2300
EnSpire™
Multilabel Reader
Valid for instruments with software version 3.0
Warning

This equipment must be installed and used in accordance with the manufacturer's recommendations. Service must be performed by personnel properly trained and authorized by PerkinElmer.

Failure to follow these instructions may invalidate your warranty and/or impair the safe functioning of your equipment.
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Introduction
Introduction

Product family

The EnSpire™ product family consists of a 2300 EnSpire Multilabel Plate Reader and a 2390 EnSpire Alpha Plate Reader. Both instruments are based on the same standard base unit, but the 2390 EnSpire Alpha is designed to be a dedicated Alpha technology reader and does not support options like the 2300 EnSpire Plate Reader. The measurement technologies of the EnSpire product family are based on proven EnVision technology.

This manual is for the 2300 EnSpire Multilabel Plate Reader. The 2390 EnSpire Alpha instrument is not covered in this manual. More detailed information concerning the 2390 instrument can be found from the separate manual.

General description

The EnSpire microplate reader from PerkinElmer is an easy-to-use platform for quantitative detection of light-emitting or light-absorbing markers in research and drug discovery applications. EnSpire consists of a base unit and optional modules according to customer needs.

EnSpire has several detection technologies to choose from. The measurement technologies are filter absorbance (F-Abs), quad-monochromator based fluorescence intensity (FI) (reading from above and
Introduction

below), luminescence (Lum) and Alpha technology. The technologies can be used in different measurement modes like single point, on-the-fly, kinetic, well area scan, spectrum scan etc., depending on the technology. Single point reading with extremely fast and accurate mechanical movement allows reading of plates up to 384 wells. Flexible spectrum scan enables measurements of absorption and emission spectra.

EnSpire is a very compact, small footprint bench top unit with features such as shaking, scanning and plate barcode reading. Barcodes can be read from any of the four sides of the plate. Other characteristics of EnSpire include a network and network servers, data capture, software for data conversion and robot control, and human operators.

EnSpire software is easy to use and provides a clear view of all relevant information on the screen. For reliability and convenience, protocols and results are stored in a database. There is a protocol explorer for quick access and editing of protocols. Example protocols are included as a starting point for users to make their own application specific protocols. The software is a 32-bit application running under Windows Vista® or Windows 7®. Result files are stored as a Microsoft Excel® compatible .csv file or as graphical format .mht file. Files can also be printed or exported into a network or USB memory stick. The easy and intuitive user interface is operated by means of a touch screen.

EnSpire can be used independently as a stand-alone manually loaded system or as a robot-controlled subsystem in an automated laboratory. Different kinds of robots can be used as the main system in an automated laboratory. Data generated by EnSpire can be transferred via the Windows operating system and a computer network to other systems.
Technologies

EnSpire has four possible technologies and the absorbance technology is available with two different implementations, filter and double-monochromator based. The measurement technologies are described in more detail in what follows:

**Fluorescence intensity with quad-monochromator (230-850 nm)**

The light source is a xenon flash lamp. The polychromatic light is directed into the excitation monochromator unit where there are two diffraction gratings. These separate the incident polychromatic beam into its constituent wavelength components, sending each wavelength in a different direction so that a narrow band of wavelengths can be collected. Wavelength selection is performed by turning a diffraction grating to the desired position with a stepper motor. The excitation wavelength is selectable from 230 nm to 1000 nm. The excitation light is then directed into the sample.

The fluorescence from the sample enters the emission monochromator. This has a similar structure to that of the excitation monochromator module. The emission wavelength is selectable from 230 nm to 850 nm.

Although monochromators relieve you of the need to have filters for every label, a broad waveband cut-off filter is still required in order to block harmonic multiple orders of the wavelength chosen. Three cut-off filters cover the entire range of wavelengths supported by the instrument. The software automatically ensures that the correct filter is used for each wavelength.

The light from the monochromator passes through the appropriate cut-off filter on the filter wheel. A side-on photomultiplier tube is used as a detector and it is located so as to maximize the efficiency. The detector is used in gated analog mode with optimized gain (high voltage) setting.

A signal from a reference photodiode is always read after every flash. The reference signal is then compared to the original reference value and the results are corrected for the same excitation energy.

Fluorescence intensity readings can be taken from above or below. Reading from above is the most efficient way when no seal is used because no plastic surface has to be penetrated. For adherent cells and lidded plates, reading from below provides superior efficiency. It is a true epimode, in the sense that both excitation and emission are from below. Switching between reading from above to reading from below and vice versa is controlled purely via the software and both above and below reading can be used in the same run.
Introduction

The FI measurement head consists of two channels, one for the excitation light and another for the emission light. This measurement head base can be moved up and down in order to adjust the focal point of the excitation and emission optics.

Absorbance technology with a double-monochromator (230-1000 nm)

For absorbance measurements the same light source is used as for FI measurement.

Using the monochromator option, light passing into the sample comes from the excitation double monochromator.

The light is directed from the top and measurement made from below.

The intensity of the light directed through the sample is measured using a reference photodiode. The light is then focused into the sample. The focal plane is the same as for FI measurements.
The Absorbance measurement head focuses the excitation light into the sample. This measurement head, can be moved up and down controlled by the software in order to adjust the focal point of the excitation optics.

The reference intensity of the light is first measured without the sample and then the samples in one plate are measured.

The light intensity is measured by a photodiode placed at an optimal position directly below the plate. The light path for absorbance measurements is thus different than for FI measurements.

The absorbance value is calculated by the equation

\[ A = -\log \left( \frac{I}{I_0} \right) \]

where \( I_0 \) is the light intensity without any sample and \( I \) is the intensity after an absorbance.

**Absorbance technology with filters (230 – 1000 nm)**

The same excitation light source is used as for the monochromator model. The wavelength of the light is selected by an optical filter placed in the filter,
wheel and it can be in the range 230-1000 nm. Several absorbance filters are available.

The light intensity is measured by a photodiode.

The intensity of the light is first measured without any sample and with the samples in the light path.

The absorbance value is calculated by the equation

\[ A = -\log \left( \frac{I}{I_0} \right) \]

where \( I_0 \) is the light intensity without any sample and \( I \) is the intensity after an absorbance.

**Alpha technology (520-620 nm)**

The Alpha technology enables very high sensitivity measurements with very low background and very high signal to background ratio. The Alpha technology uses a semiconductor laser to excite the sample. This produces high optical power at 680 nm. The light from the laser chip is guided to the sample by a light guide.

*Note!* The laser is connected to sensors placed in the plate door and in the instrument cover. The laser will not operate if the plate door or the instrument cover is open.

*Warning!* Only personnel specially trained and authorized by PerkinElmer may open the laser module or any other module for that matter.

The Alpha technology donor beads are excited by the laser beam. A photosensitizer in the donor bead converts ambient oxygen to a more excited singlet state. These oxygen molecules diffuse to the bound acceptor bead where they react with a thioxene derivative generating chemiluminescence at 370 nm. This activates fluorophores in the bead which emit fluorescence light in the range 520 to 620 nm. The long half-life of the signal permits the measurement to be time-resolved to reduce the contribution of background fluorescence. The fact that the excitation wavelength is longer than the emission, further reduces the background, as does the fact that wavelength itself is long.

The detector is a very high sensitivity photomultiplier (PMT). The PMT is located right above the sample and reads the well adjacent to the one excited by the laser. Light passes through an aperture and into the detector. This aperture is fixed in size.
Both excitation and emission occur from the top of the sample.

Only one measurement per well is recommended because the sample is partially bleached by the excitation light.

Crosstalk is a factor that must also be taken into account. There are three components: bleaching caused by the excitation of adjacent samples, afterglow from a previously excited adjacent sample and glow from an adjacent well at the time of measurement. A crosstalk correction wizard allows the setup and measurement of the contribution to the signal from a sample well due to the glow and afterglow of adjacent samples and the effect of bleaching. The software then corrects for these effects in the calculation of the final results.

**Luminescence technology (400-650 nm)**

Luminescence uses a very high sensitivity luminescence PMT as the detector. It has extremely low background, high dynamic range and spectral response from 400 nm up to 650 nm. The emission light is collected directly from the top of the well in order to maximize the efficiency.

The detector can be lowered so that it is just above the plate, thus reducing the crosstalk between wells. The detector has an aperture to define the area of the plate it can view. There is one aperture size: for 384 well plates but which can be used with 96 well plates as well. The aperture is optimized to give the highest possible signal and minimize crosstalk between wells for 384 well plates.

There is a sensor which allows automatic precise plate height determination to allow the aperture to come very close to the plate without hitting it.
Applications

EnSpire supports several kinds of assays.

Reporter gene assays

When you need to measure either the level of expression or the functional effect of a drug candidate in terms of transcriptional activity of cells, EnSpire provides the features you need for reliable detection of reporter gene expression.

The instrument has comprehensive and versatile scanning and kinetics capabilities. It supports GFP assays with reading from below.

Enzyme assays

Kinase, Protease, Helicases or Caspase assays, are examples of enzyme assays that can be run on EnSpire. The design of the plate conveyor enables these applications to be run in stabilized conditions. The instrument's kinetics facility allows you to work fast and effectively.

Receptor ligand binding assays

One of the most common molecular targets for drug discovery are G protein-coupled receptors (GPCR). Fast-reading EnSpire is ideal, for example, for B2-Bradykin, MC3, MC4 and MC5.

Cellular assays

Reading from below, scanning and kinetics are some of the features that make EnSpire the ideal tool for cellular assays such as cAMP, Ca$^{2+}$ or any ADME/tox assays.

Genotyping assays

A feature of single nucleotide polymorphisms (SNPs) research is the need for fast results. With its detector configuration, plate barcode reader, as well as factory set protocols to cover all labels and plates, EnSpire provides a complete facility for fast detection of SNPs.

Alpha technology assays

The Alpha technology is an ideal tool for screening a broad range of targets. The technology provides an easy and reliable means to determine the effect of compounds on biomolecular interactions and activities. The Alpha technology offers the possibility to assay many biological interactions including low affinity interactions as well as enzymes, receptor-ligand interactions, second messenger levels, DNA, RNA, proteins, peptides, sugars and small molecules.
Image FlashPlate™ assays

Luminescence option enables measurement of Image FlashPlate assays. This assay measures radioactive samples. The radioactivity is detected using energy transfer via a scintillant and a europium chelate (emission at 615 nm). The Image FlashPlate is a 384 shallow well microplate, coated with scintillant emitting in the red range of the spectrum, thus eliminating most of the interference from colored compounds.
Installation
Installation

Usage environment requirements

Normal clean laboratory conditions usually provide a satisfactory operational environment, but the following points should be taken into consideration.

- Ventilation should be adequate for all conditions of use
- The temperature should be reasonably constant at about 22 ºC
- Relative humidity should not be excessive
- Direct sunlight should not be able to reach the instrument
- If possible, there should be a separate power line for the instrument itself with an isolation switch and fuse box.
- If excessive fluctuations in the mains voltage are anticipated, a mains stabilizer may be necessary.

Note! Make sure that when the instrument is at its place of operation it is exactly level.

Unpacking and connecting the hardware

The EnSpire is delivered in a single carton box. All the necessary cables and accessories are delivered with the instrument. Follow the instructions on the document attached to the instrument. The EnSpire Unpacking & Installation Instructions can be also found from the EnSpire Support CD. Make sure that the instrument, PC and Touch Screen turned off before connecting any wires.

If you are using a printer, install it according to the instructions accompanying the printer. All Windows Vista and Windows 7 compatible printers are suitable (USB or network connection).

Each instrument is supplied with an integrated PC and it can be found from the right back corner of the instrument. The touch screen is a separate unit that can be placed on top of the instrument.

Figure 2.1. Location of the integrated PC
Installation

Computer system requirements

The following computer hardware and operating system software are required to ensure proper operation of the EnSpire system.

- Windows Vista, Service Pack 1 or Windows 7
- Intel Core2Duo Processor 2.26 GHz
- 4 GB Memory
- 17” Color touch screen, 1280 x 1024 resolution
- 80 GB Hard Drive
- 4 x USB
- CD-ROM / DVD
- Ethernet card

Software installation

No software installation is necessary because the EnSpire software is factory installed to the integrated PC. In case of software update follow the Software Upgrade Instructions found from the EnSpire Support CD.

The EnSpire software consist of

- EnSpire Manager
- EnSpire Database Tool
- Filter Changer Tool
- EnSpire Service

The EnSpire Manager software is the user interface used to control the EnSpire instrument. The software is designed so that it can be control through touch screen. The EnSpire Database tool enables backup or repair of current database, restore of an old database, creating of a new database. Also the reference and inventory information can be cleared using the database tool. The filter changer tool can be used overrun the barcodes of the filters. This can be useful if for an example the barcode of the filter is managed. The EnSpire Service software is used by the instrument production and the service engineers. The Service software is password protected.

This manual only covers the EnSpire Manager software. If you feel that you need to use the parts of the software please contact the PerkinElmer technical support.

You can find the EnSpire Manager icon from the desktop or from the start menu under Start -> All Programs -> PerkinElmer Life Sciences -> EnSpire Manager Software -> EnSpire Manager. The EnSpire program itself is installed in the folder C:\Program Files\PerkinElmer Life Sciences.
Instrument Overview
Instrument Overview

Information about user instructions and warnings

There are several forms of user instructions:

Installation instructions
Follow the instructions at the Chapter 2 of this manual.

EnSpire Enhanced Security Manual
In addition to this manual, there is a separate manual for the Enhanced Security Option. This manual can be found from the EnSpire Support CD.

On-line help
This is supplied with the software and can be accessed by press the Help button on the touch screen.

Routine maintenance
This is maintenance intended to be performed by the user and is described in a separate chapter of this manual. Any other maintenance than what is described there should be performed by a service person trained and authorized by PerkinElmer.

Warnings
Regarding connection of the instrument to the mains:

Note! The instrument must be connected to a mains supply having a protective earth.

On the side of the instrument:

Warning! Disconnect supply before servicing

On the back of the instrument:

Warning! CLASS 1 LASER PRODUCT IEC 60825-1:2007
Safety symbols used

<table>
<thead>
<tr>
<th>Power ON</th>
</tr>
</thead>
</table>

| Power OFF |

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Light sources

EnSpire employs a high stability, short-arc flash lamp as a light source in measurements for fluorescence intensity or absorbance with monochromators or absorbance with filters. The high-efficiency light source has a high repetition rate for high throughput applications and it allows you to perform faster multi-flash measurements. You can select the number of flashes used. To ensure both the long-term and short-term stability of measurements, the excitation energy is monitored after every flash using a reference photodiode.

In the case of Alpha technology a high power semiconductor laser emitting light at wavelength of 680 nm is used.

The instrument cover has sensors to protect in case the user opens the lid during operation.

Filter wheel

The excitation filter wheel has place for eight filters. In filter absorbance measurements, pass-band filters are placed on the wheel whereas in the monochromator absorbance measurements automatically controlled cut-off filters are used. EnSpire can use the same filters as EnVision. There is large selection of filters to choose from. If you cannot find a suitable filter from the existing selection, the PerkinElmer Custom Optics help you to order a custom filter specially designed for your application. Please contact your local PerkinElmer representant for further information.
You can easily change the filters by pulling down the handle on the side of EnSpire. This will expose the filter wheel so that you can take it out and change filters. Please see chapter 8. Maintenance, for more instructions on how to change filters.

