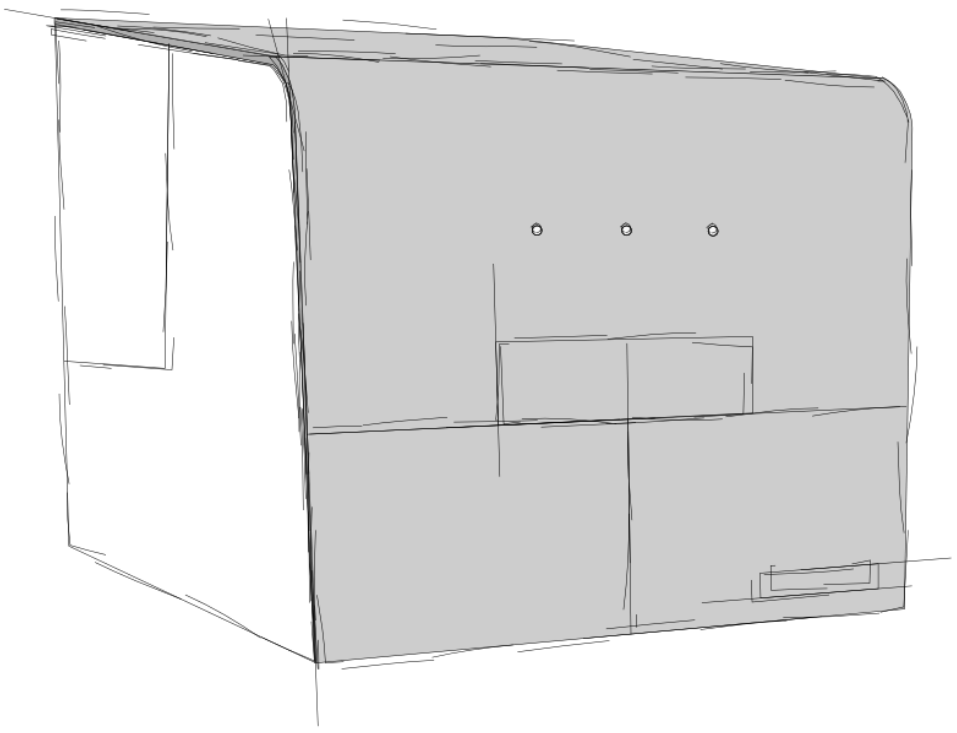
trinean

DropSense 96 and DropQuant v1.5

User manual

June 2015



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This manual is published by TRINEAN NV/SA.

Questions or comments regarding the content of this manual can be directed to the address below or to your TRINEAN representative.

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TRINEAN reserves the right to make technical improvements to this equipment and documentation without prior notice as part of a continuous program of product development.

This manual supersedes all previous editions.

Trinean warranty statement

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Trinean will provide spare parts of DS-series instruments and related items will be made available at least 7 years, directly or through third parties (e.g. distributors).

Furthermore, TRINEAN shall not be liable for incidental or consequential damages. Failure of the Customer to notify TRINEAN of a claimed defect by registered mail within thirty days of the discovery thereof shall constitute a waiver of any claim for breach of warranty. When a product is required by TRINEAN to be installed by a TRINEAN certified engineer, the period of this Warranty shall begin on the date of such installation, provided however, that any use of the product prior to such installation shall, at sole election of TRINEAN, void this Warranty. When installation by TRINEAN personnel is not required, the period of this Warranty shall begin on the date of shipment from TRINEAN. The period of this Warranty shall begin as described above whether or not the product has been installed pursuant to a purchase order, and any trial period shall be deducted from the Warranty period that would otherwise apply under a subsequent placed purchase order for that product.

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Warranty statements may differ in specific countries.

Introduction

Thank you for purchasing the DropSense96. This is a unique system for the quantification of microliter samples, consisting of DropPlate-Disposables, a DropSense96 instrument and DropQuant software.

This manual is intended for all DropSense users, and it describes the installation, operation and measurement procedures. An electronic copy of this manual can be opened from the DropQuant program by clicking the Help button.

Warning: Situations that can damage the instrument or your experiment.

Caution: Situations that can potentially affect the results of your measurements.

Note: Notes, usage tips, or additional information.

Warning

The DropSense system is designed for UV/Vis quantification of small samples. Using the system for other applications doesn't guarantee the user's safety and proper functioning of the instrument.

This instrument is intended to be used by persons having a basic knowledge of UV/Vis spectroscopy and the handling of nucleic acids and protein samples.

Before starting the installation or use of the system, read this manual carefully and make sure to understand the instructions to avoid safety issues.

Keep the manual close to the instrument for reference. The content of this manual is subject to change without prior notice.

Safety Information

Before using the DropSense 96 system, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the DropSense 96 and to maintain the DropSense 96 in a safe condition.
The following types of safety information appear throughout this user manual.
Details about these circumstances are given in a box the one below.
The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

Handling and positioning
Always make sure the instrument is placed on a steady surface, allowing easy access to its back. ***It is extremely important that both the power button and the power cable at the back are always easily accessible.***
Before moving/repositioning the instrument, always make sure that there's enough space for that. Failing to provide adequate work space can cause material damage or injury to the handler.

Risk of personal injury
When moving the instrument, always make sure to use a 2 person lift as the apparatus weights more than 18kg.

Risk of personal injury and material damage [W1]
Improper use of the DropSense 96 may cause personal injuries or damage to the instrument. The DropSense 96 must only be operated by appropriately trained and experienced personnel.
Servicing of the DropSense 96 must only be performed by Trinean trained service personnel or service technicians of an authorized agent.
Using the apparatus in a manner which is not specified in this user manual can impair the protection provided by the equipment.

Risk of personal injury and material damage [W2]
Do not attempt to move the DropSense 96 during operation.

Explosive atmosphere [W3]
The DropSense 96 is not designed for use in an explosive atmosphere.

Damage to the instrument [C1]
Direct sunlight may bleach parts of the instrument and cause damage to plastic parts. The DropSense 96 must be located out of direct sunlight.

Damage to the instrument [C2]
Avoid spilling water or chemicals onto the DropSense 96. Damage caused by water or chemical spillage will void your warranty.
In case of emergency, switch off the DropSense 96 at the power switch and unplug the power supply from the power outlet.

Moveable parts
While operating the instrument, please beware of any moveable parts.

Electrical safety

Note: Disconnect the line power outlet before servicing.

Electrical hazard [W4]
Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.
Intentional interruption is prohibited.
Lethal voltages inside the instrument
When the instrument is connected to line power, terminals may be live. Opening covers or removing parts is likely to expose live parts.

Electrical hazard [W4]
Only use the provided power supply and supply cord. For replacements contact your local distributor.
Use of wrong power supply may cause fire due to overheating.

To ensure satisfactory and safe operation of the DropSense 96 follow the guidelines below:

- The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground).
- If the instrument becomes electrically unsafe, prevent other personnel from operating it, and contact your local distributor or Trinean Service support. The instrument may be electrically unsafe when:
    - The line power cord appears to be damaged.
    - It has been stored for a prolonged period of time in conditions which are outside of the normal storage conditions.
    - It has been subjected to severe transport stresses.

Biological safety

Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store, and dispose of such samples according to the required safety regulations.

Samples containing infectious agents [W5]
Some samples used with this instrument may contain infectious agents. DropSense96 is not intended to be used for biological samples or infectious agents within the WHO risk class 3 and 4. Handle such samples with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, 2 pairs of gloves, and a lab coat.
The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of infectious agents as defined in the applicable Safety Data Sheets (SDSs) or OSHA, ACGIH,† or COSHH‡ documents.*
Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).
† ACGIH: American Conference of Government Industrial Hygienists (United States of America).
‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Chemical safety

Toxic fumes

If working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

Waste disposal

In case plasticware contain hazardous chemicals, or contagious/biohazardous materials. Such wastes must be collected and disposed of properly according to local safety regulations.

Mechanical

To ensure satisfactory and safe operation of the DropSense 96, follow these guidelines:

- Use only recommended consumables/slides.
- When powering down the instrument, or when powering it up, please do not stand or support your weight on the instrument. Rotate the instrument or try to access it from behind by rotating the instrument.

Maintenance safety

Perform the maintenance as described in Annex B. Your local distributor and/or Trinean charges for repairs that are required due to incorrect maintenance.

Risk of personal injury and material damage [W6]
Only perform maintenance that is specifically described in this user manual.

Risk of electric shock [W7]
Do not open any panels on the DropSense 96.

Damage to the instrument [C3]
Do not use solvents, or reagents containing acids, alkalis, or abrasives to clean the DropSense 96.

Specs for DS96

Technical data

Trinean reserves the right to change specifications at any time.

Environmental conditions

Operating conditions	100–240 V AC, 50/60 Hz
Power	Mains supply voltage fluctuations are not to exceed 10% of the nominal supply voltages External 24 V power supply designed for wide range of input voltages; maximum power consumption approximately 15 W
Fuse	T1A
Overvoltage category	OVC II
Data output	USB
Air temperature	15ºC to 35ºC (59ºF to 95ºF)
Relative humidity	Max. 75% (noncondensing)
Altitude	Up to 2000 m (6500 ft.) above mean sea level (MSL)
Place of operation	For indoor use only

Transportation conditions

Air temperature	–25ºC to 60ºC (–13ºF to 140ºF) in manufacturer's package
Relative humidity	Max. 75% (noncondensing)

Storage conditions

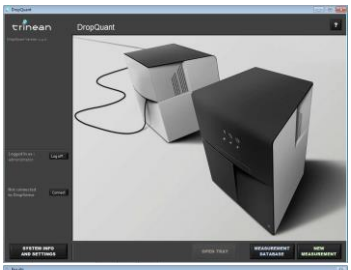
Air temperature	5ºC to 40ºC (41ºF to 104ºF) in manufacturer's package
Relative humidity	Max. 85% (noncondensing)

DropSense96 at a glance 1

DropSense96 Overview

The DropSense96 platform is designed for measuring the UV/Vis absorption spectrum of microliter-sized samples, suitable for both manual and automated workflows. From the measured absorption spectrum, the quantity and quality of small biomolecules such as DNA, RNA and proteins is calculated. This platform is the answer for many applications in life science labs.

Dropsense96
Multi-channel reader
UV/VIS spectroscopy
Full Spectrum analysis



DropQuant
Measurement software
Experiment layout
Spectral analysis
Easy data export

DropPlate 16/96
Disposable microfluidics micro-cuvettes
Anti-evaporation system

cDrop
Analytical software
Sample content
Specific quantification
Quality analysis

The DropSense96 is composed by:

- 5-channel spectrometer for reading the UV/Vis absorption spectra
- Pump and manifold unit for that allows the sample to run inside the disposable cuvette
- Mechanical stage for moving the disposables inside the instrument

The system includes the following measurement modes:

1. **Direct quantification:** the concentration of the substances is calculated from the absorption curve using Beer Lambert Law: OD = ε * C * L. With ε the (material dependent) extinction coefficient, C the concentration, L the path length of the beam passing through the sample, and OD the absorption in the sample (defined as log (P/Pref). DropQuant includes pre-defined values of the extinction coefficient of most frequently used materials. 2 groups of biomolecules are supported: nucleic acids and purified proteins. Based on the selection of the material, DropQuant gives more information on the sample, for example the 260/280 and 260/230 ratios for DNA samples, etc.

cDrop builds further on this selection.

2. **General UV/vis mode:** The result of the measurement is the full absorption spectrum as a function of wavelength (range: 230nm-750nm).
3. **Standard curves:** a basic standard curve method is available since DropQuant 1.2. The user defines the reference samples and their corresponding reference values, and the unknown samples. DropQuant constructs then the standard curve, and calculates the concentration of the unknown samples based on that information.
4. **cDrop**: a software extension that performs mathematical analysis on the UV/Vis spectra to extract information on the content of the samples (in contrast to the direct quantification method that uses the absorption information at a single wavelength to calculate the concentration of the sample). cDrop is a software add-on. It is sold as a separate product but it integrates perfectly on the DropQuant software.

The small sample requirement using the DropPlates makes the DropSense96 the perfect solution for many applications:

- Measuring the concentration (A260 nm) and purity of nucleic acid samples (DNA/RNA/oligos)
- Fluorescent dye labeling density of nucleic acid microarray samples
- Purified protein analysis (A280 nm)
- Quantification of fluorescent dye labeled proteins
- API quantification (Active Pharmaceutical Ingredient)
- General UV-Vis spectrophotometry

Trinean offers a complete system to the customer, including a number of add-ons. Contact your local distributor to get more information on these extensions to the DropSense system.

In order to assure an optimal functioning, the DropSense96 uses our proprietary DropPlates, described below.

Consumables

The DropSense is the only platform that can read DropPlates: a full 96-well plate can be read in less than 5 minutes.

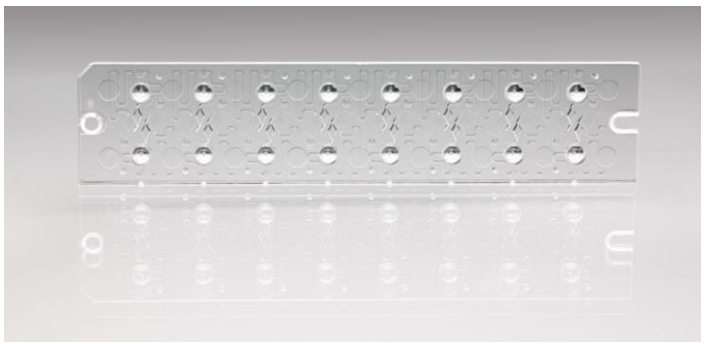
The Trinean DropPlate consumables are made of advanced technical plastics allowing high optical transmission over the full UV-VIS spectrum with a high accuracy. The basic parts of the disposable are:

- The conical-shaped input wells are positioned with a 9mm (SBS standard) pitch fit for multi-channel pipettes or robotic sample loading.
- These input wells are connected with a capillary storage channel. When dispensing a sample droplet into the input well, it is immediately transferred into the storage channel through capillary forces in order to suppress sample evaporation. This allows the user to perform the measurement on the DropSense96 within a time span of 2h after dispensing.

- The capillary storage channel is further downstream connected with one or two-measurement cuvettes for optical readout, as shown below. Those same cuvettes have a fixed path length.
- The microfluidic channel continues via a small channel towards the vent hole, to which the DropSense96 instrument connects its vacuum pressure system.

All DropPlates have an expiration date. Don't use the DropPlate after that date, as the self-filling function is no longer guaranteed.

During the measurement, the instrument simultaneously measures the UV/Vis absorption through the measurement cuvettes during the pressure-driven transport of the samples from the capillary storage channel to the measurement cuvettes. This allows the instrument to monitor the filling behavior of the measurement cuvettes and analyze the spectral absorbance of the sample. The microfluidic chip allows accurate and reproducible measurements due to fixed path lengths and elimination of sample evaporation in the input well.
There are 2 different approaches for our disposables organized in 2 different types of chips.



The plates – 2 different approaches

- DropPlate16: this small version of the DropPlate consumable has 16 input wells. The DropPlate16 must be mounted on an aluminum carrier: the DropFrame. Up to 6 DropPlates can be mounted on a DropFrame, allowing measuring 1 to 96 samples simultaneously. The DropFrame has the same outer dimensions as a standard 96well plate, assuring compatibility with liquid handling robots.

- DropPlate96: this 96well microtiter plate is designed for high-throughput use. This consumable is ideal for automated workflows as it is easy stackable and it includes a bar-code for sample tracking. The DropPlate96 is compatible with liquid handling robots (SBS standards) and it allows easy sample loading, preservation and measurement of the droplet optical absorption.

It is not necessary to fill all 16 input wells to perform a measurement. A single sample measurement can be done and the unused positions on the DropPlates can be filled in another measurement until all input reservoirs have been filled.

DropPlates are single-use disposables. Don't try to re-use a plate. The meander's self-filling behavior disappears and pipetting will result in air-bubbles.

The frames

Up to 6 DropPlates16 can be placed on a **DropFrame**, creating a **full 96well** plate with standard 96well plate dimensions (SBS standard). The **DropFrame** includes mechanical features defining a unique position of the DropPlates. This plate can only be placed in one way on the DropFrame due to the alignment pins and orientation triangles on the frame.
It is recommended to put the DropPlates16 on the frame before loading the liquid samples. The DropFrame is black colored to aid the visual inspection of loaded or empty input wells and associated sample reservoirs. This specific design of the DropFrame minimizes human errors during sample loading.

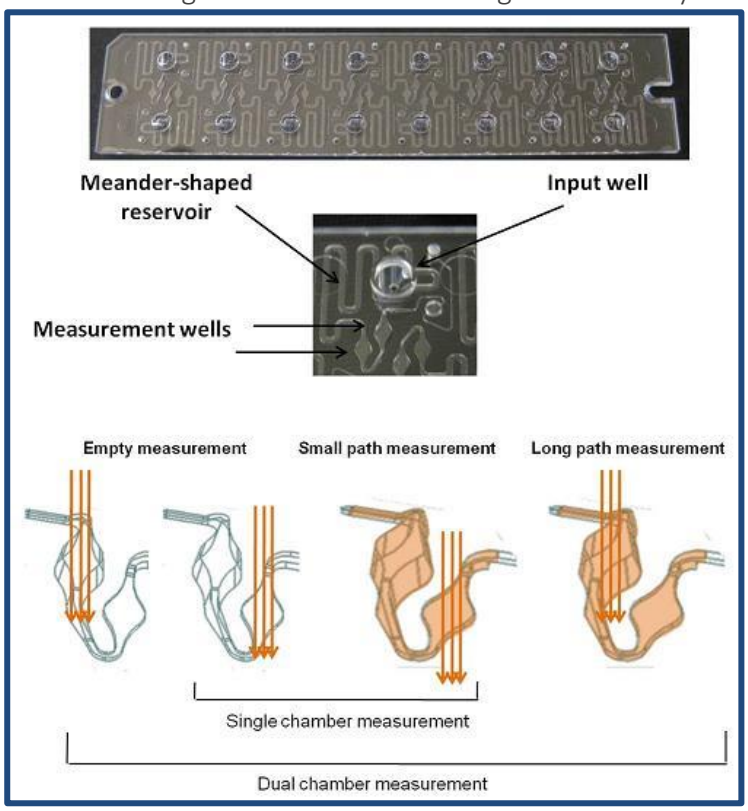
DropPlate-D+ and DropPlate-S – 2 different types for your needs

Currently Trinean has available 2 types of plates: the DropPlates S with one measurement cuvette and faster readout time, and the DropPlate-D+ with larger measurement range.
All DropPlate types have identical external dimensions and conical-shaped input wells. The input well is connected to a meander-shaped storage reservoir that uses capillary force to take up the sample from the input well. This technique protects the samples from evaporation.

Target applications	DP-S	DP-D+
Nr of measurement reservoirs (and path length)	1 (0.5mm)	2 (0.1 and 0.7mm)
Recommended sample quantity	2uL	2uL
Accepted Variation Volume	30%	20%
Sample residence time	2h	2h
Measurement range (OD10mm)	0.1 to 40 OD10mm	0.1 to 200 OD10mm
Measurement reproducibility	< 2 OD: ± 0.03 OD > 2 OD: ± 1.5%	< 2 OD: ± 0.03 OD > 2 OD: ± 1.5%
Measurement time for DP96 plate	< 5 minutes	< 8 minutes
Software and firmware requirements	All systems	DropQuant 1.2 and DropSense

The DropPlate-D+

The DropPlate-D+ (Dual New Generation) has two measurement reservoirs for optical analysis. The capillary storage chamber can store up to 2 µl sample. By sequentially filling both optical chambers, a dual path length measurement is done. The combination of both path lengths results in a large OD measurement range and thereby avoids the need of sample dilution.



