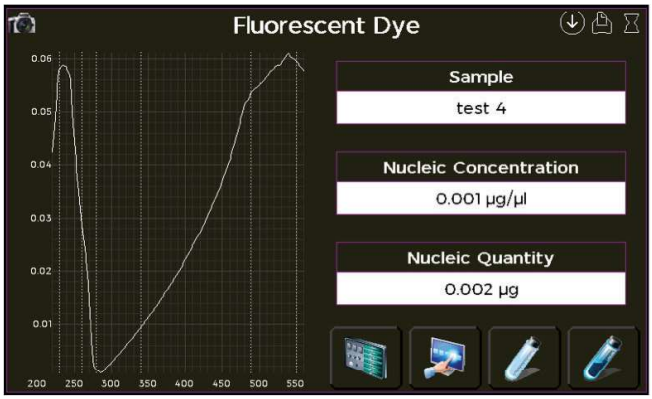
If Background Correction is set to On the Background Wavelength set in the method will be included in the left hand column and automatically subtracted from all results.



To toggle between DNA and dye parameters, press the toggle icon in the options menu to view this screen.

Options Menu

Toggle Icon



Fluorescent dye measurements are wavelength scanning measurements, to view the scan for a particular measurement press the scan button in the options menu to view this screen.

TM CALCULATION

The Tm Calculation application calculates the theoretical melting point from the base sequence of a primer. It is done using nearest neighbor thermodynamic data for each base in the nucleotide chain in relation to its neighbor (Breslauer et al, *Proc. Natl. Acad. Sci. USA*, 1986, 83, 3746). The data obtained are useful in both the characterization of oligonucleotides and in calculating Tm for primers used in PCR experiments.

The ACGT/U sequence entered in the method parameters is used to calculate the theoretical Tm, the theoretical absorbance (Absorbance units/mmol) and the conversion factor (mg/ml). This is possible as the stability of a bent and twisted sequence of bases such as an oligonucleotide is dependent on the actual base sequence. These calculated thermodynamic interactions between adjacent base pairs have been shown to correlate well with experimental observations.

The Tm Calculation application uses matrices of known, published thermodynamic values and extinction coefficients to calculate Tm and the theoretical absorbance/factor of an entered base sequence.

Tm is calculated using the equation:

$$Tm = \frac{\Delta H \times 100}{\Delta S + (1.987 \times \log_e(c/4 + 53.0822)} - 273.15 + \log [salt]$$

where

ΔH and ΔS are the enthalpy and entropy values, respectively summed from respective 2 × 4 × 4 nearest neighbor matrices

c is the Primer concentration of oligonucleotide (pmoles/ml) in the calculated Tm or the measured concentration in measured Tm. In the latter case, concentration is obtained from the equation:

$$c = \frac{\text{Abs}(260\text{ nm}) \times \text{Calculated factor} \times \text{pathlength multiplier} \times 10\,000}{\text{MW}}$$

Calculated factor and MW are defined below:

[salt] is the buffer molarity plus total molarity of salts in the hybridization solution (moles/l)

Weights for ΔS are indexed by adjacent paired bases. A similar equation applies to weights for ΔH, again indexed by adjacent bases.

> ***Note:*** *Bivalent salts may need normalizing using a multiplying factor of 100 because of their greater binding power.*

Theoretical Absorbance

The Theoretical Absorbance is based on a calculation as follows:

For each adjacent pair of bases (nearest neighbors) an extinction coefficient weight is accumulated using a 4 × 4 table (one for either DNA or RNA). This total weight is doubled and then for each internal base a counterweight is subtracted using another 1 × 4 table. The end bases are excluded from the latter summation.

Total Extinction Coefficient E = Σ (2 × aTable[base_type][base(n)][base(n+1)])- Σ(tTable[base_type][base(n)])

Conversion Factor

The Conversion Factor is given by = $\frac{\text{Molecular weight}_{ABCDE}}{\Sigma_{EABCDE}}$

where

E_{ABCDE} = [2 × (EAB +EBC +ECD +EDE) –EB –EC –ED]

MW (g/mole) = [(dA × 312.2) + (dC × 288.2) + (dG × 328.2) + (dT × 303.2.)] + [(MWcounter-ion) × (length of oligo in bases)]

(for RNA oligonucleotide, (dT × 303.2) is replaced by (dU × 298.2)

The MW calculated using this equation must be adjusted for the contribution of the atoms at the 5' and 3' ends of the oligo.

For phosphorylated oligos:	Add [17 + (2 × MW of the counter-ion)]
For non-phosphorylated oligos:	Subtract [61 + (MW of the counter-ion)]

The MW (g/mole) of the most common oligo counter ions are:

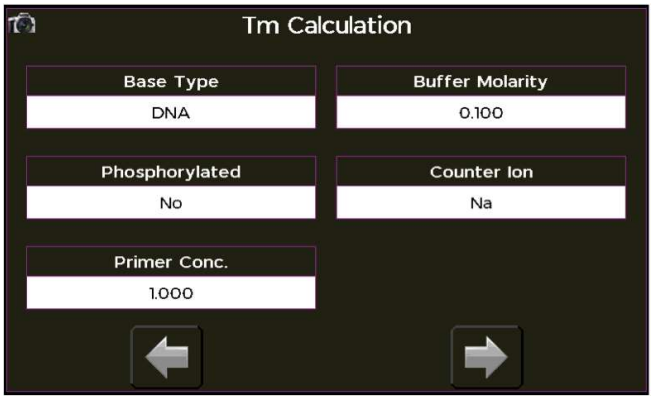
Na (sodium)	23.0
K (potassium)	39.1
TEA (triethylammonium)	102.2
Other	Defaults to 1.0 (variable 0.1–999.9)

Calculated molecular weight: a weight is added for each base looked up from a table. The weight of the counter ion is added for every base from a small table for the known ions. If phosphorylated, then the system adds 17.0 plus two counter ions otherwise it subtracts 61.0 and one ion.

Theoretical Absorbance: for each adjacent pair of bases (nearest neighbors) a weight is accumulated using a table. For each internal base a weight is subtracted using another table. Separate tables are used for DNA and RNA.

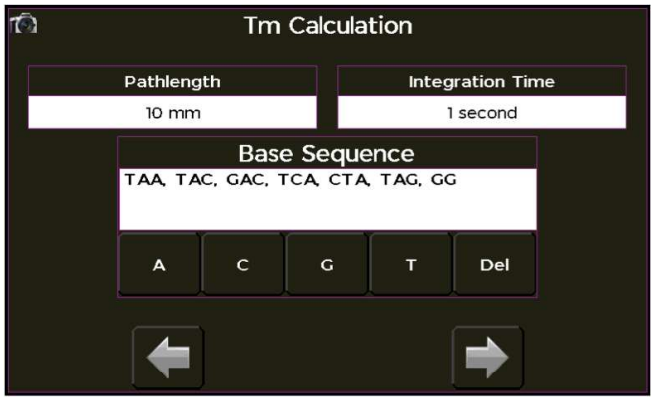
Calculated factor: this is the calculated molecular weight divided by the theoretical absorbance.

Measurement Parameters

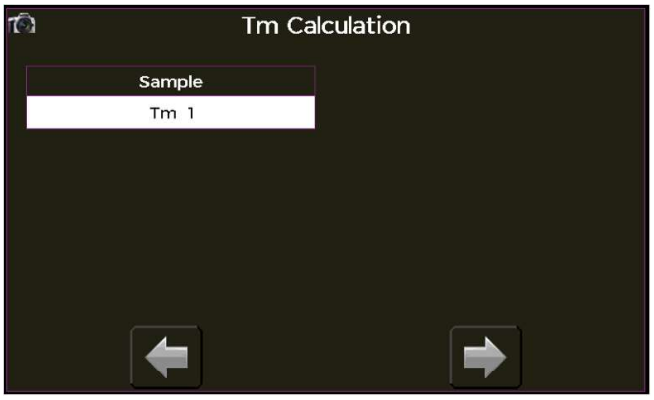


Set the required Base Type (DNA/RNA), whether the base is Phosphorylated, the Primer Concentration (pmoles/ml), Buffer Molarity and Counter Ion. Counter Ion has options for Na (sodium), K (potassium), TEA (triethylammonium) and Other, allowing the user to set the required molecular weight (MW) of the counter ion.

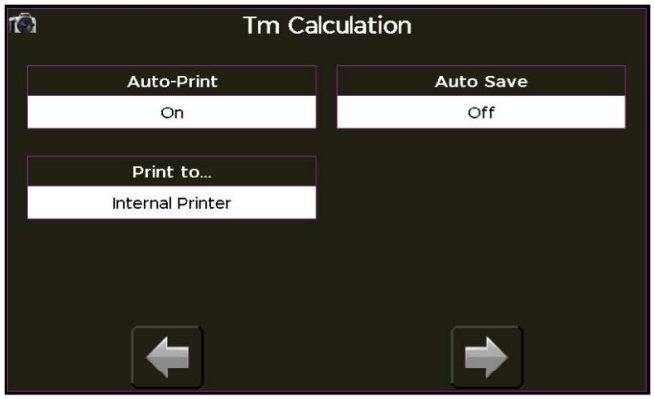
NUCLEIC ACID APPLICATIONS



Set the Pathlength and Integration Time you require. Base Sequence allows the user to enter the known base sequence triplets using the buttons A, C, G and T/U. To improve readability, a comma is added after each triplet.

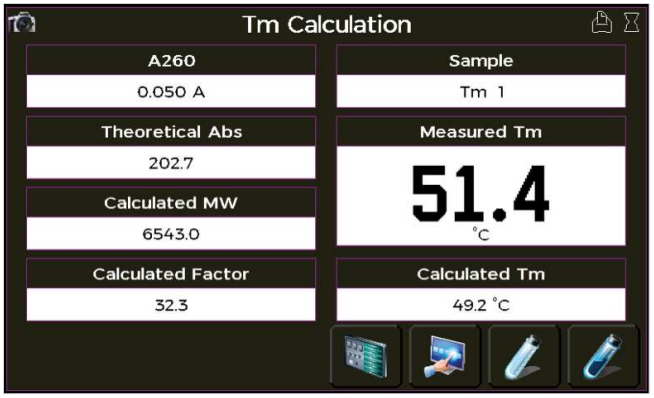


The Sample Seed entered under Sample will the filename of any automatically saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

PROTEIN APPLICATIONS

The BioDrop spectrophotometers contain dedicated methods for both colorimetric protein assays, direct UV measurements and fluorescent labelled protein.

BCA, BRADFORD, LOWRY, BIURET and PIERCE PROTEIN ASSAYS

The BCA, Bradford, Lowry, Biuret and Pierce protein assays are well established spectrophotometric methods for determining the amount of protein in a sample. The exact choice of the assay depends upon the concentration of protein being measured and the detergents/reducing agents used in purification. Detailed protocols are supplied with all assay kits and should be followed closely to ensure accurate results are obtained. An outline of the protein assays offered is provided below:

Bradford method:	Quantifies the binding of the dye Coomassie Brilliant Blue to an unknown protein and compares this binding to that of different, known concentrations of a standard protein at 595 nm. The standard protein is usually bovine serum albumin (BSA).
Biuret method:	Depends on reaction between Cu^{2+} ions and amino acid residues in an alkali solution. The resulting copper complex absorbs light at 546 nm.
BCA method:	Depends on reaction between Cu^{2+} ions and amino acid residues. In addition, this method combines this reaction with the enhancement of Cu^{+} ion detection using bicinchoninic acid (BCA) as a ligand, giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to break down cell walls.
Lowry method:	Depends on quantifying the color obtained from the reaction of Folin-Ciocalteu phenol reagent with the Tyrosyl residues of an unknown protein and comparing with those derived from a standard curve of a standard protein at 750 nm (usually BSA).
Pierce method:	Binds a proprietary die-metal complex to a protein in acidic conditions that cause a shift in the dye's maximum absorption measured at 660 nm.

Determination of Protein Concentration using Bicinchoninic Acid (BCA) Protein Assay

The principle of the bicinchoninic acid (BCA) protein assay relies on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{+}. The amount of reduction is proportional to the amount of protein present. BCA forms a purple-blue complex with Cu^{+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins. The BCA assay can be used to quantify proteins in the concentration range 0.2 to 1.0 mg/ml. It is compatible with many detergents but not compatible with reducing agents such as dithiothreitol above 1 mM.

Getting Started

It is always advisable to prepare the standard in the same buffer as the sample to minimize any interference effects. BCA assays are routinely performed at 37 °C. Color development begins immediately and can be accelerated by incubation at higher temperatures. Higher temperatures and/or longer incubation times can be used for increased sensitivity.