**Focus point adjustment**

The focus point for the incident light is adjustable. The measuring height is a parameter that can be defined in your protocol. The focus point can be located at the very bottom or at the very top of the well or anywhere between. The maximum measurement height is 26 mm and the minimum 0 mm assuming a plate thickness of less than 20 mm.

**Plate types**

Microtitration plates of 6, 12, 24, 48, 96 and 384 wells may be measured. Plates can be between 3 and 28 mm in height for absorbance and fluorescence intensity measurements and between 7 and 28 mm for Alpha technology and luminescence technologies. An optional special adapter allows cuvettes to be measured.

**Temperature control**

The EnSpire can be equipped with an optional temperature control module. The EnSpire has a uniquely isolated measurement chamber that allows the plate temperature to be controlled with high precision. The heating system is implemented with 16 resistors, eight above and eight below the measurement chamber that provide uniform temperature over the measurement area. In addition, the measurement chamber has its own fans for optimal air flow control.

The temperature control enables for an example cellular activity measurements at 37 °C and denature of the proteins at 42 °C. The performance specifications for the temperature control can be found from the specifications section of this manual.

**Measurement modes**

**Measurement (single point)**

Single point measurement performs one measurement using the selected technology and measurement parameters and then moves on the next well.

**Repeated sequences**

Repeat sequences perform several repeat measurements at selected time intervals. The number of measurement points as well time interval of the measurement among with many other measurement parameters can be defined.
Spectrum scan

In the Spectrum scan measurement several repeat measurement are performed for a well using different wavelength for each measurement. Spectra achieved often provided additional information compared to the single point measurement and therefore wavelength scan measurements are useful tool for an example when new assays are been setup.

Shaking

The shaker accommodates the wide range of plates and applications for which EnSpire is suited. Three modes are available; linear, orbital and double orbital. The duration, speed and amplitude of shaking can all be defined by the user.

Scanning

Cell applications, require several reading points in one well. Due to the heterogeneous spread of cells in a well it is important to scan the complete area of the cell culture. This can be done with EnSpire by specifying the shape of the scan area and the number of points to be scanned. The scanning function is also suitable for the reading of small membranes, chips and slides.

Barcode reading

The barcode reader allows you to load barcode labeled plates, which are identified by the barcode reader. Codabar, Code39, Interleaved 2 of 5 and Code 128 barcodes can be read. Barcodes can be read from all four sides of a plate. Plates must be over 7 mm high. The optimal position for plate barcodes is in the middle of each plate edge so that the vertical center level is at 8mm height. The maximum width of a barcode is 50mm.

Figure 3.2. Example of correct barcode positioning
Operating the instrument
Operating the instrument

Conventions used

Buttons or other software items to be pressed are in bold text e.g. Run Protocols.

The names of pages that open as a result of pressing a button are in bold italic e.g. Run Protocols.

Note! If a feature is described in this manual but it does not appear in your software then it is part of a different instrument model.

Caution! For users with the Stacker option - keep your hands away from the stacker area when the software is started or restarted. The rods in the stacker will come up during the initialization process.

Start up

1. Switch on the built-in computer
2. Wait until Windows software has booted.
3. Switch on EnSpire.
4. Press the EnSpire icon.

Once the instrument and the software have been started, the instrument will need about 15 minutes before it is operational.

If the software has an Enhanced Security Option installed the software prompts for the user. Select the user from the list and provide the password to the field below if the system is password protected.

More information regarding the Enhanced Security Option (e.g. how to create new users) can be found from the separate 2300-9050 EnSpire Enhanced Security Manual.
Shut down

1. Close EnSpire by pressing the Settings button and then the Exit to Windows button under the Options tab. Select either Only EnSpire Manager or Whole PerkinElmer EnSpire Software and press OK.
2. If you select to switch off the whole software, you can then switch off the built-in computer.
3. You can then switch off EnSpire.

Introduction to the user interface

When you start up the system, the user interface opens:

There are four large buttons and two smaller ones at the top of the page. The active button is highlighted and determines what is shown in the rest of the page:

Run Protocols – press to run protocols.
Create Protocols – press to create new protocols. You can do this by editing a copy of a protocol, create a new one from the beginning or import an existing protocol.

Edit Protocols – press to edit protocols.

Show Results – press to see a list of results.

Load/Unload plate – press to load or unload a plate

Settings – press to see and edit parameters that affect instrument operation.

Virtual keyboards

If the software requires entry of alphanumeric or just numeric information, a full keyboard or just a numeric keyboard will appear on the screen. You can then enter the information by typing with the keys.

Note! Press Shift to get a keyboard with capital letters and symbols.

The numeric keyboard has just numeric symbols.

Press OK after you have completed the information entry. The keyboard will disappear leaving the information in the appropriate field in the software.

Note! Press the cancel button (white X) if you want to close the keyboard without entering the information.
Run Protocols

Protocol selection

Protocols can be run by selecting the protocol from the protocol list, placing the plate in the plate holder and pressing the Run button to start a measurement. The live display is activated during the measurement and after the run has completed the final results are opened. See below for further details.

Make sure the Run Protocols button is activated. If it is not, press it to activate it. A list of protocols will be shown. These include protocols supplied by the manufacturer, which have a lock mark to show they cannot be changed permanently. However, you may edit the protocol and run an assay with the edited protocol, but you will not be able to save it. If you want to save an edited locked protocol, create a copy of the protocol, and then modify the copy. If a protocol is valid, there is a green mark beside it. Such a protocol can be run. The plate layout for the selected protocol is also shown. Select the protocol to be run.

Barcodes can be attached to the plate on any of the four sides and can be used to select a protocol for the assay automatically.

Note! When using barcodes for protocol selection, make sure you set the barcode mode in Settings under the Barcodes tab. You must select there the barcode to be read and then either select Define the protocol using or Split barcode for the protocol. You must also have defined the links between the barcodes and protocols. See “Barcodes” on page 110 for information on how to set barcode parameters.

Note! In Barcode mode you cannot choose the protocol since it is defined by the barcode, except when the barcode is used only for plate identification.
Note! In multiplate assays, if plates after the first one have no barcodes they will be measured with the protocol defined by the first plate barcode. If they have their own barcode then the protocol defined by that barcode will be used to measure the plate.

Plate loading

If the plate carrier is in the instrument, press the LOAD button or press **Unload** on the software toolbar and the plate carrier will come out. Under **Settings** you can choose how soon the plate carrier moves into the instrument if it is not in use.

If you are using a stacker, see “Using a stacker” on page 121.

Load a suitable plate into the plate carrier (starting with the first plate if you are running a multiplate assay).

Make sure the plate is orientated correctly with the A1 position in the left hand corner furthest from you i.e. the side that enters the instrument first.

Note! If you are using strip plates, make sure the strips are not sticking up, otherwise the plate may jam in the instrument. Results will not be correct if strips are not properly in position.
Press the **Load plate** button. The plate will be loaded into the instrument.

**Plate loading with a stacker**

If you are using a stacker, see “Using a stacker” on page 121 for instructions on how to use it, and “Stacker” on page 110 to see how to set parameters for it.

**Measuring**

Press the **Run (Barcode) Protocols** button. The run will begin.

*Note!* If you need to stop the run, press the **Stop** button (the **Run Protocols** button transforms into the **Stop** button while a run is occurring). You will be asked to confirm this action before the measurement is actually stopped. If you confirm it, the measurement will be terminated and any results will be saved in the database. They will also be displayed in the **Show Results** page.

A live display of the run will be shown in the **Measuring** page. There are two tabs: **Counts** and **Graph**.

**Counts**

If you select this view you will see a picture of the plate. The intensity of the signal from each well (or the amount of absorbance) is shown by how the well is colored. If it is gray, the sample has saturated the detector. The counts value is superimposed on each well. See “Counts/Calc” on page 44 for more information.
Press **Full Screen Plate** if you want the plate map to be fitted so that it fills the whole screen. Press the screen to switch off the Full Screen Plate view.

Select the **Track current well** checkbox if you want the current well to be always visible during measuring. This is useful when the plate is magnified and only some of the wells appear in the live display.

**Graph**

In this view you will see a miniature plot for each well position if there are repeat measurements or the well area scan, if such a measurement was selected.

When measurement of a plate has finished, results will be saved in the database and the **Measuring** page will change to the **Show Results** page. See “Show Results” on page 41 for information about result viewing.

Press **Full Screen Plate** if you want the plate map to be fitted so that it fills the whole screen. Press the screen to switch off the Full Screen Plate view.

**Track current well** – Select this checkbox if you want the current well to be always visible during measuring. This is useful when the plate is magnified and only some of the wells appear in the live display.

**Plate unloading**

When the plate has been measured, the plate carrier will bring out the plate. Remove the plate and if the current protocol has been defined as **Multiplate assay**, load the next plate and repeat the whole process until all plates have been measured for the protocol.
Editing plate map

You can modify the plate map by pressing **Edit plate map** or in the **Assay Start Wizard** when creating protocols. A window allows you to add or remove wells to the plate map. See “Create protocols” on page 54 and “Editing protocols” on page 63 for more instructions on how to do this.

Notes can be added to the **Assay notes** field. The text entered will be appended to the exported results.

*Note!* For the factory set protocols only the option **Run without saving** is available.
Show results

Current results

After a measurement has been completed the *Show Results* page will appear automatically showing the current results. See “Results page” on page 44 for more information.

*Note!* Do not press the *Show Results* button because this will cause the *Results selection* page to appear instead of the current results.

Result selection

To see results from other measurements, press the *Show Results* button. A list of protocols and their assay numbers will appear. There may also be a flag beside the protocol name.

Results can be ordered by Assay number (increasing or decreasing) or in alphabetical order (or reverse order) of the protocol name. Press one of the
column headers **Assay ID** or **Protocol** to select the type of listing. Press the same header again to reverse the order.

If you wish to display the list of results for only one protocol, press the down arrow beside the selection box.

A drop-down list of all available protocols will appear. Select a protocol. Press the **Refresh** button to see the results for that protocol.

On the right of the page you can see more details of the run as well as the plate that was used. Press the **Show Results** button under the plate (or at the top of the page) to see detailed results.
You can delete a result by selecting it and pressing the **Delete results** button.

You will need to confirm this operation by pressing **OK**.
Operating the instrument

Results page

The Results page has the following tabs:

Counts/Calc
Graph
List
Protocol info
Notifications
Recalculation
Export

Note! Deleted results can be restored from the Recycle Bin provided it has not been emptied.

The selection box near the top of the page allows you to select the results corresponding to different calculations. If no calculations have been selected then only the raw results are displayed.

Counts/Calc

The contents of the Counts/Calc tab are similar to those in the Counts/Calc tab of the Measuring page. The intensity of the signal from each well (or the amount of absorbance) is shown by how the well is colored. The counts value is superimposed on each well.

Note! Any saturated result will be highlighted with gray.

The side-bar on the right of the page gives you the following options.
View

You can select the size of the plate display. The options are 100%, 150%, 200%, 250%, 300% and Size to fit. You will need this for numerical values and higher density plates where the values are hard to read. Select Size to fit if you want the software to determine the optimum size for seeing the whole plate.

Two other controls let you choose the plate to display on screen, and the repeat number for the plate chosen. The plate number control will be disabled (grayed out) if the assay consisted of only one plate, and the repeat number control will be disabled if there was no repeat measurement.

Color scale

Logarithmic – select this checkbox if you want the scale for the signal intensity to be logarithmic, otherwise it will be linear.

The range for the scale is selected from the list box. The range is 100 to 100 million in factors of 10. The default range for most labels is 1 million and linear scale.

For absorbance the scale is 0 – 6 and logarithmic.

The scale that is selected is then used the next time a similar measurement is made.

There is also a color scale with sliders. You can move the sliders by pressing the arrows next to them or by dragging them with your finger.

The numbers by the sliders depend on the scale range chosen and the position of the slider within that range. Any results that are smaller than the lower slider will appear in e.g. violet. Any results higher than the upper slider will appear in e.g. red.

Results between the values of the two sliders will appear in a color that corresponds to the value.

Save plate as file button can be used to save the current plate map view as a jpg-image. When the button is pressed a browser opens which you can use to select a destination folder for the picture file.

Note! The type and number of colors used can be changed in Settings under the General tab.
Operating the instrument

Graph

The contents of the Graph tab are similar to those in the Graph tab of the Measuring page. There will be a miniature plot for each well position if you are doing a kinetic measurement.

The side-bar on the right of the page gives you the following options.

View

You can select Plate view or Overlaid curves.

Plate view - You can select the size of the plate display. The options are 100%, 150%, 200%, 250% 300% and Size to fit. You will need this for numerical values and higher density plates where the values are hard to read. Select Size to fit if you want the software to determine the optimum size for seeing the whole plate.

Overlaid curves – before you select this, press the wells you are interested in. Each time you have selected a well, press the Add curve button. The well coordinates will be added to the list under the Overlaid curves button.

When you have selected all the curves you want, press the arrow of the first control so that Overlaid curves reads as the control text. The selected curves will be displayed with measured values plotted against time.
To remove a curve from the display, select it from the **Overlaid curves** list and press **Remove curve**.

Press **Remove all** if you want to get rid of all curves from the **Overlaid curves** list.

**List**

This shows results in the form of a table of numerical values.

**Protocol info**

The parameters of the assay protocol are shown. There is also an alphanumeric version of the plate layout.

**Notifications**

This shows any error messages or warnings that have been produced. A yellow exclamation mark shows a warning e.g. if the signal has been too
high so that the detector is saturated. A red cross along with the Assay ID icon shows an error. Other notifications are in blue.

Recalculation

There are two tabs: Plate and Calculations. Calculations is selected by default.

The Calculations tab has two frames. In the left frame, called Formulas, are all the possible calculations with those available for the current protocol displayed in color (against grayed-out text for those unavailable). You can scroll the list using the up and down arrows located respectively on the top and bottom of the left frame.

To add a calculation, select it from the list and press the Add button between the two frames.
The selected calculation is then passed to the right frame, called **Selected calculations** and the list of available calculations in the left frame is updated. You can edit the selected calculations by pressing the **Edit** button next to it.

A page where you can edit the parameters opens. When you are done, press **Back to calculations**.

To delete a selected calculation, select it from the right frame and press the **Remove** arrow between the two frames.

If you wish to modify the order for selected calculations, you can do this by selecting a calculation and moving it up and down the list using the **Up** and **Down** buttons.

When you have edited or added a calculation, press the **Calculate** button to recalculate the results.

For information about calculations, see “Calculations” on page 82.

**Note!** If a calculation error has appeared in **Notifications**, the calculation that has been defined might disappear from the list with no results shown.

**Note!** The raw data is not changed by adding or removing calculations.

Press the **Plate** tab to see and edit the plate format. You are not allowed to delete samples but you can change the type. Press the **Calculate** button to see the result of the changes.
The functions under this tab are similar to those in the Plate tab of the Protocol editing page. See “Plate” on page 64 under “Editing protocols” starting on page 63 for more information.

Export

Pressing this button on the toolbar allows you to send results to a file or printer. Select File export or Print export. According to the option you choose, some controls will be disabled.

The Export tab has three sub-tabs: Preview, File settings and Wells. The default tab is Preview.

When you have set the parameters, press Export.