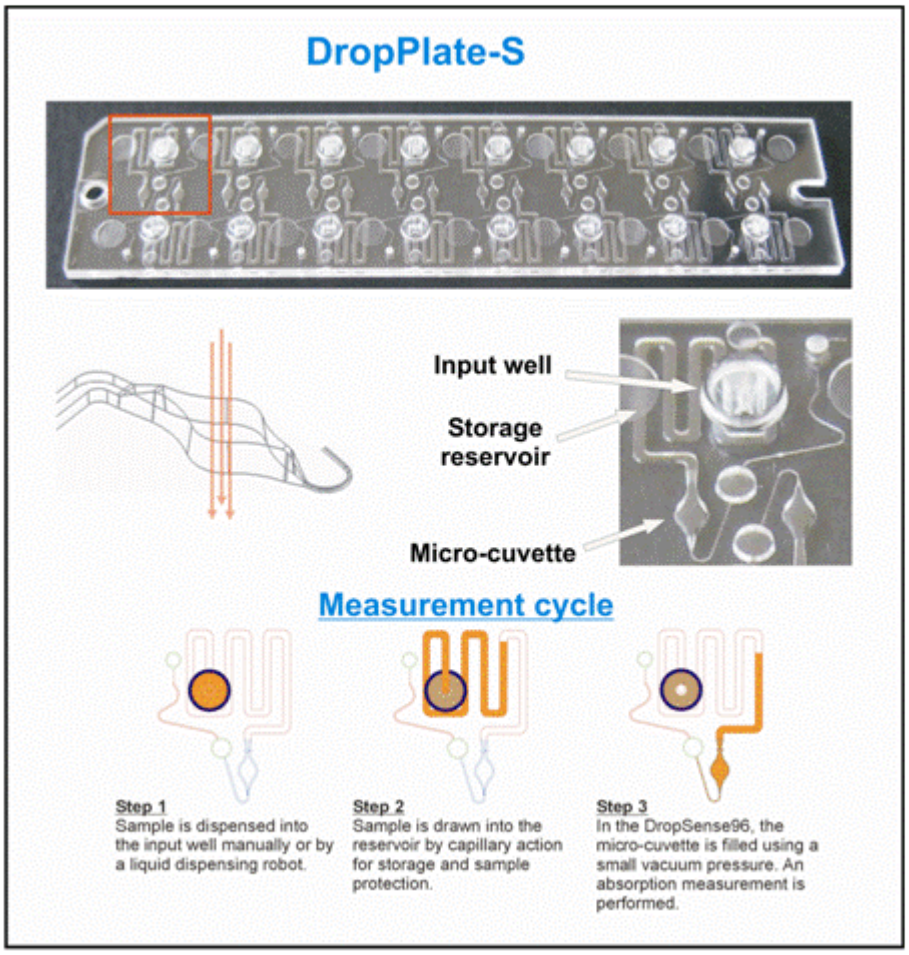
The D+ plate has 2 measurement reservoirs allowing measurements at 0.1mm and 0.7mm path length, offering a wider measurement range for higher-concentration samples.

Sample size requirements: For correct and more accurate measurements, it is essential that a minimal amount of sample is dispensed for correct filling of the measurement chambers allowing precise measurements. Although this volume range takes a pipetting error into account, it is best to use a precision pipette (e.g. 0.5-5 µl) with precision tips to ensure that the recommended sample quantity is dispensed.

The DropPlate-S

The DropPlate-S looks very similar to the DropPlate-D+, however the meander reservoir is shorter and due to having only one measurement chamber, its maximum range is lower than on the D+. The path length of the cuvette is 0.5mm, and with a 0.1-40 OD range (for a 10mm equivalent path) the DropPlate-S is ideally suited for quantification of biomolecule samples derived from automated extraction procedures with a stable yield. For example, extracted human genomic DNA tends to be within a 50-1000 ng/µl concentration range and it can be quantified using the DropPlate-S. Since the DropPlate-S contains only one micro-cuvette instead

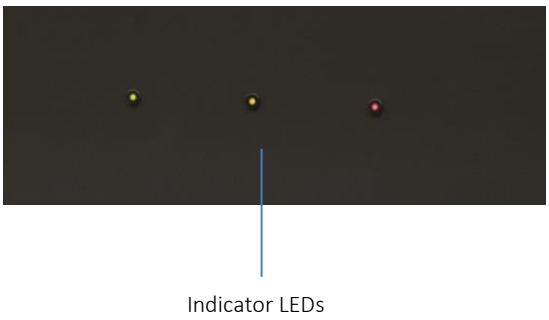
of two like the DropPlate-D+, the measuring time for a full 96well DropPlate-S is decreased by half to about 4 minutes.



Please refer to the glossary for details on OD, Sample size requirements and recommended sample quantity.

External Characteristics

The front panel of the instrument contains the centrally positioned microwell plate drawer to load and unload the **DropFrame** or the **DropPlates**, and three indicator lights: Green, Orange and Red light.



Front

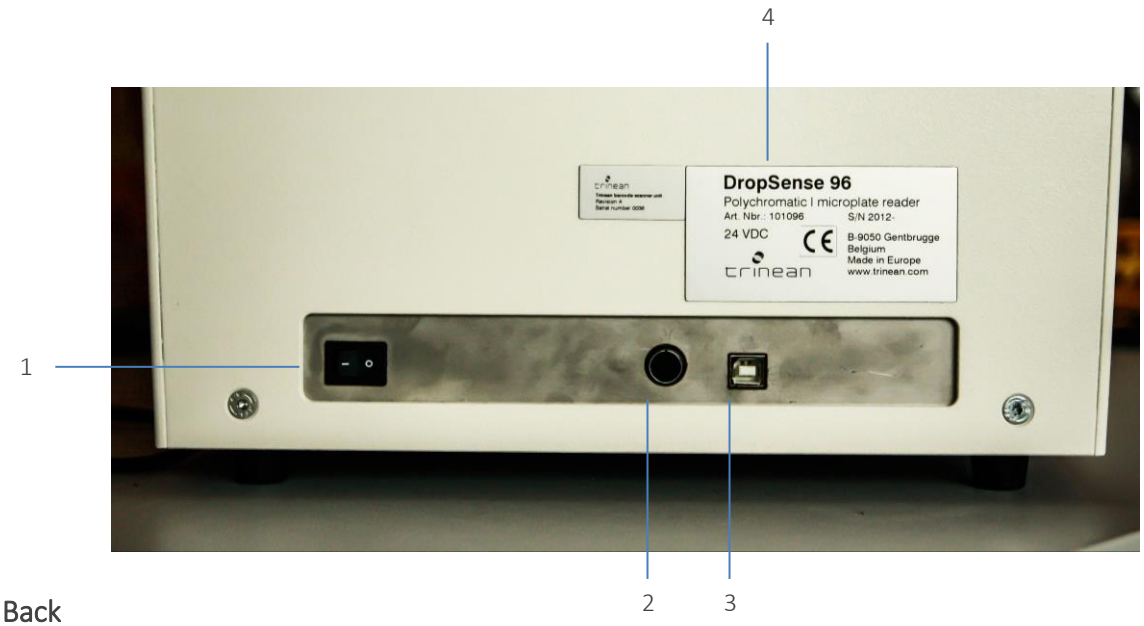
1 The indicator LEDs show the status of the instrument:

a. Green, orange and red: during the DropSense96 start-up, all three lights will flash simultaneously for about 10 seconds
b. Green and red: DropSense96 is ready but no communication with a DropQuant running on the PC has been achieved (check if DropQuant is running and if USB connection is OK)
c. Green: DropSense96 is ready and connected with the PC
d. Green and orange light: busy with a measurement
e. Red: error state. Please refer to the "troubleshooting" section or contact your local distributor

2 **Microplate drawer**

The microplate drawer moves in and outside of the instrument, to load and unload the DropPlates. A lid on the front panel closes the instrument automatically when the drawer is inside the instrument. The drawer remains in the instrument during measurement cycle.
The drawer can accept different types of carriers, as long as their outer dimensions are compatible with the SBS standard.

The plate carrier can be opened at any given time using the 'open tray' button in the DropQuant software. Do not obstruct the movement of the tray. When reading is complete, the drawer of the DropSense96 opens, allowing customers to remove the DropPlate. The drawer closes automatically after 30 seconds (this feature is not active when the DropSense96 is integrated).



Back

1 Power switch
2 Power cord receptacle: plug the power cord in here
3 USB port
4 Label with line voltage rating, cautionary information, and serial number.

It is required to use the Trinean USB cable, including the ferrite filters.
No stable operation of the instrument can be guaranteed if a different USB cable is used, for example if a longer cable or if a cable without the filtering elements is used.

The power supply

The DropSense instrument uses an external AC/DC power supply (similar to a laptop power supply), which is included in the shipment. The power supply input is compatible with the US standards (110VAC) and European standards (230VAC). Depending on the customer's location, a US type power cord or EU type power cord is included with the instrument.
This external power supply delivers 24VDC to the instrument.

Software 2

DropQuant

Trinean's DropQuant Software, is divided in 2 important packages.

- DropQuant – to define and perform experiments.
- DataViewer – to analyze the experiments.

Computer requirements

Computer requirements and software installation
The operating software will only run on a PC meeting the criteria below. No Mac versions of the software are currently available.

- Microsoft Windows XP, Vista, Windows 7, Windows 8 operating system
- Microsoft .NET Framework version 2.0 or higher
- 1.5GHz or higher processor
- 1GB or more of RAM (2GB if running Vista)
- 100 MB of free hard disk space for software installation
- Free USB port (the instrument can only be connected via the USB port)
- Microsoft Excel and Adobe pdf reader to manipulate archived data (optional but essential for that)

Installation/Updates

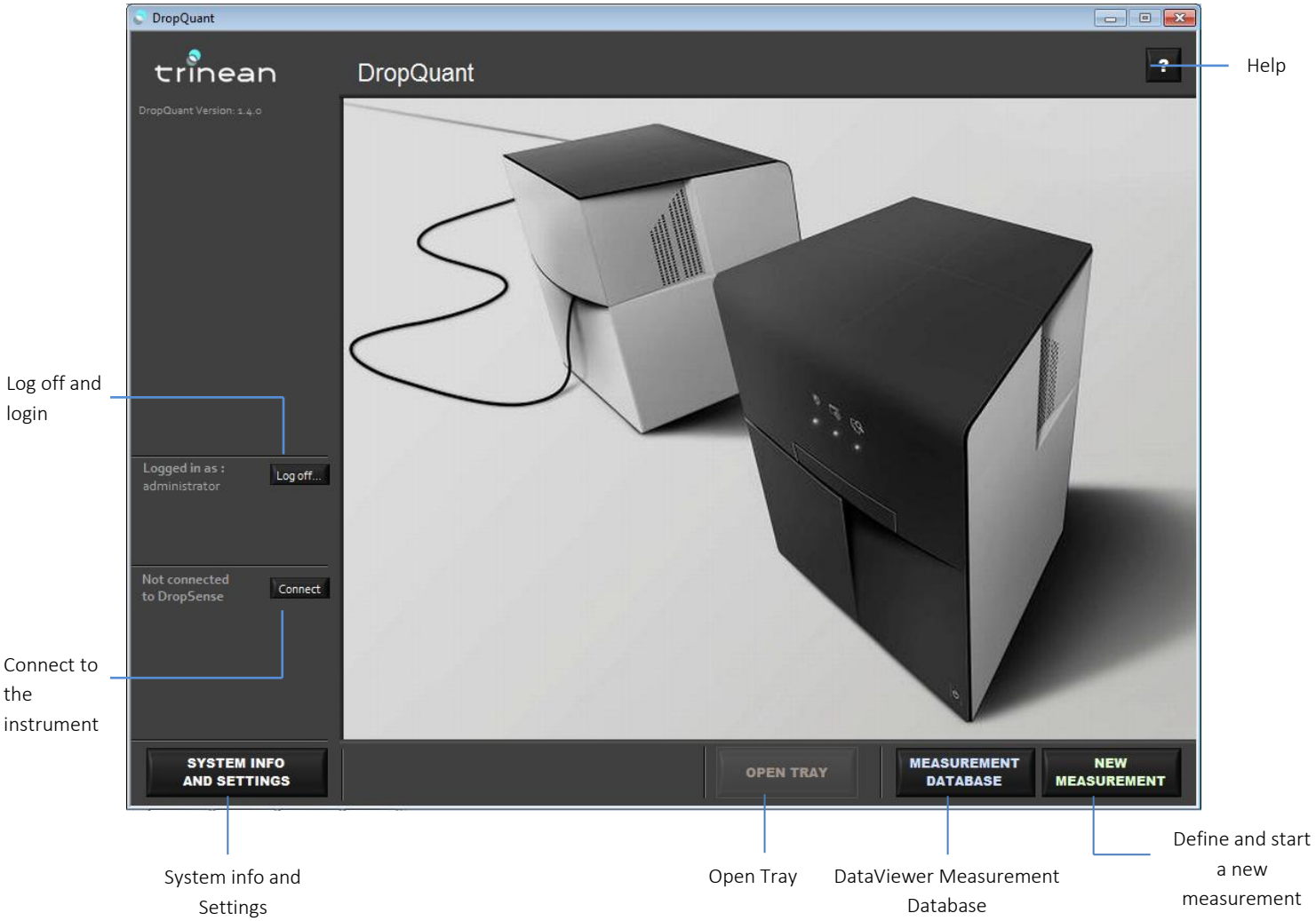
Trinean's DropQuant Software installation and update, works like many other windows software programs, and both its installation and update should work by following the same procedure.

Login

After inputting the user name and password, a series of self-tests will occur. For more details on user names, please consult the user name section in chapter 6.

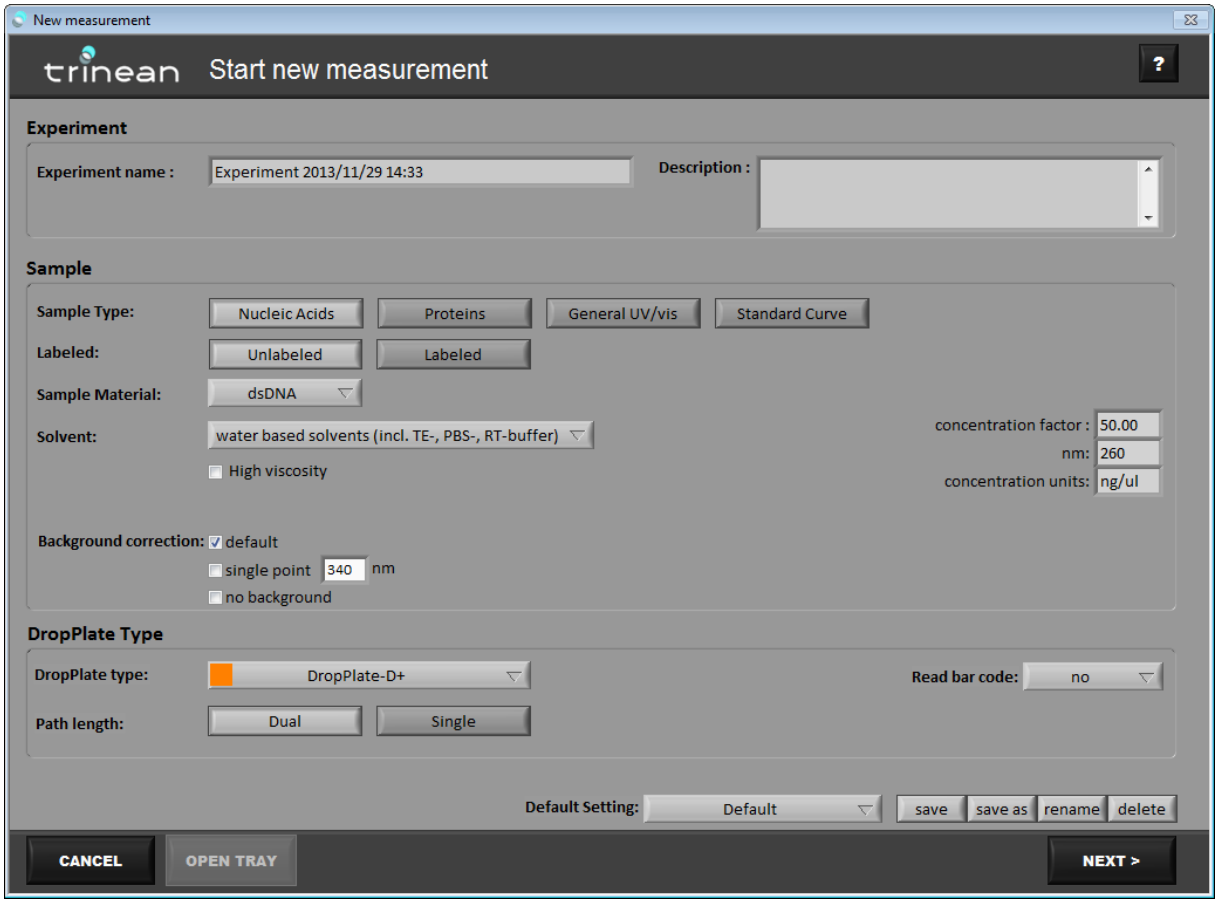
Home screen

On this home screen, it is possible to access the Measurement Database, set up a New Experiment/Measurement and to access the Info and Settings,



New Measurement

Setting up a new measurement with DropQuant, requires a few steps. On the New Measurement window, it is possible to define the following settings:



Experiment definition: Including 2 editable fields, the user can define the experiment by name and include extra information on the description box. A default name is generated for every experiment, and it includes date and time for easy chronologic storing. All the experiment information will be included in the final experiment report.

Sample definition: Several pre-configured applications have been included for quick and easy experiment definition.

- Nucleic acids
- Purified Proteins
- General UV-VIS
- Standard curve

Each of those applications has multiple sub-options that will be explained in the Applications chapter of this manual.

Solvent: Despite not having any influence on the measured spectrum, this option could influence pumping times of the samples.
High viscosity – In some cases where the samples are rather viscous, it might be necessary to activate this mode. The high viscosity mode, allows the pumps to work for some seconds longer than normal, allowing the viscous sample to reach the measurement chambers.

Background selection: Choice between the default background correction using the spectral range of 400-600 nm or a single wavelength of choice.

DropPlate type: Depending on the application or sample concentration range, a choice of DropPlate-S or DropPlate-D+ can be made. Both DropPlates are explained in Chapter 1. Alternatively, a standard 96well microtiter plate can be read. This selection requires additional information such as path length, sample volume and plate brand.

Path length selection: When selecting the DropPlate-D+, the user can choose between a single or dual path length measurements. When using a DropPlate-S, only a single path length measurement is possible. This selection requires a sample volume of 2 µl and generates a measurement range of 0.1-40 OD (for a 10mm path).

Save as Format: When all the above selections have been made, the user can save these in a new format for future use. These formats are username dependent. Only the user administrator has the possibility to make a format that is available for all users. The formats can also be deleted and renamed.

Plate Layout

After defining the experiment, it is time to set up the plate settings.
On this screen, it is possible among other options, to set up multiple plates.

Despite multiple plate selection is possible, only ONE 96 well plate can be loaded and measured at a time into the DropSense 96. Loading multiple plates will damage the instrument.