PROTEIN APPLICATIONS

Materials Required

- Bicinchoninic Acid Kit for Protein Determination
- Suitable tubes with caps to hold and mix 2.1 ml samples and to heat at up to 60 °C
- Plastic disposable cuvettes
- Standard protein solution of known concentration (1 mg/ml)
- Incubator or block heater to heat sample tubes

Preparation of the BCA Working Reagent

BCA reagents A and B are available commercially from a number of different sources. Instructions given here are for the kit supplied by Sigma Aldrich, other methods will be similar. Always refer to the manufacturer's instructions.

1. Mix 50 parts of Reagent A (a solution containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1N NaOH, pH 11.25) with 1 part of Reagent B (4% (w/v) $CuSO_4.5H_2O$), preparing sufficient reagent for all the standards and samples. 2ml of working reagent is required for each sample.

2. Mix until the solution is a uniform light green color. The solution is stable for 1 day.

Standard Preparation

1. Prepare a series of protein standards ranging in concentration from 0.2 to 1.0 mg/ml such that the final volume for the assay is 0.1 ml. The BioDrop spectrophotometers can measure up to 9 standards and up to 3 replicates.

2. Add 2.0 ml of the BCA working reagent to each standard, vortex gently and incubate using one of the following parameters: 60 °C for 15 minutes, 37 °C for 30 minutes or room temperature from 2 hours to overnight.

3. If required, allow the tubes to cool to room temperature.

Sample Preparation

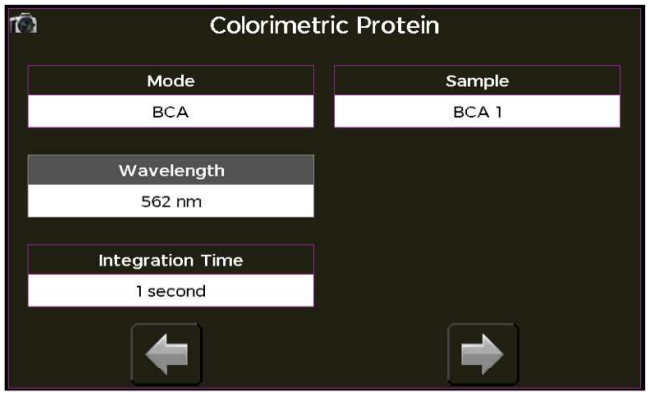
1. Prepare the unknown samples as described above ensuring that the final volume is 0.1 ml.

2. Add 2.0 ml of the BCA working reagent to each sample, vortex gently and incubate using one of the following parameters: 60 °C for 15 minutes, 37 °C for 30 minutes or room temperature from 2 hours to overnight.

3. If required, allow the tubes to cool to room temperature.

Creating a Standard Curve

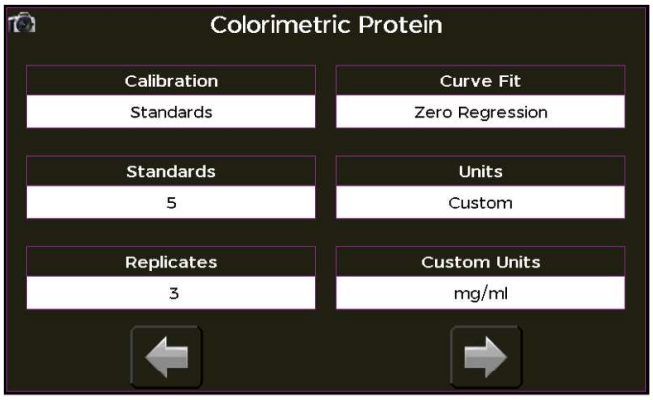
Enter the Colorimetric Protein application under Protein in the Life Science group.



Select the mode for BCA measurements and the Wavelength is set to 562 nm. Integration Time and Sample ID can be set as you wish.

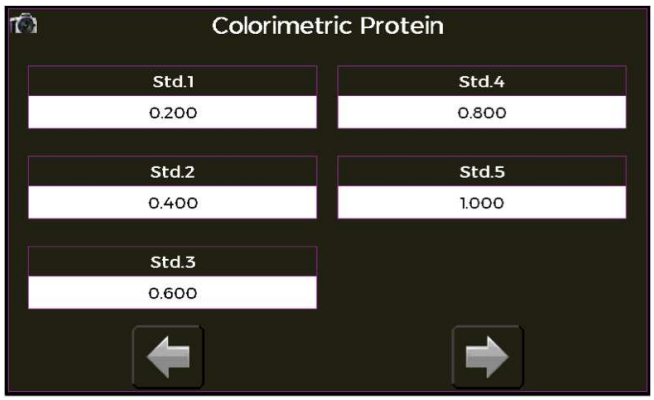
You may advance to the next screen at any time by pressing the forward arrow and return to the previous screen by pressing the back arrow.

PROTEIN APPLICATIONS

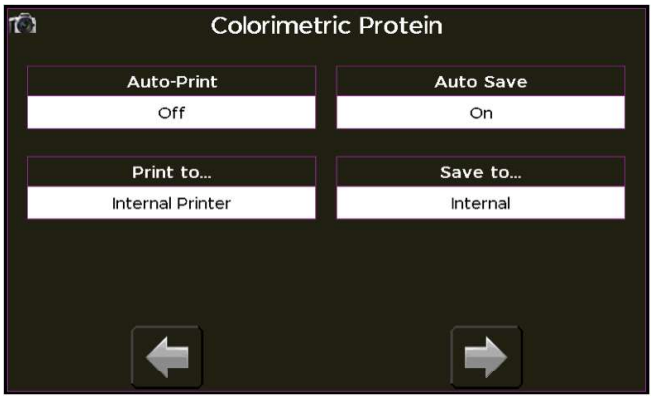


Set Calibration to Standards, Curve Fit to Zero Regression and enter Units of mg/ml, choose Custom for Units and use the alphanumeric entry box. The number of standards and replicates can be set as you wish (for optimum accuracy, it is recommended that the number of standards is ≥4 and replicates is >1).

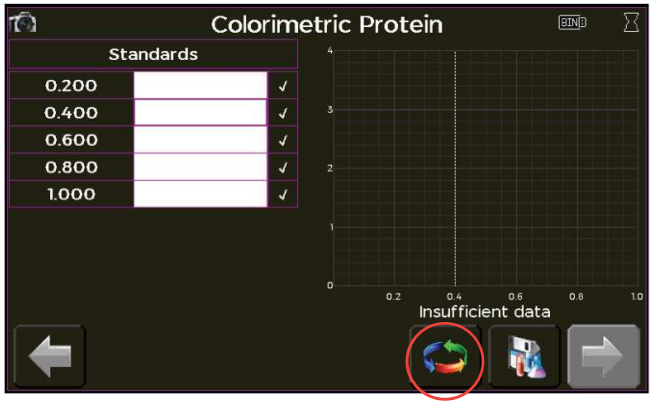
Note: *With Calibration set to Standards, the user is required to prepare and measure standards. With Calibration set to Manual, the user inputs both the standard concentrations and absorbencies.*



Enter the concentration of prepared standards using the numeric keypad.



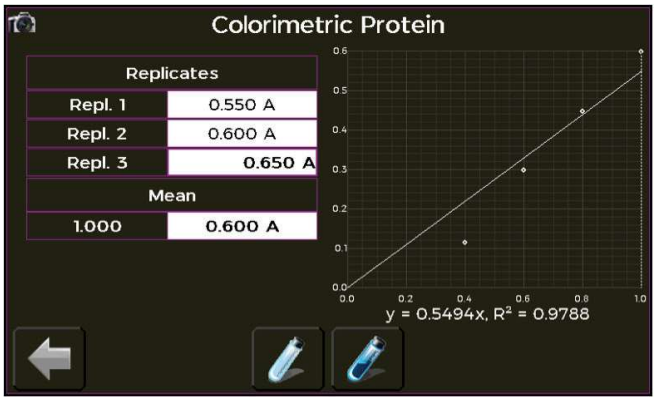
Set the outputs required in your method. For more information see the section Saving and Printing.



To create the standard curve when using replicates, press the Replicates button to take you to the screen shown. With Replicates off standards can be measured directly.

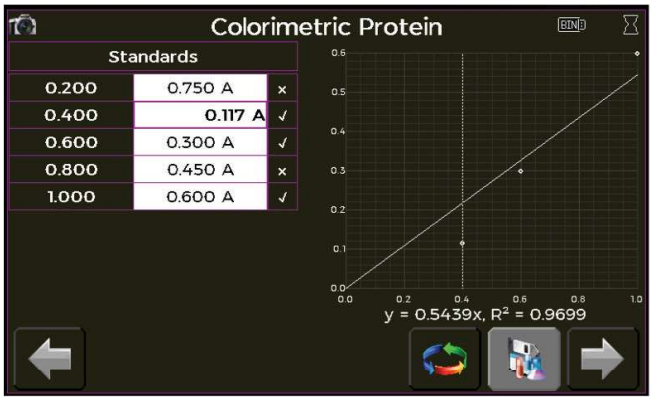
Note: After all replicates have been taken for a standard pressing the replicates icon takes the user to the next standard that was specified in the method.

PROTEIN APPLICATIONS



To create a standard curve, insert the cuvette containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the first standard/replicate in the series and press the Take Measurement button. A single reference suffices for standard curve creation.

Continue recording all standards/replicates until the standard curve has been completed by pressing the forward button after each replicate. After the standard curve has been collected press the back arrow to proceed to the below screen.

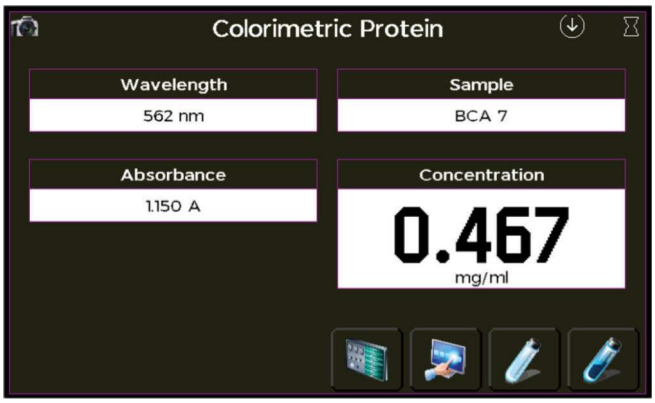


To ignore any outlying standard measurements press the tick in the appropriate row to toggle it to an "X". Any ignored measurement will be automatically removed from the standard curve. These can be reinstated by pressing the cross.

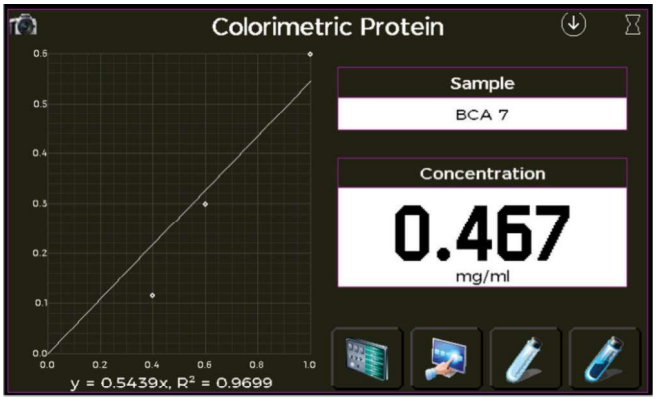
Press the forward arrow to proceed to the sample measurement.

Note: *Pressing the save method icon before any standards have been measured will save the method parameters only. Recalling a method containing method parameters only will require the user to construct a standard curve before measuring samples.*

Taking a Measurement



To perform a measurement, insert the cuvette containing the reference solution in the cuvette holder and press the Take Reference button. Remove and replace with a cuvette containing the sample and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.



To view the standard curve whilst on the sample measurement screen, simply press the 'View Curve' icon that appears under the options menu.

Note: *Saving the method using the Save Method icon that appears on the options menu will save both the method parameters and the standard curve. Recalling a method containing method parameters and standard curve allows the user to measure samples directly.*

Determination of Protein Concentration Using Direct UV Methods

The direct UV method of protein determination has a number of advantages over traditional colorimetric assays, in that it does not rely on an external protein standard and the sample is not consumed in the assay. However, the presence of nucleic acid in the protein solution can have a significant effect due to strong nucleotide absorbance at 280 nm. This can be compensated by measuring A260 and applying the equation of Warburg and Christian for the protein crystalline yeast enolase (Equation 1).

$$\text{Protein concentration (mg/ml)} = (1.55 \times \text{Abs280}) - (0.76 \times \text{Abs260}) \quad \textbf{1}$$

$$\text{Protein concentration} = (\text{Factor 1} \times \text{Abs280}) - (\text{Factor 2} \times \text{Abs260}) \quad \textbf{2}$$

The BioDrop spectrophotometers use default A260 and A280 factors of 0.76 and 1.55, respectively. These factors can be edited so that the equation can be applied to other proteins (Equation 2). Compensation for background, dilution and pathlength can also be entered.