File settings tab

- **File name** (File export only): There is a default file name you can use or you can create your own.
  Press Browse to choose where to save the file. The folder name and path will be added to the beginning of the file name.
  Press Keyboard to access a keyboard so that you can enter the file name.
  Press Placeholders to see a list of placeholders which can be appended to the file name and select the one you want.
  The placeholders are:

  - **Default folder**, this allows files to be written to it even if the user of the operating system has read only rights. There is a shortcut to the Default data folder on the Desktop. The location of this folder is C:\Documents and Settings\All Users\Application Data\PerkinElmer Life Sciences\EnSpire. If you select some other folder and you do not have write rights to
Operating the instrument

it an error message will appear when you have pressed Next. You can create sub-folders by putting backslashes (\) between place holders. E.g. "<DefaultDataFolder>|<Assay ID>" means that in the default data folder, sub-folders are created each with an Assay ID. You can also connect placeholders together e.g. "<DefaultDataFolder>|<Date>_<Time>" would define a sub-folder with a name combining date and time.

- **Assay ID**, a unique numeric identifier for the measurement
- **Protocol ID**, a unique numeric identifier for the protocol.
- **Protocol Name**, the name of the protocol. Specified in the ID tab in the protocol name edit box.
- **Plate barcode**, the barcode on the plate.
- **Date**, date when the assay was run in the format YYYYMMDD.
- **Time**, the time when the assay was run in the format hhmmss.
- **_**: this insert the underscore character to the file name.

- **File type** (File export only): the first control you can choose the file type. You can choose between an Excel file (.csv), a text file (.txt) and an MHT file. The latter produces an output in “mht” format. This format is suitable for reading by a web browser and can include pictures as well as text. When you use this output type and you have a kinetic measurement, a graph of the results will appear as part of the output. If you want to include the pictures, make sure you tick the Show picture checkbox. Ticking the Show picture checkbox while csv or txt format is chosen will cause the file type to change to MHT. The second control lets you choose the kind of separator you want to use and the field next to it displays the separator. You can use a tab, or you can use the default separator for your PC. If you want to choose your own, select User defined list separator and type the separator you want in the field. This is useful when results are output with more than one item in a row. Use the default separator if you are going to import results into a spreadsheet and you want them arranged in columns.

- **Additional information**: you can choose to export protocol information and/or plate/background information.
Preview tab

- **Export format** (File export and Print export): select the format you want from the drop down list. The options are:
  - **Plate** - results are in plate format
  - **List** – results are in a list.
  - **List, each repeat in separate row**: each individual result is output on a separate row
  - **List, all repeats in one row**: all the results for one well are output on the same row
- If you tick the **Show old export formats (pre-EnSpire 3.0)** checkbox, then you also get the following in the list:
  - **Old plate format**: output in old plate format
  - **Old list format**: output in old list format
  - **Old list format (one row for each well)**: output in old format
- **Add all measurements and calculations** button: If you press this, all the measurements and calculations will be shown in separate plate maps.

- Preview: on each item of the preview you can find a – (minus) button that lets you remove the corresponding item from the export. You can also add items by pressing the + (plus) button. You can also choose what you want each item to display by pressing the title and choosing from the list that appears.
Wells tab

The well tab contains a plate map. You can choose individual wells or group of wells (or select or deselect them all using the Select all and Clear all buttons) and choose the number of repeated assays (when applicable) to export.
Create protocols

Press the Create Protocols button. The button then gets the focus and the icon on it changes.

When you want to create a new protocol you can either modify the parameters of an existing protocol (Copy selected) or you can define all the parameters yourself (Create new).

Copy selected

Select a protocol and press the Copy selected button. A new protocol will be created with the parameter values of the one you selected. The Edit Protocols button will light up. Follow the procedure described in “Editing Protocols” starting on page 63.

Create new

Select technology

Press the Create new button. The Select technology page will open.
Note! During protocol creation you can go back in the sequence of pages by pressing the Back button.

Select a technology for the new protocol: Absorbance, Alpha technology, Luminescence or Fluorescence intensity.

Note! The technologies available depend on the instrument model you have.

Press Next. The Select plate page will open.

Select plate

Select the plate type to be used. Press the Up or Down arrows to scroll the list of plate types so that you can see the one you want. Information about
the selected type is shown in the Plate information box. For more information about plate types see “Plates” on page 116.

Press **Next**. The **Select protocol operations** page will open.

**Select protocol operations**

![Image of Select protocol operations](image)

Select a protocol operation and press **Add**. It will be moved to the Measurement sequence list.

![Image of Measurement sequence list](image)

Repeat this for each operation you want.

**Note!** If you select **On-the-fly** you cannot select any other operations. It is only available for Absorbance measurements.

You can remove an operation from the list by selecting it and pressing **Remove**.
You can change the position of an operation in the list by selecting it and then pressing the appropriate Up or Down Change order arrow on the right of the page. Each time you press this, the operation moves by one position up or down respectively.

When you have selected all the operations you require and put them in the right order in the measurement sequence, press Next. The Specify operation parameters page will open.

Specify operation parameters

Select each operation in turn to see its parameters. Default values are proposed but you can edit them using the virtual numeric keyboard that appears when you press the area where the parameter value is displayed. See the “Protocol operations” chapter for details of the parameters in each operation.

Press Next when the parameters are as you want. The Data analysis page will open.

Data analysis

If you just want raw results press the No data analysis button and then press Next.

If you want to analyze the data by including calculations in the results, press the Add data analysis button. The Select calculations page appears with a list of calculations. To see more calculations press the Up or Down arrows.
Select a **calculation** and press **Add**. It will be moved to the Measurement sequence list.

Repeat this for each calculation you want.

You can change the position of a calculation in the sequence by selecting it and then pressing the appropriate **Up** or **Down Change order** arrow on the right of the page. Each time you press this, the calculation moves by one position up or down respectively.

For details of the calculations see “Calculations” on page 82.

When you have selected all the calculations you require and put them in the right order in the measurement sequence, press **Next** and you can see the calculations and their parameters.
When you have checked the calculation parameters, press Next. The Select well options page appears to allow you to define the type and format of the wells on the plate.

Select well options

Note! The Assay Start Wizard does not let you create multiplate assays. If you wish to use multiplate assays, you will need to finish your protocol creation first and then edit it with the Edit Protocols function.

If a sample type is required it will appear in the Required samples area.

You can choose the size of the picture of the plate with the View list box. The range is from 100% to 300% or Size to Fit.

Use the list on the right of the page to select the well type you want.

Select the wells to which you want to apply the type. You can select:

Well

An individual well can be selected by pressing it.

Row
A row can be selected by pressing the letter on the plate frame corresponding to the row.

**Column**

![Diagram of a column selection]

A column can be selected by pressing the number on the plate frame corresponding to the column.

**The whole plate**

![Diagram of the whole plate selection]

The whole plate can be selected by pressing the upper left corner of the plate frame.

**An area of wells**

![Diagram of an area of wells selection]
Mark an area of wells by dragging your finger across the touch screen from the first to the last well.

When you have assigned the types to all the wells, press Next. The Protocol name page will open.

Select the protocol name

![Protocol name page]

Enter the protocol name. When you press the area where the name has to be entered, the keyboard will appear enabling you to type in the name.

Press Next when you have finished entering parameters. The Optimization plate preparation page will open. The Select optimizations page will open.

Select optimizations

![Select optimizations page]
Select the **optimizations** to be done for the protocol. You can select more than one of these.

For details of optimizations see “Optimizations” on page 93.

When you have selected the optimization(s) you want, press **Next**.

**Optimization plate preparation**

An example of the plate will appear showing where the various samples should be placed. You also need to make sure the parameter values for the samples are correct.

Prepare the plate and set the parameters.

Load the plate in the plate carrier and press **Next** to start the optimization.

You may have to repeat this process if there are several optimizations and they cannot be combined on the same plate.

When optimization is completed the results will be shown. If the optimization was successful, the protocol can be seen on the protocol list (press **Run Protocols** to see the list). The green mark beside it shows that the protocol is valid for use.
If the mark is grey then the protocol is not valid and you must repeat the optimization or change some parameters. Do this using **Edit protocols**.

**Editing protocols**

**Select protocol**

Press the **Edit protocols** button to begin editing.

A list of protocols that can be edited will appear. Select the protocol you want to edit and press **Edit Protocols**.

A page will open with a number of tabs referring to the different parts of the protocol to be edited. The same page will appear if you have previously selected **Create protocol** and then the **Copy protocol** option.

**Note!** Locked protocols will not appear in this list because they cannot be edited. You can however, copy a locked protocol and edit the copy. A protocol in this list can be deleted by pressing the **Delete protocol** button or it can be exported to an external PC by pressing the **Export protocol** button.
Plate

Above the picture of the plate there is a selection box with which you can select the type of samples you want on the plate. These are:
- Delete selected samples
- Undefined – Measured
- UNK - Unknown
- STD – Standard
- BL – Blank
- CTL – Control
- S – PL sample
- ZL – Z_Low
- ZH – Z_High

First select the sample type by unfolding the dropdown list and selecting the sample type from it.

Then mark the area on the plate that you want this sample type for. See “Select well options” on page 59 for examples of how to select an area of wells. You can select:

- **Individual wells** – press the well you want
- **A row** – press the letter of the row on the left of the plate
- **A column** – press the number of the column on the top of the plate
- **Whole plate** – press the area at the top left hand corner of the plate
- **An area of wells** - mark an area of wells by dragging your finger across the touch screen from the first to the last well.

The Multiplate assay checkbox lets you create a protocol that has a first plate different from the subsequent ones. If you tick it, two sub-tabs will appear in the Plate tab: First plate and Other plates. Choose one of the two tabs to edit the corresponding plate map.

*Note!* If you already have different plate maps under First plate and Other plates and you deselect the Multiplate assay checkbox, only the First plate map will be kept.
On the right of the page there are additional functions to help in defining the plate format.

**View** selection control – select the scale for the picture of the plate: *Size to fit, 100%, 150%, 200% 250%, 300%*

**EC50 curve index** – set the number of the main index for the next EC50 sample that will be placed on the map. This control is enabled when you select EC50 Sample from the drop down list.

**Replicates** – set the number of replicates for the next sample type

**Sample index** – set the number of the index for the next control, standard or sample type

*Note!* Both these parameters are only enabled for sample types that have indexes i.e. UNK, STD, CTL and S

**Show advanced options** - press this button to see options to allow you to set plate formats using the autofill function.

**Fill start** – select where you want the autofill to begin: *Top left, Top right, Bottom left, Bottom right*

**Fill style** – select how the autofill will happen:
- **By rows** (always starting from the left)
- **By rows bi-directional** (left to right, right to left etc.)
- **By columns** (always starting from the top)
- **By columns bi-directional** (top to bottom, bottom to top etc.)

Press **Autofill** and the plate will be filled according to your selection.

*Note!* Autofill only functions if the plate is empty. Press the **Clear all** button to empty it first.

**Change plate type** button can be used to change the plate format or the preset plate type. For an example you change the plate type from 96 OptiPlate to 384 OptiPlate. Please note changing the plate type will reset plate map.

When you have finished plate formatting press the **Save changes** button to save the format.
Measurement

The Measurement tab is divided into three areas. The first one contains the available operations (themselves subdivided into measurements and other operations like shaking, etc.). The second area contains the sequence in which the chosen operations take place in the current protocol. The third area lets you set various parameters for each of the selected operations.

Available operations

This area contains all available operations for the protocol. It is itself subdivided into two categories: measurements and other operations (which include shake, delay, etc.).

Sequence and Repeated sequence

This area contains two columns: Sequence and Repeated sequence. Select a protocol operation from the available operations on the left. A right arrow button is displayed between the two areas.

Press it to add the operation. If you selected a measurement, you may have to choose from a list that will be displayed. The operation is then moved to the column that had the focus.

To remove an operation, select it and press the left arrow button besides it.

Note! If the operation is in the Repeated sequence column, it will first be moved to the Sequence column. You will then need to press the left button arrow to delete it.

Although the main sequence can be repeated, you can add smaller repeated sequences within the main sequence by moving the operations to the Repeated sequence column. Use the up, down, left and right arrows to place the operations where you want them.
You can set the repeat parameters for the sequence by pressing the **Operated: by xxx Repeats: yy** sequence title (where xxx is how the sequence happens and yy is the repeat number). The parameters for the repeated sequence are displayed in the right panel:

- Sequence: this can be by plate, by one well or by a number of well you determine (n number)

- Duration: you can set the number of repeats (range is 1 – 999, default is 10) and how long (in seconds) to wait between two repeats (range is 0 – 3600, default is 0)

**Note!** Remember there are restrictions when using On-The-Fly measurement or when using the **Alpha** technology.

**Note!** There must be at least one operation on the list. If there is only one you cannot remove it. To remove it, first add a new operation, then select the one you want to remove and press **Remove**.

**Operation specific parameters**

This area lets you see and edit parameters related to the operation selected in the **Sequence or Repeated sequence** column. Default values are proposed but you can edit them. See “Protocol operations” on page 71 for details on the parameters in each operation.
The **Optimize** button allows you to re-optimize the protocol you are editing. See “Optimizations” on page 93 for more details.

![](image)

**Setting plate repeats**

Repeats for the whole plate or for a sub-sequence are also set in the **Measurement** tab.

To set the repeat for the main sequence, select the **Assay/plate repeat** button that bridges over the two columns, and the **Repeat** parameters will be displayed in the third area, where you can edit them. You can set the **Number of assay repeats** as well as the time between two assay repeats (Start repeat each). Set the time unit using the left and right arrows. You can also set the **Number of plate repeats** and the time between two plate repeats (similar to the time settings for the **Number of assay repeats**).

To set the repeat for a sub-sequence, press the button on the top of the sequence (where it reads **Operated: by xxx Repeats: yyy**) and the **Repeat sequence** parameters will be displayed in the third area, where you can edit them.

**Calculations**

Press the **Calculations** tab to list the calculations selected for the protocol. In the **Formulas** column, all the possible calculations are listed, with those unavailable grayed out. To see more calculations press the **Up** or **Down** arrows.
Select a calculation from the list and press **Add**. It will be moved to the **Selected calculations** list.

Repeat this for each calculation you want.

You can change the position of a calculation in the sequence by selecting it and then pressing the appropriate **Up** or **Down** arrow on the right of the page. Each time you press this, the calculation moves by one position up or down respectively.

You can edit the calculations in the **Selected calculations** column by selecting it and pressing **Edit**.

When you have edited or added a calculation, press the **Back to Calculations** button if you want to edit or add or edit other calculations.

For information about calculations, see the “Calculations” chapter.

**Settings**

This lets you view and edit the name of the protocol and any notes that have been added, as well as a number of other protocol-specific settings.

You can edit all the parameters that are available. Any parameter that cannot be edited will appear greyed out.

**Gripper height** - defines the height at which the gripper takes the plate
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**Measurement mode** - select how the plate will be measured: **By rows**, **By columns**, **By rows bi-directional**, **By columns bi-directional**.

**Use rotated plate** - select **No** if you want the row beginning with the A01 well to be the first to enter the instrument and hence where the measurements begin. Select **Yes** if you want the plate rotated so that the A01 well is on the last row to enter the instrument.

**Change plate type** - press this button to prompt a window to appear where you can select the plate type.

**Name** - this is just for informational purpose and cannot be changed. You can create a new plate type from the **Settings Inventory Plates** display, and give it the name you want.

**Height** - give the height of the plate in units of mm.

**Well diameter** - give the diameter of the well in units of mm.

**Well volume** - give the volume of the well in micro liters.

**Column coordinate of top left corner well** - give the X position of the top left corner well. This is the distance of the center of the A1 well measured from the left edge of the plate.