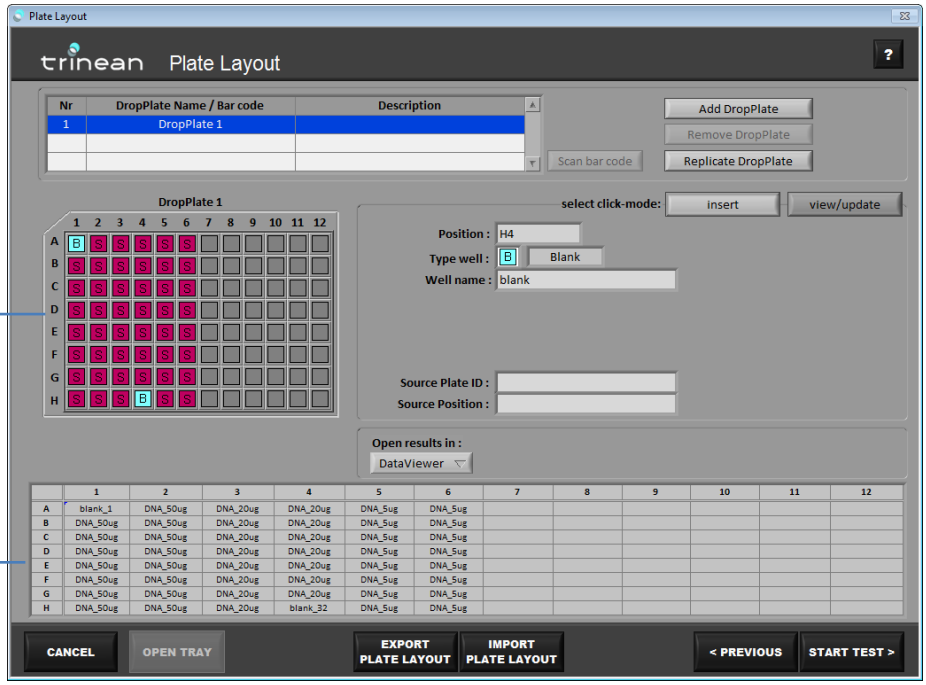


Plate layout definition

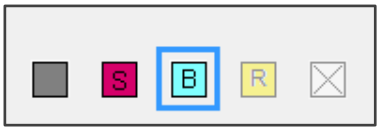
Sample name definition table

DropPlate Name/Barcode and Description – It is possible to set up different plate names for each plate. In case of a DropPlate 96 with a barcode, it is possible to use an internal or an external barcode reader. In case of an internal barcode reader, available at Trinean, is installed, no manual scanning is necessary.
Scanning a bar code also has the advantage of having the system saving all the available wells on an plate into a database.

Select click-mode – These two buttons, allow the user to change between the **insert mode**, where it is possible to add new wells or replace existing ones, to **view/update** mode, where it is possible to navigate through pre-defined wells, and redefine information such as Well name and Type well.

Plate layout definition – here you can define the plate layout, being possible to select several options.

- Type well – Sample, Blank, Reference (for reference curves).
  Note that on sample selection, it is possible to define replicates.



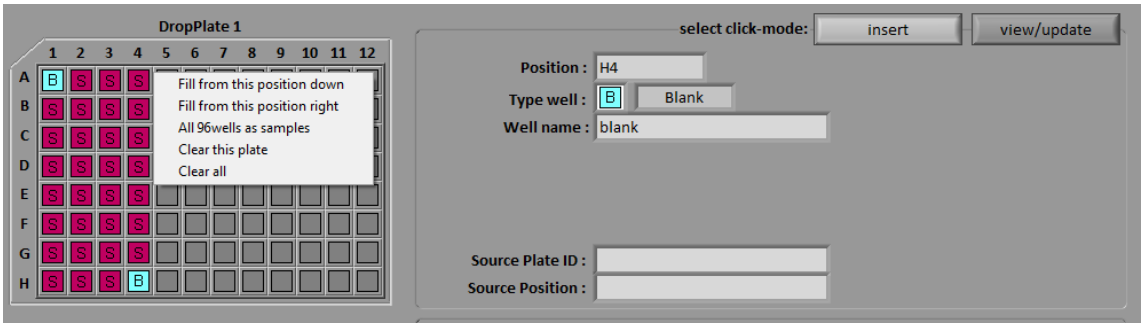
  Blanking information is also available when selecting samples.
  - Autoblank – calculated by software
  - Blank – Average of blanks or specific blank
- Well name – It will be reflected on the table at the bottom of the screen.
- Source plate ID – In case of pipetting the samples from a standard 96 well plate to our plates, it is possible to add a common identifier such as plate ID or a bar code.
- Source plate Position – In case of pipetting the samples from a standard 96 well plate to our plates, it is possible to add a common identifier that allows to track the position of this sample on the plate of origin.

Open results in – for customers with cDrop, it is possible to open the results after the measurement immediately with cDrop for content analysis. For all the other customers, the default setting will be DataViewer. This option must be selected before defining the sample locations(s) on the plate. It is also possible to select between multiple protocols by selecting the sample first and defining it on the plate layout, and then the protocol.

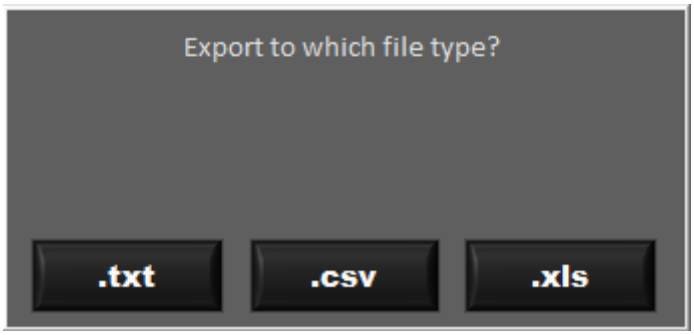
Sample name definition table – Despite being possible to define a plate layout and the sample name on the menus above, it is also possible to define them here using a table format. Simply input the desired name(s) on the table after the sample is defined above.
It is also possible to copy values from a chart in Excel to this one simply by dragging the table or copy/pasting it. This is quite useful when working with complex layouts.

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank											
B												
C												
D												
E												
F												
G												
H												

Additional Settings – Right clicking on the Plate Layout definition field, it is possible to access a set of options that allow the user to fill plate positions in a serial mode.



Export/Import Plate Layout – In order to speed up the process of starting a measurement, it is possible to pre-define a plate layout. Several formats are accepted, such as txt, csv and xls.



trinean

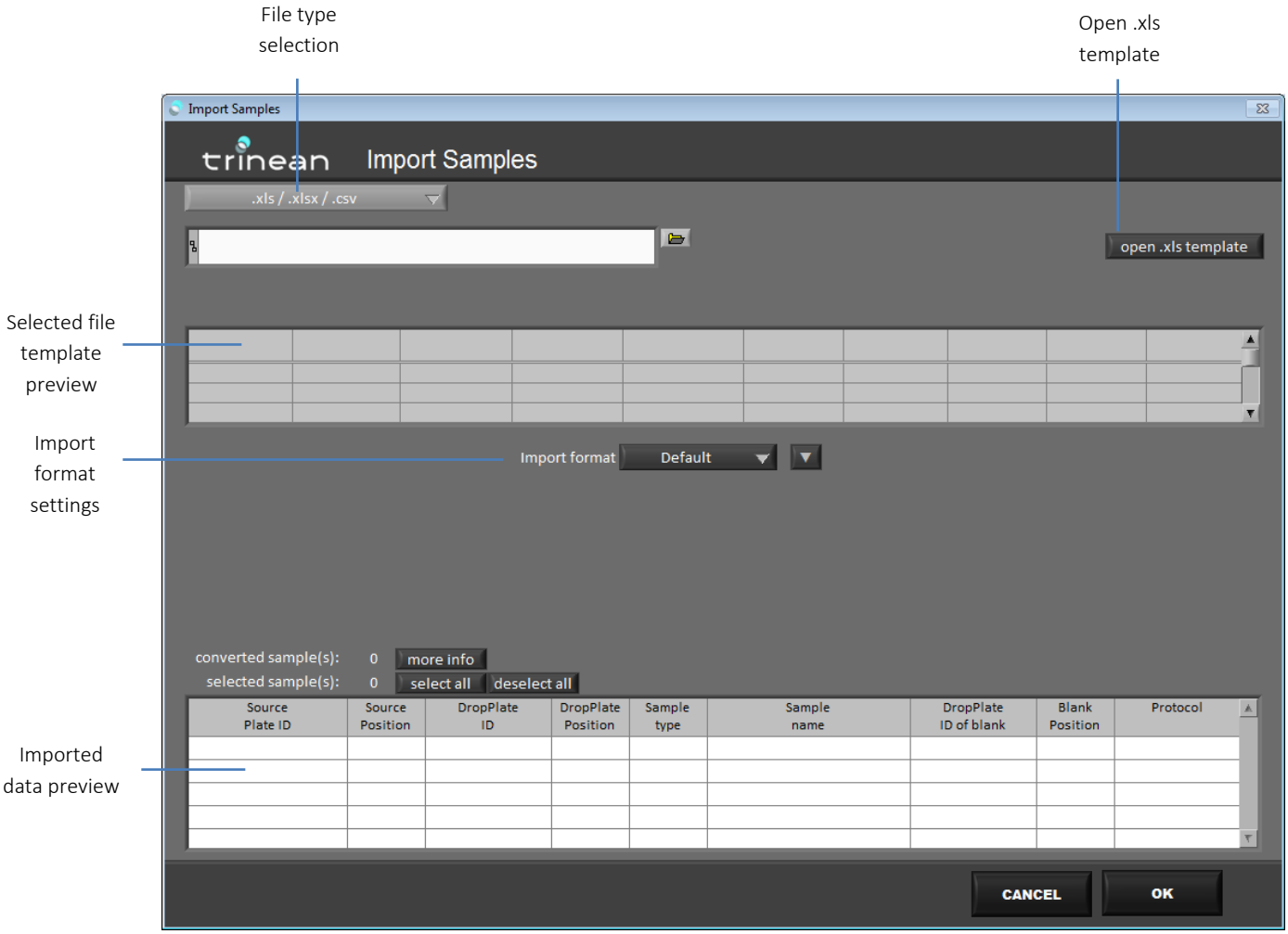
Plate Layout

DropPlate 1

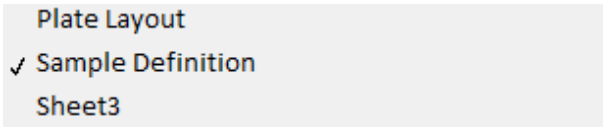
	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
B	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
C	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
D	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
E	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
F	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
G	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	dsDNA_50ug								
H	dsDNA_100ug	dsDNA_100ug	dsDNA_50ug	Water								

Import

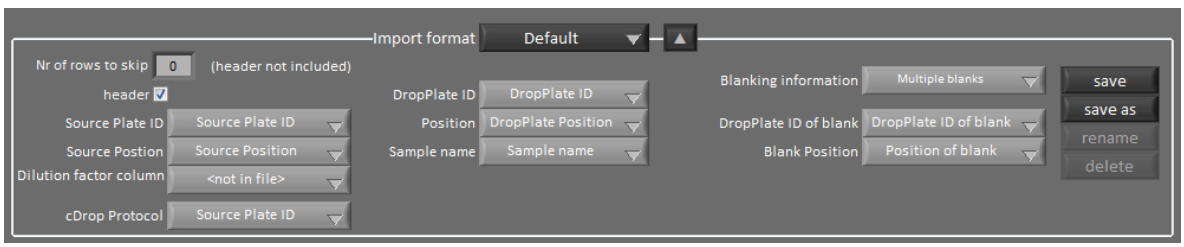
Import of plate layout – An import function ('Import Plate layout') is foreseen at the bottom of the 'Plate Layout' screen for fast and easy entering of plate layout information. Several types of files (excel, txt and csv) can be imported and the data can be reformatted into a dedicated DropQuant software table for quick plate layout configuration.



File type selection – Select between the available file types. It is also possible to select between spreadsheets on a particular file.

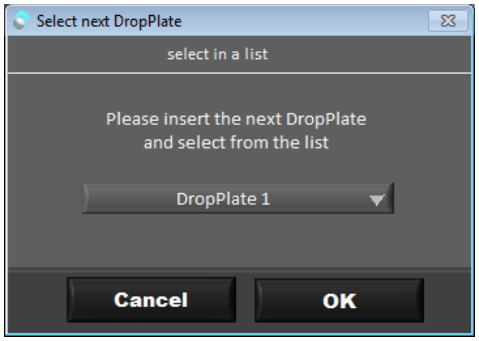


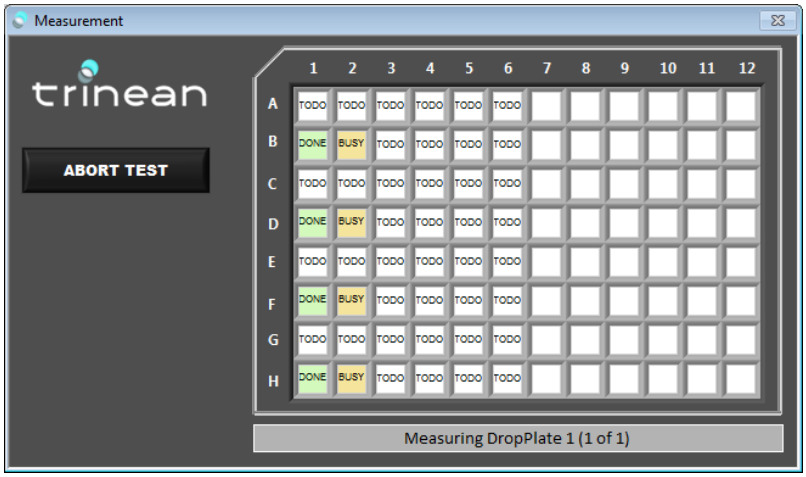
Import format settings – After pressing the arrow next to "Default" it is possible for the user to define the format to be imported. Please note that if errors are shown on the information displayed on the converted samples/selected samples info, they are most likely to be related to something being not correctly defined here.

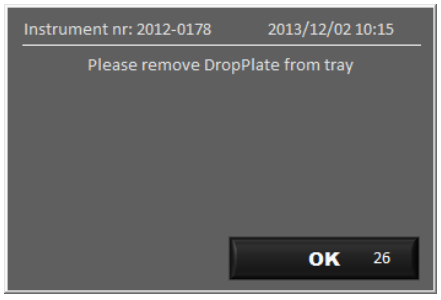


Measurement

While measuring the plate, DropQuant has a succession of windows with plate loading instructions and information that allow the user to see the measurement progression.







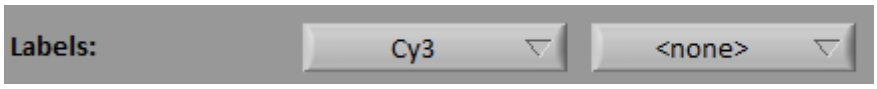
Applications 3

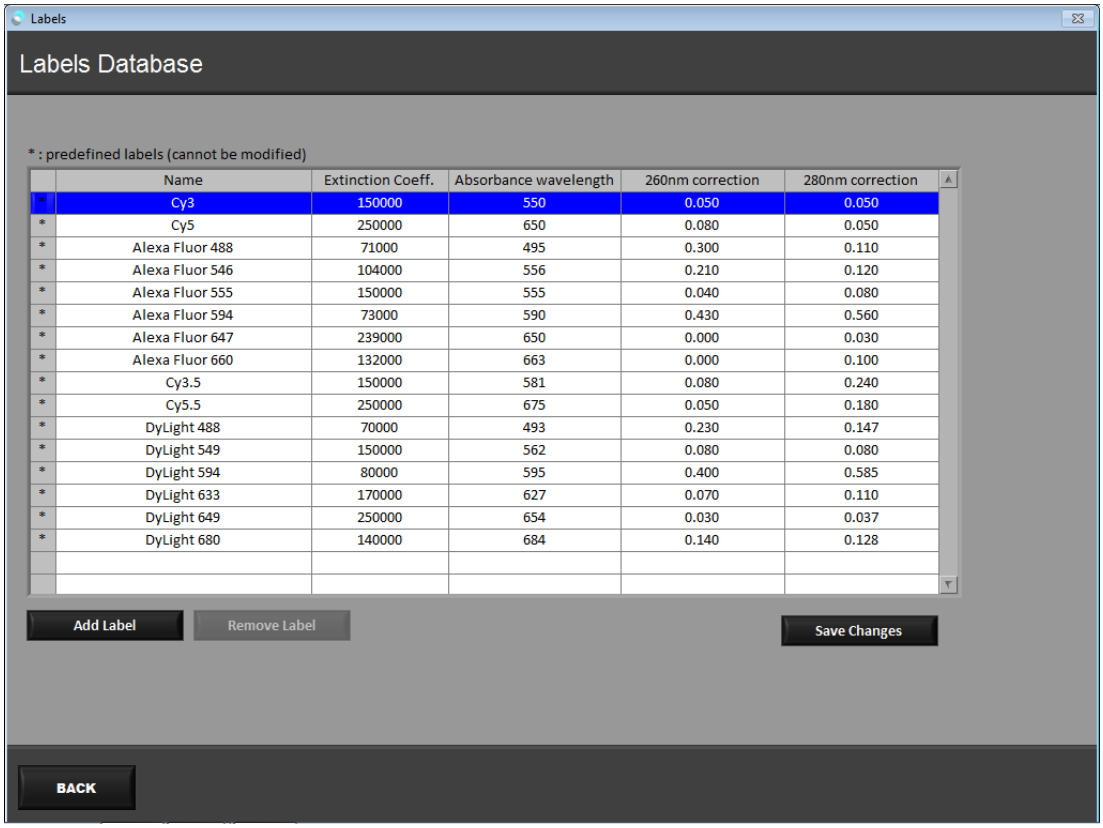
There are some important differences and extra options when selecting the 4 available **"Sample Types"**. For extra information about DNA/RNA, Protein concentration and purity determination, as well as General UV/Vis spectrophotometry, please consult Annex 1: Theory.

Nucleic Acids

Labeled - Measuring the labeling efficiency of fluorescent-tagged probes before micro-array hybridization eliminates potentially flawed samples and improves experiment effectiveness. The full spectrum analysis with the DropSense96, allows measurement of nucleic acid absorption at 260 nm while detecting the fluorescent dye on their absorption peak. In a single measurement, the DropQuant software calculates the nucleic acid concentration expressed in µg/µl and the dye concentrations in pmol/µl.

Labels – Here it is possible to choose between several types of predefined labels. Selecting this option, activates the Labels database screen, where it is possible to add or remove labels.



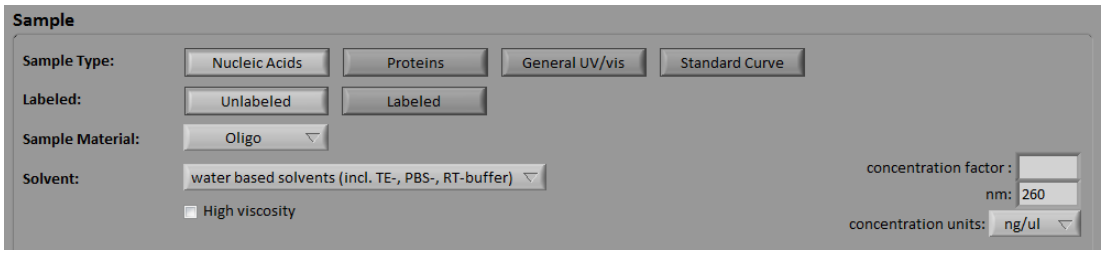


Oligo measurements

An oligo is defined by its sequence. Its characteristics such as the molecular weight and molar extinction coefficient can be calculated from the sequence, and they can be used in the concentration calculation. These equations are well described in literature.
DropQuant allows defining the sequence of each sample, and to use this information on the post-processing.
Concentration is expressed as ng/uL or pmol/uL. Conversion between both depends on the molecular weight (which can be calculated from the sequence)
Nucleic acids such as DNA are characterized by a concentration factor, which is the concentration as function of the absorption [ng/uL /OD]. The extinction coefficient is the absorption as function of the mol [pmol/uL / OD], the concentration and the mol are related by the molecular weight [g/mol].