To customize Equation 2 for a particular protein, the A260 and A280 values should be determined at known protein concentrations to generate simple simultaneous equations, which, when solved provides the two coefficients. In cases where Factor 2 is found to be negative, it should be set to zero since it means there is no contribution to the protein concentration due to absorbance at 260 nm.

The A260/A280 ratio also gives an indication of protein purity; a ratio of 0.57 can be expected for pure protein samples.

Background Correction

- To compensate for the effects of background absorbance caused by turbidity, high absorbance buffer solutions and the use of reduced aperture cuvettes, the BioDrop spectrophotometers can use background correction at a 320 nm.
- When used A320 is subtracted from A260 and A280 prior to use so that:

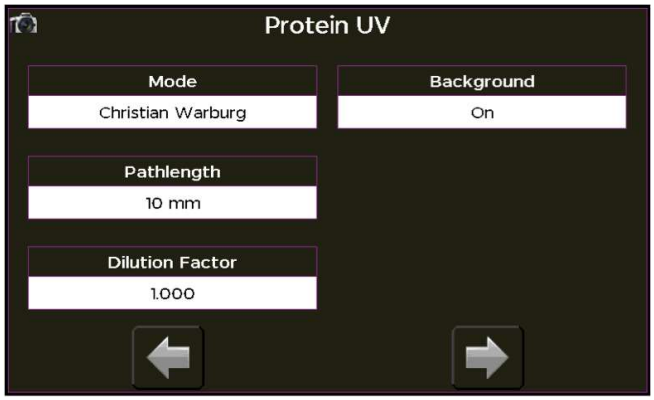
$$\text{Protein concentration} = [\text{Factor 1} \times (\text{Abs 280} - \text{Abs 320})] - [\text{Factor 2} \times (\text{Abs 260} - \text{Abs 320})]$$

$$\text{Ratio} = (\text{Abs 260} - \text{Abs 320}) / (\text{Abs 280} - \text{Abs 320})$$

- The use of background correction can remove variability due to handling effects of low volume disposable cuvettes.

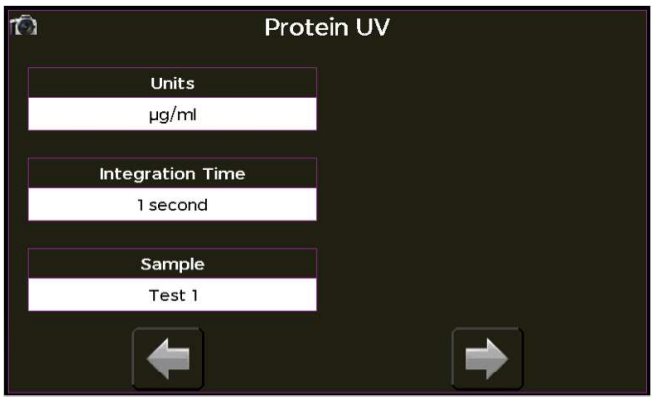
Protein UV

Measurement Parameters

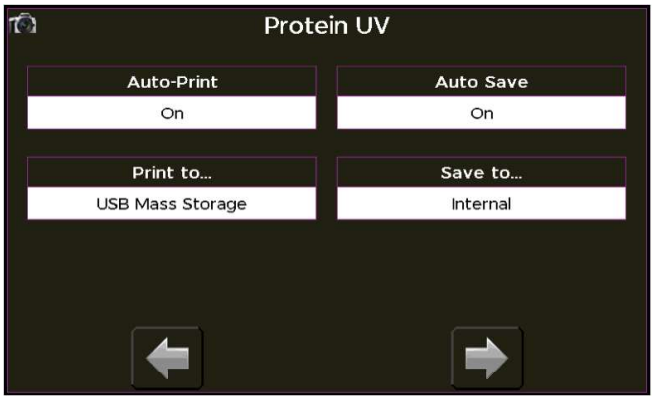


Select the Mode required, the options are Christian Warburg, BSA, IgG, Lysozyme, Molar Extinction, Mass Extinction,E1%, and Custom (for Molar Extinction the user will also be required to enter the molar extinction coefficient, and molecular weight and atomic units for Mass Extinction).

Set Pathlength and Dilution Factor to the required values. Check above to see if background correction is required.

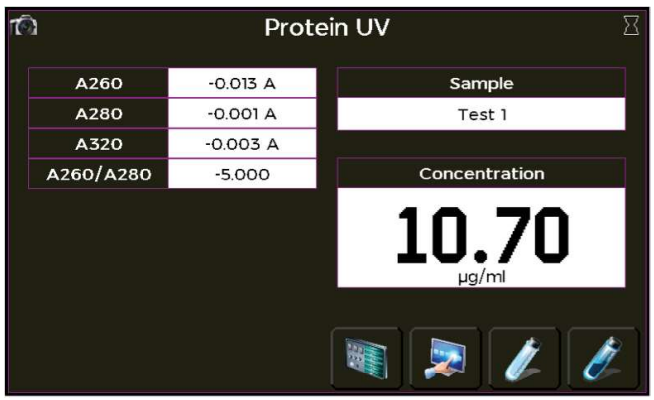


Set Units to encompass the expected concentration of your samples. Integration time can be set as you wish. The Sample Seed entered under Sample will the filename of any saved file.



Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



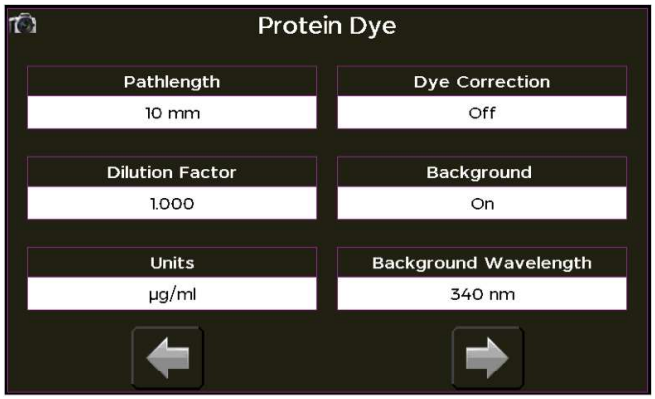
To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series. If Background is set to On, the A320 result will be included in the left hand column and automatically subtracted from the displayed A260, A280 and A260/A280 results. To view the Survey Scan, toggle on the View Scan icon which is accessible within the Options Menu.

PROTEIN APPLICATIONS

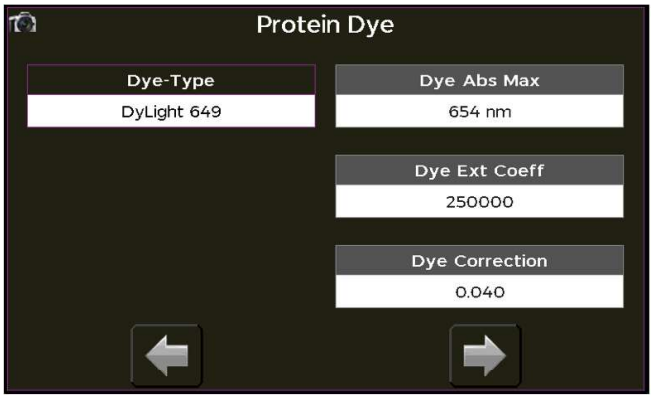
Protein Dye

This application measures the concentration of protein that has been fluorescently labelled. The reported values in this application are absorbance at 260 nm, 280 nm, 340 nm, and the specified dye. Protein and Dye concentration is calculated and displayed, along with the degree of labelling.

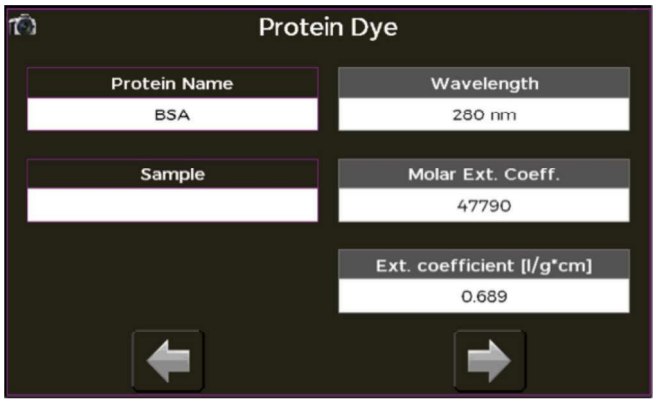
Measurement Parameters



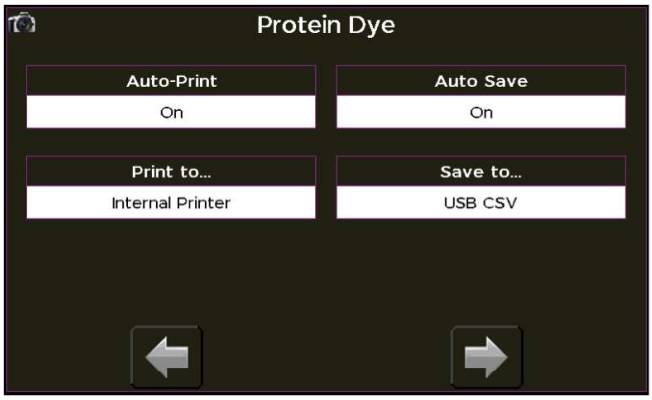
Select the appropriate Pathlength, enter the Dilution Factor if applicable, select Units and choose if Dye Correction is on or off. If the Background is turned On, enter the appropriate Background Wavelength.



Choose the appropriate Dye-Type. If Custom is chosen, Dye Abs Max, Dye Ext Coeff and Dye Correction values can be entered, otherwise they will remain greyed.

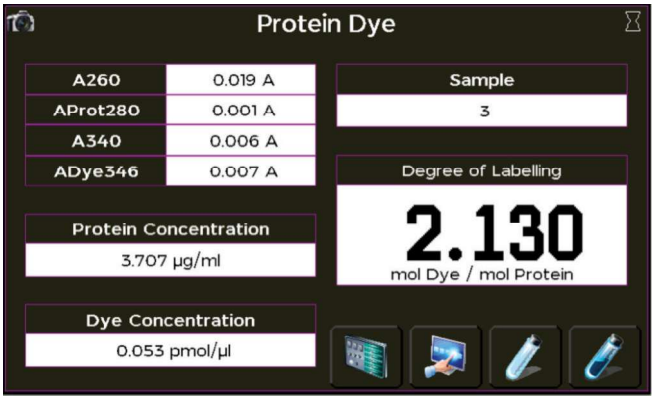


Select your Protein. If Custom is chosen, Wavelength, Molar Ext. Coeff., and Ext coefficient [l/g*cm] values can be entered, otherwise they will remain greyed.



Set the outputs required in your method. For more information see the section Saving and Printing.

Taking a Measurement



To perform a measurement, insert a cuvette (or load directly onto the µLite+ sample port) containing the reference solution in the cuvette holder and press the Reference button. Remove and replace with a cuvette containing the sample (or load sample directly onto the µLite+ sample port) and press the Take Measurement button. A single reference suffices for subsequent analysis in the same series.

SAVING & PRINTING

The BioDrop spectrophotometers allow users to save and print sample data. This can either be included automatically as a method parameter or performed manually from the sample measurement screen.

Saving Sample Data

The BioDrop spectrophotometers allow users to save sample data in three different formats:

Internal

The sample data is saved to the instrument's internal memory format. See the Sample Manager section for details on saving and recalling data from the internal memory.

> ***Note:*** *To ensure the optimum performance of the BioDrop, it is recommended that unwanted data be deleted from the instrument's internal memory at regular intervals.*

USB

The sample data is saved to a USB memory stick in a format that can be read by BioDrop spectrophotometers only. Files in this format cannot be opened by Microsoft Excel or similar programs.