**Row coordinate of top left corner well** - give the Y position of the top left corner well. This is the distance of the center of the A1 well measured from the edge of the plate that enters the instrument first.

**Column coordinate of top right corner well** - give the X position of the top right corner well. This is the distance of the center of the last well on the first row measured from the left edge of the plate.

**Row coordinate of top right corner well** - give the Y position of the top right corner well. This is the distance of the center of the last well on the first row measured from the edge of the plate that enters the instrument first.

**Column coordinate of bottom left corner well** - give the X position of the bottom left corner well. This is the distance of the center of the first well in the last row of the plate measured from the left edge of the plate.

**Row coordinate of bottom left corner well** - give the Y position of the bottom left corner well. This is the distance of the center of the first well in
the last row of the plate measured from the edge of the plate that enters the instrument first.

**Column coordinate of bottom right corner well** - give the X position of the bottom right corner well. This is the distance of the center of the last well in the last row of the plate measured from the left edge of the plate.

**Row coordinate of bottom right corner well** - give the Y position of the bottom right corner well. This is the distance of the center of the last well in the last row of the plate measured from the edge of the plate that enters the instrument first.

### Output

![Output tab screenshot](image)

The **Output** tab is divided into three tabs that let you specify how the results from a measurement with this protocol should be output: to a file, to a printer, as an Assay export or to some or all of these. To select the output type(s) you want, go to the corresponding tab and tick the corresponding checkbox.

The **Assay export** tab lets you export to an ESA assay file to enable result import to a separate workstation, allowing data analysis, added calculations, recalculation, etc.

Select the **File** tab or **Print** tab to set the corresponding parameters.

These parameters are the same as those under the **Export** tab in the **Result** page. See “Export” on page 50 for more information.

### Protocol operations

**Measurement technologies**

The measurement technologies available with EnSpire depend on the configuration of the instrument. The possible measurement technologies are:

1. Fluorescence Intensity
2. Absorbance (using filters or monochromators)
3. Alpha technology
4. Luminescence

**Fluorescence intensity with monochromators**

Light of a particular wavelength is selected by a monochromator and used to excite the fluorochrome in the sample. This produces prompt fluorescence at a different wavelength. This emission light is then directed through the emission monochromator to the detector. The excitation can be from top or bottom of the well.

**Absorbance with monochromators**

Light of a particular wavelength is selected by a monochromator and passed through the contents of the well from above where part is absorbed. This emission light is then directed through the emission monochromator to the detector. The ratio of the transmitted light intensity to the reference intensity is determined. A reference measurement is made before the plate is moved to the measurement position. This enables the absorbance to be calculated. The equation used is:

\[ A = - \log \left( \frac{I}{I_0} \right) \]

Where \( I \) is the intensity of the light through the sample and \( I_0 \) is the intensity of the reference measurement.

*Note!* Even though monochromators are used, a broad waveband cut-off filter is still required in order to block harmonic multiple orders of the wavelength chosen. Three cut-off filters cover the entire range of wavelengths supported by the instrument.

**Absorbance with filters**

Light of a particular wavelength is selected by a filter and passed through the contents of the well from above where part is absorbed. The emission light is then direct through a filter to the detector. The ratio calculation is the same as described for absorbance with monochromators.

*Note!* This option is only available in instruments with Alpha technology and no monochromators.

**Alpha technology**

The Alpha technology provides a very high sensitivity method of detecting molecular interactions. It is based on the laser excitation of special Alpha technology donor beads and the detection of emission light from bound acceptor beads.

The donor beads are generally coated with molecules allowing capture of the sample. The acceptor beads are coated with appropriate molecular binding partners. In the direct method, addition of sample promotes binding of the donor and acceptor beads. In competitive assays the sample reduces the amount of binding.
In the Alpha technology configuration there is a laser light source specifically for Alpha technology measurements. The laser illuminates the sample wells at a wavelength of 680 nm exciting molecules in the donor beads. The excitation time is adjustable within limits of 1 s total measurement time per well. This energy is then transferred to any bound acceptor beads which then emit the energy in the range 520 to 620 nm. The emitted light is then detected by the detector. The intensity of the signal allows determination of the sample.

When working with Alpha technology the following points are important:

The measuring chamber of EnSpire is well insulated and reduces the possibility of temperature fluctuation but you should still avoid an environment in which big temperature fluctuations may occur. because these will reduce the reproducibility of your results.

Only use white opaque plates such as OptiPlate plates from PerkinElmer.

Avoid bright light (especially red light) in the area of the instrument and any other associated sample processing or plate handling equipment. Green filters are recommended for light fixtures.

Cover the sample plates with opaque plate covers (you can use an opaque or black plate as a cover) at all times except when dispensing or measuring.

Pipetting should be started from the uppermost right corner (like measuring does) and be done row by row. This permits the fastest measurement performance.

_Note!_ For Alpha technology in the case of 384-well plates continuous excitation is used. It is recommended that you only measure complete rows.

The Crosstalk Correction optimization deals with the problem of crosstalk between wells.

**Luminescence**

Measurement is made of light produced in the sample as a result of e.g. chemical process instead of excitation by a light source.

**Measurement operations**

_Note!_ The measurement operations available depend on the selected technology and installed options.

Measurement (found under _Single_ when editing protocols)
Well area scan
Kinetic measurement (only available from the _Assay Start Wizard_)
Spectrum scan measurement
Shake
Delay
Temperature
On-the fly measurement
Dispense
Dispense measurement (only available from the Assay Start Wizard)

Note! Remember there are restrictions when selecting On-The-Fly measurement.

Selecting measurement operations is described under “Select protocol operations” starting on page 56.

Due to differences between the measurement technologies and instrument models some of the parameters may not be visible for a measurement. These differences are noted in what follows.

Measurement (Abs, Fl or Lum)

Note! See the separate section for the Alpha technology measurement parameters.

For fluorescence intensity a single excitation wavelength is selected with the excitation monochromator and the resultant fluorescence is measured at the wavelength selected with the emission monochromator.

For absorbance in an instrument with monochromators a single incident light wavelength is selected with the excitation monochromator and the amount of transmitted light is measured.

For absorbance in an instrument without monochromators a single incident light wavelength is selected with a filter and the transmitted light is measured.

For luminescence detection fixed filter is used and the relative amount of the emission light is measured.

Excitation/Emission (Fl)

Select either Top for excitation from above or Bottom for excitation from below.

Excitation wavelength (nm) (Fl)

Set the wavelength of the excitation light.

Wavelength (nm) (Abs with monochromators)

Set the wavelength of the incident light

Excitation filter (Abs with filters)

Select the filter type to be used. All filters defined in Settings can be seen in the selection box by pressing the Right or Left arrows. Only those filters
with the green mark are actually loaded in the filter wheel and available for use.

Press the **Link** button to the right of the **Right** arrow to see more details of the filter. Press the **Back** button to return to the Measurement parameters.

If you want a filter not currently in the filter wheel you must access the filter wheel and load the filter as described in the Instrument manual – “Routine maintenance – Changing filters”. The **Inventory** tab in **Settings** shows a picture of the filter wheel and the filters that are loaded.

**Measurement height (mm)**

Set the focus height (in millimeters) for the optics. This is measured from the bottom of the plate.

*Note!* If you optimize the measurement height, the result of that optimization will be used instead in place of the value set for this parameter.

**Number of flashes**

Set the number of flashes for one measurement of the sample.

**On-The-Fly measurement (Abs)**

With this operation the plate does not stop at the measuring position but is measured as it is moving past. Only one flash is used. This speeds up the measuring process but requires adequate signal from the sample.

**Wavelength (nm) (Abs with monochromators)**

Set the wavelength of the incident light

**Excitation filter (Abs with filters)**

Select the filter type to be used.

**Measurement height (mm)**

Set the focus height (in millimeters) for the optics. This is measured from the bottom of the plate.

*Note!* If you optimize the measurement height, the result of that optimization will be used instead of the value set for this parameter.

**Number of flashes**

Set the number of flashes for one measurement of the sample.

**Well Area Scan Measurement**

Scanning can be used for all cell-based assays. Especially for those using adherent cells, for example Green Fluorescent Protein (GFP) assays.
you can define the number of measurements from 1 to 100 per well. By defining the distance between points you define the scanning area. You can also define the shape of the scanned area, which can be either round or rectangular.

The parameters are:

**Number of horizontal points**

Number of points in the X-direction (1-10).

**Number of vertical points**

Number of points in the Y-direction (1-10).

**Distance between points**

Distance between each point in the well where a measurement is made. The distance between points can be: 0.1 - 7.15 mm and the maximum value depends on the total number of points and the well size.

**Scan Mode**

This is the shape of the array of measurement points over the well. It can be either a rectangle or circle.

**Excitation/Emission (FI)**

Select either *Top* for excitation from above or *Bottom* for excitation from below.

**Excitation wavelength (nm) (FI)**

Set the wavelength of the excitation light.

**Wavelength (nm) (Abs with monochromators)**

Set the wavelength of the incident light

**Excitation filter (Abs with filters)**

Select the filter type to be used.

**Measurement height (mm)**

Set the focus height (in millimeters) for the optics. This is measured from the bottom of the plate.

*Note!* If you optimize the measurement height, the result of that optimization will be used instead in place of the value set for this parameter.
Number of flashes

Set the number of flashes for one measurement of the sample.

Kinetic Measurement

If you have a fast kinetic assay such as Ca\(^{2+}\) measurement, flash luminescence etc, you should use the kinetic measurement mode. In Kinetic measurement only one well at the time is measured, for example 20 times with 3 s delay between each measurement, then the next well is measured etc. When the measurements have been done for one well they are then done for the next well. The **Graph** feature can be used in the **Measuring** and **Results** pages to see a plot of results of a kinetic measurement.

The kinetic measurement properties are:

Number of measurements

The number of times the measurement is to be repeated (up to 300).

Interval between measurement start times (0 – 5400 s)

The time between the end of one repeat measurement and the beginning of the next (0 - 5400 s).

Excitation/Emission (FI)

Select either **Top** for excitation from above or **Bottom** for excitation from below.

Excitation wavelength (nm) (FI)

Set the wavelength of the excitation light.

Wavelength (nm) (Abs with monochromators)

Set the wavelength of the incident light

Excitation filter (Abs with filters)

Select the filter type to be used.

Measurement height (mm)

Set the focus height (in millimeters) for the optics. This is measured from the bottom of the plate.

*Note!* If you optimize the measurement height, the result of that optimization will be used instead in place of the value set for this parameter.

Number of flashes

Set the number of flashes for one measurement of the sample.
Spectrum Scan Measurement (Abs or Fl)

*Note!* You must have an instrument with monochromators for this operation to be available.

For fluorescence intensity you can choose a range of excitation wavelengths over which the excitation monochromator will scan in steps. The resultant fluorescence is measured at the wavelength selected with the emission monochromator. Alternatively, you can choose a single excitation wavelength with the excitation monochromator and measure the resultant fluorescence over a range of wavelengths scanned in steps with the emission monochromator.

For absorbance you can choose a range of incident light wavelengths to be scanned by the monochromator in steps. The amount of transmitted light is measured.

**Excitation scan/Emission scan (Fl)**

Select either an excitation or emission scan.

**Min wavelength (nm)**

Set the starting wavelength for the scan.

**Max wavelength (nm)**

Set the ending wavelength for the scan.

**Step (nm)**

Set the size of the steps to be used during the scan.

**Excitation/Emission wavelength (Fl)**

Set the wavelength.

*Note!* The software will automatically set this parameter type to be **Emission wavelength** if you selected the type of scan to be Excitation. It will set it to be **Excitation wavelength** if you selected Emission scan.

**Measurement height (mm)**

Set the focus height (in millimeters) for the optics. This is measured from the bottom of the plate.

*Note!* If you optimize the measurement height, the result of that optimization will be used instead in place of the value set for this parameter.

**Number of flashes**

Set the number of flashes for one measurement of the sample.
**Shake**

*Note!* You must select some other operation in addition to this i.e. Measurement, Scan, Kinetic or Spectrum scan.

Shaking can be used to mix the solution in the wells. The parameters associated with **Shake** are:

**Shake mode**

Select the path of the shaker motion - straight line, circular or figure of eight (linear, orbital or double orbital respectively). The setting for **Shake Mode** affects the range for the **Speed** parameter.

**Duration**

Set the duration of the operation in seconds (0.1 - 6000 s).

**Speed**

Set the speed is the number of revolutions per minute. The range for this depends on the **Shake mode** and the **Diameter**.

**Diameter**

Set the distance between the extremes of the movement of the center of a well in the plate. The units are millimeters (0.1 - 10 mm). The 0.1 in the example here means that the shaking moves the center of the plate + or - 0.05 mm. The setting for **Diameter** affects the range for the **Speed** parameter.

**Plate location**

Select the position for the plate: **Inside** or **Outside**. The former means that the shaking occurs when the plate is at the measuring position. The latter when the plate carrier is extended outside the instrument.

**Delay**

*Note!* You must select some other operation in addition to this i.e. Measurement, Scan, Kinetic or Spectrum scan.

**Delay** is used to introduce a time gap between measurements. You can use a delay to follow slow kinetic measurements. This way you can measure a whole plate, wait, and measure again.

**Duration**

Set the length of the delay between the completion of the preceding operation and the start of the next one. The range is 0.1 s to 6000 s.
Plate location

Select the positions for the plate: Inside or Outside. The former means that the delay occurs when the plate is at the measuring position. The latter when the plate carrier is extended outside the instrument.

Temperature

When the temperature operation is initiated the instrument starts to heat (or cool) towards the target temperature. When the target temperature is reached the instrument stays in the temperature set by last temperature operation until a new temperature operation is started or until the protocol is completed. Please note that the general temperature setting for the instrument (See section 4.16 Settings) is temporarily disable if a measured protocol includes a temperature operation.

Temperature (15-65) °C

Set the temperature in which you want the instrument to be adjusted. The maximum temperature is +65°C and the minimum temperature is dependent for the ambient conditions. The approximate minimum temperature is ambient temperature +2°C. The lowest allowed ambient operating temperature for the instrument is +15°C.

Condensation prevention for sealed plates

Tick this checkbox when using sealed plates in order to avoid condensation on the cover. This is explained in more details on page 114.

Upper heater temperature is ... than lower heater

Set the temperature difference (warmer, colder) between the upper and lower heater.

Fast start (Don’t wait for temperature stabilization before next operation)

When the fast start is activated the temperature operation is completed as soon as the instrument reaches the target temperature for the first time. If the fast start is not used, the software waits until the temperature is stabilized so that the maximum drift within one minute is ±0.3 °C.

Fast cooling (plate door open)

When Fast cooling is enabled, the plate door opens and an extra fan blows the hot air outside the instrument. This boosts the ventilation within the instrument which leads to better cooling.
Set target and continue immediately

This function acknowledges that there is a target temperature that the instrument tries to set, but it continues with the next operation immediately and does not wait even for the target temperature to be reached.

Switch off temperature adjustment

When there are temperature operations in a protocol, this can be added as a separate temperature operation, e.g. at the end of the protocol, so that heating/cooling is switched off after the protocol was run.