Defining the sequence

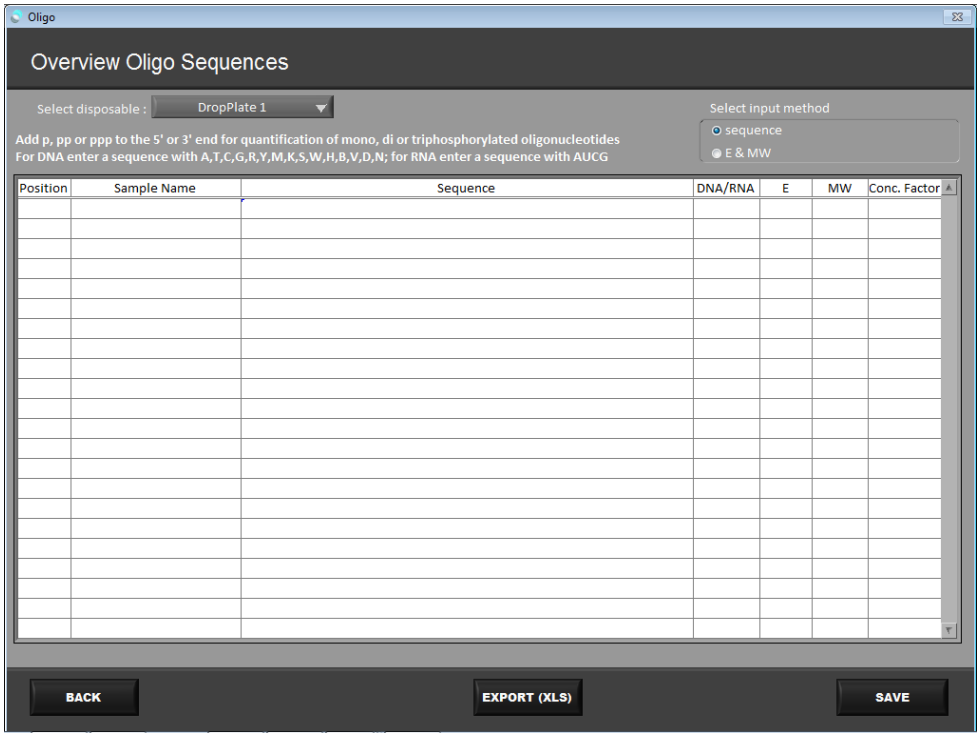
1. On the experiment definition screen, select Sample Type>Nucleic >Sample Material>Oligo.
2. Select the unit of concentration: µg/µl or pmol/µl. The selected value will be used on the post-processing window. Default value is µg/µl.



Other options like solvent, labels and background correction can be selected as for other nucleic acid samples.

The sequence can be inputted from the sample definition screen, using the “Enter/check sequences” button.

1. First define the samples including the blank, source plate definition and other information. After the samples are defined, the sequence information can be defined.
2. Click the “Enter/check sequences’ button and a table with the wells defined on the plate will appear.



Available, we have 2 different input methods.

Sequence – Type in the oligonucleotide sequence, or copy/paste them from an excel table. The software will calculate the properties such as the extinction coefficient, the molecular weight and the concentration factor.
All the information can be saved in excel by pressing the 'export excel' button at the bottom of the screen. Phosphorylation at both the 5' and 3' end can also be included by adding the right number or the letter 'p' to the sequence. Press 'Save' to return to the 'Plate layout' screen.

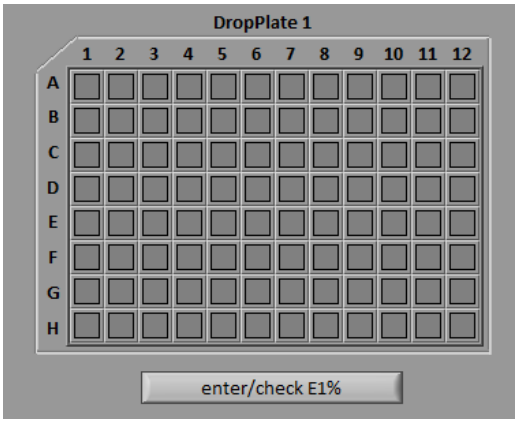
E&MW – Here it is possible to enter individual or replicated extinction coefficients and molecular weight information.

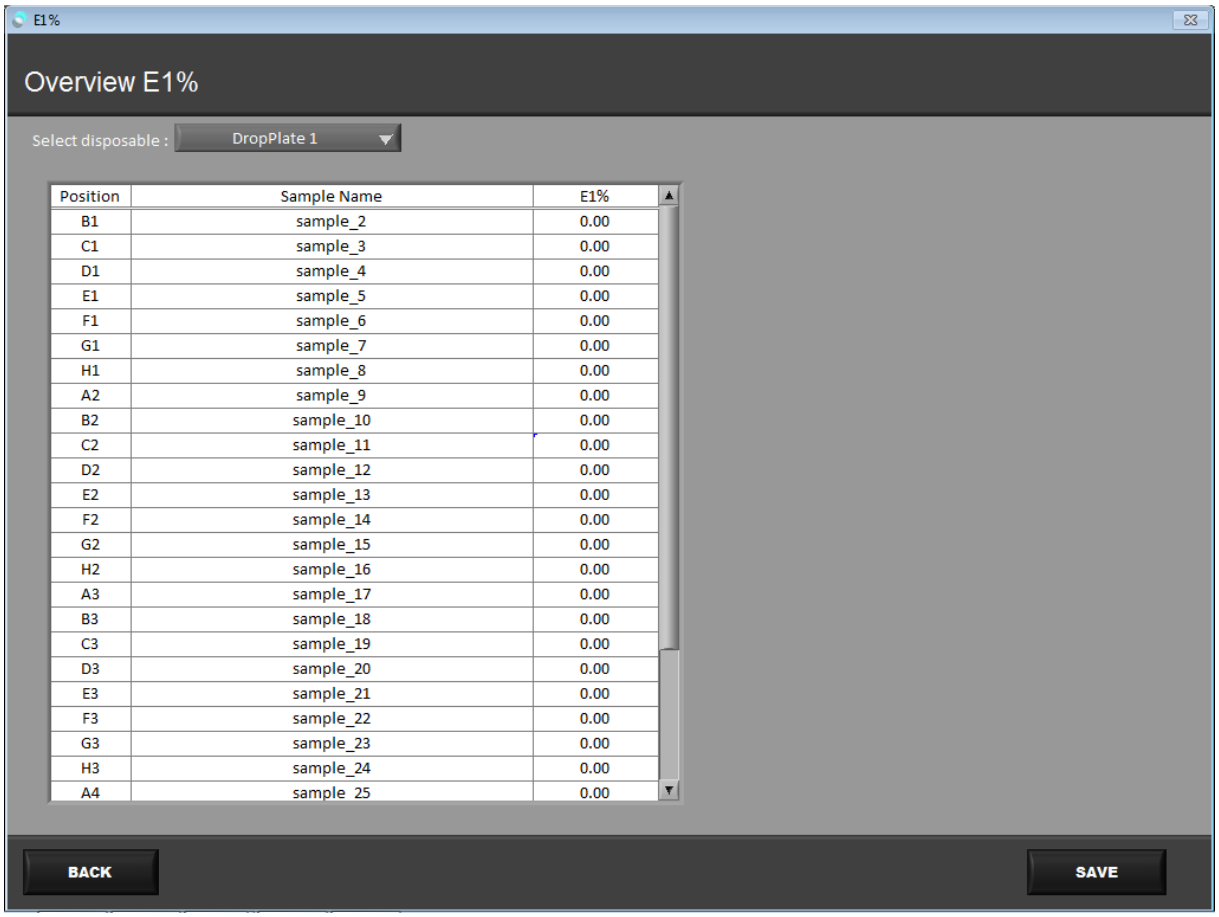
When using the **"Replicates"** function entering the sequence of one sample will automatically leads to the fill in of the sequence and data for all its replicates.

Please don't confuse Replicates with the R *button available on the same screen. The last one is meant to be used on the Standard Curves application.*

Proteins

Measuring multiple proteins with different extinction coefficients – when measuring several proteins, it is possible to set that information before the measurement.

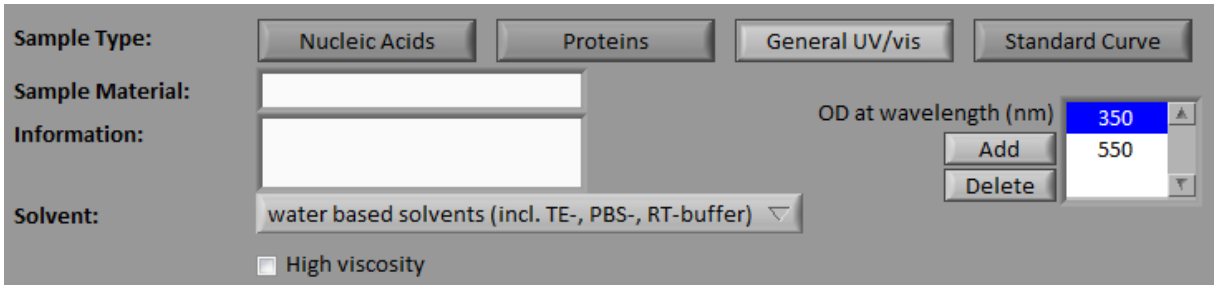


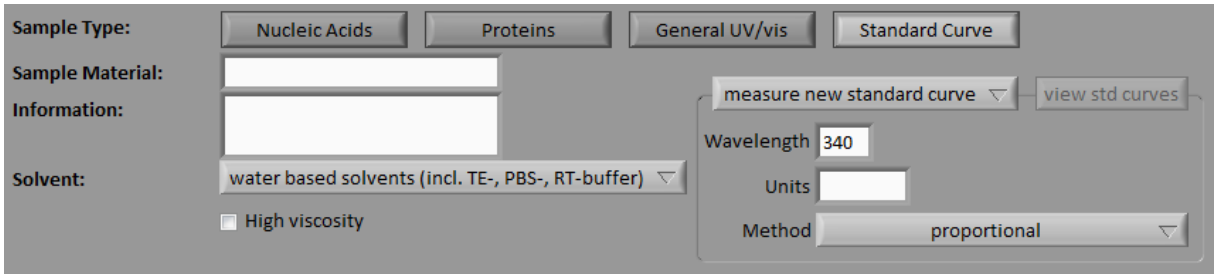


Position	Sample Name	E1%
B1	sample_2	0.00
C1	sample_3	0.00
D1	sample_4	0.00
E1	sample_5	0.00
F1	sample_6	0.00
G1	sample_7	0.00
H1	sample_8	0.00
A2	sample_9	0.00
B2	sample_10	0.00
C2	sample_11	0.00
D2	sample_12	0.00
E2	sample_13	0.00
F2	sample_14	0.00
G2	sample_15	0.00
H2	sample_16	0.00
A3	sample_17	0.00
B3	sample_18	0.00
C3	sample_19	0.00
D3	sample_20	0.00
E3	sample_21	0.00
F3	sample_22	0.00
G3	sample_23	0.00
H3	sample_24	0.00
A4	sample_25	0.00

General UV/Vis and Standard Curve

In addition to the other sample type options, it is also possible to perform a General UV/Vis measurement, or a Standard Curve.





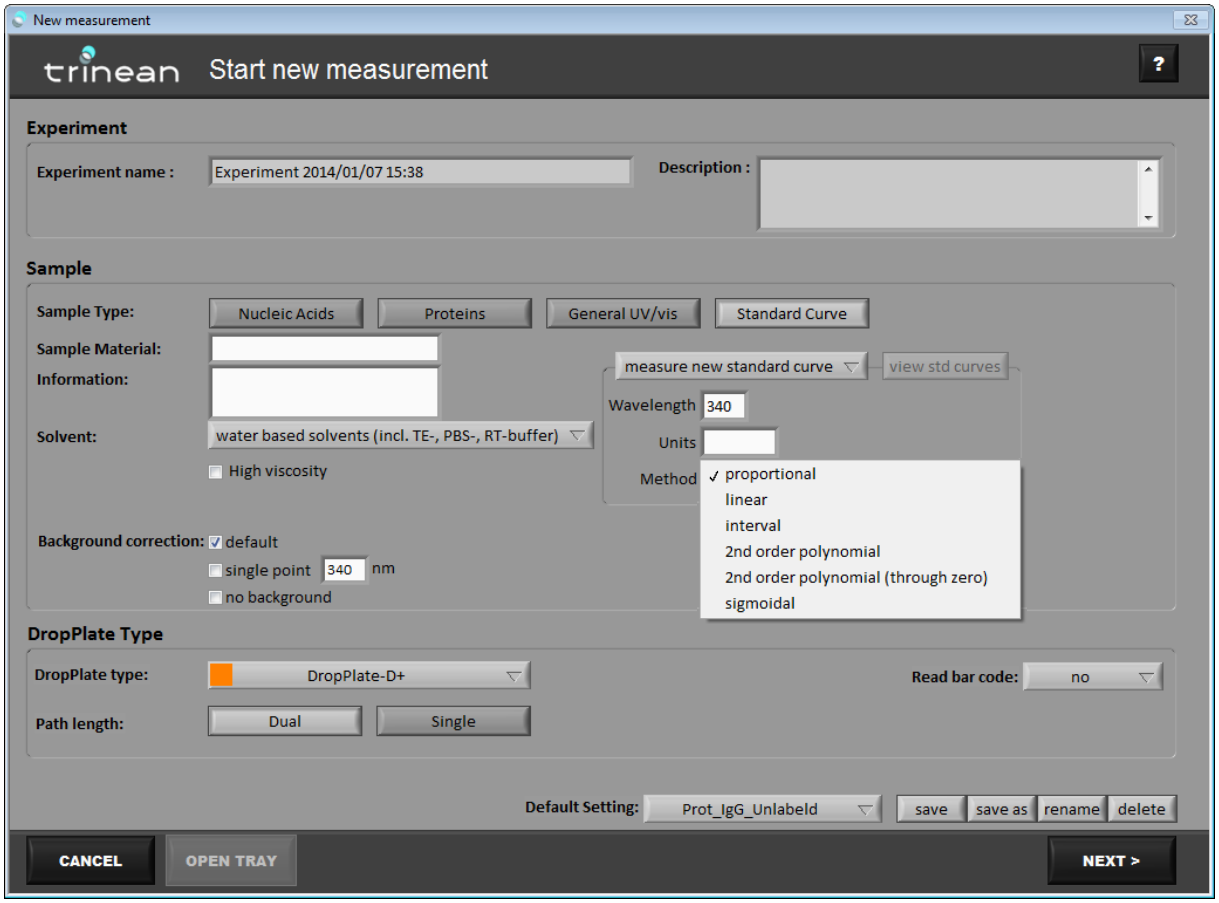
Standard curves

For more information on standard curves, please read the extra information on the Annex 1: Theory.

Defining standard curve experiment

As explained in the introduction, standard curves can be defined inside the experiment or by selecting a standard curve from an earlier experiment. Both methods are explained in this paragraph.

Case 1: the standard reference samples are part of the experiment.
Click on “new experiment” and on the start experiment window, select standard curve.



Next input fields are available to define the parameters of the standard curve method:

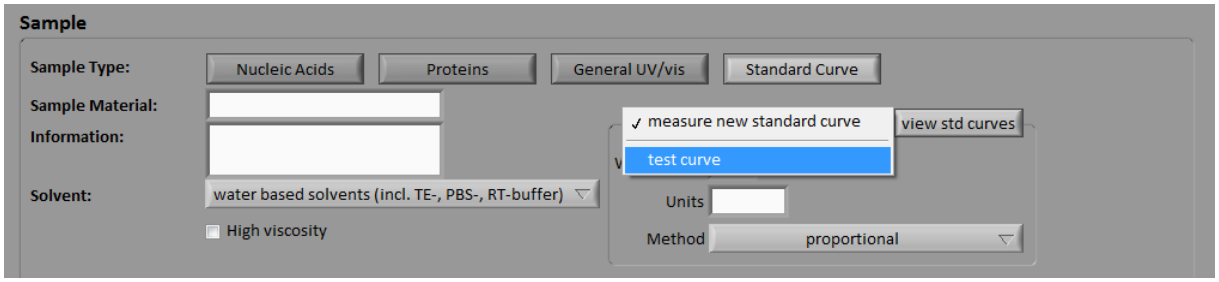
- Sample material – for information purposes only (required field).
- Information.
- Wavelength – the wavelength at which the standard curve is calculated.

This cannot be changed by the customer after the measurement is done.
Still, keep in mind that the ***Method can be*** *changed later.*

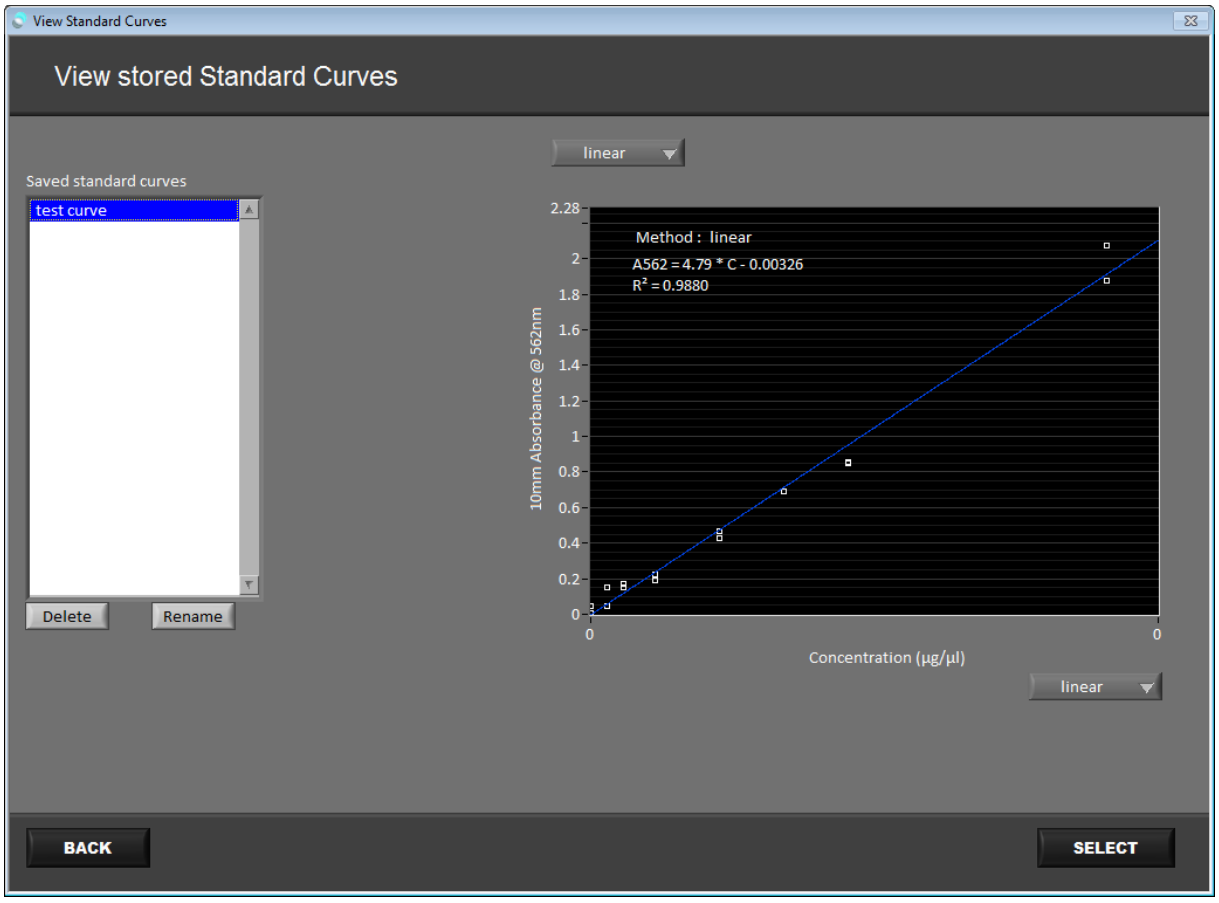
- Unit – the unit of the reference value.
- Method
  - Proportional – $OD = A*Conc$
  - Linear – $OD = A*conc + B$
  - Interval – $OD = A_i*conc+B_i \text{ if } OD_i<OD<OD_{i+1}$
  - 2nd order polynomial – $OD = A*conc^2+B*conc+C$
  - 2nd order polynomial through zero – $OD = A*conc^2 + B*conc$
  - sigmoid – $OD = A / \{ 1 + exp [- (Conc – B) / C] \}$
  - 4 parameter curve fit – $OD=[(A-D)/(1+\{conc/C\}^B)]+D$

Case 2: use a pre-existing standard curve in the experiment
It this approach, it is assumed the DropQuant user has already performed an experiment with a standard curve before, and saved the standard curve of that experiment for future use.