> ***Note:*** *Sample data will be saved to the BioDrop Samples directory on the USB memory stick; if this directory is not present it will be created.*

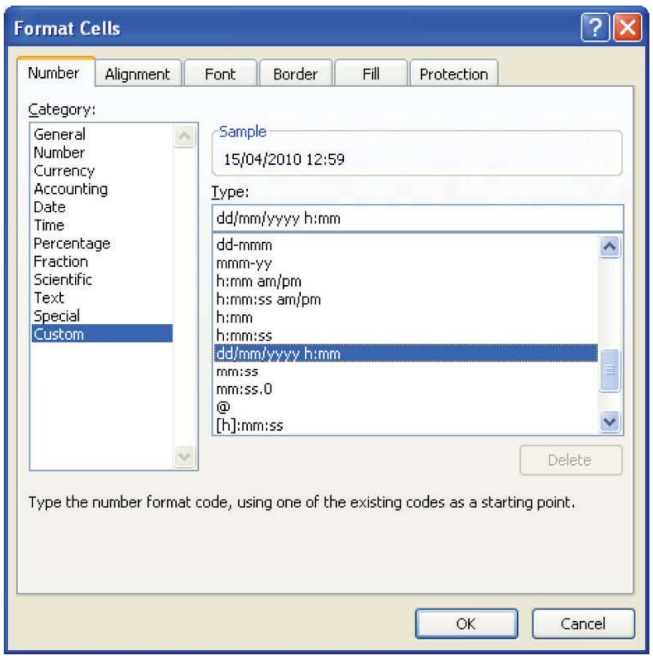
SAVING & PRINTING

USB CSV

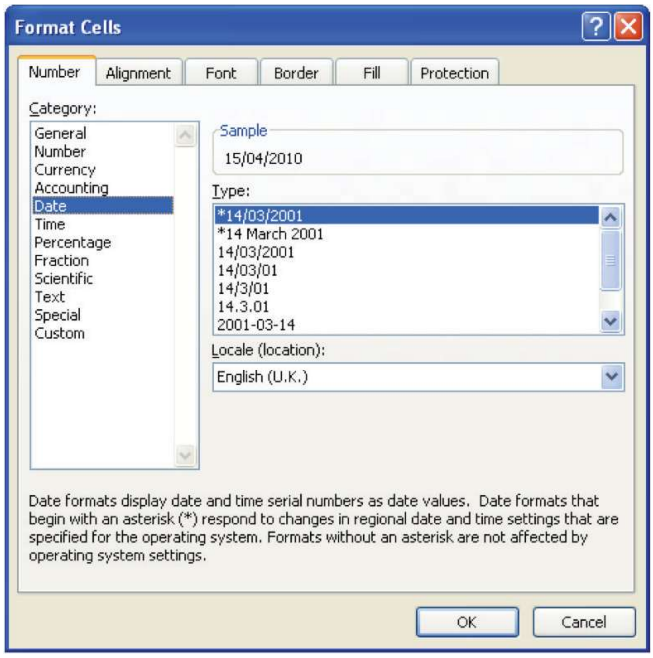
The data is saved to a USB memory stick in comma separated variable (CSV) format allowing it to be opened directly using Microsoft Excel or other similar programs. Files in this format cannot be opened using the instrument.

> ***Note:*** *To view the data display name in a recognizable format, the cells for File Created and Date and Time will need formatting as described below.*

File Created: Right click in the appropriate cell and select Format Cells from the list, select Custom in Category list, select dd/mm/yyyy h:mm in the Type list and select ok.

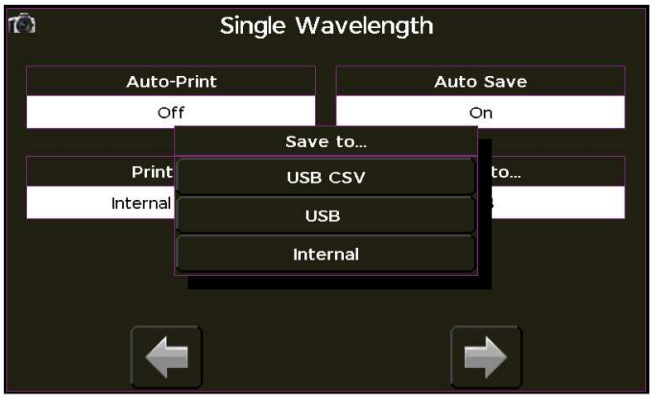


Date / Time: Right click in the appropriate cell and select Format Cells from the list, under Category select Date or Time and the desired format from the list on the right hand side (see below) and select ok.



SAVING & PRINTING

Automatic Saving



The option to save sample data automatically is set under method parameters. With Auto Save set to On, the save location can be set to USB CSV, USB, or Internal (the USB options are only available if a USB memory stick is inserted).

The filename given to an automatically saved file will be either the Sample Seed entered in the method parameters or, if the user chooses not to enter a Sample Seed, Default. The instrument will only save one 'Default' file per application; subsequent saves of files without a sample seed will overwrite the previous default file.

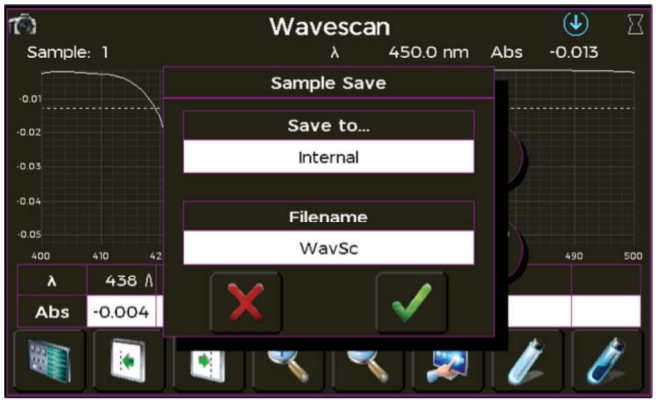
As sample data is saved when the user exits the application, removing a USB memory stick before exiting will result in loss of data.

> ***Note:*** *All files are appended with a unique time and date stamp, it is therefore possible to create two or more files sharing the same name.*

> ***Note:*** *With Sample Overlays ≥2 in Wavescan and in Kinetics, the overlaid data will always be saved automatically to the instrument's internal memory.*

Manual Saving

If a method does not require sample data to be saved each time a measurement is taken, it is possible to manually save sample data in one of the formats outlined above. This procedure is described below:



After collecting all required sample measurements, select Save Sample Data from the options menu on the sample measurement screen to display the dialogue box shown left. The save location and filename are set by pressing the Save To and Filename boxes, respectively. If no Filename is entered the file will be titled Default.

> ***Note:*** *Any sample data saved manually will override the auto save function.*

SAVING & PRINTING

Screen Capture

When USB memory stick is inserted, a camera icon will appear on the top left corner of the instrument. This can be pressed at anytime to capture an image of the current screen and saved to your USB memory stick as a bitmap image.

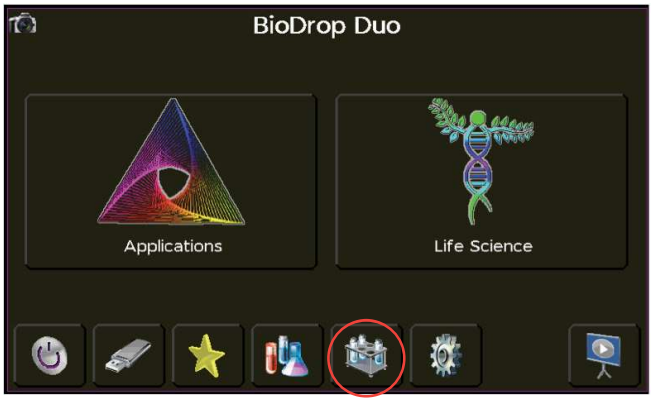
Exporting Data

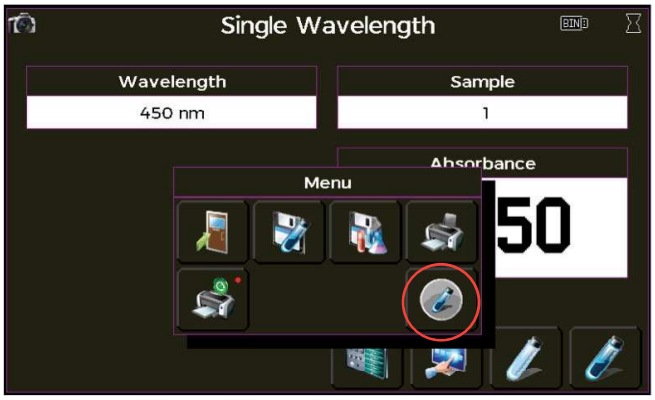
The BioDrop spectrophotometers allow users to recall saved sample data from the internal memory or a USB memory stick and save this in another format. This is done as follows:

Recall saved data using Sample Manager and press the Save Sample Data button on the options menu to display the save sample dialogue box. Set the desired save location and filename using the Save to and Sample Name boxes, respectively and press the check mark to confirm the data export.

SAMPLE MANAGER

Sample Manager is the application used for saving and recalling data from both the instrument's internal memory and the instrument's USB format. Sample Manager can be accessed from either the main screen on the bottom tool bar (below left) or from within an application using the Load Sample icon on the options menu (below right).





To recall a saved file, highlight by pressing on the desired file and press the Sample icon in the right hand corner of the screen. Data will be displayed on the Sample Measurement screen (see Recalled Files below).

With a USB memory stick inserted it is possible to toggle between the internal and USB memories using the icon in the left hand corner of the screen. The location of the displayed data will be indicated by the icon in the top right hand corner.

Sample Manager has been designed to make finding saved files as simple as possible. Therefore, it is possible to arrange files alphabetically, by application or by date/time saved by pressing the column headers Filename, Application and Date, respectively.

If there are too many saved data files to fit on a single screen, this is signified at the top of the screen, e.g. Page 1 of 2. Scrolling through the screens is achieved using the up and down arrows at the bottom of the screen.

Note: *Sample Manager can only display the first 100 sample data files; if the internal memory or USB stick contains >100 files these can be viewed be deleting or moving (USB only) unwanted data.*

Deleting Data from the Internal Memory

To ensure that the internal memory of the instrument does not contain too many unwanted data files, Sample Manager allows you to delete files. This can be done in one of three ways:

Deleting a single file:	Highlight the file for deletion and press the delete button
Deleting multiple files:	Highlight multiple files and press the delete button
Deleting all files:	Press the 'Delete all' icon at the bottom of the screen.

Note: *It is only possible to delete internal data using Sample Manager. Sample Manager does not allow a user to delete sample data files from a USB memory stick; this must be done using a PC.*

SAMPLE MANAGER

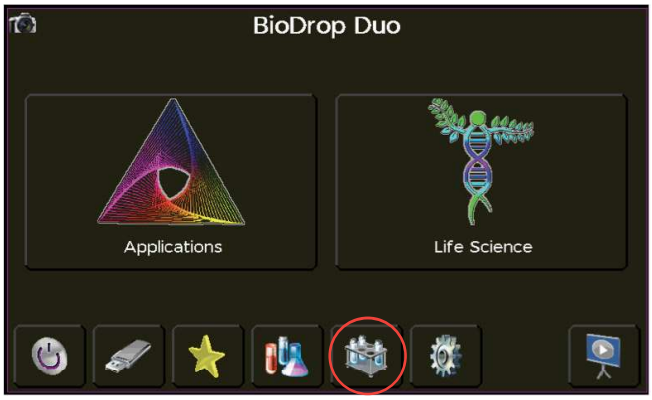
Locking Files

Sample Manager allows the user to lock files saved to the internal memory to prevent the accidental deletion of files containing precious data. To lock files, highlight the required data and press the lock icon at the bottom of the screen. Locked files are signified by the lock icon in the right hand column. Once a file is locked, selecting 'Delete' or 'Delete All' does not clear this data from the instruments' internal memory. To unlock a file, highlight the appropriate locked data and press the lock icon at the bottom of the screen.

> ***Note:*** *As sample data files saved to a USB memory stick must be deleted using a PC, it is not possible to lock USB sample data using Sample Manager.*

To exit from Sample Manager press the 'Exit' icon.

Accessing Sample Manager from the Main Screen



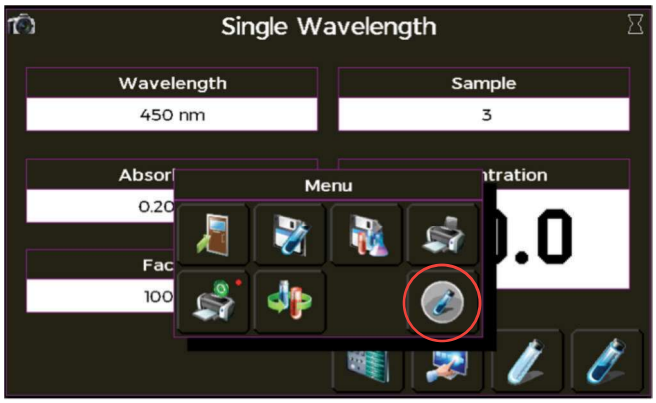
When accessed from the main screen, Sample Manager will display all files held on the internal memory or USB memory stick and allows the user to lock and delete files saved to the internal memory. However this option is disabled for Limited users.