Dispense

Used pump

Use the arrows to select the pump(s) you want to use. You can choose between: Pump 1; Pump 2; Both (Pump 1 first); Both (Pump 2 first)

Dispensing speed (for corresponding pump)

This lets you set the pump dispensing speed. The range is 50 – 500 µl/s and the default value is 200 µl/s

Dispensing volume (for corresponding pump)

This lets you set the volume to be dispensed by the pump. The range is 1 – 300 µl and the default value is 120 µl.

Syringe filling volume (for corresponding pump)

This lets you choose whether the syringe is filled only with the volume that will be dispensed (dispensing volume) or if it should be filled up (full).

Assay/plate repeat affected

Select the assay or plate repeat to which the dispense operation will be applied.

Alpha technology parameters

The Alpha technology parameters are:

Distance between upper surface of plate and detector (mm)

This is normally 0 for Alpha technology

Total measurement time (ms)

Set the total measurement time. This comprises the excitation and emission times.
Operating the instrument

**Excitation time (ms)**

This is the length of time the laser is used to excite the sample. The percentage figure next to the excitation time shows how much of the total measurement time is used for excitation.

*Note!* The allowed range for the total and excitation times is 10 - 1000 ms. The resolution is 1 for 10 - 100 ms, 5 for 100 - 500 ms and 10 for 500 - 1000 ms.

*Note!* The following three parameters are normally obtained from the crosstalk correction optimization and do not need entering here. However, the software allows you to manually enter values if you have not made an optimization but you have values from another source i.e. from a crosstalk made on another EnSpire. This is only valid if the crosstalk correction factors are optimized for the same plate type (i.e. the distance between wells is equal) and the **Total measurement time** and **Excitation time** are the same. If no values are entered and no optimization done, a zero correction is applied.

**Afterglow correction factor**

Afterglow is crosstalk from an excited sample well into an adjacent well. This crosstalk decreases with time so there are a number of values for this parameter to allow a decay curve to be determined. When this crosstalk correction is applied for a sample measurement, the system calculates the time since the adjacent sample was excited and subtracts the appropriate crosstalk contribution from the measured signal.

**Glow correction factor**

When a sample is excited, adjacent wells will be affected and make a contribution to the emission light entering the detector. The amount of this contribution is determined in the crosstalk correction optimization so that it can be subtracted from the measured signal.

**Bleach correction factor**

When a sample is excited it degrades (bleaches) adjacent samples thus reducing the emission from those samples when they are actually measured. Depending on the position of the sample well on the plate it may be subject to bleaching due to the excitation of several adjacent samples. Up to three levels of bleaching are calculated and the appropriate correction is applied to the measured signal.

**Calculations**

**Introduction**

This chapter describes the calculations that can be done with data measured with EnSpire.
Calculations can be defined in Protocol creation (Assay Start Wizard) or Protocol editing (Calculations tab) or can be added in Results (Recalculation tab).

Only those calculations that are valid for the protocol operation selected are enabled.

Choose the calculation you want from the left frame (Formula). If needed you can scroll the list using the up and down arrows located respectively above and below the Formula frame to find the calculation. Select the calculation and press the Add button between the two frames. The calculation is added.

Add all the calculations you want, and then you can rearrange the order in which they are applied. To do this, select a calculation from the Selected calculations frame and move it up or down the list one place at the time using the up and down arrow located to the right of the frame. Repeat this operation for all calculations you want to move until the calculation sequence suits your needs.

You can edit the calculations by selecting an existing calculation and changing its parameters.

When you have edited or added a calculation, press the Back to Calculations button if you want to edit or add more calculations.

Note! You can remove a calculation from the list by pressing the Remove left arrow button between the two frames.
### Types of calculations

The following table describes the calculations available.

<table>
<thead>
<tr>
<th>Calculations</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank correction (blanks on each plate)</td>
<td>( X - B ), where ( X ) = Measured value, ( B ) = Average of blanks on current plate</td>
</tr>
<tr>
<td>Curve fitting UNK calculation</td>
<td>A curve is fitted to the results of the measurements of standards on each plate i.e. each plate has a separate curve. This curve is used to evaluate unknowns.</td>
</tr>
<tr>
<td>Curve fitting (Blank corrected)</td>
<td>Same as “Curve fitting UNK” but the calculated results have been blank corrected (subtracted) with a value taken either from wells marked as Blank in the plate map, or Standard sample with 0 concentration (set in the calculation settings).</td>
</tr>
<tr>
<td>Curve fitting UNK calculation (Standards on first plate)</td>
<td>Same as Curve fitting UNK calculation, but with standards only on first plate.</td>
</tr>
<tr>
<td>Curve fitting (blank corrected)(standards on first plate)</td>
<td>Same as Curve fitting (blank corrected), but with standards only on first plate.</td>
</tr>
<tr>
<td>Average of sample type</td>
<td>Average of the samples</td>
</tr>
<tr>
<td>%CV of sample type</td>
<td>( 100 \times \text{SD/AVG} )</td>
</tr>
<tr>
<td>Standard deviation of sample type</td>
<td>Standard deviation of the samples</td>
</tr>
<tr>
<td>Dilution correction</td>
<td>Operation that corrects for the dilution factor of unknown and control samples. You can select the source numbers on which to apply the correction (usually a calculation) and you can set the dilution factor for each unknown and control sample.</td>
</tr>
</tbody>
</table>
| Dose-response curve (EC50_IC50)                        | A curve fitting calculation where the result is the IC50/ED50 concentration (the half maximal concentration). A single output value is given for the curve and its sample wells. In the plate map S – sample type (S1.1, S1.2, S2.1,....) is used to differentiate between the replicate samples (indicated by the last number in S2.1) and different standard curves (indicated by first
### Slope for EC50_IC50 curve
When curve fitting (EC50) is added to calculation, the slope value at the halfway point of the curve (at the IC50/EC50 concentration) is shown as a result output.

<table>
<thead>
<tr>
<th>Calculation Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of sample type</td>
<td>Sum of the samples</td>
</tr>
<tr>
<td>Peak of sample type</td>
<td>Maximum value of the samples</td>
</tr>
<tr>
<td>Minimum of sample type</td>
<td>Minimum value of the samples</td>
</tr>
<tr>
<td>Generic between sample types</td>
<td>Allows you to make calculations between different samples types.</td>
</tr>
<tr>
<td>General</td>
<td>Allows you to make your own calculation. See the text for more information.</td>
</tr>
<tr>
<td>Ratio</td>
<td>X/Y, where X = Measured value 1, Y = Measured value 2. Requires at least two labels for meaningful result</td>
</tr>
<tr>
<td>Addition</td>
<td>X + Y</td>
</tr>
<tr>
<td>Substraction</td>
<td>X – Y</td>
</tr>
<tr>
<td>Peak within well</td>
<td>Maximum of values obtained from kinetic or scan measurement</td>
</tr>
<tr>
<td>Minimum within well</td>
<td>Minimum of values obtained from kinetic or scan measurement</td>
</tr>
<tr>
<td>Result at nm</td>
<td>Signal response from a user-defined wavelength. This allows you to select a wavelength and get the signal as an output per well. The wavelength has to be within the range already measured with the Spectrum scan operation.</td>
</tr>
<tr>
<td>Area under curve</td>
<td>Area under curve drawn from points obtained from kinetic measurement or from spectrum scan</td>
</tr>
<tr>
<td>Well time (s)</td>
<td>Time in seconds related to the first measurement of a well in kinetic and plate/assay repeat. The default MeasTime output item is given in 1/1000 seconds starting from the beginning of the plate.</td>
</tr>
<tr>
<td>Dispense time (s)</td>
<td>Time in seconds elapsed before starting the dispense operation related to the first</td>
</tr>
<tr>
<td>Measurement Type</td>
<td>Description</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Kinetic slope</td>
<td>Measures the slope between data points in kinetic measurements. You can choose the calculation page, unlike in the Max slope calculation. Also the result is according to linear fitting between the selected data points.</td>
</tr>
<tr>
<td>Slope R² value</td>
<td>By default this calculation comes automatically when Slope calculation is selected but you can select that it does not come. The result output is the R² (R square) that indicates how well the data points and linear regression fitting co-align (output values between 0 and 1).</td>
</tr>
<tr>
<td>Maximum slope</td>
<td>Maximum slope of (overlapping) sets of points on a curve obtained from kinetic measurement e.g. for sets of 3 points and a 20 point curve the slope would be measured for points 1,2,3;2,3,4;...;18,19,20. The maximum slope is determined according to the absolute value of the slope and shown with the current sign.</td>
</tr>
<tr>
<td>Start point of Max Slope</td>
<td>This calculation can only be used when the Max slope –calculation is added first. The calculation reports the starting point of the Max slope found in Maximum slope calculation.</td>
</tr>
<tr>
<td>Maximum velocity (Vmax)</td>
<td>Michaelis-Menten equation enzyme reaction velocity, Vmax calculation is enabled, when &quot;Curve fitting (EC50)&quot; calculation is used above with &quot;Michaelis-Menten&quot; fitting method. Used in enzyme kinetic assays.</td>
</tr>
<tr>
<td>Michaelis constant (Km)</td>
<td>Michaelis-Menten equation substrate affinity constant, KM calculation is enabled, when &quot;Curve fitting (EC50)&quot; calculation is used above with &quot;Michaelis-Menten&quot; fitting method. Used in enzyme kinetic assays.</td>
</tr>
<tr>
<td>Average within well</td>
<td>Average of values obtained from scan measurement</td>
</tr>
<tr>
<td>Standard deviation within well</td>
<td>Standard deviation of values obtained from scan measurement</td>
</tr>
<tr>
<td>%CV within well</td>
<td>$100 \times \frac{SD}{AVG}$, where $SD = \text{Standard deviation}$, $AVG = \text{Average of values obtained from scan}$</td>
</tr>
<tr>
<td>Operating the instrument</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>measurement</td>
<td></td>
</tr>
<tr>
<td>Sum within well</td>
<td>Sum of the values obtained from kinetic or scan measurement</td>
</tr>
<tr>
<td>Crosstalk correction</td>
<td>Measured crosstalk correction factor</td>
</tr>
<tr>
<td>Flatfield correction</td>
<td>Well-specific correction factors obtained by optimization and applied to the measured values. Correction is technology and plate specific.</td>
</tr>
<tr>
<td>Validation</td>
<td>Allows you to determine if a result is inside or outside a selected range.</td>
</tr>
<tr>
<td>Z’</td>
<td>Z’ determines the assay quality using the standard deviation and average of high and low (or positive and negative) samples in the plate. At least two replicates from both are needed. The formula is: ( Z' = 1 - \frac{(3 \cdot SD(\text{high}) + 3 \cdot SD(\text{Low}))}{\text{Abs}(\text{Avg}(\text{High}) - \text{Avg}(\text{Low}))} )</td>
</tr>
<tr>
<td>Copy</td>
<td>Copy of X (the measured value)</td>
</tr>
</tbody>
</table>

**Creating your own calculation**

Select the **General** calculation option. You can then create a calculation involving up to three factors labeled X, Y and Z.

The drop-down list boxes allow you to select one of the four arithmetic operators +, -, *, /.
Operating the instrument

The three option buttons below allow you to choose the order in which the operations happen.

The final three pairs of option buttons allow you to select the value to be used for each of the three factors. Each value can be a result or a number.

*Note!* If you only want two factors, set the third factor to be 1 and either multiply or divide by it.

**Example: Ratio calculation**

To calculate a simple ratio of two results select \((X/Y)/Z\). Choose X and Y to be the results you want. Set Z to be 1.

**Example: Mean calculation**

To calculate a mean of two results select \((X+Y)/Z\). Choose X and Y to be the results you want. Set Z to be 2.

**Accessing curve fitting**

If you have a curve fitting calculation selected you can display the curve. The following examples are taken from *Show Results*.

When first selected the *Show results* page will display the Raw results under the *Counts/Calc* tab.

Select the *Graph* tab.

Select the curve results from the drop-down selection box.

Change the View from *Plate view* to *Overlaid curves* in order to see the curve.
Operating the instrument

If you want to hide the information related to the curve fitting, you can remove the **Show fitting info** selection.

Recalculation for curves

If you want to modify the curve fitting calculation, press the **Recalculation** tab, then press **Edit** for the appropriate calculation.
You can inactivate points on the curve if you want to see how this affects the curve fit. Press the **Active points/Inactivate points** button.

A window opens showing the settings for each point. Press the points you want to inactivate.
The selected points will be inactivated in the plot (they will be replaced by squares). The curve fit will be modified to fit the new configuration of points.

*Note!* Points are not actually removed from the data, only the curve display and the calculation results are affected.

You can also change parameters connected with the curve fitting e.g. from Linear to Spline.

If you want these changes to be included in results, press the **Calculate** button.

If you do not want changes to be saved, press **<< Back to Calculations**. The original curve will be displayed and the results will not be changed.

**Fitting parameters**

The curve fitting parameters are:

**Source**: Select the calculation for which you want to set the fitting parameters

**Fitting**: select from the following types:
Linear regression
Spline – in this case the Spline smooth parameter is enabled
4PL
5PL
MichaelisMenten
Sigmoidal response
Sigmoidal response, asymmetrical

**Weighted**: select Weighted or Unweighted fit.
Operating the instrument

**X-axis**: select Linear or Logarithmic

**Y-axis**: select Linear or Logarithmic

**Concentration unit**: enter the concentration unit to be used.
Optimizations

Introduction

This chapter describes the optimizations that can be performed.

>Note! Although they are described separately here most of the plate preparation can be done by combining samples on the same plate and then running the optimizations at the same time. Where this is not possible you can still select all the optimizations but you will be guided to do plate preparation separately.

Monochromator wavelength and Baseline optimization (Abs)

Wavelength optimization requires that the Baseline optimization be done also so when you select Wavelength, Baseline will also be selected automatically.

>Note! Baseline optimization can be selected independently of Wavelength optimization.

This optimization determines the wavelength at which maximum absorbance occurs. One sample is used to determine the wavelength and one for the baseline.
Select the lower and upper limits of the wavelength range. Select also the step length between wavelength measurements and the number of flashes.

Load the plate. Press **Next** to run the optimization.

After the optimization, the results of the Baseline optimization will be displayed.

Press **Next**.

A plot of wavelength against counts will be displayed and the wavelength for the lowest counts value is selected.
You can adjust the result manually if required by pressing the screen at the wavelength you want. The red line will move to that position.

When you are done press **Next**. See the section “After an optimization” on page 105.

**Monochromator wavelength optimization (Fl)**

This optimization determines the wavelength at which maximum emission occurs. A single sample is used. Press **Next** to see the *Plate preparation* page.
Operating the instrument

Select the lower and upper limits of the wavelength range. Select also the step length between wavelength measurements and the number of flashes.

Press Next.

A plot of wavelength against counts is displayed for the emission monochromators. The wavelength for the highest counts value is selected. You can adjust the result manually if required by pressing the screen at the wavelength you want. The red line will move to that position.

Press Next.
A plot of wavelength against counts is displayed for the excitation monochromators. The wavelength for the highest counts value is selected. You can adjust the result manually if required by pressing the screen at the wavelength you want. The red line will move to that position.

Press **Next**. See the section “After an optimization” on page 105.

**Plate dimension optimization (All technologies)**

This enables EnSpire to determine the exact positioning of the plate being used. The position of each of the four corner wells of the plate is measured. It requires a plate with samples in each of the four corner wells using the label you intend for normal measurements. E.g. a full plate is suitable.
Operating the instrument

Set the **Number of flashes** and the **Size of the scanned area** i.e. the size of the edges of the area of the wells to be scanned. See the table for typical size settings.