The standard curves available for selection, are directly available when clicking the “measure new standard curve button”.



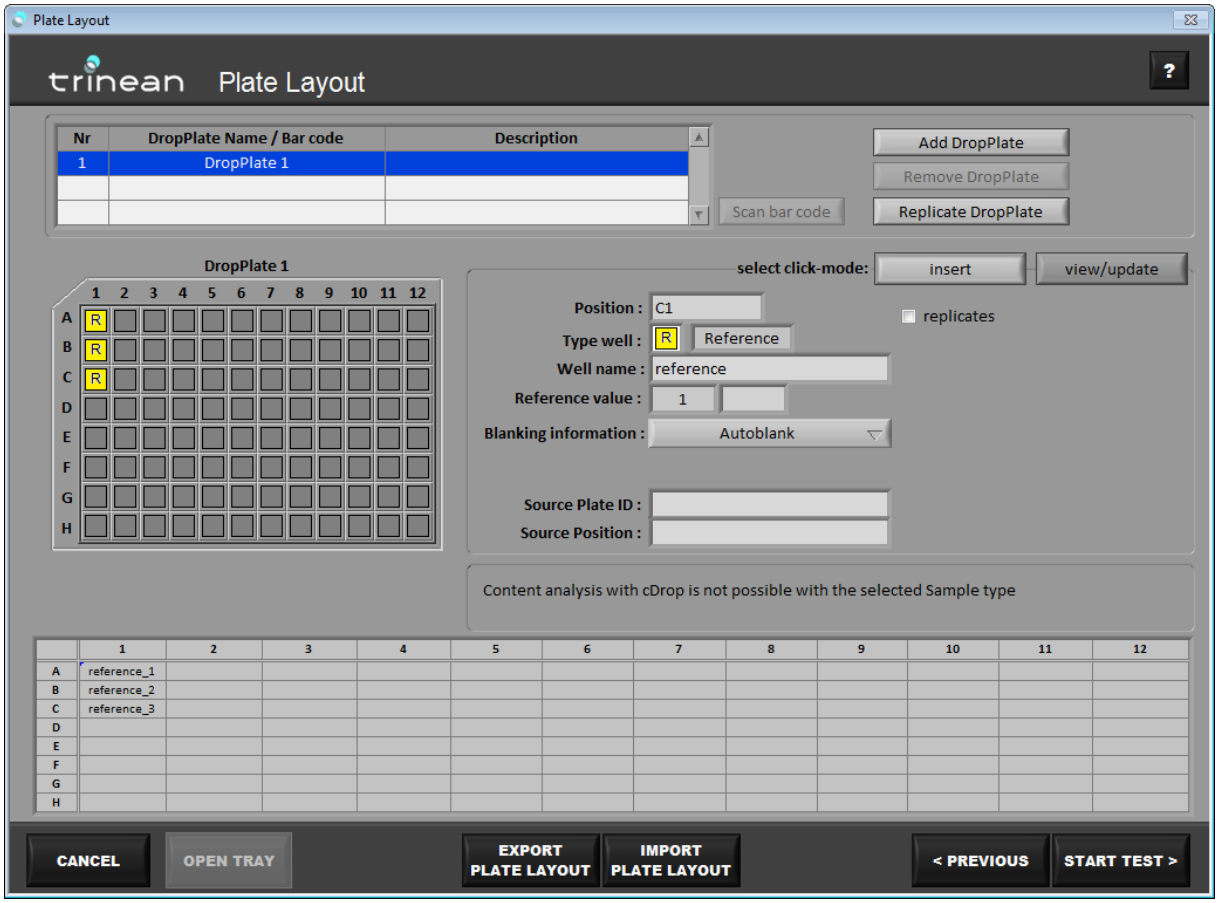
The user can also browse the available standard curves by clicking the “view std curves” button. This opens a window with all standard curves available for use.



Once the user selected a pre-defined standard curve, it is not possible to add new standard reference samples in the experiment.

Samples definition

Input via graphical user interface

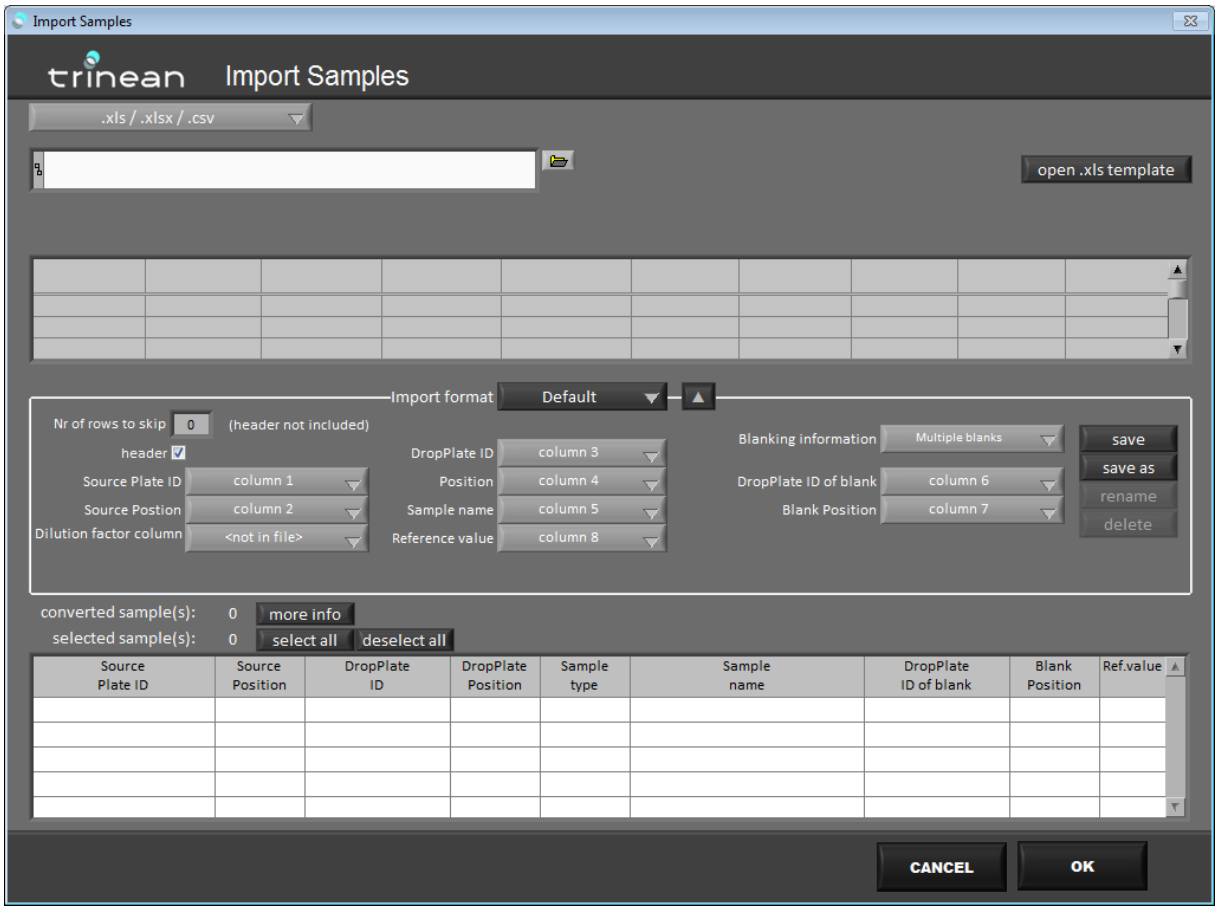


- *There is one set of reference samples to define one standard curve which is used for all unknown samples in the experiment. It is not possible to define multiple standard curves in one experiment.*
- *An experiment can contain multiple plates, the reference samples can be on any location on one or multiple plates.*
- *The reference samples can be defined on any plate inside the experiment. However, if the measurement is stopped (by customer interaction, instrument failure, power breakdown etc) before the reference samples are measured, no concentrations will be calculated.*

It is not possible to define extra standard curve samples if a pre-defined curve is selected.
It is recommended to define replicates of the references samples, to improve the overall accuracy of the method. This is highly recommended if the OD values of the references are at the limits of the detection range (0.1 OD).

Sample definition

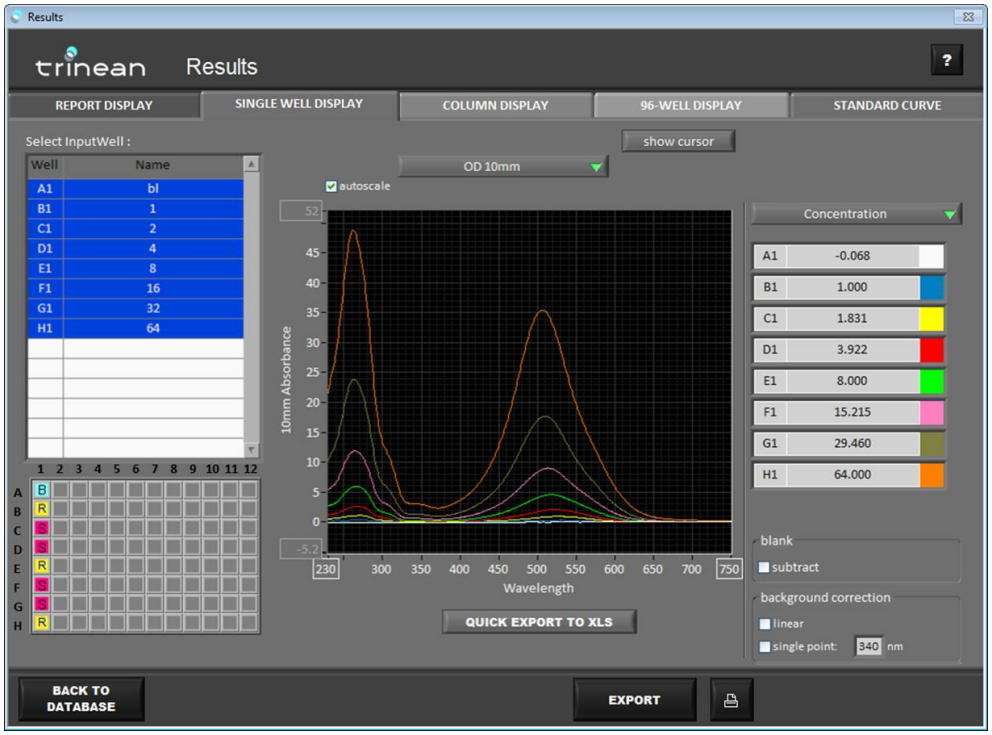
The import section is extended, allowing definition of standard curve measurements. There is one extra column for the definition of the reference value. If this column contains a number, then the sample is by default a reference. If it is empty, then this well is an unknown sample.



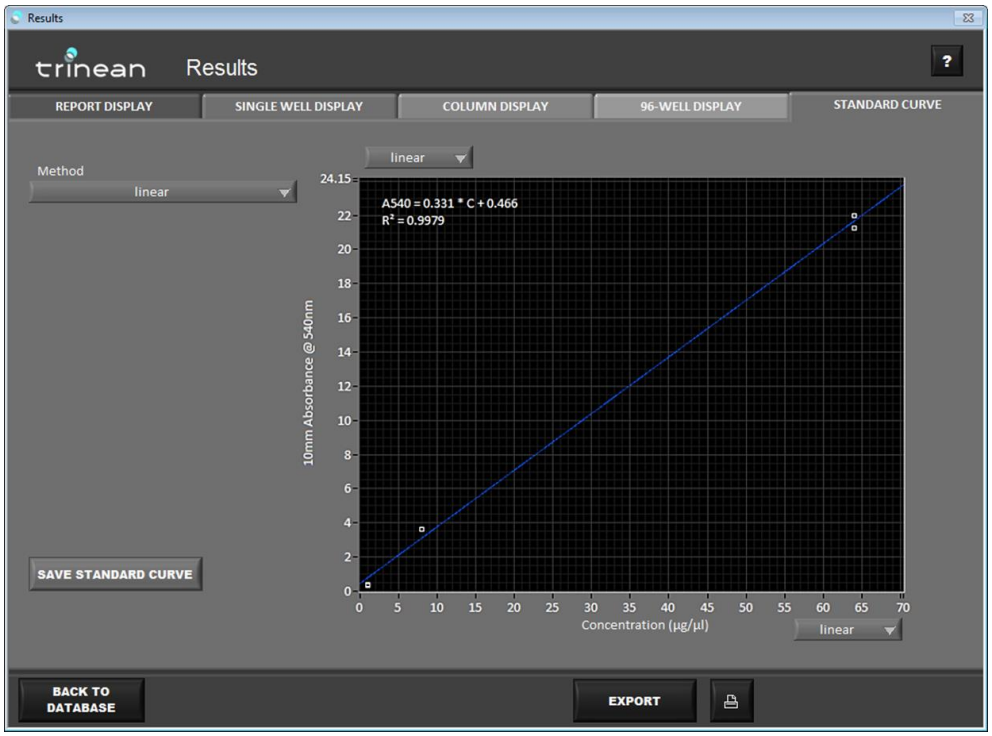
The settings of the standard curve are part of the format, and are thus not part of the import file.

The results window

Standard curve experiments can be selected from the measurement database and opened in the post-processing window just as any other DropQuant measurement. The results window shows the measured spectra of all samples (references and unknown samples). The concentration of the unknown samples is calculated and shown in the tables.



There is an extra tab showing the standard curve.



It is possible to change the method from the post-processing window.

Saving a standard curve for future use

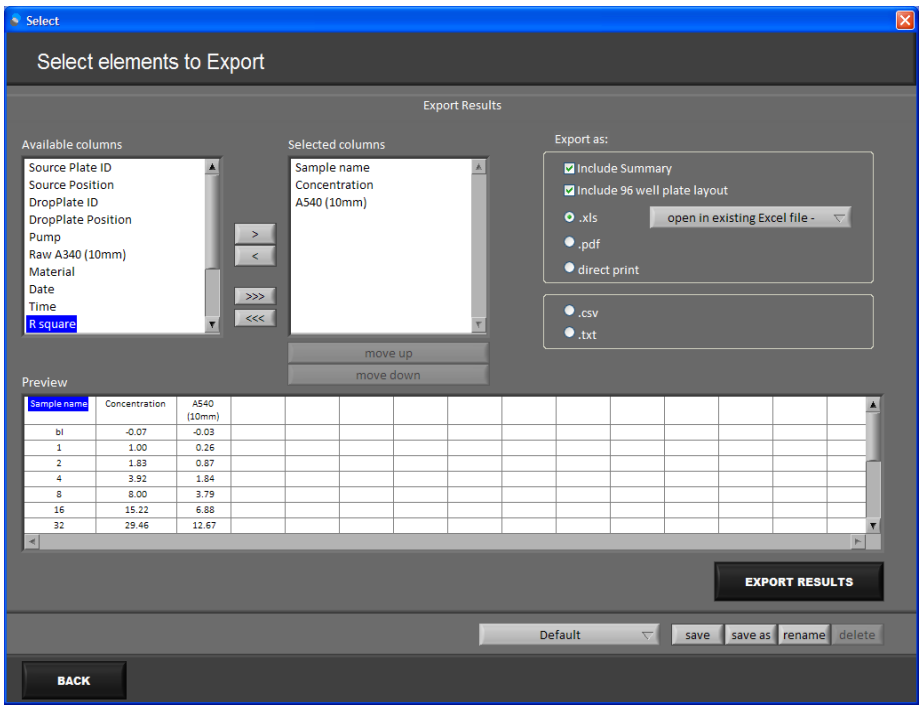
On the standard curve tab, the user can click the “save standard curve” button to save the standard curve for future use. The user has to fill in a name for the curve.

It is important to keep in mind that for some types of experiments, a new standard curve should always be used, instead of a previously saved one.

Exporting the results

The export data function includes a few extra columns in case of a standard curve experiment:

- “A540 (10mm)” (if 540 is the wavelength chosen for building the standard curve): the measured OD used for creating the standard curve.
- “Concentration”: the calculated concentration. The syntax depends on the unit defined in the experiment definition phase. For example : if unit is ng/uL, then the name of the column should be “Concentration (ng/uL)”



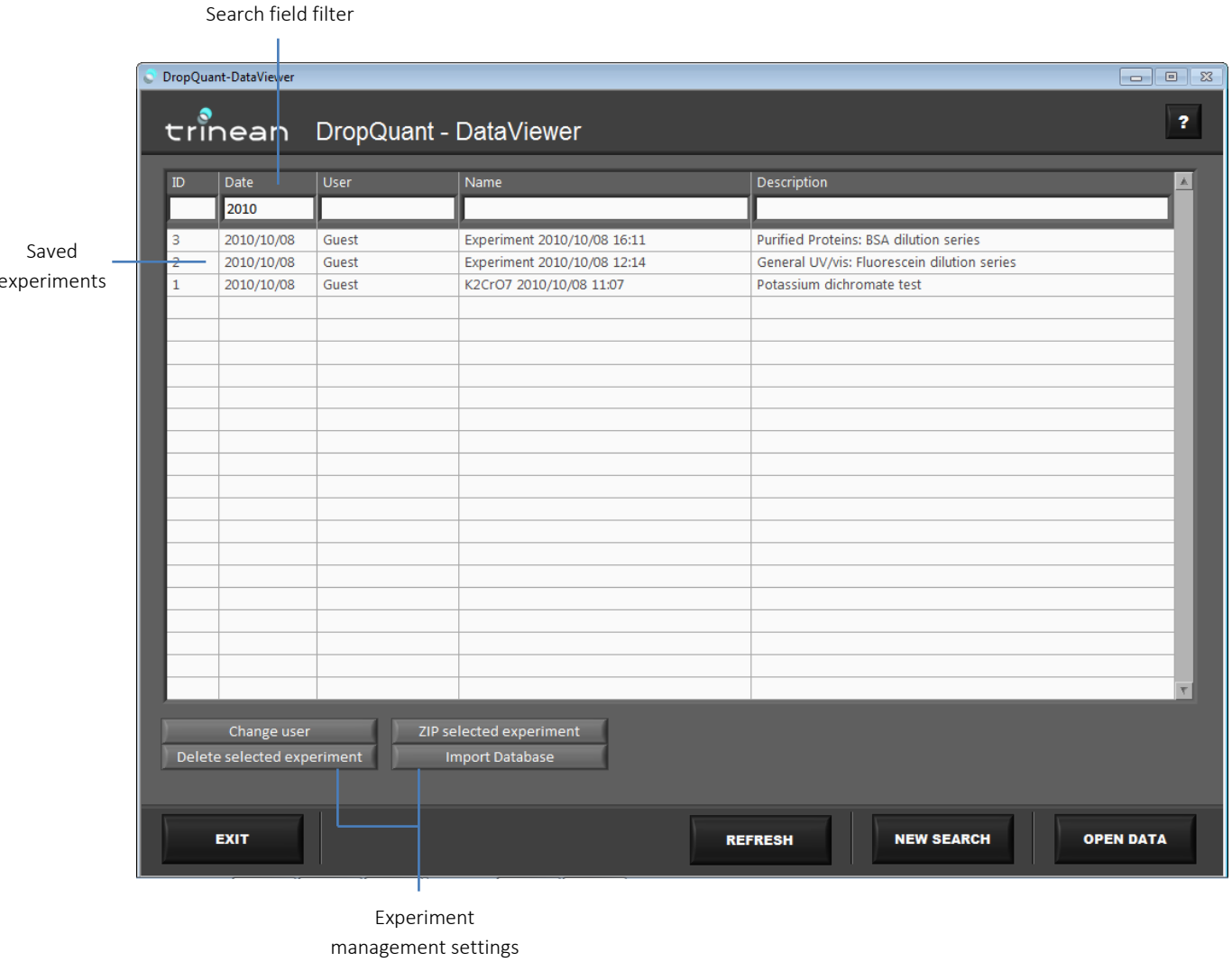
Typical applications

The applications for the standard curve include the concentration measurement of proteins with unknown extinction coefficient, readout of colorimetric assays, etc.