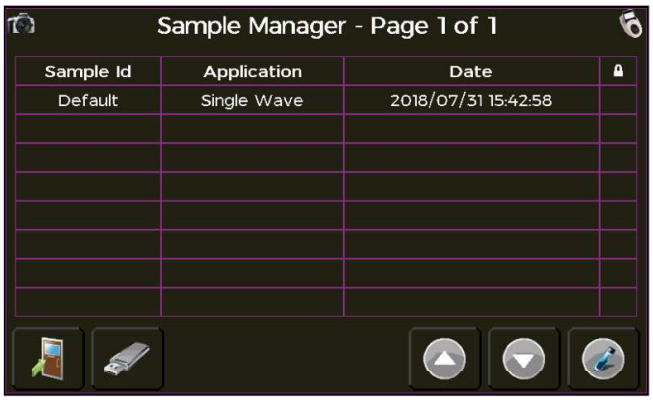
To allow post scan manipulation of saved Wavescan and kinetics data, files loaded from Sample Manager are loaded directly into Trace Manager (see Trace Manager section for details).

> ***Note:*** *With large numbers of files held on the internal memory there may be a short delay before Sample Manager opens.*

SAMPLE MANAGER

Accessing Sample Manager from within an Application

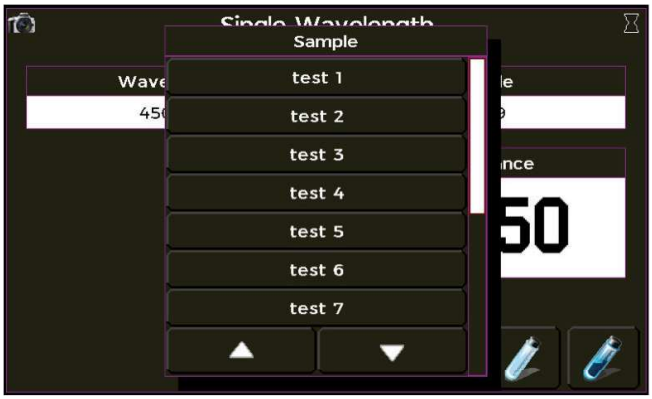




When accessed from within an application, Sample Manager will only display files belonging to that specific application and only allows users to recall saved data and lock files.

Recalled Files

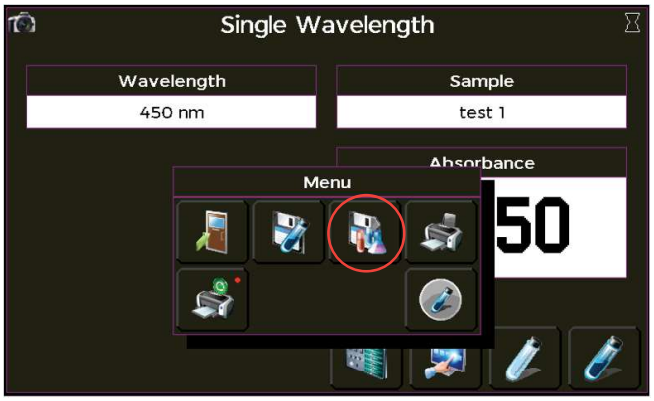
When recalled, sample data files will display the first sample recorded in a measurement. To access all sample data within a recalled file, press the Sample box to display a list of all samples contained within the file. Pressing on the desired file will populate the boxes on the sample measurement screen with the saved data. Choosing to measure another sample with an old sample's data displayed simply updates the sample measurement screen and the appropriate boxes.



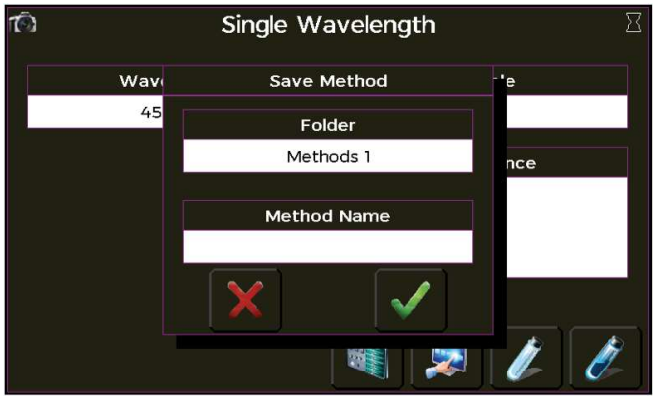
Note: *Wavescan and kinetics sample data is recalled using Trace Manager. For details see the Trace Manager section for details.*

SAVING METHODS

Methods can be stored to both the instruments internal memory and to USB memory sticks. The procedure for saving methods is described below:



After selecting the desired application and setting the required method parameters select the Save Method icon from the options menu on the sample measurement screen. Set the desired file name and save location using the dialogue box shown below.

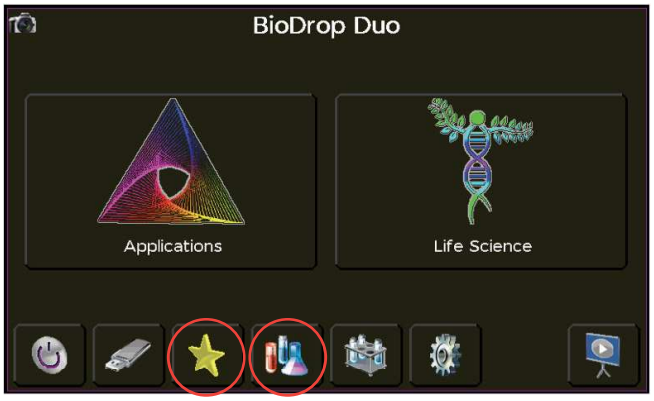


Pressing the Folder box produces a list of available save locations, choose one. USB will only appear on the list if a USB memory stick is inserted.

Pressing the Method Name box allows the user to set the desired method name using alphanumeric text entry.

Press the check mark to save and exit or the “X” to exit without saving.

Methods Saved to the Internal Memory



Methods saved to the instrument’s internal memory are stored in either the Methods or Favourites folders, both of which are accessible via the toolbar located at the bottom of the screen. The BioDrop spectrophotometers are capable of storing up to 90 methods on the instrument’s internal memory.

SAVING METHODS

Methods Folder

The Methods folder is made up of 9 folders, each capable of storing up to 9 methods. The method folder icons have been designed to give the user an indication of the number of methods that are stored in that folder.

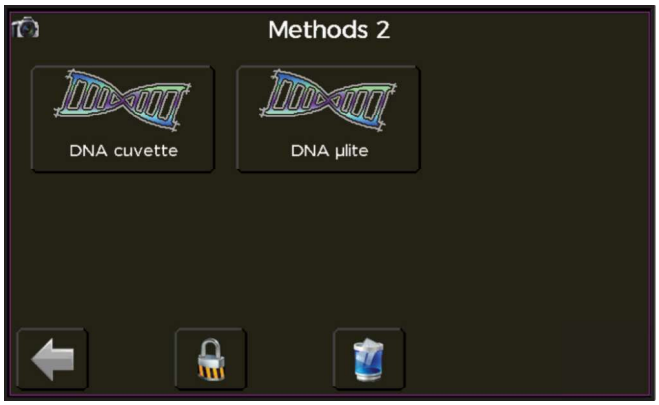
Renaming Method Folders

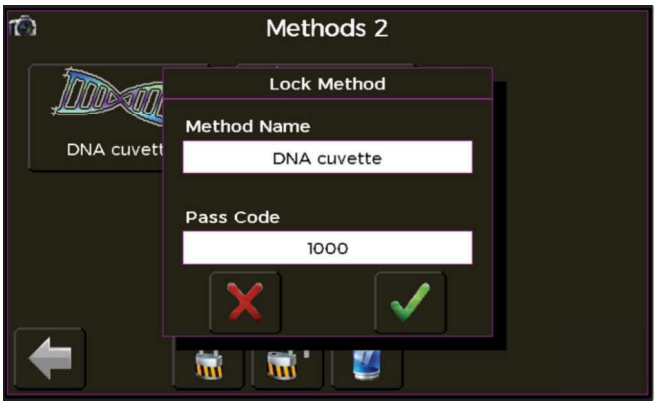
Method folders can be renamed using the Edit Icon located in the toolbar at the bottom of the screen.

Locking Saved Methods

Method folders can be locked using the lock icon located in the toolbar at the bottom of the screen. Choose the method to lock and add a pass code to protect the method folder. Locked folders cannot be renamed and are indicated by a padlock.

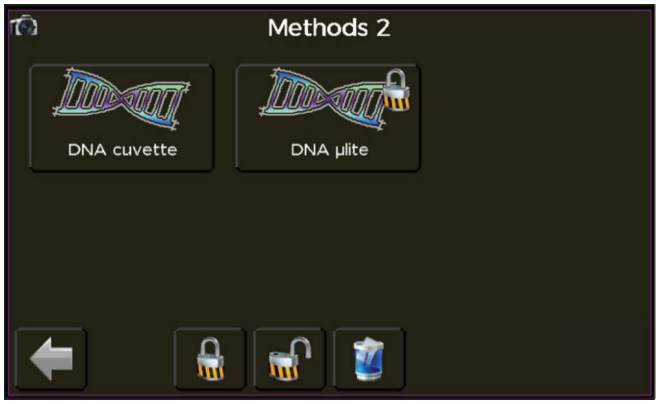
SAVING METHODS

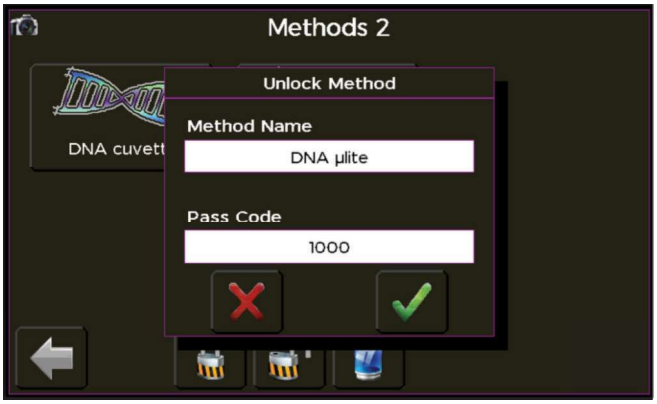




Within a methods folder, it is possible to add pass codes to individual methods and to lock them from deletion using the lock icon located in the toolbar at the bottom of the screen.

Choose the method to lock and add a pass code to protect the methods. Locked methods cannot be renamed and are indicated by a padlock.

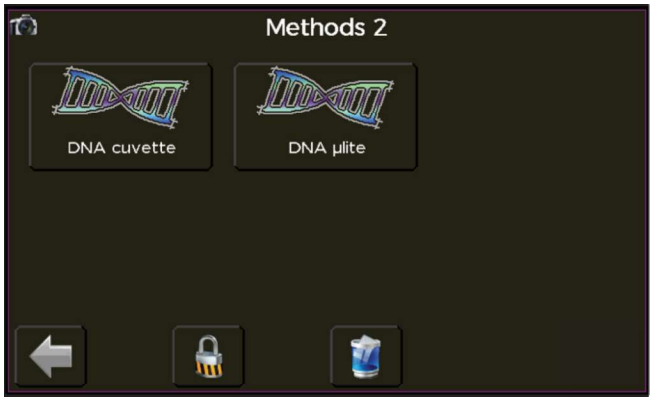




Locked methods can be unlocked by using the unlock icon, also located in the toolbar at the bottom of the screen. selecting the desired method and entering the correct pass code.