**Note!** There is no **Number of flashes** parameter if the technology of the protocol is Alpha technology.

<table>
<thead>
<tr>
<th>No. of wells</th>
<th>Spacing centers (mm)</th>
<th>Typical size setting (mm)</th>
<th>Allowed range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>384</td>
<td>4.5</td>
<td>2.7</td>
<td>1 - 9.0</td>
</tr>
<tr>
<td>96</td>
<td>9</td>
<td>6.3</td>
<td>1 - 18</td>
</tr>
</tbody>
</table>

**Note!** There are 10 movements in the scan (in both the horizontal and vertical directions producing a measurement of 121 points in an 11 x 11 array).

Press **Next** to start the optimization. After the optimization measurement a page like the following will be displayed.
This page has four tabs each with a picture showing the distribution of signal intensity across a corner well.

The order of the wells is:
Well 1 - upper left corner
Well 2 - upper right corner
Well 3 - lower left corner
Well 4 - lower right corner

The crosshairs should be in the centre of the well. If they are not, you can mark the center of each well using the X and Y selection boxes on the left of the page. You can also select the size of the step.

Choose the area with the most intense signal (in the case of absorbance the signal will be lowest here). Color coded contours show the signal intensity with red the most intense.

The picture shows the result of adjusting the position.
Note! In fluorescence intensity measurement, the signal should not exceed 500,000 RFUs for a single flash, otherwise saturation of the detector may occur and you will not be able to be sure of the position of greatest intensity. In this case, and if the red area is not symmetrical, you should select the center of the corner well as indicated by the general shape of the contours.

When you are satisfied with the position for each well, press Next. See the section “After an optimization” on page 105.

Measurement height optimization (FI)

This optimization enables EnSpire to determine the optimum focal point for measurement. A single sample is required for this.

Set the Number of flashes. Scan plate for strongest sample is activated as default. If you prefer to select the well used for the optimization manually, uncheck the box and select the desired well.

Press Next to start the optimization. The selected well is measured over a range of vertical positions and the one giving the highest signal is determined. After the optimization measurement a page like the following will be displayed.
A plot of height against measured results is displayed. You can adjust the result manually if required by pressing the screen at the wavelength you want. The red line will move to that position.

Press **Next**. See the section “After an optimization” on page 105.

**Z' optimization (Abs, Fl and Lum)**

This optimization determines how well the sample results are separated from the background when the scatter of results is taken into account. At least two high samples and two lows samples are required.
You can select the target Z’ factor, the default is 0.85.

Press **Next** to do the optimization.

In the results, the minimum number of flashes to achieve the Z’ target is selected. You can adjust the result manually if required by pressing the screen at the flash number you want. The red line will move to that position.

Press the **Raw results** tab if you want to see the raw results.
Press Next. See the section “After an optimization”.

**Flatfield correction (AS, FI and Lum)**

You can select this correction for Alpha technology (shown above), Fluorescence or Luminescence.

This corrects for variation in the signal across a plate due to e.g. plate curvature. In such a case the signal at the edges may be less than at the center due to the difference in distance from the detector.

Press Next to see the **Plate preparation** page.

This optimization allows the differences between each part of the plate to be determined. Set the **Number of flashes** or **Measurement time** depending from the technology.

*Note! In the case of Alpha technology the **Number of flashes** nor the **Measurement time** parameter is available.*

Press Next to start the optimization.
The correction factors calculated are applied to subsequent plate measurements.

Press **Next**. See the section “After an optimization” on page 105.

**Crosstalk correction optimization (AS and Lum)**

For the Alpha technology this optimization allows three types of crosstalk to be measured and corrected for. These are: glow, afterglow and bleaching. In order to measure these different types of crosstalk several configurations of samples are needed on the plate. See the chapter “Protocol operations” and the section “Alpha technology parameters” for more details of these three factors. For the luminescence technology this optimization allows the glow effect from the adjacent wells to be measured and corrected for.

Press **Next** to see the **Plate preparation** page.

**Plate preparation for Alpha technology**

**Plate preparation map for luminescence**
An optimization plate with the specified plate map must be used.

Press **Next** to start the optimization.

Press the **Raw results** tab if you want to see the raw results.

Press **Next**. See the section “After an optimization” on page 105.

**After an optimization**

After an optimization has been run you are then given a choice of whether you want to run the protocol now the optimization is complete or if you just want to save the optimization results.
Press **Next** when you have made your choice. If you chose **Only save protocol** the list of protocols will be displayed and the **Edit** or **Create** process will be finished. If you select **Save and run protocol**, you can load a plate and measure it with the optimized protocol. Results will be shown in the normal way at the end of the measurement.

Note! This page is common to all optimizations.
Settings

Press the Settings button to open the Reader settings page. This allows you to set some general parameters and shows what options are installed.

Information under each tab is described in this chapter. The tabs are: Options, General, Database, Stacker, Dispenser, Barcodes, Temperature, Inventory, Recycle bin and Normalization.

Options

This page allows you to give a name for your instrument to distinguish it from other instruments in the same laboratory or network.

The instrument serial number will be shown. In simulation mode this is not available (N/A).

A list of the options installed will appear. You can view this information but not change it.

The Exit to Windows button is to allow you to exit the EnSpire manager or the whole software. Press Exit to Windows, choose which of these two options you want and press OK. To cancel the exit operation press Cancel.

The Minimize window shrinks the EnSpire Manager window to the taskbar and allows the access e.g. to the desktop.
General

Plate settings

**Soft plate movement** – if the plate wells are very full, this option allows you to select that the plates will be moved more slowly than normal in order to avoid spillage.

**Automatically load plates into instrument after** – if you select this you can give a time in minutes. The plate carrier will automatically be moved into the instrument when this time has elapsed.

**Reader control scale colors**

When a plate is measured the results are shown in the *Measuring* and *Results* pages using colors to show the intensity of the signal (or amount of absorption).

The default color range is from red (strongest) to blue (weakest). If you want to change the color you can do it with this parameter. Press **Clear colors** then press **Add color**. Select the color you want for the weakest signal and press **OK**. Repeat this for the color for the strongest signal. You must choose at least two colors but you can have more than two.

If you want to reset the original rainbow colors, clear all colors, and then add only one color.

**Default export data folder**

The current default export data folder is shown. You can change this if needed. A browse button helps you define the new folder by showing the existing folders.

*Note!* You must have rights to write to the folder otherwise an error message will appear when you try to exit from **Reader settings**.
Operating the instrument

Unload plate after instrument initialization

Select this if you want to make sure that any plate left in the instrument is unloaded after initialization. If you have a robotic system you may not want a plate to be unloaded after initialization, in that case leave the check box unselected.

Suppress warning messages while starting assay

Select this to prevent warning messages appearing when an assay is started.

Use floating menu bar

Select this to use a floating menu bar that gives you some control over the software main window and provide additional gadgets. The checkboxes besides the Show floating menu bar let you set whether or not you want the menu bar and the gadgets to display when starting the whole software.

Prepare transport

Before moving the instrument, press Prepare Instrument for Transport. Confirm this by pressing OK. See the Routine maintenance chapter of the instrument manual for more instructions.

Create error report

Press this button if you have encountered an error that you cannot solve. The software will gather basic information about the software and create the error report. This will be of help when contacting your local PerkinElmer Technical support. By default, the software will place the file on your desktop.

Database

EnSpire can make a backup of all the results and parameter settings. You can specify where this folder should be stored if you want a location different from the default. You can select if you want the backup file to be compressed and how often you want the back up to be made. You can
choose that it will happen automatically or if you will be asked before it happens.

The date of the most recent back up is given. If you want to make another backup immediately, press the **Create Backup Now** button.

*Note!* Enhanced Security users data is not backed up with this function, but all audit data is always included. If you wish to backup also Enhanced Security users data, use EnSpire DataBase Tools.

**Stacker**

This tab contains options for use with the stacker. When not using barcodes, you can choose to always start the new assays by using the last protocol by ticking the appropriate checkbox. This ensures that all the plates in the stacker will be analyzed without the need for user input.

You can also set the instrument so that it ends the assay when the stacker is out of plates by ticking the checkbox.

The plate gripper height can be set here.

Set from which stack the instrument should load the plates. Default is right.

Press **Reset Stacker** if you want to remove the magazine table. This will lower the stacker rods and so free the magazine table.

**Dispenser**

The content of this tab is described in a separate chapter. See “Dispenser control” starting from page 123.

**Barcodes**

This tab is sub-divided into two tabs: **Barcode reading** and **Protocol starting**.

**Barcode reading** tab:
• **Read barcode from the**

Specify where on the plate the barcodes are to be attached; all four sides are possible.

If you choose to use more than one barcode position then you need to specify which barcode is to be the primary one, which the secondary etc.

• **Protocol definition by barcodes**

This field allows you to specify what the barcodes are to be used for.

If you select **Use barcodes as plate ID only** the primary barcode will be used for the plate ID.

If you select **Define the protocol using** then you can select which barcode is used to select the protocol. In addition you can select if another barcode is to be used as for the plate ID and which position that barcode is in.

• **Split barcode**

This allows you to have a single barcode for both protocol selection and plate ID. Press this option button if you require this feature.

You can select if the **First** or **Last** of the number of digits you set define the **Protocol** or **Plate ID** barcode.

Then you need to tell what the other digits refer to. Choose whether **None of** or **All** or **Rest of** the digits define the **Plate ID** or **Protocol** barcode respectively.
• Plates without ID barcodes

This field allows you to specify what happens if a plate ID barcode is missing. You can either replace the plate ID barcode with a time stamp or you can enter the text you want to appear e.g. "No barcode".

Protocol starting tab:

You can define the protocol to be used for measuring a plate carrying a particular barcode. Acceptable barcode types are listed in the Specifications in the Instrument manual. Set the following parameters:

• Type

Select the barcode type. There are three possibilities:

- Fixed – Select this if you want this barcode to always select this protocol.

- Temporary - Select this if want this barcode to only once select this protocol. After it is used once the definition is deleted. A different protocol can then be linked with this barcode

- End code – Select this if you want measurement to stop when this barcode is read.

• Barcode

Press this field to activate the keyboard and then type in the actual code to be used for the barcode (maximum 30 characters).

• Protocol

Select the protocol to be used when the barcode is read.
• **Add**

The **Add** button will be activated when you have defined the previous parameters. Press **Add** to enter the definition. It will appear in the table below.

• **Delete**

The **Delete** button will be activated when you have added a barcode to the table. Select a barcode and press **Delete** to remove it.

*Note!* If you are using simulation mode and you select barcodes then you cannot simulate starting a run.

**Temperature**

The **Temperature** tab allows you view temperatures and adjust the temperature control. This tab is only visible if your instrument has Temperature Control Option installed.

Checking the **Temperature adjustment** box activates the heating or cooling of the instrument depending from the current settings. The temperature option can be turned off by unchecking the **Temperature adjustment** box.

*Note!* When the Temperature adjustment is off the instrument does not actively affect the temperature of the instrument. For an example if the instrument was heated into an elevated temperature before the temperature adjustment was turned off, instrument will slowly start cool near the ambient temperature.

The target temperature is typed in the **Set measurement chamber to** field. If the current chamber temperature is lower than the target temperature the instrument will start the actively heat the instrument. If the target temperature was lower the instrument will start to actively cool the instrument.
Note! The instrument can’t reach temperatures lower than the ambient temperature.

The **Condensation prevention for sealed plates** checkbox allows you to set the amount by which the temperature of the heater that is above the assay plate differs from the temperature of the heater below the plate. The amount of this difference can be a maximum of 4 °C. Keeping the upper heater at a higher temperature than the lower heater avoids the formation of condensation droplets on the under surface of the seal when using a sealed plate.

The **Set heating off after next assays** checkbox turns the temperature control off when the next assays is measured.

The **Show raw data** checkbox bring separate temperatures for upper and lower sensors graphs and values visible.

The **Use fast cooling** enables faster cooling of the instrument. The fast cooling is available when the target temperature is higher than current chamber temperature. The fast cooling activates the chamber fan and opens the instrument door. It is recommend to remove the plate from the plate carrier before the fast cooling is activated in order to avoid unnecessary evaporation, contamination or light exposure of the samples. The fast cooling is active until the target temperature is reached, temperature stabilizes to its minimum temperature (i.e. ambient temperature +2 °C) or **cancel** is selected.

**Inventory**

There are three tabs: **Visual, Filters** and **Plates**.

**Visual**

This tab shows picture with the positions and types of the filters in the filter wheel. Pressing on one of the filter positions will take you to the corresponding filter in the **Filters** tab.
Filters

This tab shows a list of the filters defined in the software. Each filter has a unique barcode so that the instrument can positively identify which filter is loaded. Press the **Filter Name** column heading to order the filters according to the name. Press the **Barcode** column heading to order the filters according to the barcode number. The parameters of the selected filter are shown.

**Add**

If you press this, the selected filter type will be copied so that you can edit its parameters and thus create a new filter type.

**Delete**

If you have added a filter, you can also delete it by selecting it and then pressing **Delete**. You cannot delete filters defined by the manufacturer.

*Note!* Parameters of a user-defined filter can be edited but those of a manufacturer-defined filter can only be viewed.

**Filter parameters**

The filter parameters are the same for all filters.

**Barcode** - This barcode identifies the filter. It is supplied automatically and you cannot change it.

**Name** - Enter the name of the filter. Use a consistent naming system so that you can easily recognize the filter.

**Description** – This gives details of the filter and its type.

*Note!* The description is just a text field and does not update automatically. If you change a parameter, you must type in the new description.

**Center wavelength** - (CWL) This is the middle of the range of wavelengths that will pass through a filter. The units are nanometers.

**Bandwidth** – (BW) This is the "Full width at half maximum" (FWHM): the width of a bandpass filter between specific absolute transmission points, i.e. 0.5 x peak transmission. The units are nanometers.
Transmittance value (%) – (Tmin) This is the percentage of the incident light that passes through the filter.

Slot - This is not a parameter you can edit. In the case of a newly added filter it will show N/A, (not available). When the instrument detects a filter with the barcode given above, it will automatically fill in the slot number.

Use with absorbance – This box will be checked if the filter can be used with absorbance.

Plates

This tab shows a list of the plates defined in the software. The parameters of the selected filter are shown.

Add

If you press this, the selected plate type will be copied so that you can edit its parameters and thus create a new plate type.

Delete

If you have added a plate, you can also delete it by selecting it and then pressing Delete. You cannot delete plates defined by the manufacturer.

Note! Parameters of a user-defined plate can be edited but those of a manufacturer-defined filter can only be viewed.

Plate parameters

Name - Press the name area so that the keyboard appears and then type in the name of the plate.

Note! Press Del first to delete the default plate name.

Use a consistent naming system so that you can easily identify the plate.

Number of rows – Set the number of rows for the plate.

Number of columns – Set the number of columns for the plate.
The remaining parameters are the same as those described in the “Settings” section of the “Protocol editing” chapter. Set them as needed.

**Recycle bin**

The **Recycle Bin** folder stores deleted results, protocols, plates etc.

Press the **Restore** button to restore the selected item. The item will disappear from the **Recycle Bin** and will be restored to the location from which it had been deleted.

*Note!* When a protocol is deleted, all results measured with that protocol are also deleted. When you restore a deleted protocol, any results that were deleted with it are not restored.