Interpreting data 4

DropQuant DataViewer

To analyze previous measurements, we have available a DropQuant DataViewer.



The DataViewer front page shows all the measurements ran on your DropSense 96. It is important to note that some experiments might not be visible to certain users due to access rights. For more information, please read the Users section in this manual.

Search field filter – To help the user searching for previous measurements based on a specific field, it is possible to filter the desired measurements, by specifying relevant data.

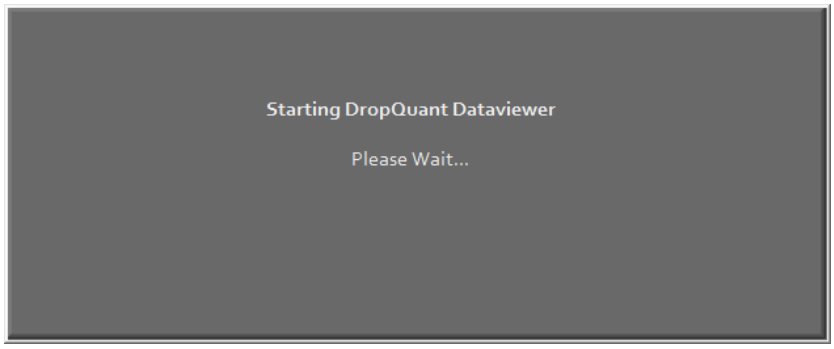
Saved Experiments – All measured experiments accessible to the current user, will be displayed here. It is important to while setting up an experiment, to input as much information as possible so it can be searched while filtering results.

Experiment management settings – This set of buttons allows the user to export and import experiments or databases.

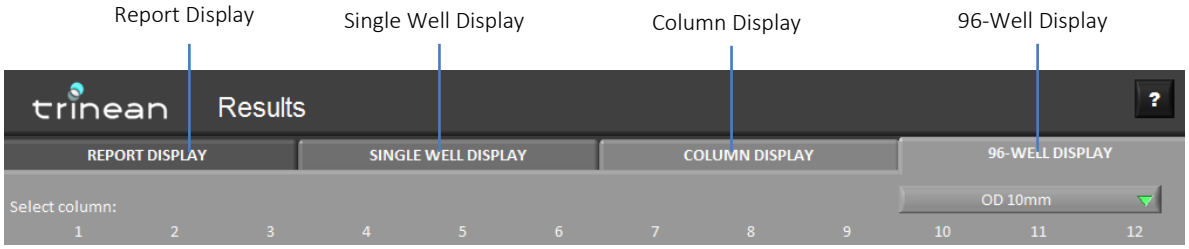
- **Change user –** Change the user assigned to that experiment.
- **Delete selected experiment**
- **Zip selected experiment –** Useful for diagnostics. Whenever facing issues with the DropSense 96 it is important to send the experiments where the issue first occurred to your local distributor.
- **Import Database –** As it is possible to use both DropQuant and DataViewer as standalone software, you can also import experiments saved on a different computer.

DataViewer results window

After a measurement, two options are possible depending on the selected method on DropQuant's experiment definition.
As default, DropQuant DataViewer starts, allowing the user to access options designed to help interpret the measurements performed with the DropSense 96.

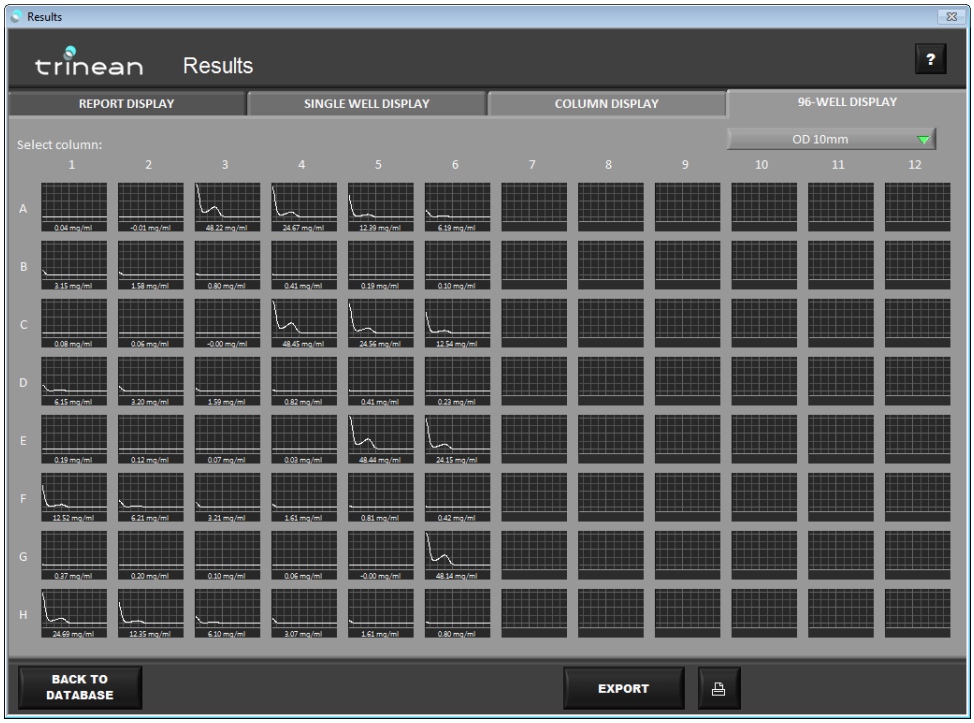


When opening an experiment, 4 tabs are visible to all users.



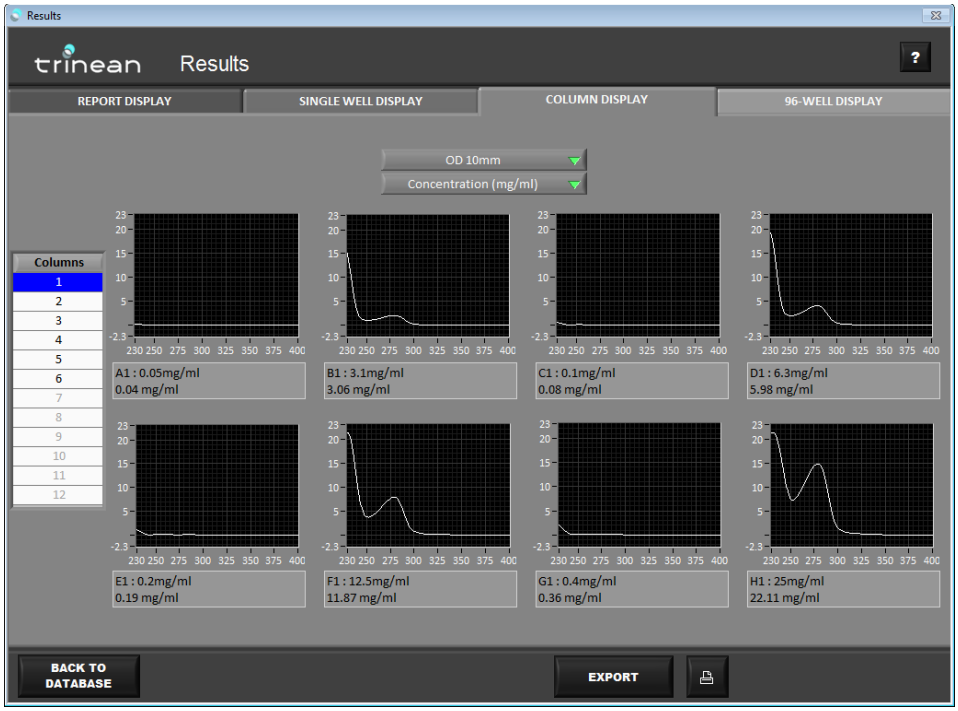
96-Well Display

This window allows the user to have a classical overview of the measured samples.



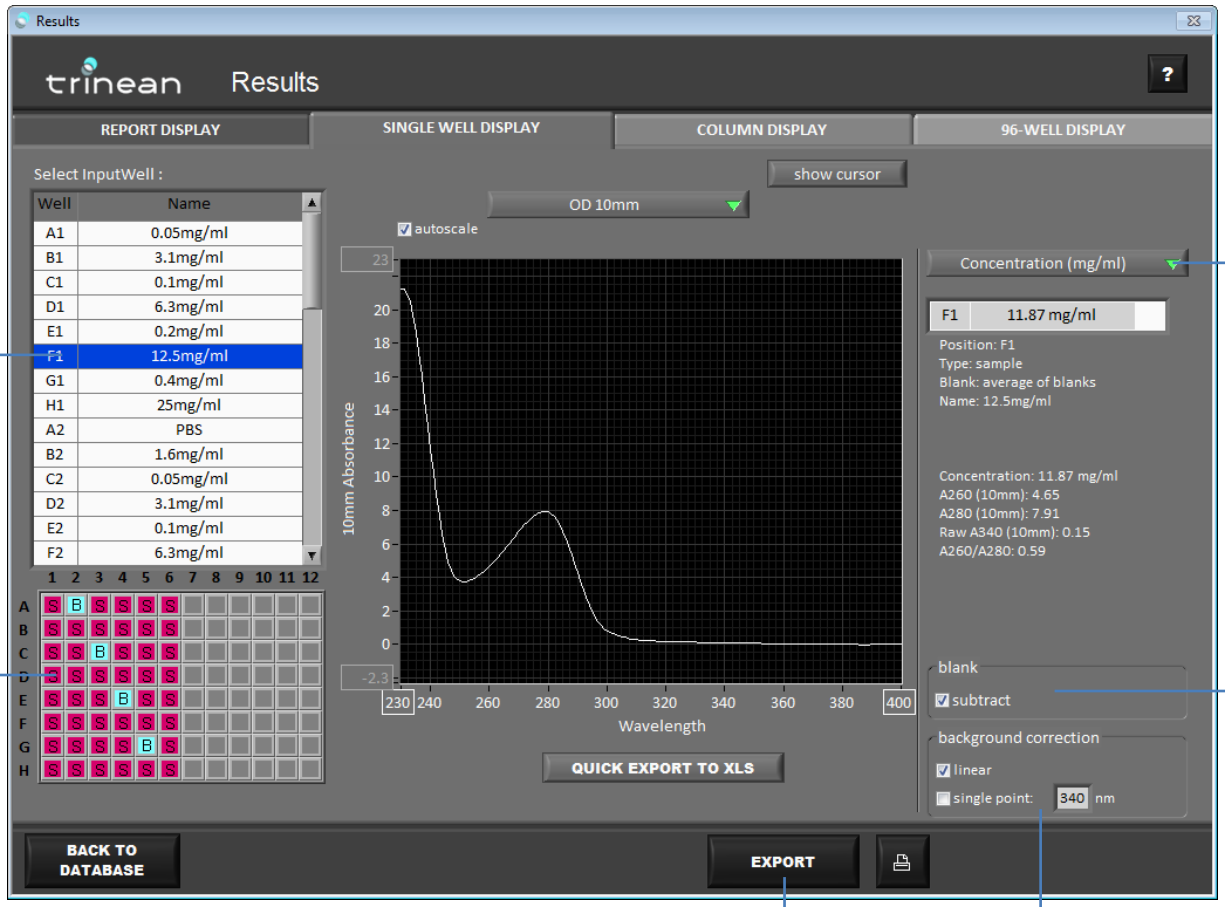
Column Display

Similar to the 96-Well display but with a column view instead.

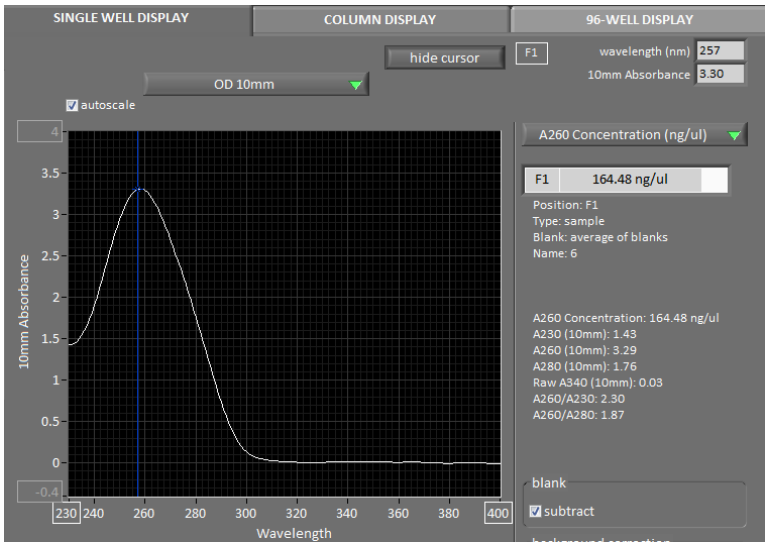


Single Well Display

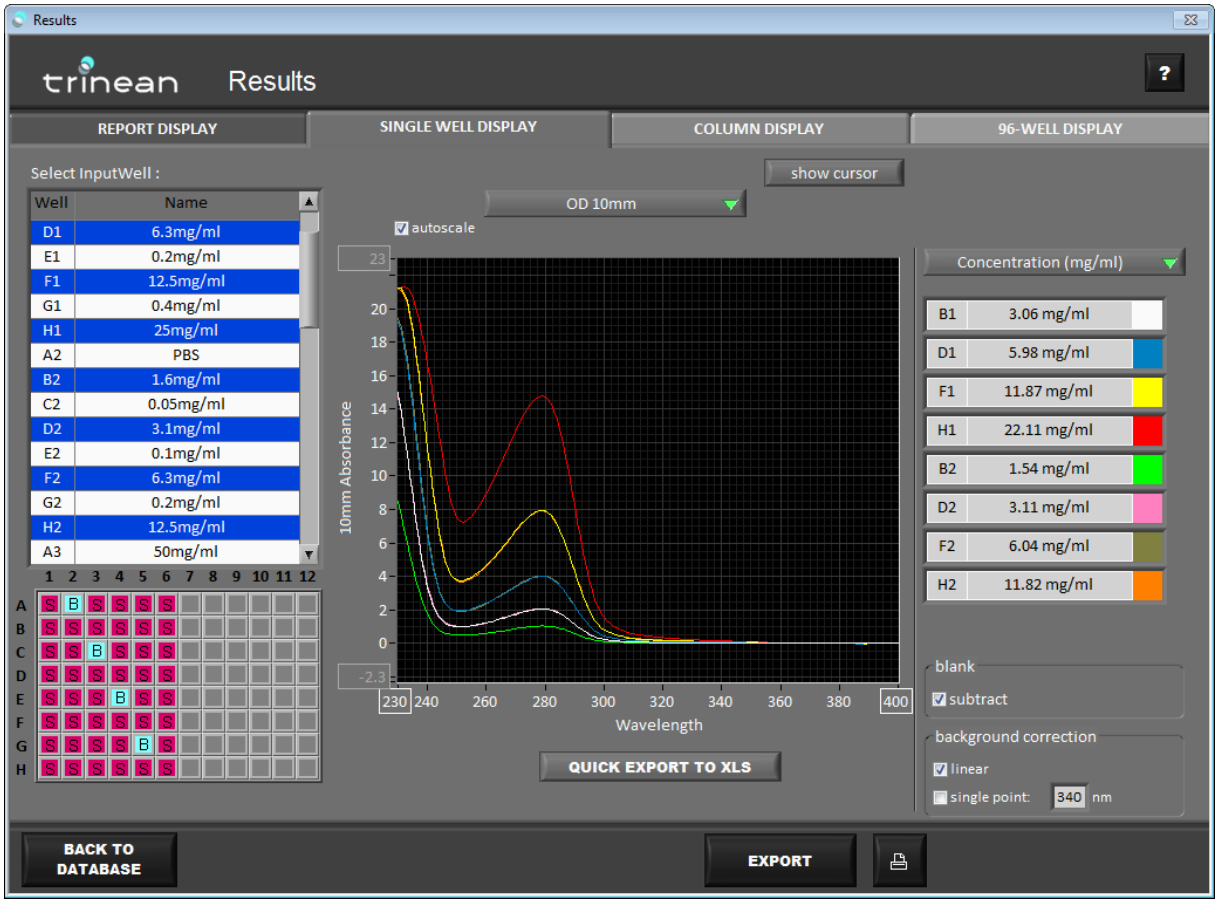
Probably the most interesting view on the results window, as it allows the user to change parameters in order to analyze the results. It also allows the user to have a more detailed view, that it's not possible with other tabs.



At the top, the customer can select the "show cursor" option. This will show a cursor on the spectrum view window, allowing readout of the OD at any wavelength.



Input Well Selection – as it is possible to select a single well to be visualized on this window, it's also possible to select multiple wells, using either the Ctrl or the Shift key.



Blank – When using a blank sample, it is automatically subtracted from the sample absorbance before concentration calculation. This feature can be turned off so the spectral shape and concentration calculation is shown without blank compensation. In this mode, the shape of the blank spectrum itself can be analyzed. When the blank subtraction is turned off, all other screens will generate data without blank subtraction.