SAVING METHODS

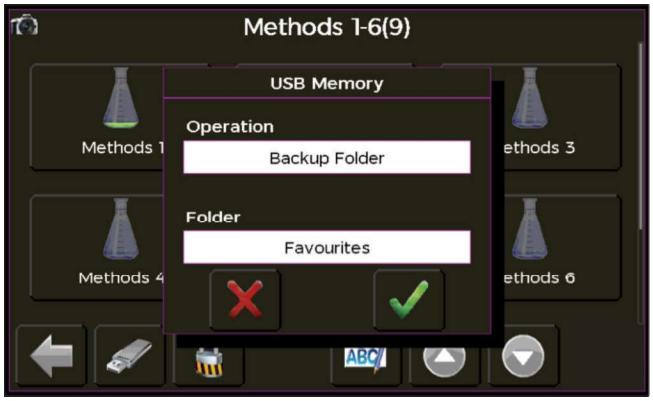
Deleting Saved Methods



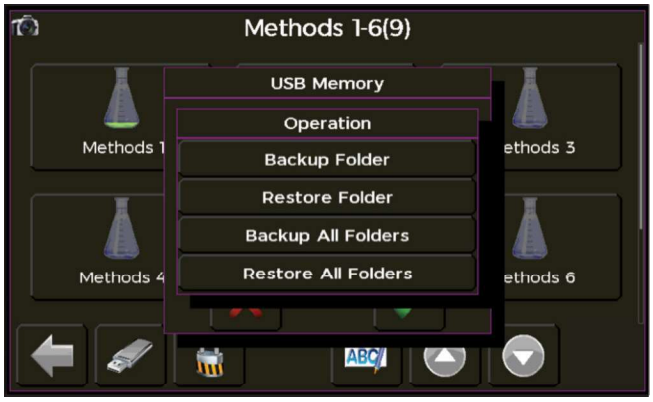
Within a methods folder, it is possible to delete saved methods using the Delete icon located in the toolbar at the bottom of the screen and selecting the desired file.

Note: *Locked methods must be unlocked to allow deletion.*

Backing Up Method Folders to USB



With a USB stick inserted it is possible to use the USB icon located in the toolbar at the bottom of the screen



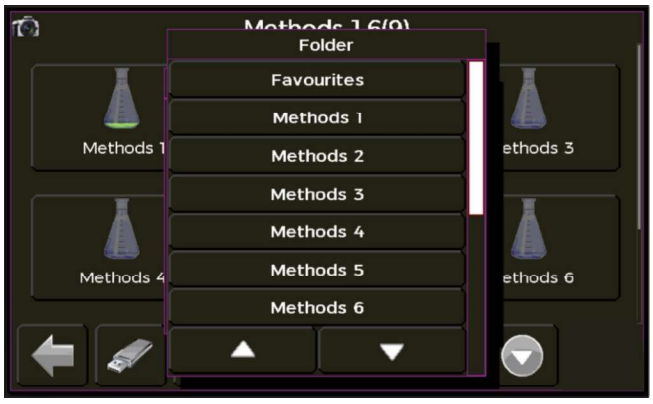
Choose the type of operation:

Backup Folder: Copies all methods from a specified folder to a USB memory stick

Restore Folder: Copies a backed up method folder from the USB memory stick to the internal memory

Backup All Folders: Copies all method folders from the internal memory to a USB memory stick

Restore All Folders: Copies all backed up method folders from the USB memory stick to the internal memory



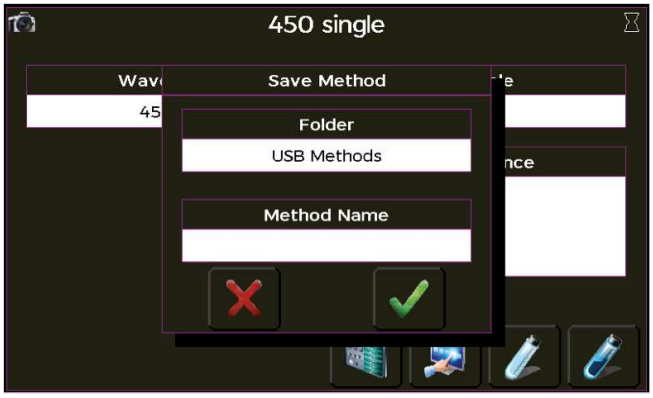
Choose the folder to have your methods uploaded to.

SAVING METHODS

Favourites Folder

The Favourites folder is capable of storing up to 9 user defined methods. Methods stored in the Favourites folder can be locked and deleted as described above. Select the Favourites Icon (shown below) located in the toolbar to access the favourites folder.

Saving Methods to USB



To save files to a USB memory stick, follow the procedure described above and select USB in the Folder box. The USB option will only appear in the list if a USB memory stick is inserted.

Methods saved to a USB memory stick will appear in the BioDrop Methods folder in the root directory.

Note: *Although it is possible to save an unlimited number of method files to a USB memory stick, only 9 can be displayed on the instrument at any one time. As these will only be read from the BioDrop Methods folder, additional files can be stored in other locations.*

PRINTING

Printing Sample Data

The BioDrop spectrophotometers allows users to print sample data in the following ways:

> ***Note:*** *Only available printers will be shown in the Print to… options box.*

Internal Printer

Data can be printed to the built in printer where fitted. Data is printed with method header, instrument serial number, time/date and all sample results. If numerical data is being shown on the display, only this data will be printed, if graphics are displayed on the screen these will be printed as well as numerical data.

The built in printer is available as an accessory and can easily be fitted to existing instruments — see instructions on page 77.

Print Via Computer (PVC)

Print via Computer (PVC) is an application running under Microsoft Windows™ to enable the instrument to transfer data into a PC environment. From there, the data can be printed or saved in a variety of formats, including graphics and text formats or as an Excel™ file. PVC can store data to a common directory or be configured to save to independent directories by both file format and instrument serial number.

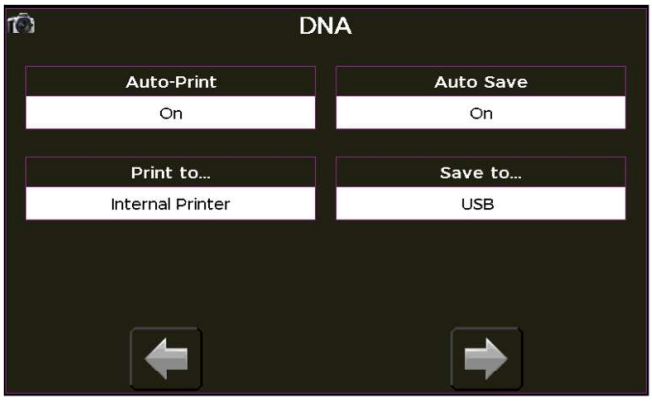
PVC is capable of supporting several instruments simultaneously, limited only by hardware and the speed of the host system and operates via USB cable.

Installation and operating instructions for PVC can be found on the media supplied for the respective spectrophotometer.

USB Mass Storage

Requires a USB memory stick to be installed in the side port for this option to be available. Data can be sent directly to a USB memory stick as a .pvc file and can be viewed using PVC software.

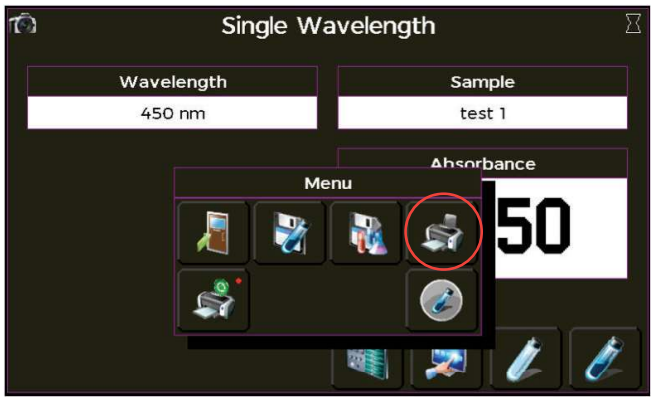
Automatic Printing



The option to print sample data automatically is set under method parameters. With Auto Print set to On, the print location can be set to one of the available options as described above.

PRINTING

Manual Printing



If a method does not require sample data to be printed each time a measurement is taken, it is possible to manually print sample data.

After collecting all required sample measurements select the Print icon from the options menu on the sample measurement screen.

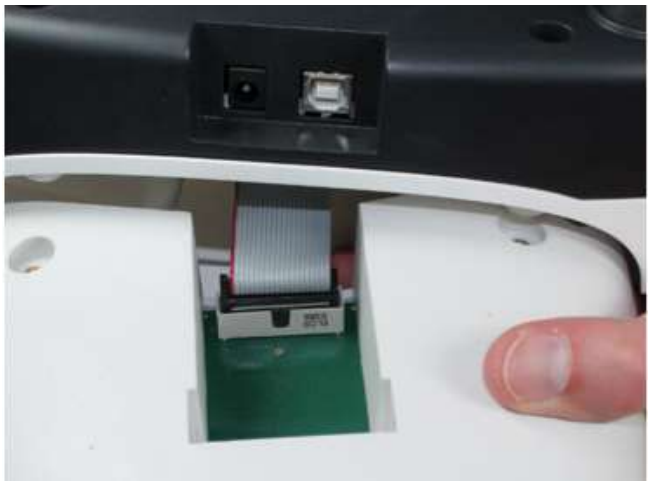
Auto print can also be enabled \ disabled by pressing the auto print button.

BUILT IN PRINTER

Installation Guide

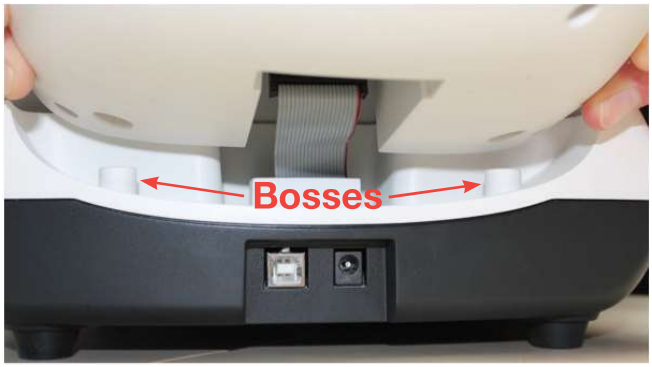
1. Turn the instrument over and place on a soft surface. Remove screws from positions A and B.

   Turn the instrument back over and lift the accessory cover vertically upwards to remove. Remove the tie-wrap from the printer cable.

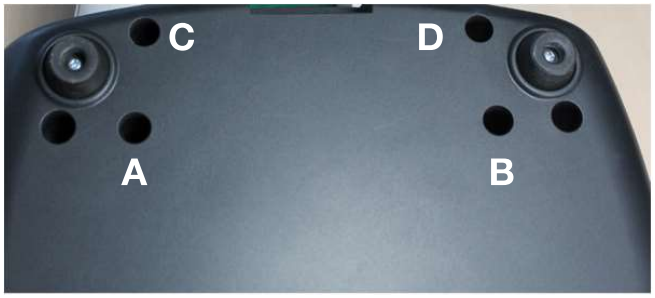


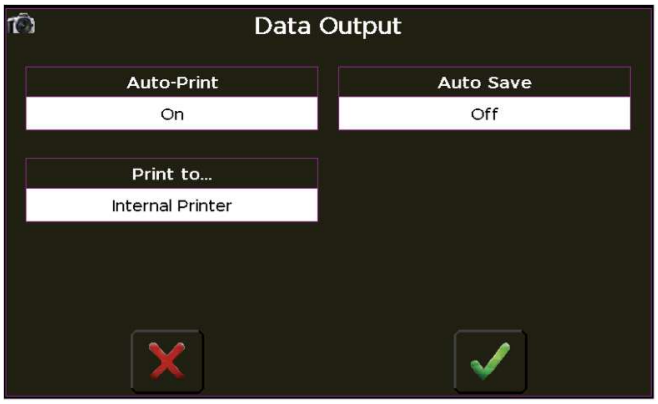
2. Plug the accessory cable into the printer, noting the alignment lug.

3. Place the accessory cover on top of the printer and then lower the printer onto the locating bosses and push down firmly.



4. Invert the instrument and replace the accessory cover screws at A and B and add the printer mounting screws at positions C & D.





Set the built in printer by pressing Settings on the main screen in the bottom toolbar and Data Output icon.

BUILT IN PRINTER

Refilling the Printer Paper

1. Place hands on the side of the printer cover and gently lift off the paper cover.

2. Place paper roll into printer with paper feeding from the bottom. Feed the paper into the slot by turning the green knob clockwise.