Press the **Empty** button to permanently delete all items from the **Recycle Bin**. Press **OK** to confirm that you want to permanently delete all items or press **Cancel** to cancel this operation.

**Normalization**

If you have several EnSpire instruments and you want to eliminate the effect of differences between the absolute value of results, you can normalize results using this page. Enter a normalization factor to be applied to the results of the instrument you are using.

To determine this factor, you must first choose one of the readers as the reference instrument. Measure the same samples in each reader. Compare the results obtained with each of the readers with those obtained with the reference instrument. Calculate the factor needed to multiply the results for each reader to get the same results as with the reference instrument. This is the factor you need to enter here. Examples of these factors could be 1.05 or 0.97. All results obtained with a reader will then be multiplied by the factor entered under **Normalization**.
Using a stacker
Using a stacker

Getting the stacker ready

Before you can use the stacker, you need to place the magazine table on top of the stacker in front of the plate loading door.

*Note!* If the rods of the stacker are up, press the **Reset stacker** button in **Settings/Stacker** so you can put the magazine table into place.

You also need to set the parameters in the **Stacker** tab of the **Settings** page. For more information, see “Stacker” on page 110.

*Caution!* For users with the Stacker option - keep your hands away from the stacker area when the software is started or restarted. The rods in the stacker will come up during the initialization process.

Preparing magazines

Magazines are available in two sizes: 20 plate magazine and 50 plate magazine. Plates are usually loaded into the magazine before the magazine is placed on the stacker.

Plates are inserted into the magazine from the top. Make sure the plates are orientated correctly with the A1 position in the left hand corner furthest from you.

*Note!* Check that the handles of both magazines are down during a run so that the plates can move upwards without hindrance.

The plates should be loaded in the order in which you want them processed by the instrument, i.e. the first plate should be on the bottom of the loading magazine.

Loading magazines

When all the plates have been loaded, place the loading magazine at the loading position of the stacker (defined in the **Stacker** tab) and place an empty magazine at the unloading magazine position of the stacker.

If you have set a repeat for all the plates, the instrument will return them automatically to the loading magazine once they have all been processed and the repeat will be started automatically after the delay you have set. This ensures that the order of the plates in the repeat measurements is the same as that during the first measurement.

Run protocols

Running protocols occurs in the same way as described under “Run protocols” on page 36.
All the plates in the stacker will be measured. If you have selected **Multiplate assay** for the protocol, the first plate will be measured according to the **First plate** map, and all subsequent plates will be measured according to the **Other plates** map.

**Using protocol barcodes with the stacker**

When using barcodes for protocol definition, the instrument will look for a barcode on each plate and run the corresponding protocol. If a plate does not have a barcode, it will be processed with the protocol defined by the barcode on the previous plate. If that previous plate happens to have a barcode defining a multiplate assay protocol, then the plate without barcode will be measured according to the **other plate** map.

**Manual plate loading**

If you want to load plates manually, you need to remove the magazines and upper part of the stacker - the magazine table.

Start by lifting off the magazines.

Pull forward the magazine table and lift it off.

*Note!* If the rods of the stacker are up, press the **Reset stacker** button in **Settings/Stacker** to get the magazine table out. Reverse the procedure to reinstall the magazine table and magazines.
Dispenser Control
Dispenser Control

In the *Settings* window, press **Dispenser** to access functions and parameters involved in the operation of the dispenser. There are two tabs: Maintenance and Settings.

**Maintenance**

You can perform maintenance operations with the dispenser by selecting the pump and the operation.

**Initialization**

Select the pump or pumps to be initialized then press the **Init** button to perform initialization.

Initialization resets the pumps by setting the valves and syringes to their home positions. This may involve liquid being expelled through the aspiration tube into the liquid reservoir. If you are not sure what liquid was used last, direct the aspiration tube into an empty reservoir to avoid accidentally mixing liquids.

*Note!:* the other maintenance operations are disabled until **Init** has been pressed and initialization performed.

**Rinse**

This operation allows you to dispense a selected volume of liquid into the waste container. You can use this operation to empty tubing if you perform it with the inlet tube in air. This operation also allows you to rinse the tubing of the selected pump or pumps.

Press **Next** to continue.
Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Press **Next** to continue or **Back** to return to the **Maintenance** page.

Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. Rinsing will then take place.

An animation will show what is happening.
Press **Finish**. The **Maintenance** display will reappear.

*Note!* If any PUMP button is pressed while the dispenser is operating, that operation will be terminated immediately and dispenser initialization must be done again.

**Fill tubing**

This operation aspirates enough liquid to fill the tubing. A small extra amount (about 150 µL) will be aspirated to ensure there are no air bubbles in the tubing. The excess will be dispensed into the waste container.

Press **Next** when you have selected this option.

This operation allows you to fill the tubing of the selected pump or pumps.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Press **Next**.
Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. Tubing will be filled.

An animation will show what is happening.

Press **Finish**. The **Maintenance** display will reappear.

**Retrieve liquid**

Returns liquid from the syringe and tubing to the liquid reservoir (use this if the liquid is expensive reagent!).

Press **Next** when you have selected this option.
Select the pump or pumps from which you want to retrieve liquid.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Press **Next**.

Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. Liquid will be retrieved to the liquid reservoir.

An animation will show what is happening.
Press **Finish**. The **Maintenance** display will reappear.

**Test dispense**

This operation allows you to dispense a set volume into the waste container.

Press **Next** when you have selected this option.

**Note!** When the instrument lid is open, you can, by hand or with a tool, move the tip mount outside the instrument so that you can observe the flow of liquid through the tip. In this case the tip mount used must be identified.

Select the pump or pumps with which you want to do a test dispense.

Select the tip mount to be used. This must be the one in the instrument as shown by the * mark.

See **Show advanced options** for some extra parameters you can edit.

Press **Next**.
Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. Liquid will be dispensed to the waste container.

An animation will show what is happening.

Press **Finish**. The **Maintenance** display will reappear.

**Empty syringe**

This operation drives the syringe to the empty position. Liquid will be expelled into the liquid reservoir.

Press **Next** when you have selected this option.
Select the pump or pumps with the syringe(s) you want to empty.

See **Show advanced options** for some extra parameters you can edit.

Press **Next**.

Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. The syringe will be emptied into the liquid reservoir.
Press **Finish**. The **Maintenance** display will reappear.

**Fill syringe**

This operation drives the syringe to the full position. Liquid will be aspirated from the liquid reservoir to fill the syringe.

Press **Next** when you have selected this option.

Select the pump or pumps with the syringe(s) you want to fill.

See **Show advanced options** for some extra parameters you can edit.

Press **Next**.
Press **Start** and the operation will start for the pumps selected on the previous screen. Alternatively, you can press the PUMP button on the dispenser for the pump where you want to carry out the operation. In this case, remember to press both buttons if you want the operation to be applied to both pumps. The syringe will be filled from the liquid reservoir.

Press **Finish**. The **Maintenance** display will reappear.

**Show advanced options**

Select **Show advanced options** if you want to see or change certain basic parameters. If you do not select this option then the default values will be used. There are two sets of identical parameters for Pump1 and Pump2.
(Total) Volume

This parameter determines the volume of liquid aspirated.

For **Rinse** the default is 2000 µl and the range 500 – 10000 µl. The parameter name is **Total volume** because several dispensings occur and this parameter is the total volume used for all the dispensings.

For **Fill** the default is 50 µl and the range 50 – 10000 µl. The parameter name is **Volume**.

For **Test** the default is 50 µl and the range 2 – 475 µl. The parameter name is **Volume**.

**Speed 50 – 500 µl/s**

This parameter determines the volume per second of liquid aspirated. The default is 200 µl/s. The more viscous the liquid the higher the speed you should use.

**Tip tube volume 50 – 2000 µl**

The volume of the tube between the syringe and the tip mount is known for a particular tip type and this volume appears as the default parameter value. The software allows you to change this value if it is necessary.

**Aspiration tube volume µl**

The value of this parameter is fixed here but can be changed under the **Settings** tab.

**Total tube volume µl**

This is the sum of the **Tip tube volume** and the **Aspiration tube volume**.

If you change these parameters and want to save the changes, select **Remember settings**.
Settings

Select the **Setting** tab to see these parameters.

*Note!* the following settings only take effect if you press the **Set** button.

### Stirring

Select this check box and then select the speed to be used for stirring the liquid in the liquid reservoir. The range is 100 to 500 revolutions per minute.

### Heating

Select this check box and then select the temperature for the liquid in the liquid reservoir. The range is 25 to 60 ºC.

### TipMount in instrument

Select the tip mount present in the instrument.

### Advanced settings

Select this to see extra parameters for both Pump1 and Pump2.

**Connected to tip** – This is just for information, it shows you which tip is connected to which pump (1, 2 or None).

**Aspiration speed is x% of dispensing speed** – the range is 5 to 100% and the default 70%.

**Aspiration tube volume** – set the volume for the aspiration tube if it is different from the default 270 µl. The allowed range is 50 to 700 µl. This value is used in **Show Advanced options** for the various dispense operations to calculate the **Total tube volume** by adding it to the **Tip tube volume**.
Robot integration
Robot integration

EnSpire can be accessed remotely over network (intranet, internet). Usually EnSpire is accessed by a robot to enable continuous loading and unloading of microplates. The EnSpire supports Windows Communication Foundation (WCF) application programming interface. These provide the basis for seamless integration and instrument control. The robot and the counter work as a unit.

The robot integration is discussed in more detail in a separate *Integrating EnSpire™ instrument to laboratory automation*. This manual can be found from the EnSpire Support CD as well as sample applications and code examples.
Maintenance
Maintenance

All maintenance, other than that described here, must be performed by service personnel authorized by PerkinElmer.

Routine maintenance for dispenser

Before daily work

At the beginning of a working day we recommend that you perform the following operations depending on the situation:

- if the tubing has been rinsed and emptied when operation finished the previous time, (recommended procedure) then Fill the tubing with reagent.

- if reagent has been left in the tubing (not recommended), some may have been lost through evaporation. First Retrieve the reagent and then Fill the tubing.

- if rinse liquid has been left in the tubing, then you have to get rid of this before dispensing reagent. You can Retrieve the rinse liquid and then Fill the tubing. Alternatively you can empty the tubing using Rinse without aspirating any liquid i.e. with the inlet tube in air. Then replace the inlet tube in the reagent reservoir and Fill the tubing.

After operation

Empty syringe then Retrieve reagent back into the reagent reservoir. Replace this reservoir with one containing rinse liquid. Rinse the tubing. Either leave the tubing filled with rinse liquid if you are planning to use it again soon, or empty it i.e. Rinse with the inlet tube in air.

All these operations, as well as others, can be found under the Maintenance tab when you press Dispenser Control.

Clean tubing

It is good practice to clean the dispenser tubing on a regular basis.

Start by rinsing with de-ionized water. Then, repeat the rinse operation, but this time with a 50% ethanol solution. Finally, rinse again with de-ionized water.

If necessary, fill the tubing with a 50% ethanol solution and leave it there for a while, then rinse with de-ionized water.

Cleaning the instrument

The plate carrier should be kept clean to avoid dust and dirt entering into the optics at the measuring position.
You can remove dust by the use of very clean and dry compressed air or special canned air for optics cleaning.

The plate carrier should be cleaned at least once a week using a soft cloth or tissue paper soaked in a mild detergent solution or alcohol.

Filters should be free of finger prints. Fingerprints on filters should be removed with 99.8 vol. % Alcohol (Ethanol Aa) on microfiber cloth.

In general all the dust and dirt in the system can increase the measurement background of the system especially if wavelengths in the ultraviolet region are used.

**Changing filters**

Filters can easily be changed.

*Figure 8.1. Opening the filter wheel holder*

Pull down the filter module handle on the side of the instrument.

Remove the filter wheel.

Remove from the wheel any filters that are not needed.
Insert into the wheel the filters you need.

Load the filter wheel into the filter module.

Lift the handle to move the filter wheel into its operational position.

The instrument will read the filter barcodes and identify the filters. This information is displayed on the Settings page under the Visual sub-tab of the Inventory tab.

**Prepare transport**

Before moving the instrument, press **Prepare Instrument for Transport**. This button is under the General tab on the Settings page.
Load on the plate carrier the special plate for transport as shown in the figure. Press OK when you have done this. The plate will be taken in and the door closed. You will then be told to shut down the software, screen and instrument.

When you restart the instrument having moved it, the plate carrier will come out so that you can remove the transport plate. After this the instrument is ready for normal use.
Frequently asked questions
Frequently asked questions

What is EnSpire?

EnSpire is a multilabel reader that can measure the following leading technologies: Fluorescence Intensity, Absorbance, Luminescence and Alpha technology.

What features does EnSpire have?

The Alpha technology option includes a laser. The Absorbance option with filters uses a flash lamp and has a photodiode as a detector. It can be combined with the Alpha technology option but not with monochromators. The monochromator option includes monochromators in the excitation and emission paths and can be used for fluorescence intensity and absorbance measurements. Also a high sensitive Luminescence option is available. The instrument always has four barcode readers for plates. A dispenser is available as an option: it has two pumps (1-475 µl, step 0.5 µl, speed 50-500 µl/s). Another optional equipment is a stacker that can be used with magazines for 20 and 50 plates. The Enhanced Security software should be used if you need CFR 21 Part 11 certification.

Can EnSpire be integrated with robotic systems?

Yes, it can be integrated through a Windows Communication Foundation (WCF) application programming interface.

What modes does EnSpire use for different measurements?

It uses an analogue measurement mode for Fluorescence Intensity and Absorbance measurements and photon counting for Alpha technology and Luminescence.

What does analogue measurement mode mean?

In analogue measurement mode the photons from the sample are detected and the photo current is amplified in the detector (photomultiplier tube). The current from the detector is then integrated and converted into a digital signal. The instrument does not count individual photons but a current level caused by multiple photons. Results are Relative Fluorescence Units (RFUs).

What does photon counting mean?

In photon counting, when each photon hits a detector it causes a current pulse. Photon counting electronics counts individual pulses. In addition the
counting electronics includes a discriminator by which the so-called dark pulses (noise) can be effectively eliminated. Results are Counts.

The difference between photon counting and analogue measurement can be described as follows. If you want to count the amount of dripping water then photon counting is equivalent to counting each drop and analogue measurement to counting the amount of water in the sink.

**Why use analogue measurement mode in FI and Absorbance?**

In these measurements the photons are emitted by the sample almost instantly after the lamp has flashed a light pulse into the sample. The photon rate is usually so high that the photon counting mode would be saturated. By using the analogue measurement mode, higher photon levels can be measured from a sample.

**What does the measurement height mean?**

Measurement height is the distance of the light beam focus point from the bottom of the plate (not from the bottom of the well). There is a measurement height wizard which automatically adjusts this value. In Alpha technology the measurement height is the distance between the top of the plate and the detector.

**What is a quad-monochromator?**

Monochromator function relies on the direction of a beam of polychromatic light onto a diffraction grating. The grating separates the incident polychromatic beam into its constituent wavelength components, sending each wave-length into a different direction so that a narrow band of wavelengths can be collected. Double monochromators contain two diffraction gratings. There are two double monochromators in the quad-monochromator version of EnSpire. The use of monochromators provides the benefit that wavelength can be selected continuously, i.e. without steps, through the workstation software.

Using the quad monochromator for fluorescence intensity excitation light from the lamp is directed through the excitation double monochromator into the sample. The emission light is then directed through the emission double monochromator to the detector.