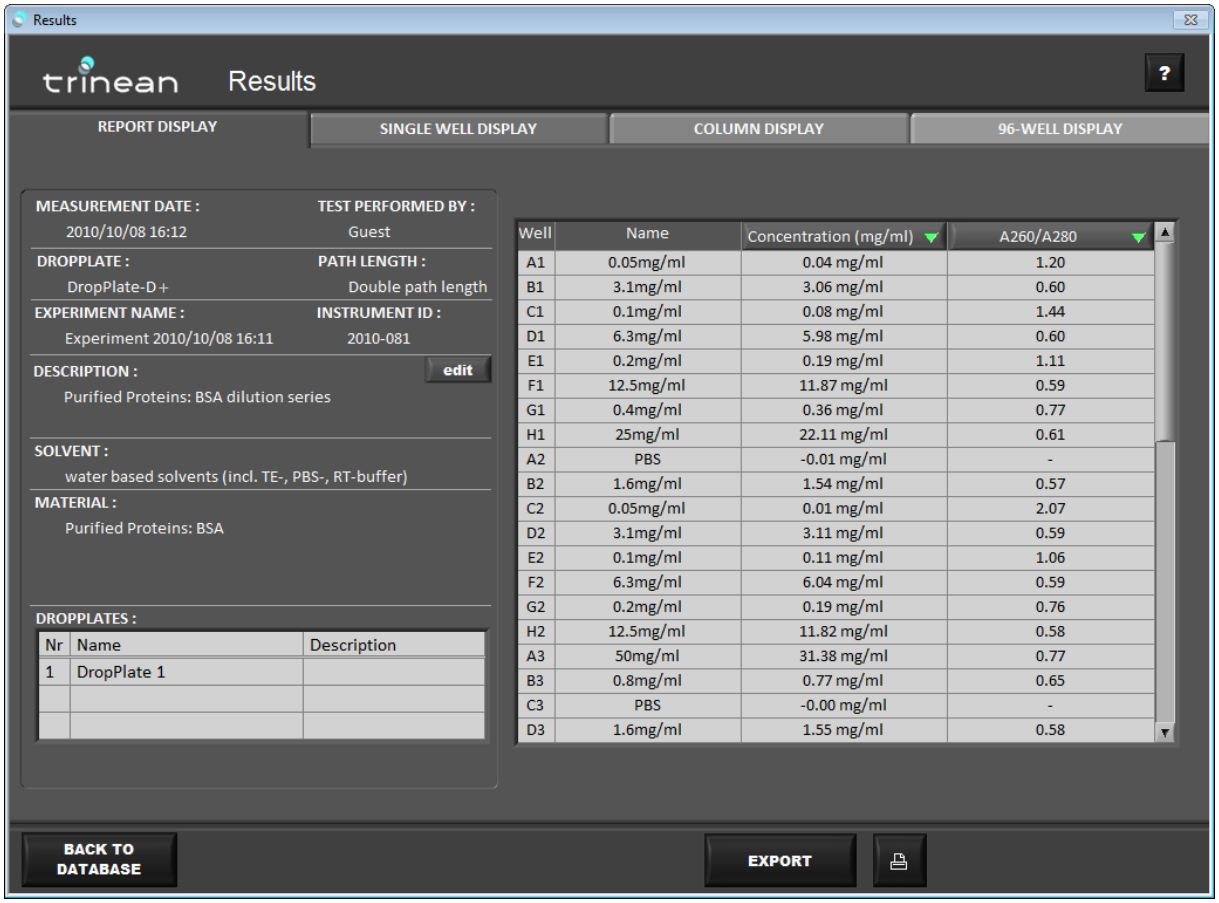
These settings are not stored to the DropFiles: next time the DropFile is opened, the data is shown using the original settings at time of defining the experiment.

It is also important that ***all blanks contain MQ (or similar) water only****.*

Background correction – the background correction method, can be selected on the "Start new measurement" pane, while setting up a new experiment. The background information is derived from the RAW OD spectrum measured. On its default setting, a linear fit through the 400-600 nm region of the transmission spectrum is subtracted from the full spectrum. On the single point mode, the background level of a wavelength of choice is subtracted from the full spectrum.

Report Display

This window shows a small report including all the necessary information for a classic measurement. Here it is also possible to change the experiment description.
Some extra information such as Measurement date, DropPlate type used, Performed by, among others, can also be found on this screen.
This information might also be important for diagnostic purposes when an issue appears.

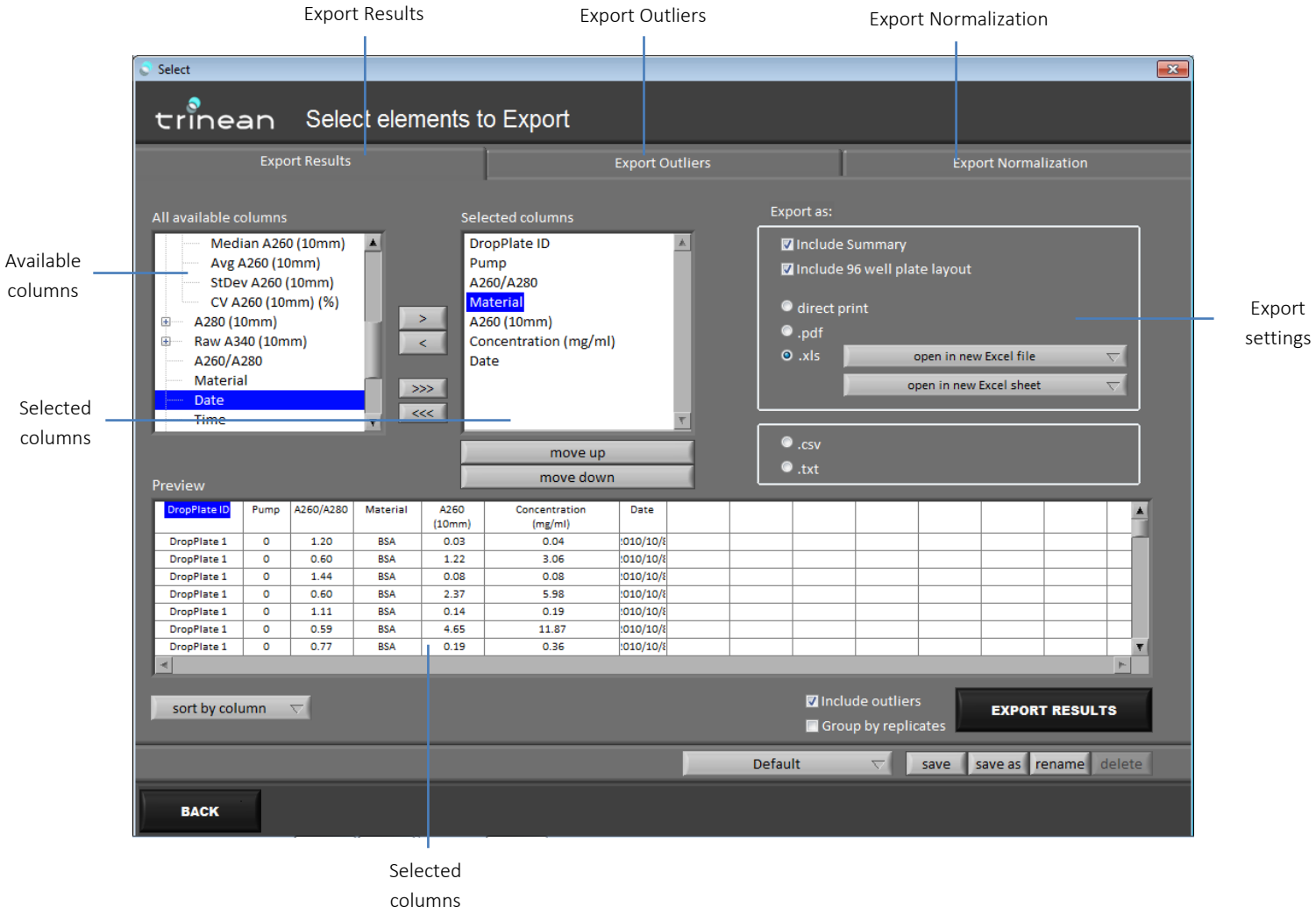


Exporting data 5

On DropQuant DataViewer, exporting couldn't be easier. Almost on every screen it is possible to export the measured data, either to excel or one of the many other file types we have available, or into a LIMS system.

Export Results

When exporting results, it is possible in advance to select which information to export.

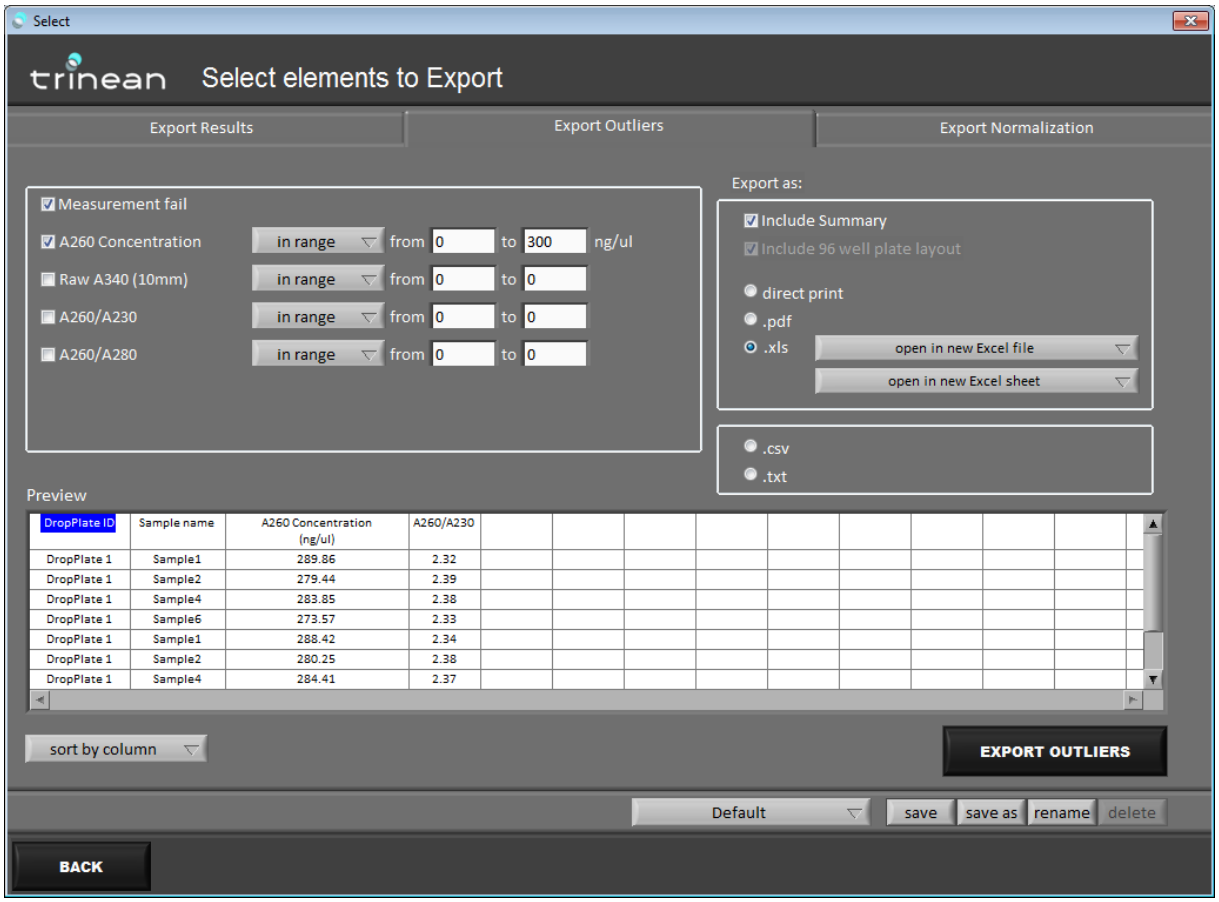


All available columns – Here it is possible to find several fields with the data available to be exported. It is also possible to move the fields up and down, in this way organizing the selected columns table above.

Export Settings – It also possible to define some extra settings regarding the final file format. It is possible to choose between 4 file types and direct printing, to include a summary and the plate layout, and some extra information related to the Excel file.

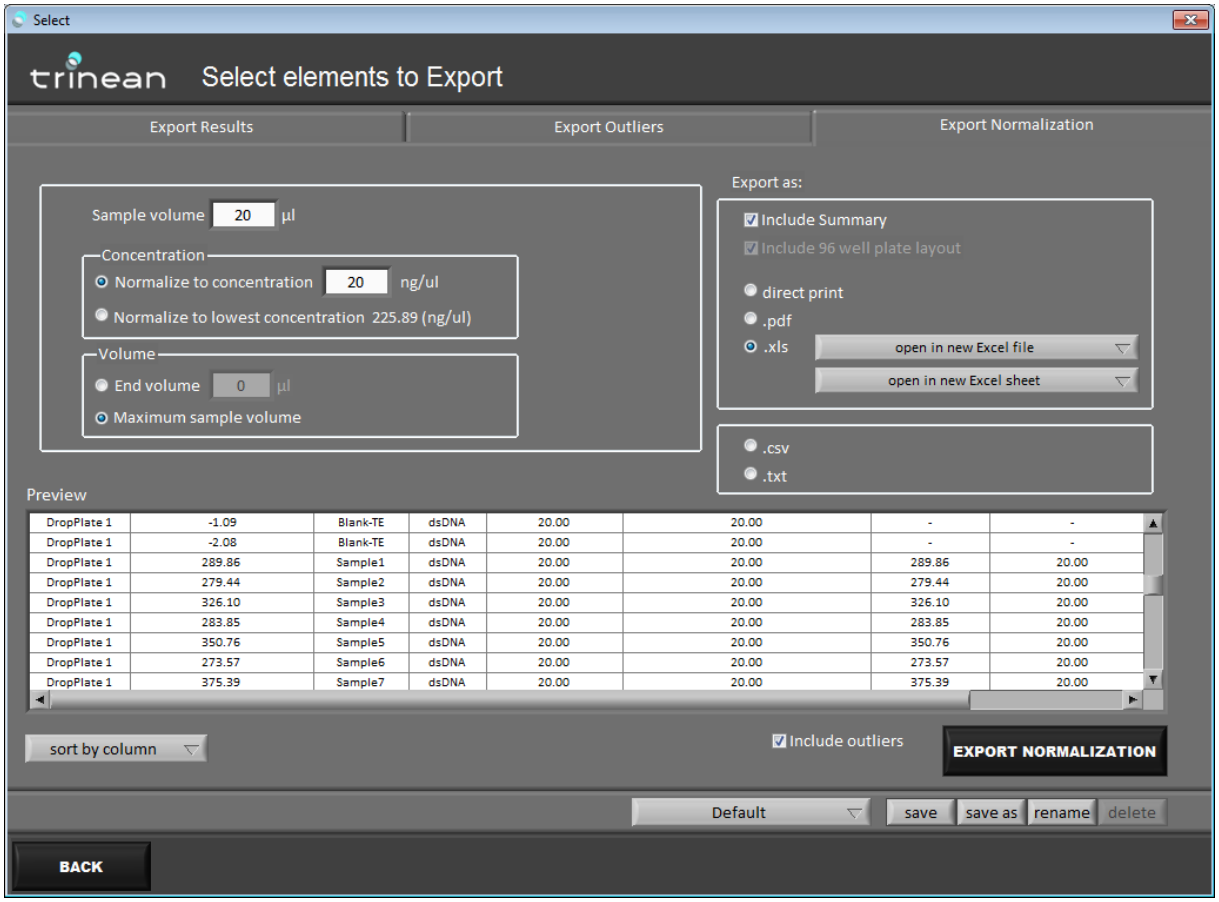
Export Outliers

It is also possible to export outliers from your experiment. On this screen, it is possible to define what to export, and which are the ranges you want to use as a filter.



Export Normalization

The last tab on the export window allows the customer to calculate the quantities of sample and buffer to be mixed to achieve normalization: the goal is to dilute the samples to equalize the concentration of all samples to the same concentration (and optionally also same volume).



There are two different operation modes.

Add buffer to the sample to achieve a target concentration – It is assumed all samples are available in the same volume. For this mode, select maximum sample volume. Note that normalization cannot be done if the sample concentration is smaller than the target concentration. In that case the value of the added buffer volume on the table will appear as a dash (-). To have a solution the normalized concentration must be smaller than the smallest concentration measured in the set of samples. DropQuant includes the option to set the target concentration to the smallest concentration of the set of samples. In that case, the smallest sample needs no dilution. Then, the added buffer volume should be 0.

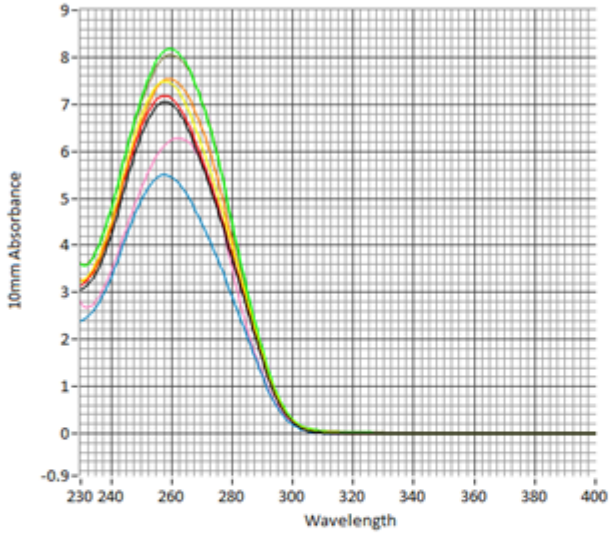
Take aliquot of the sample and mix with buffer to achieve normalized sample with target volume and target concentration – The aliquot volume taken from the sample is here identified as the used sample volume; the buffer volume to be added is the added buffer volume. For this mode, set the end volume to the desired value. Here, there is no solution if the target sample volume is too large and/or if the target concentration is too large.
Once this is selected, the required sample and buffer volumes will be automatically calculated.

Quick Export

For the user's convenience DropQuant DataViewer also allows to quickly export the data to .xls format. Please note that this function is only available on the single well display window, and that in order to export more than one measurement/well, the left column has to have the desired measurements selected.

DropSense REPORT

trinean



	DropPlate ID	DropPlate Position	Sample name	Pump	A260 Concentration (ng/ul)	A230 (10mm)	A260 (10mm)	A280 (10mm)	Raw A340 (10mm)	A260/A230	A260/A280
	DropPlate 1	E4	Sample5	0	349,91	3,07	7	3,73	-0,01	2,28	1,88
	DropPlate 1	F4	Sample6	0	272,67	2,4	5,45	2,91	-0,01	2,27	1,87
	DropPlate 1	G4	Sample7	0	371,6	3,22	7,43	3,98	0,01	2,31	1,87
	DropPlate 1	H4	Sample8	0	356,03	3,13	7,12	3,79	-0,02	2,27	1,88
	DropPlate 1	A5	Sample9	0	408,33	3,59	8,17	4,49	-0,02	2,27	1,82
	DropPlate 1	B5	Sample10	0	312,06	2,78	6,24	3,53	-0,01	2,25	1,77
	DropPlate 1	C5	Sample11	0	402,2	3,61	8,04	4,42	0,02	2,23	1,82
	DropPlate 1	D5	Sample12	0	376,77	3,27	7,54	4,11	0,01	2,3	1,83

Software - extra functionalities 6

Users

The functionality of the DropQuant software depends on the user level at login.

- Guest – minimal functionality: can perform measurements, no password required.
- User – this is the default user level, offering the full functionality of the software to start measurements and analyze the results, including using formats.
- Administrator (in older versions **lab manager**) – has more rights for changing the settings such as the user database and the location of the measurement database. Adding more administrator level users requires service login.
- Service – login level for servicing and troubleshooting the DropSense instrument. This level requires a "service dongle", available to trained service engineers only.

User level	Default user (available at installation)	Password
Guest	guest	None
User	user1	None
Administrator	administrator	JC7uD7E (default password)
Service	service	*Non available for users*

At startup, a number of default users are created. It is recommended to add more administrator level users during the installation process and to change the default password. Don't forget to write it down on a secure place.
The user is the "owner" of his own measurements; this has an impact on the visibility of the experiments. By default experiments are visible only to the owner of the measurement, and are thus not visible to other users. This protects the results of the experiment.
There are two exceptions:

- Users can always open and analyze measurements owned by a user of a lower level user. For example: everyone can analyze measurements done by guest, service engineers can open and analyze measurements done by guest, standard user and administrator, and so on.
- It is possible to give a user access to all measurements owned by all other users up to administrator level.

Bar code scanner

The 96-well disposables all have a barcode with a unique label. The DropSense instrument can be extended with an internal barcode scanner and the DropQuant software includes a lot of functions related to the barcode.