3. Replace paper cover.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE
Negative absorbance readings	• Sample measurements will be negative absorbance reading if the absorbance value of the reference is higher than the sample. • Negative readings can also result if reference and sample are inter-changed or if the sample is very dilute and close to the absorbance of the reference. • Contact your supplier for advice on the minimum concentrations that can be measured.
Unexpected results	• Bubbles or contamination in the sample or reference can result in considerable errors. If using BioDrop CUVETTES check using the bubble viewer provided. • Incorrect cuvette orientation. Rotate by 90˚ and repeat. • Incorrect cuvette material for UV measurement wavelengths. • Wrong path length selected in software. • For Duo+ models, sample placed in cell holder and on micro-volume sample platform at the same time.
Absorbance higher than expected	• Incorrect sample reference. • Incorrect cuvette orientation. • Incorrect cuvette material for measurement wavelengths. • Wrong path length selected in software. • For Duo+ models, sample placed in cell holder and on micro-volume sample platform at the same time. • Contamination in sample or on cuvette. • For DNA applications check 320 nm background, if higher than 0, select background correction in method set up. • Possible incorrect optical alignment. Contact technical support.
Absorbance lower than expected	• Incorrect sample reference. • Check sample and reference for contamination. • Check sample and reference samples are not the same. • Incorrect cuvette material for measurement wavelengths. • Wrong path length selected in software. • For standard cuvettes, ensure the beam goes through the sample (fill cuvette with sample to 20 mm from the base). • For micro-volume sample platform check size and position of droplet. • For DNA applications check that the measurements at 230 nm and 320 nm are near 0. • Possible stray light issue. Contact technical support.
Poor reproducibility	• Insufficient sample in cuvette. • Cuvette in wrong orientation. • Cuvette material unsuitable for wavelengths used. • Concentration of sample too low or too high. For best results the measured sample absorbance using a 10 mm path length cuvette should ideally be between 0.1 and 2.0 A. If absorbance is >2 A, measurement is no longer in the linear range. • Particulates in sample. Absorbance measurements will not be accurate with turbid samples. • Possible noise or measurement stability issue. Contact technical support.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE
Instrument start up reported failure	• Check all sample paths are clear and clean — dried on DNA/Protein sample on the Micro-volume head on the Duo+ and µLite+ units may cause start up calibration errors. • Check original 18V dc supply is connected and is fully engaged. • Report persistent failures to technical support.
Absorbance readings stable but different than expected	• Check that the Absorbance displayed is being normalized to a path length of 10 mm if a micro-volume device (like BioDrop CUVETTE) is used. • Note that with a 0.5 mm path length the ideal measurement range becomes equivalent when normalized to 2A to 50A and for a path length of 0.125 mm it becomes 8A to 200A. • For unresolved Absorbance issues contact technical support.

CONTACTS, TECHNICAL SUPPORT, SERVICE, REPAIR OR RETURN

If you have any problems using your instrument in the first instance please refer to the trouble shooting guide. If you require further assistance, please contact us for assistance.

Web	www.biodrop.co.uk
Telephone (US)	+1-508-893-8999
Telephone (UK)	+44 (0) 1223 423723
General Enquiries	enquiries@biodrop.co.uk
Technical Support	support@hbiosci.com

TECHNICAL SPECIFICATIONS

PARAMETER	BioDrop Duo+	BioDrop µLite+
Display	7" colour display with capacitive touch panel	
Configuration	Split Beam	
Lamp	Pulsed Xenon lamp with 3 year warranty	
Sample Compartment	Cuvette port and micro-volume sample port	Micro-volume sample port
Pathlength	10 mm cuvette port; 0.5 mm micro-volume sample port	0.5 mm micro-volume sample port
Wavelength Range	190 nm-1100 nm	
Wavelength Accuracy	±2 nm	
Wavelength Reproducibility	±1 nm	
Spectral Bandwidth	5 nm	
Stray Light	<0.5%T @ 220 nm NaI, <0.5%T @ 340 nm $NaNO_2$	
Photometric Range	-0.3A to 2.5A, 0 to 199%T	
Photometric Accuracy	±0.01A + 1.5% of the reading @ 546 nm	
Photometric Reproducibility	±0.003A (0 to 0.5A), ±0.007A(0.5 to 1.0A)	
Noise	0.005A peak to peak, 0.002A RMS	
Power Input	120 to 240V~ 50/60Hz 40VA Max	
Dimensions	Height 190 mm x Width 280 mm x Depth 410 mm (421 mm with printer)	
Weight	Approx. 3.55kg (4kg with printer)	
Software	Resolution Software (included)	
Life Science Applications	DNA, RNA, Oligo, Fluorescent Dye, T_m Calculation, Protein Dye, Protein UV and Colorimetric protein methods	
Applications	Single Wavelength, Concentration, Wavescan, Kinetics, Standard Curve, Substrate, Equation Editor	
Languages	English, French, German, Spanish, Italian, Japanese, Chinese	

TABLE OF ICONS

ICON	TITLE	FUNCTION
	Back Arrow	Returns the user to the previous screen.
	Forward Arrow	Advances the next screen in a sequence.
	Open Options Menu	Displays the relevant options menu, the exact content of the menu will depend upon the application.
	Page Down	Allows the user to navigate to the next page.
	Page Up	Allows the user to navigate to the previous page.
	Cursor Left	Used in the wavescan and kinetics applications to move the cursor left. The position of the cursor and the corresponding x and y values are displayed above the scan.
	Cursor Right	Used in the wavescan and kinetics applications to move cursor right. The position of the cursor and the corresponding x and y values are displayed above the scan.
	Toggle Data Viewed / Reset	When used in the Fluorescent Dye in the Nucleic Acids application, this toggles the data displayed between sample and dye data. Under Instrument Settings, this resets a value.
	Delete All	Deletes all saved data/methods. Delete all is a two stage process.
	Delete	Deletes the highlighted data/method. Delete is a two stage process.

TABLE OF ICONS

ICON	TITLE	FUNCTION
	Thresholds	Used in Equation Editor. This allows users to add thresholds (pass/fail) limits to their results.
	OK / Accept	Used to confirm / accept any changes.
	Cancel	Exits from an application or screen. If cancel is selected without saving, any changes will be lost.
	Lock	Locks sample data file, method folder and methods against accidental deletion.
	Unlock	Unlocks a locked sample data file, method folder and method.
	Instrument	Used in Sample Manager to access sample data files stored on the instrument's internal memory or to indicate that the data being displayed is from the internal memory.
	Linear Section	Used in the kinetics applications to view a line of best fit on the scan. The red dot will turn green when being used. It can be toggled on and off.
	Section	Used in kinetics applications to view data in a specific section. It is possible to add t0 through to t7. The red dot will turn green when being used.
	Method Folder	Methods saved internally can be stored in one of nine method folders (each capable of storing up to nine individual methods).
	Rename Method Folder	Accessed on the methods screen, this allows the method folder to be renamed.

TABLE OF ICONS

ICON	TITLE	FUNCTION
	Toggle View Scan On or Off	Used in the nucleic acid applications DNA, RNA and Oligo. This allows the user to view a survey scan of the last sample run (in the region 220–320 nm). The red dot will turn green when being used.
	Toggle Auto Print On or Off	Accessed via the options button on the sample screen. The red dot will turn green when being used.
	View Method Parameters	Accessed on the sample measurement screen. Pressing this button takes the user back to the method parameters.
	Print Data	Accessed via the options button on the sample measurement screen. Pressing this button prints the sample data.
	Save Method Parameters	Accessed via the options button on the sample measurement screen. This allows a method to be saved to a location specified by the user.
	Save Sample Data	Accessed via the options button on the sample measurement screen. This allows sample data to be saved to a location specified by the user.
	Load Sample	Accessed via the options button on the sample measurement screen. Displays the sample data held in either the internal memory or on a USB memory stick.
	Instrument Information	Accessed via instrument settings. This displays instrument information such as product name, serial number etc.
	Instrument Settings	Accessed via instrument settings. This allows the user to set the default bandwidth, save new baselines and view the date of the last service.
	Lamp Settings	Accessed via instrument settings details of the lamps.

TABLE OF ICONS

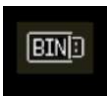
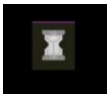
ICON	TITLE	FUNCTION
	Instrument Reset	Accessed via instrument settings. User has the option to reset the instrument and delete all samples, users, and methods.
	Auto Print — Internal Printer	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to the internal printer.
	Auto Print — USB Mass Storage	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to a USB memory stick.
	Auto Print — PC via USB	Displayed on the status bar. This indicates that the instrument will automatically print all sample data to PVC via USB cable.
	Auto Save — USB CSV	Displayed on the status bar. This indicates that the instrument will automatically save all sample data to the USB memory stick as a csv file.
	Auto Save — USB	Displayed on the status bar. This indicates that the instrument will save sample data to a bin file that can be opened by the instrument.
	Auto Save — Internal	Displayed on the status bar. This indicates that the instrument will automatically save all sample data to the internal memory.
	Xenon Lamp Failed	Displayed on the status bar. This indicates that the xenon lamp has failed.
	Instrument Busy	Displayed on the status bar. This indicates that the instrument is performing a measurement.
	Printing to Internal Printer	Displayed on the status bar. This indicates that the instrument is printing to the internal printer.
	Printing to USB Mass Storage	Displayed on the status bar. This indicates that the instrument is printing to the inserted USB memory stick.

TABLE OF ICONS

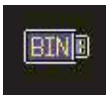
ICON	TITLE	FUNCTION
	Printing to PC via USB	Displayed on the status bar. This indicates that the instrument is printing to PVC via USB cable.
	Saving to USB as CSV File	Displayed on the status bar. This indicates that the instrument is saving as a CSV file to USB memory stick.
	Saving to USB Memory as Bin File	Displayed on the status bar. This indicates that the instrument is saving sample data to a bin file that can only be opened by the instrument. These may be loaded via sample manager.
	Saving to Internal Memory	Displayed on the status bar. This indicates that the instrument is saving the sample data to the internal memory.
	View Graph	When used in standard curve applications i.e. Lowry protein assay, this toggles the sample measurement screen to display or hide the standard curve.
	Replicates	Used in standard curve applications to collect data from a group of replicates.
	Take Sample Measurement	Commences a sample measurement. A reference measurement must be taken prior to a sample measurement
	Take Reference Measurement	Commences a reference measurement.
	Backspace / Delete	Used in text entry mode to move the cursor backwards (left) and delete any unwanted characters.

TABLE OF ICONS

ICON	TITLE	FUNCTION
	Symbols	Selects symbols when in text entry mode.
	Accented Characters	Selecting this icon will display accented characters for use when in text entry mode.
	Toggle Case	Toggles between lower case and upper case letters when in text entry mode.
	Trace Manager	Used in the wavescan and kinetics applications. This allows the user to overlay up to 8 samples data files and to choose what type of data is displayed i.e. raw data, smoothed data, 1st derivative.
	USB Memory Stick	Used on the Sample Manager screen to access data stored on a USB memory stick. Used in the options menu in the methods folder to allow the user to backup methods to a USB memory stick.
	Add User	Displayed under User Access, this allows anyone with Administrator privileges to add another user to the instrument.
	Delete User	Displayed under User Access, this allows anyone with Administrator privileges to delete users from the instrument.
	Edit User	Displayed under User Access, this allows anyone with Administrator privileges to edit currently users' parameters.
	Zoom In	Used in the wavescan and kinetics applications. This allows the user to zoom into a specific region of a scan.
	Zoom Out	Used in the wavescan and kinetics applications. This allows the user to zoom out and return to the original scan.
	Favourites	Located on the toolbar at the bottom of the screen. Where most commonly used methods can be saved for quick and easy access.

TABLE OF ICONS

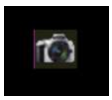
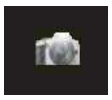
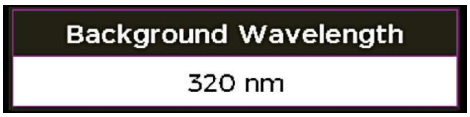
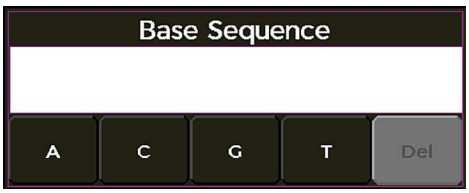
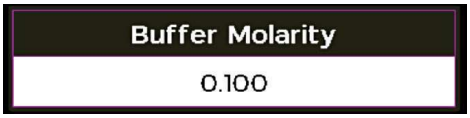
ICON	TITLE	FUNCTION
	Methods	Located on the toolbar toolbar of the menu screens at the bottom of the screen. Where stored methods are saved for quick and easy access.
	Sample Manager	Located on the toolbar at the bottom of the screen. A quick and easy access to view saved samples.
	Settings	Located on the toolbar at the bottom of the screen. A quick and easy access to instrument settings.
	Screenshot	To save a bitmap image of the screen to the USB memory stick which must be inserted for the icon to be displayed.
	Camera Taking A Screenshot	Displayed on the status bar. This indicates that the instrument is taking a screenshot to the USB memory stick.
	Exit	Exits the application or screen without saving any changes.
	Rerun Standard Button	Available in the options menu when running in Standard Concentration mode and the application status is Sample or Standard. Pressing the button causes the Run Standard dialog to be displayed.
	Open Trace Selector	Located in Wavescan and Kinetics at the right-hand side of the graph. Opens the trace selector control panel.
	Open Trace Hide	Located in Wavescan and Kinetics at the right-hand side of the graph. Opens the trace hide panel.
	Open Trace Delete	Located in Wavescan and Kinetics at the right-hand side of the graph. Opens the trace delete panel.
		When the trace selector control panel is open, traces are identified by a line of the same color. Selected trace is shown in the down or checked position. Only one trace can be selected at any given time. Where there is no trace available, or the trace is hidden, the button is greyed out.