In the case of absorbance the input light comes from a double monochromator but the output light does not go through the second double monochromator.

Although monochromators relieve you of the need to have filters for different wavelengths, a broad waveband cut-off filter is still required in order to block harmonic multiple orders of the wavelength chosen.
cut-off filters can cover the entire range of wavelengths supported by the instrument.
Specifications
Specifications

This section contains information about the safety standards and provides the EnSpire technical information.

Safety standards

Certification:

- IEC-CB, CE and NRTL-TUV Rheinland of North America

The instrument fulfills the requirements of:

- IEC 61010-1:2001
- IEC 60825-1:2007
- EN 61326-1:2006
- UL 61010-1:2004
- CAN/CSA-C22.2 No. 61010-1-04

Class 1 Laser product

**Caution!** Use of this instrument other than specified in the user instructions may result in exposure to hazardous laser radiation.

The laser is classified according to standard EN 60825-1:2007

The safety specifications are met also under the following environmental conditions in addition or in excess to those stated in the operating conditions:

Altitude: up to 2000 m

Temperature: +15°C to +30°C

Relative humidity: 10 - 80%

Mains supply fluctuations: ±10%

Installation category (overvoltage category): II according to IEC 664-1 (Note 1)

Pollution degree: 2 according to IEC 664-1 (Note 2)

**Note!** Installation category (overvoltage category) defines the level of transient overvoltage which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example in CAT II which is the category used for instruments in installations supplied from supply comparable to public mains such as hospital and research laboratories and most industrial
laboratories the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.

*Note!* Pollution degree describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only non-conductive pollution such as dust occurs with the exception of occasional conductivity caused by condensation.

Both of these affect the dimensioning of the electrical insulation within the instrument.

### Conformance to EU directives

| The CE mark conforms to the following EU directives | 2004/108/EC relating to Electromagnetic Compatibility  
2006/95/EC Low Voltage Directive |
| Performance specifications used to verify conformance to the Directives above | EN 61326 class B Requirements  
IEC 61010-1:2001 |

### Environmental conditions

Operating conditions: +15°C to +30°C, Relative humidity 10 - 80%.

Operating conditions for Alpha technology: +20°C to +25°C, Relative humidity 80%.

Transportation conditions: -20°C to +50°C, Relative humidity 5 to 90%, IEC 68-2-56 as guidelines packed in transportation package

Storage conditions: -20°C to +50°C, Relative humidity 5 to 90%, IEC 68-2-56 as guidelines packed in transportation package

### Power requirements

Power consumption: Maximum 300 VA, normal operating conditions 150 VA.

Mains voltage: 100...240 V, 50/60 Hz

### Physical dimensions

**Reader**

Height: 420 mm  
Width: 500 mm  
Depth: 508 mm
Weight: 37.8 kg

Reader with stacker and dispenser option installed
Height: 420 mm
Width: 665 mm
Depth: 727 mm
Weight: 54.2 kg

Input and output connections

Instrument:
- Type B USB
- 9-pin D-sub, female (CAN In)
- 9-pin D-sub, male (CAN Out)

PC:
- DVI-I video connector
- Ethernet connector (RJ-45 10/100 Mb/s)
- At least 4 x USB

Printers:
- Connected to PC, USB or Ethernet

Integrated PC

The workstation software is run under Windows Vista SP1 or Windows 7 on an Intel Core™ 2 Duo (2.6 GHz), minimum 4GB memory, with >80 GB hard disk.

Touch screen

The instrument is controlled by means of a touch screen display. This has a touch sensitive color display with minimum resolution of 1280 x 1024 pixels.

Plates

6, 12, 24, 48, 96 and 384-well plates are compatible with the instrument. The maximum outer dimensions are 86.0 x 128.2 x 28.0 mm. Both opaque and clear plates are suitable (for photometric measurement a clear bottom is required).

Plate barcode specifications

Barcode length: max. 50 mm, 6 – 20 characters
Barcode height: min. 5 mm
Empty space at the ends of barcode label: min. 10 mm
Minimum bar width min. 0.25 mm
Bar-space ratio 1/3
Non-fluorescent label material
Code types (variable number of digits, no check digit):

- CODE39
- INTERLEAVED 2/5
- CODABAR
- CODE128

Barcode reading from all four sides of a microplate.

**Light sources**

The flash light source used for measurements is a UV xenon flash tube, spectral range 230 - 1000 nm. In Alpha technology a 680 nm semiconductor diode laser is used.

**Detection units**

Absorbance: Photodiode, range 230-1000 nm

Fluorescence Photomultiplier tube (in accordance of the monochromator), range 230 - 850 nm

Alpha technology: very high sensitivity, photomultiplier tube, range 400 - 650 nm.

Luminescence: very high sensitivity, photomultiplier tube, range 400 - 650 nm.

**Measurement directions**

- Fluorescence intensity from top
- Fluorescence intensity from bottom
- Absorbance from top to bottom
- Alpha technology from top
- Luminescence from top

**Dispenser Specifications**

Number of injectors Two
plate formats 6 to 384-well plates
Volume range 1 - 475 μl per stroke (Stroke volume is volume dispensed in one syringe cycle. Up to four dispense strokes per well can be programed.
Minimum dispense volume 1 μl
Specifications

Dispense increments 0.5 μl steps

Accuracy
0.1 % @ 350 μl
1.1 % @ 10 μl
< 3 % @ 1 μl

Precision
0.05% @ 350 μl
1.0 % @ 10 μl
< 3 % @ 1 μl

Speed 50-500 μl/sec

Dead volume <400 μl (reagent volume needed to prime the system)

Temperature control Software controlled for reagent containers (up to 65 °C)

Stirring Software controlled magnetic stirrer 100 to 500 rpm

Plate shaking

Three plate shaking modes are available: linear, orbital and double orbital. Three speed levels can be selected and the amplitude of the movement is adjustable. The plate shaking is available for all technology.

Stacker

The stacker can hold magazines for 20 or for 50 plates.

Temperature controller

Minimum temperature: ambient temperature +2 °C
Maximum temperature: +65 °C
Temperature gradient @ 37 °C: ±0.5 °C
Heating time for room temperature to 37 °C: < 15 min
Temperature accuracy: ± 1 °C
Condensation prevention: Upper heating element can be adjusted to the higher temperature than the lower element (max. difference 4 °C).

Scanning

Scanning of wells (several measuring points within a well) are available for most technologies.

Application wavelength ranges

- Photometric application range 230 – 1000 nm
- Photometric spectrum scan 230 – 1000 nm
Specifications

- Fluorescence intensity application range 230 - 850 nm
- Fluorescence intensity excitation & emission spectrum scan measurement 230 - 850 nm
- Luminescence application range 400 - 650 nm.
- Verification of spectral properties of a fluorescence label prior to measurement with fixed excitation and emission wavelengths

**Photometric performance with monochromators**

- Wavelength range: 230-1000 nm
- Wavelength selection: monochromator, tunable in 0.1 nm increments
- Photometric resolution: 0.001 OD

**Fluorescence intensity performance with monochromators**

- Wavelength selection: monochromators, tunable in 0.1 nm increments

**Measurement times and performance**

*Typical throughput times (time per plate)*

<table>
<thead>
<tr>
<th>Technology</th>
<th>Time for 96 wells</th>
<th>Time for 384 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs with quad-monochromator</td>
<td>42 s</td>
<td>1 min 28 s</td>
</tr>
<tr>
<td>FI with quad-monochromator</td>
<td>1 min 3s</td>
<td>2 min 38 s</td>
</tr>
<tr>
<td>Alpha technology</td>
<td>54 s</td>
<td>1 min 48 s</td>
</tr>
<tr>
<td>Abs with filters</td>
<td>31 s</td>
<td>1 min 6 s</td>
</tr>
<tr>
<td>Luminescence</td>
<td>49 s</td>
<td>1 min 47 s</td>
</tr>
</tbody>
</table>

*Minimum throughput time (time per plate)*

<table>
<thead>
<tr>
<th>Technology</th>
<th>Time for 96 wells</th>
<th>Time for 384 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs with quad-monochromator</td>
<td>22 s</td>
<td>27 s</td>
</tr>
<tr>
<td>FI with quad-monochromator</td>
<td>38 s</td>
<td>1 min 5 s</td>
</tr>
</tbody>
</table>
### Specifications

<table>
<thead>
<tr>
<th>Technology</th>
<th>41 s</th>
<th>1 min 15 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs with filters</td>
<td>22 s</td>
<td>27 s</td>
</tr>
<tr>
<td>Luminescence</td>
<td>42 s</td>
<td>1 min 13 s</td>
</tr>
</tbody>
</table>

### Measurement Performance

<table>
<thead>
<tr>
<th>Technology</th>
<th>Feature</th>
<th>Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence intensity,</td>
<td>Detection limit (96-plate)</td>
<td>&lt;1 fmol/well</td>
</tr>
<tr>
<td>Fluorescence intensity, monochromator</td>
<td>Detection limit (384-plate)</td>
<td>&lt;1 fmol/well</td>
</tr>
<tr>
<td>Fluorescence intensity, monochromator</td>
<td>Bandwidth (Excitation / Emission)</td>
<td>&lt; 8nm</td>
</tr>
<tr>
<td>Absorbance Filter / Monochromator</td>
<td>Measurement range (96-plate, 200 µL)</td>
<td>0-4 OD @ 405 nm</td>
</tr>
<tr>
<td>Absorbance Filter / Monochromator</td>
<td>Measurement range (384-plate, 50 µL)</td>
<td>0-4 OD @ 405 nm</td>
</tr>
<tr>
<td></td>
<td>Accuracy @ 2 OD</td>
<td>&lt; 2% @ 405 nm</td>
</tr>
<tr>
<td></td>
<td>Precision @ 2 OD</td>
<td>&lt; 0.1 % @ 405 nm</td>
</tr>
<tr>
<td>Absorbance Monochromator</td>
<td>Bandwidth</td>
<td>&lt;8 nm</td>
</tr>
<tr>
<td>Absorbance Monochromator</td>
<td>Wavelength accuracy</td>
<td>±2.0 nm</td>
</tr>
<tr>
<td>Absorbance Monochromator</td>
<td>Wavelength precision</td>
<td>±0.2 nm</td>
</tr>
<tr>
<td>Technology</td>
<td>Feature</td>
<td>Performance</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Alpha technology</td>
<td>Detection limit (384-plate, 50 µL) (25 µL, phosphorylated bio-peptide, kinase assay)</td>
<td>&lt; 100 amol</td>
</tr>
<tr>
<td>Luminescence</td>
<td>Detection limit: ATPlite glow luminescence (384-plate, 50 µL)</td>
<td>&lt; 10 pM</td>
</tr>
</tbody>
</table>
WEEE instructions for PerkinElmer products

A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, according to the regulatory guidelines in your area.

The objectives of this program are to preserve, protect and improve the quality of the environment, protect human health, and utilize natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the customer’s environment.

Requirements for waste collection, reuse, recycling, and recovery programs vary by regulatory authority at your location. Contact your local responsible body (e.g. your laboratory manager) or authorized representative for information regarding applicable disposal regulations. Contact PerkinElmer at the web site listed below for information specific to PerkinElmer products.

Web address:
http://las.perkinelmer.com/OneSource/Environmental-directives.htm

Customer Care: 1-800-762-4000 (inside the USA)  
(+1) 203-925-4602 (outside the USA)  
0800 40 858 (Brussels)  
0800 90 66 42 (Monza)

Products from other manufacturers may also form a part of your PerkinElmer system. These other producers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these producers directly before discarding any of their products.

Consult the PerkinElmer web site (above) for producer names and web addresses.
Glossary
### Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha technology option</td>
<td>detection unit designed for AlphaScreen and AlphaLISA assays, consists of a very high sensitivity detector and a semiconductor laser. The Alpha technology is based on the use of coated beads. Donor beads are excited by laser light. Energy is transferred to bound acceptor beads which then emit light</td>
</tr>
<tr>
<td>barcode reader</td>
<td>component that scans the ID barcode mounted on any of the four sides of the sample plate</td>
</tr>
<tr>
<td>bottom measuring head</td>
<td>measuring head positioned below the x-y conveyor</td>
</tr>
<tr>
<td>bottom measuring head body</td>
<td>body section of the bottom measuring head; it embodies dedicated positions for different modules</td>
</tr>
<tr>
<td>detector</td>
<td>component that intensifies and measures the signal from the emission channel. Photomultiplier tubes and photodiodes are used as detectors in the EnSpire.</td>
</tr>
<tr>
<td>dispenser unit</td>
<td>device for dispensing to a microtitration plate in EnSpire. One or two pumps can be installed.</td>
</tr>
<tr>
<td>diffraction grating</td>
<td>a device for dispersing light, used as the basis for a monochromator</td>
</tr>
<tr>
<td>filter (optical)</td>
<td>component letting only a certain bandwidth range of light pass through</td>
</tr>
<tr>
<td>filter wheel</td>
<td>support into which the filter is fitted</td>
</tr>
<tr>
<td>high throughput light source</td>
<td>flash lamp producing wide-spectral excitation light</td>
</tr>
<tr>
<td>instrument case</td>
<td>assembly outer cover protecting the instrument</td>
</tr>
<tr>
<td>laser</td>
<td>a laser emitting light at a wavelength of 680 nm and used to excite samples in Alpha technology measurements</td>
</tr>
<tr>
<td>lid</td>
<td>opening in the upper instrument case providing access to the instrument</td>
</tr>
<tr>
<td>Terms</td>
<td>Definitions/Description</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>lid sensor</td>
<td>sensors in the instrument case to ensure that the lid and the door are properly closed</td>
</tr>
<tr>
<td>light guide</td>
<td>optical fiber to guide light</td>
</tr>
<tr>
<td>loading door</td>
<td>light-tight sliding door and the related mechanism</td>
</tr>
<tr>
<td>Luminescence</td>
<td>a detection mode for bio- and chemiluminescent assays.</td>
</tr>
<tr>
<td>measurement height changer</td>
<td>module to adjust the height of the measuring heads from the point of measuring</td>
</tr>
<tr>
<td>monochromator</td>
<td>device for separation of a narrow band of light wavelengths from a broader range of wavelengths</td>
</tr>
<tr>
<td>multilabel reader</td>
<td>entity that enables measuring different applications</td>
</tr>
<tr>
<td>multilabel test plate</td>
<td>a special microplate for the user to test the operation of the instrument. It has samples suitable for testing the operation of optical components for various labels</td>
</tr>
<tr>
<td>photometric detector</td>
<td>part located below the sample; for signal measurement it contains a photodiode</td>
</tr>
<tr>
<td>photometric optics</td>
<td>assembly to reconstruct the emission light from the light cable into a beam that passes through the sample well bottom</td>
</tr>
<tr>
<td>plate carrier</td>
<td>mechanical part on which the sample is conveyed in the instrument</td>
</tr>
<tr>
<td>stacker</td>
<td>device to enable automatic transfer of sample plates to/from the instrument</td>
</tr>
<tr>
<td>top measuring head</td>
<td>measuring head positioned above the x-y conveyor</td>
</tr>
<tr>
<td>very high sensitivity detector</td>
<td>a separate special detector used for luminescence and Alpha technology measurements</td>
</tr>
<tr>
<td>x-y conveyor</td>
<td>mechanism that moves the plate carrier in the horizontal plane between different functional positions including from the loading position to the position in the instrument where measurement occurs</td>
</tr>
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