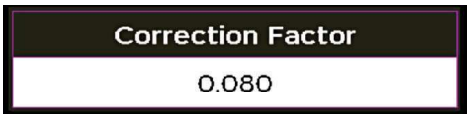
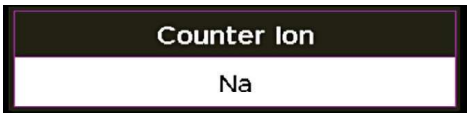
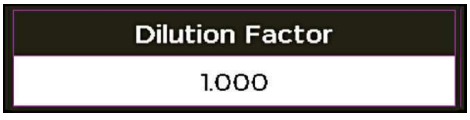
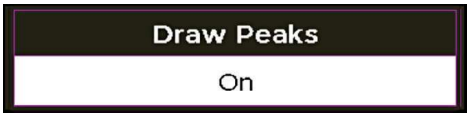
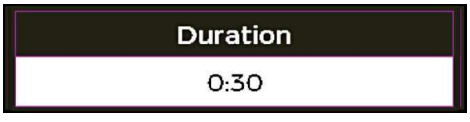
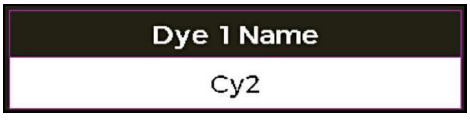
TABLE OF ICONS

ICON	TITLE	FUNCTION
		When the trace hide control panel is open, traces are identified by an eye icon of the same color. Hidden traces are identified as a button in the down or checked position. Multiple traces can be hidden at the same time, hiding a selected trace causes the nearest visible trace to be selected. Where there is no trace available the button is greyed out.
		When the trace delete control panel is open, traces are identified by a bin icon of the same color. Where there is no trace available the button is greyed out.

GLOSSARY OF CONTROLS

BOX	FUNCTION
	Toggles between on and off. Used in all applications to set whether sample data is printed automatically or not.
	Toggles between on and off. Used in all applications to set whether sample data is saved automatically or not.
	Can be toggled on and off. Used in the life science application measurements to subtract the absorbance value at 320 nm. This is done to allow for the effects of turbidity, high absorbance buffer solutions and the use of reduced aperture cuvettes. Note, absorbance wavelength can vary.
	Set by numeric entry. Used in Fluorescent Dye measurements only and enables the user to specify the wavelength of background correction.
	Used in Tm calculation to set the sequence of bases. The bases A, C, G, T can be added in DNA mode and the bases A, C, G, U can be added in RNA mode. Bases are grouped in threes to improve readability.
	Used in Tm calculation to toggle between DNA and RNA base types.
	Used in User Interface in Settings to set the brightness of the screen.
	Numeric entry, used in Tm calculation only. Buffer molarity = buffer molarity + total molarity of salt (moles / L).

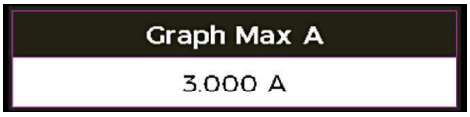
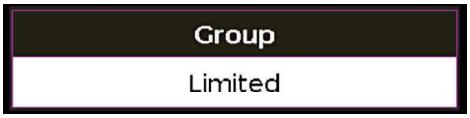
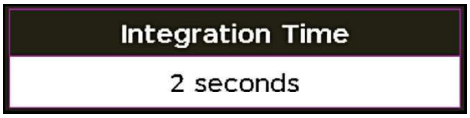
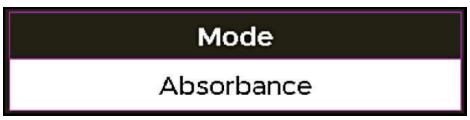
GLOSSARY OF CONTROLS

BOX	FUNCTION
	Used in all standard curve applications to set the method used to collect the standard curve. Options are for Standards or Manual.
	Set by numeric entry, used in the Fluorescent Dye application only. This is the correction factor applied to the absorbance of the dye at a specified wavelength.
	Used in Tm calculation only and allows the user to add the type of counter ion used. The options are sodium (Na), potassium (K), triethyl-ammonium (TEA) or other. If other is selected, the molecular weight of this counter ion must be added using Other MW.
	Used in all standard curve applications this is the curve fit that will be applied to the standards' absorbance values. Options are Zero Regression, Regression and Substrate, Interpolation and Cubic Spline.
	Alphanumeric entry for custom dye name, used in Fluorescent Dye application only.
	Numeric entry. This is the delay required before a kinetic and substrate measurement commences.
	Numeric entry. This is used in nucleic acid and protein applications to compensate for the absorbance of highly concentrated samples.
	Used in wavescan only, this switches display of peak cursors on and off. Peak cursors show vertical dashed lines displaying the measured peak height and horizontal dashed lines showing the peak width.
	Numeric entry. This is the duration over which a kinetic and substrate measurement is performed.
	Used in Fluorescent Dye application only. This is the first dye type used in the measurement.

GLOSSARY OF CONTROLS

BOX	FUNCTION
Dye 2 Name Alexa Fluor 350	Used in Fluorescent Dye application only. This is the second dye type used in the measurement (where applicable).
Dye Abs Max 346 nm	Used in Protein Dye, and if Dye-Type of Custom is chosen, set the maximum absorbance wavelength for the dye.
Dye Ext Coeff 19000	Used in Protein Dye, and if Dye-Type of Custom is chosen, set the Ext Coeff for the dye.
Dye Correction On	Used in Protein Dye, it corrects the sample measurement for any offset caused by the dye.
Dye-Type Alexa Fluor 350	Used in Protein Dye, select pre-defined dye or custom. Dye Abs Max, Dye Ext Coeff, and Dye Correction will be greyed when selecting a pre-define Dye-Type.
Extinction Coefficient 150.0 E+3	Numeric entry for Fluorescent Dye application only. This is the extinction coefficient of the specified dye. Note for pre-defined dyes, these values are not editable and the box will be greyed out.
Ext. coefficient [l/g*cm] 1.400	Used in Protein Dye, set the Ext Coefficient [l/g*cm] of the Custom Protein sample.
Factor 1.000	Numeric entry to 3 decimal places. In concentration and nucleic acid measurements multiplying absorbance readings by this factor gives the concentration value. In kinetic measurements the result is calculated by multiplying this factor by absorbance, delta absorbance or the slope.
Feature Detection Sensitive	Used in wavescan measurements only. This determines the sensitivity of the peak or valley detection i.e. sensitive will detect more peaks or valleys than course. Options are; Off, Coarse, Sensitive or Custom (when custom is selected the minimum peak height and width must be entered).

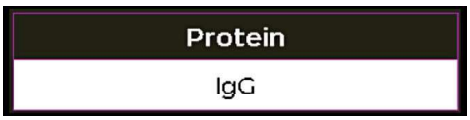
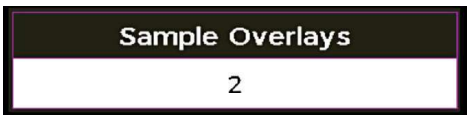
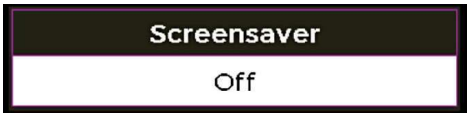
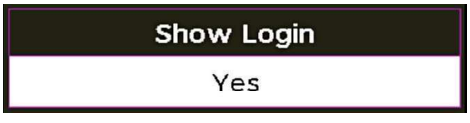
GLOSSARY OF CONTROLS

BOX	FUNCTION
	Toggles between wavelength and absorbance, used in wavescan measurements only. This determines how features will be ordered in the peak/valley table below the scan.
	Toggles between peaks and valleys, used in wavescan measurements only. This determines what feature type will be detected.
	Used in Substrate application, set the minimum absorbance value in the graph.
	Used in Substrate application, set the maximum absorbance value in the graph.
	Used in User Access to set the group a user will belong to and what access they will be granted.
	Used in all applications. This is the duration the instrument will take a reading at an individual wavelength. The longer the integration time, the greater the signal to noise ratio and the greater the accuracy.
	Numeric entry. This is the interval at which serial kinetic readings and substrate measurement will be taken.
	Numeric entry. This is the upper limit of a wavescan measurement.
	Numeric entry. This is the lower limit of a wavescan measurement. *Note: The max wavelength must always be greater than the min wavelength by at least the step value.*
	Used in the Single Wavelength, Kinetics and Protein applications to set the required measurement mode.

GLOSSARY OF CONTROLS

BOX	FUNCTION
Molar Ext. Coeff. 210000	Used in Protein Dye, set the Molar Ext Coeff of the Custom Protein sample.
Nucleic Acids dsDNA(260nm)	Used in Fluorescent Dye application only, this sets the nucleic acid used in the measurement.
Number of Dyes 2	Toggles between 1 and 2. Used in Fluorescent Dye application only to set the number of dyes used in the measurement.
Other MW 1.000	Numeric entry. This option is only used if the counter ion type in the Tm calculation is set to Other.
Password 1000	Used in User Access to set a password for new users
Pathlength 10 mm	Used in all life science applications expect for Colorimetric Protein. This is the pathlength of the cuvette used in the measurement. Options are for 10 mm, 5 mm, 2 mm, 1 mm, BioDrop 500, BioDrop 125 and µLite+ 0.5 mm.
Phosphorylated No	Toggles between yes and no. Used in Tm calculation to set if the sample to be measured is phosphorylated or not.
Post Run Autoscale Off	Used in Substrate application, toggles on and off to automatically fit the data into the graph after the measurement.
Primer Conc. 1.000	Numeric entry to 3 decimal places. Used in Tm calculation to sets the primer concentration in pmole/mL.
Print to... Internal Printer	Used in all applications to set the desired print location. Only available targets are displayed.

GLOSSARY OF CONTROLS

BOX	FUNCTION
	Toggles between On and Off, used in Equation Editor only. With Prompt on the measurement will proceed as follows: measure wavelength 1, prompt for sample, measure wavelength 2, prompt for sample etc.
	Used in Protein Dye, select the Protein of interest. Wavelength, Molar Ext Coeff, Ext Coefficient will be greyed is a pre-defined Protein is selected. If chosen Custom, set all other parameters.
	Used in all standard curve and substrate applications. This is the number of times a standard measurement is repeated before the mean of these values is plotted on the standard curve. Options are off (1 measurement), 2 or 3.
	Determines how many samples will be overlaid on the graph. Options are off, 2 to 8.
	Sets the number of samples that will be measured during the method.
	Used in the Substrate application to set the number of times a sample measurement is repeated for the mean of the values.
	Set the desired save location. Only available locations are displayed.
	Used in User Interface in Settings to set the time before the BioDrop screensaver will be displayed
	Used by the Default Administrator in User Access to set if user login will be displayed or not.

GLOSSARY OF CONTROLS

BOX	FUNCTION
Standards 5	Used in all standard curve applications. This is the number of standards that will be used to create the standard curve; options are from 1 to 9.
Standard Replicates Off	Used in the Substrate application to set the number of times a standard measurement is repeated for the mean of the values.
Std.1 10.00	Numeric entry to 2 decimal places. Used in all standard curve applications this is the concentration of the standard.
Text Entry Mode QWERTY	Used in User Interface in Settings to set the text entry mode used for alphanumeric text entry.
Units µg/ml	Used in applications where a concentration is the end result. Units are entered via either alphanumeric entry or from a list of options.
User Name 	Used in User Access when creating a new user.
Volume (µl) 1.000	Numeric entry to 3 decimal places, this is used in the Fluorescent Dye application and is the volume of the probe in µL.
Wavelength 450 nm	Numeric entry to 0 decimal place and is used in all fixed wavelength applications to determine the wavelength at which the measurement will be performed.
λ Max 500 nm	Numeric entry to 0 decimal place. Used in the Fluorescent Dye application, this is the wavelength at which the absorbance of the dye will be measured. Note for pre-defined dyes, values are not editable and this box will be greyed out.
Y Max 1.500	Numeric entry to 3 decimal places. This is the maximum value of the Y axis shown during a kinetics measurement. *Note the graph will automatically rescale at the end of the measurement to give the optimum Y max.*
Y Min 0.000	Numeric entry to 3 decimal places. This is the minimum value of the Y axis shown during a kinetics will automatically rescale at the end of the measurement to give the optimum Y min.

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