The barcode on the DropPlate

The type of barcode on the DropPlates is a universal code, type CODE 128, 2 dots, and 14 characters. This code can be read by all barcode scanners.
The syntax of the unique code is ABCCCDDEEFFFFF; with

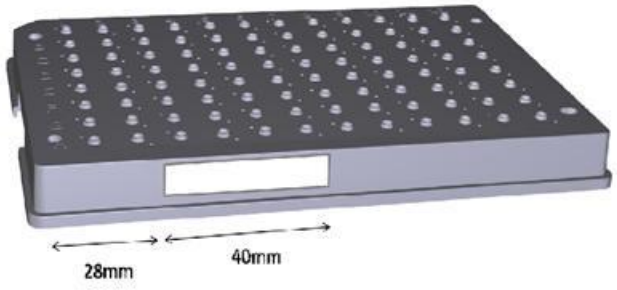
- A is the product number
- B is engineering revision number
- CCC is supplier batch number
- DD expiry date (encodes as the number of weeks since 01/01/2010)
- EE is the coating run number
- FFFFF unique disposable number within lot

Trinean defines this code; there is no possibility to order DropPlates with user-defined codes.

The software reads the barcode and performs a number of checks:

- Type of the plate should match the experiment definition
- Expiration date
- Check if the plate has been used before, and disable the wells that have been used before.

The position and size of the barcode on the frame is shown below.

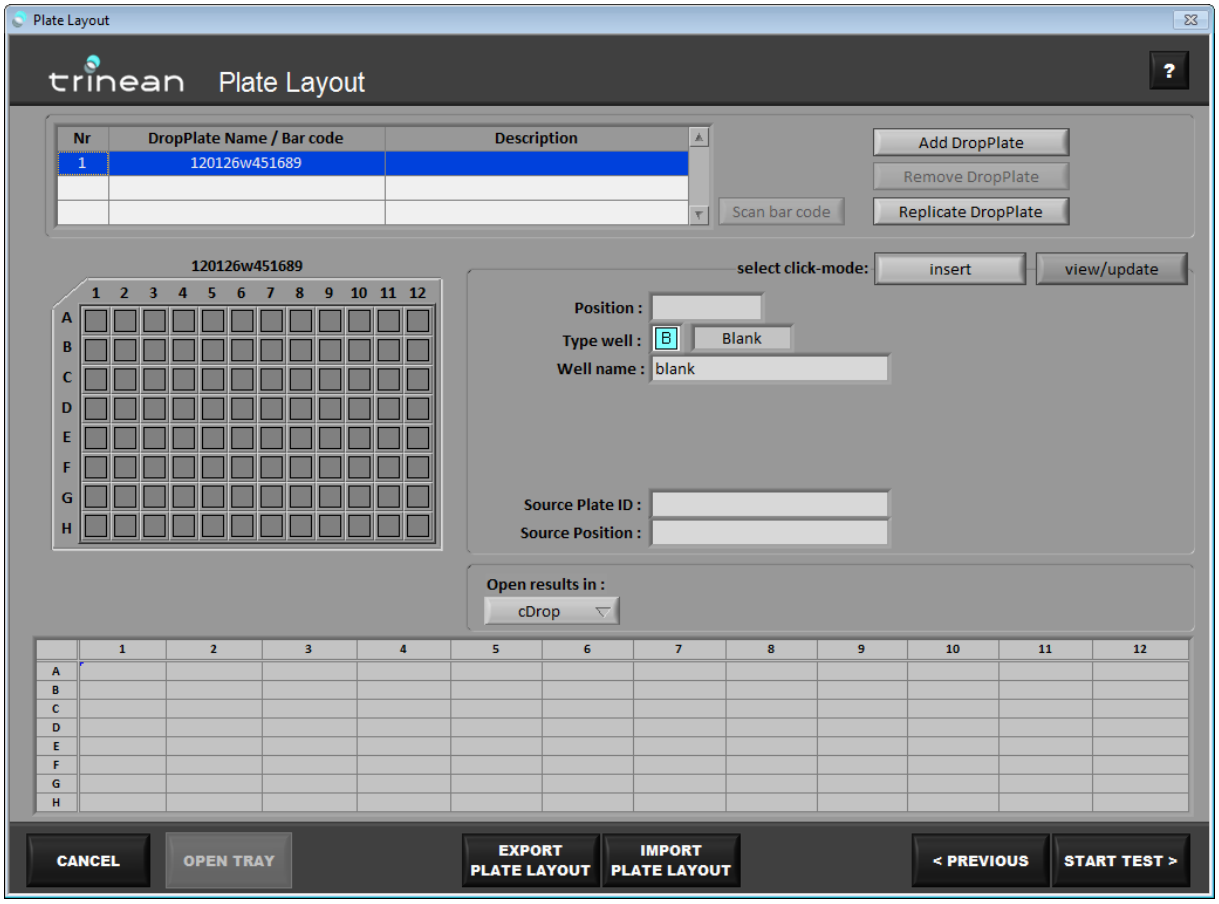


The experiment definition

When defining an experiment, the customer can select to use the internal barcode scanner or not. When this option is selected, the DropQuant software will automatically read the barcode when the disposable is loaded, and check if this agrees with the plate definition (or add the barcode to the plate definition).
When defining the plates in the experiment, the user must input the barcode label as name for the plate. There are 3 options:

- Input via the keyboard: double-click on the default name and edit the name.

- The internal barcode scanner: click on the “scan bar code” button and the code will be scanned and automatically filled in the table. If there is no plate in the instrument, the tray opens automatically and the customer can load the plate.
- Use an external barcode scanner connected to the PC. This is explained in the next paragraph.



In case the barcode labels are not known at the definition of the experiment, the customer can use the default names. During the measurements the program will scan the code, detect that the name does not correspond to the plate names defined in the experiment, and ask the customer to overwrite the plate name with the code scanned. Note that in this case the DropQuant will check the DropPlate type and expiry date during the measurement (not before the measurement starts).

In that case, it is the responsibility of the customer to connect the results of the DropQuant measurement with the source plate/position information. It is assumed the customer has saved this information during the dispensing.

The internal barcode scanner is an option of the DropSense instrument. Contact the service engineer to install the barcode scanner on the instrument.

Use of an external scanner for scanning the barcode for use in the plate definition

It is possible to use part of the barcode functionality when using an external barcode scanner that acts as an external keyboard. This offers next functionality:

- The name can be scanned in a convenient way and the DropQuant program will check the type of disposable and expiry date of the DropPlate
- The name can be scanned and the available wells database will be checked to disable the wells on that plate that have been used before.

It is not possible to check the order of the plates inserted in the instrument when doing an experiment with multiple DropPlates. This check is available for internal barcode scanner only.

There are many types of external barcode scanners acting as a keyboard: first click with mouse on the input field in the DropQuant software, so that the cursor is at the good position. Then scan the barcode: the code will be inserted automatically at the cursor position.

Be sure to set the correct parameters for the scanner (type of barcode, type of language for the keyboard, etc).

Available wells database

In case the user uses DP96 format plates and an internal barcode scanner, DropQuant is ready to keep track of the plates and wells used by the system. DropQuant includes the software to keep track of the wells used.
After scanning a bar coded plate, DropQuant will immediately lookup this barcode in the used well database and show a cross-icon for the wells that are already used.

This function is also available for manual barcode scanner operation.

Limitations

- This function is available for DP96 plate only. For customers using DP16 format plate on aluminum DropFrame there is no way for unique identification of the wells used.
- If customer uses multiple PCs connected to DropSense, then these PCs must share the same available wells database. Selecting the used well database is explained in next paragraph.

Managing the available wells database

(please check Chapter 7)

Software - advanced settings 7

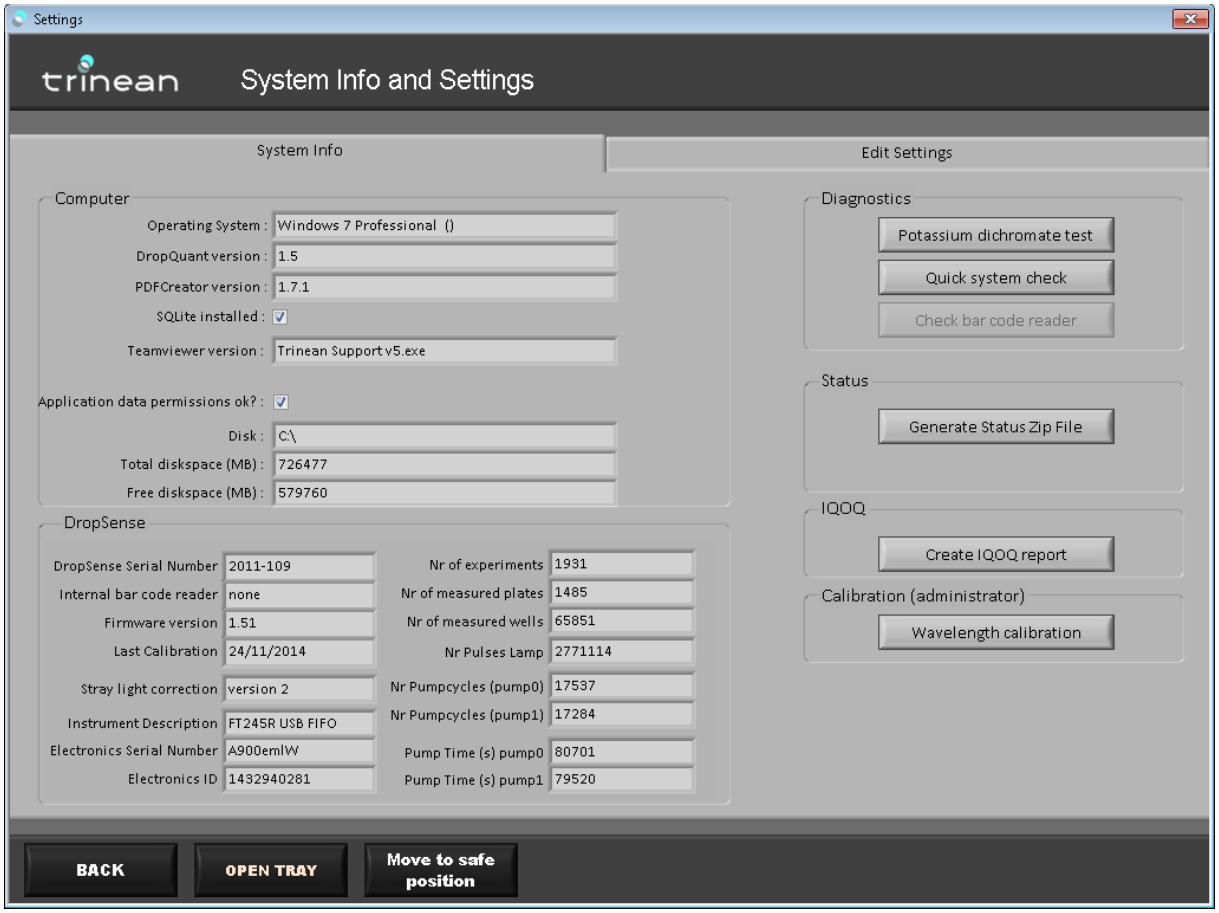
The system info and settings window

Administrator accounts have access to the 'Systems info and Settings' window. On the main window, there is a button on the left bottom corner.

This button opens a new window containing two tabs for system info, diagnostic tools and software setting options.

System info

The system info window gives information about the installed Trinean software and the instrument connected to the PC.



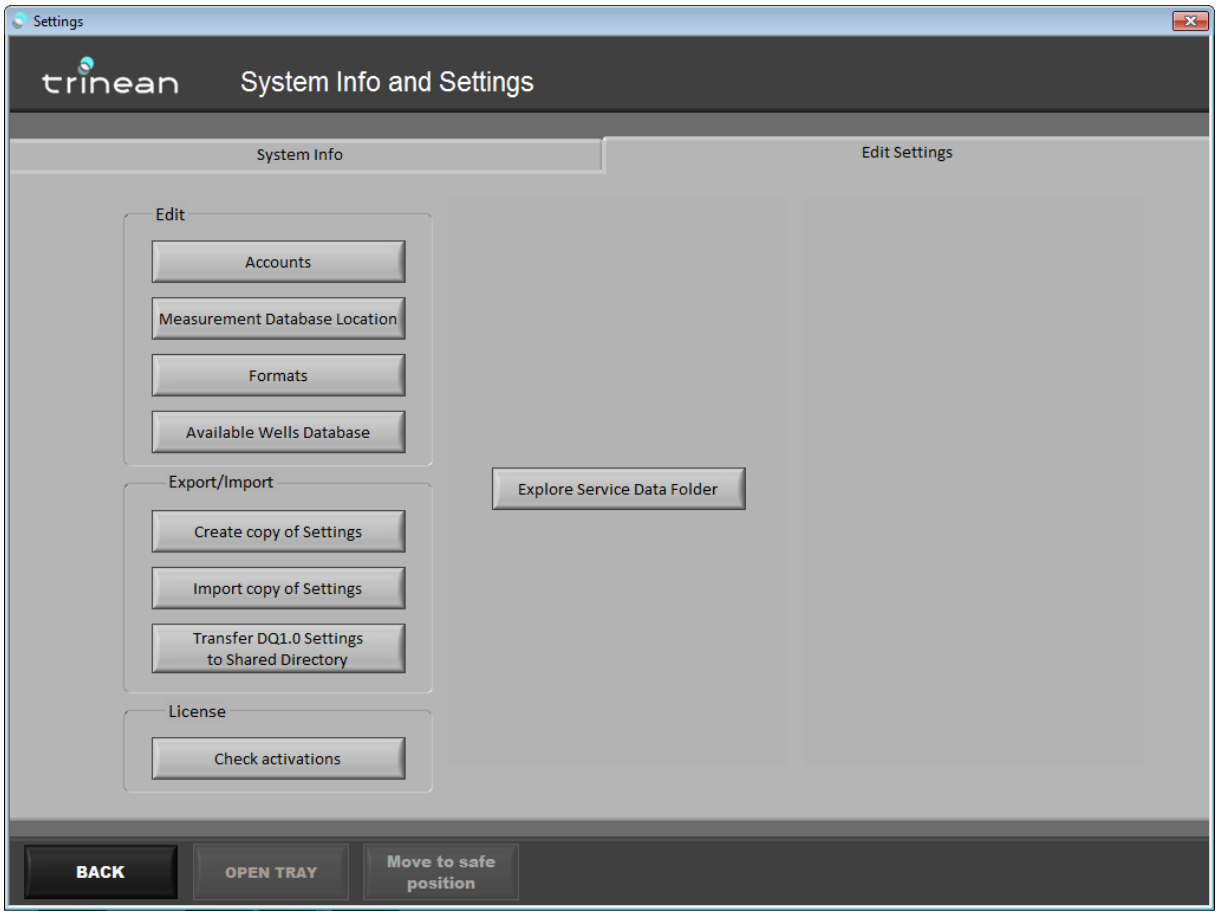
Diagnostics / Status

Check Chapter 8: Diagnostic tools

IQOQ

On the screen above, you can find a "Create IQOQ report". The software automates the IQOQ as much as possible, guiding the user through the validation and creating a report. After pressing the button, a screen will popup asking for the IQOQ code that can be purchased at your local distributor.

Edit Settings



The settings of the software are stored in a number of different .ini files. The administrator user can edit, export and import these settings, to keep the system up to date.

Accounts

The edit user database allows adding new users and editing the settings of current users.

Only administrator level users can create new users. The only information required is the user name, the password, and the option to give the new user access to all experiments.
When the “access to all experiments” is selected, that user can open all experiments by user guest, user and administrator levels. Also reports can be created from these experiments.
Service level is required to create new administrator users. Contact your technical support.

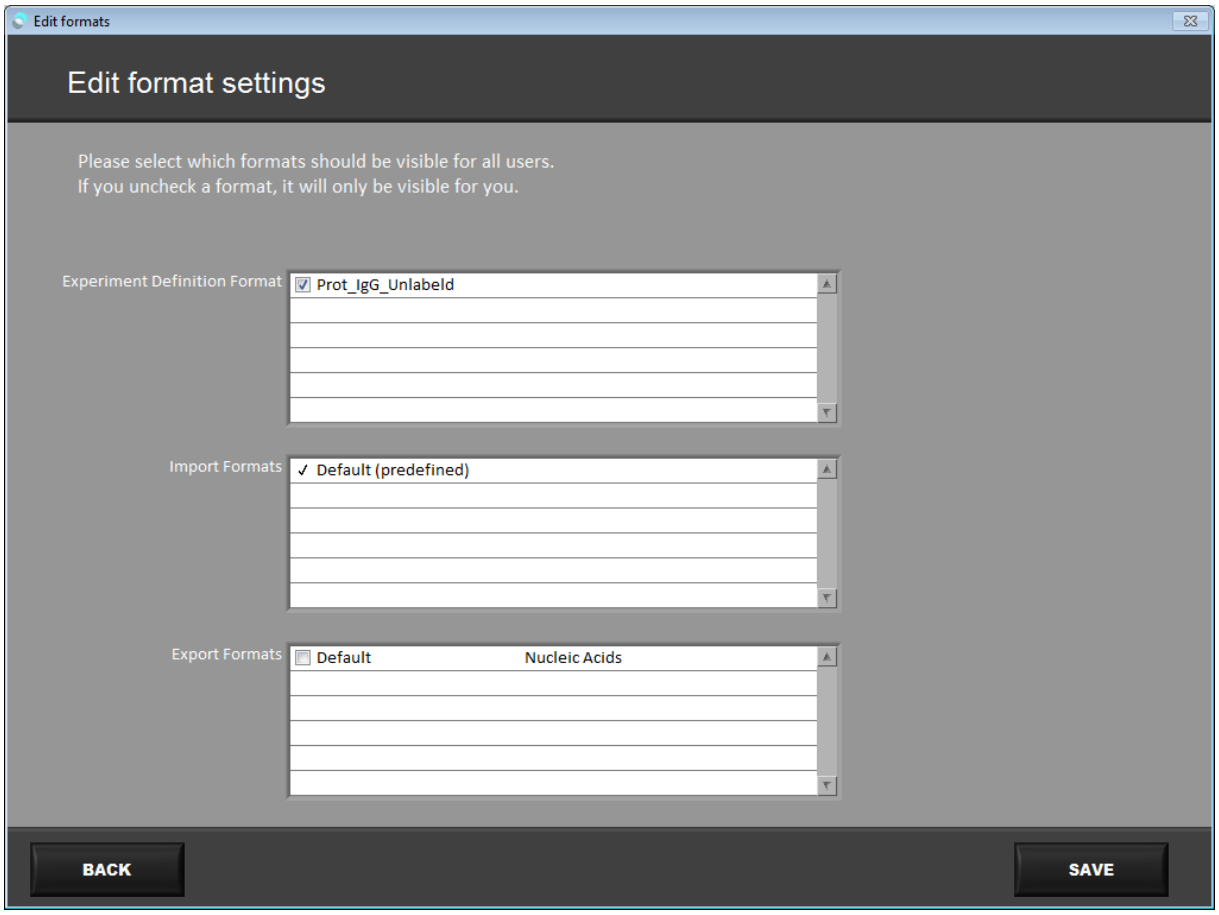
Measurement Database location

The experiments’ raw data is stored in a “measurement database location”. This directory contains a subdirectory per experiment, with all raw data. The location of this directory can be changed by clicking the “measurement database location” button.

Indexing of large databases can take a long time, especially when the database location is on a network drive.

Formats

The formats are not common for all DropQuant users. Each user defines his formats and these are by default not visible to other DropQuant users. However, the administrator can make one or more of his formats visible to all DropQuant users.



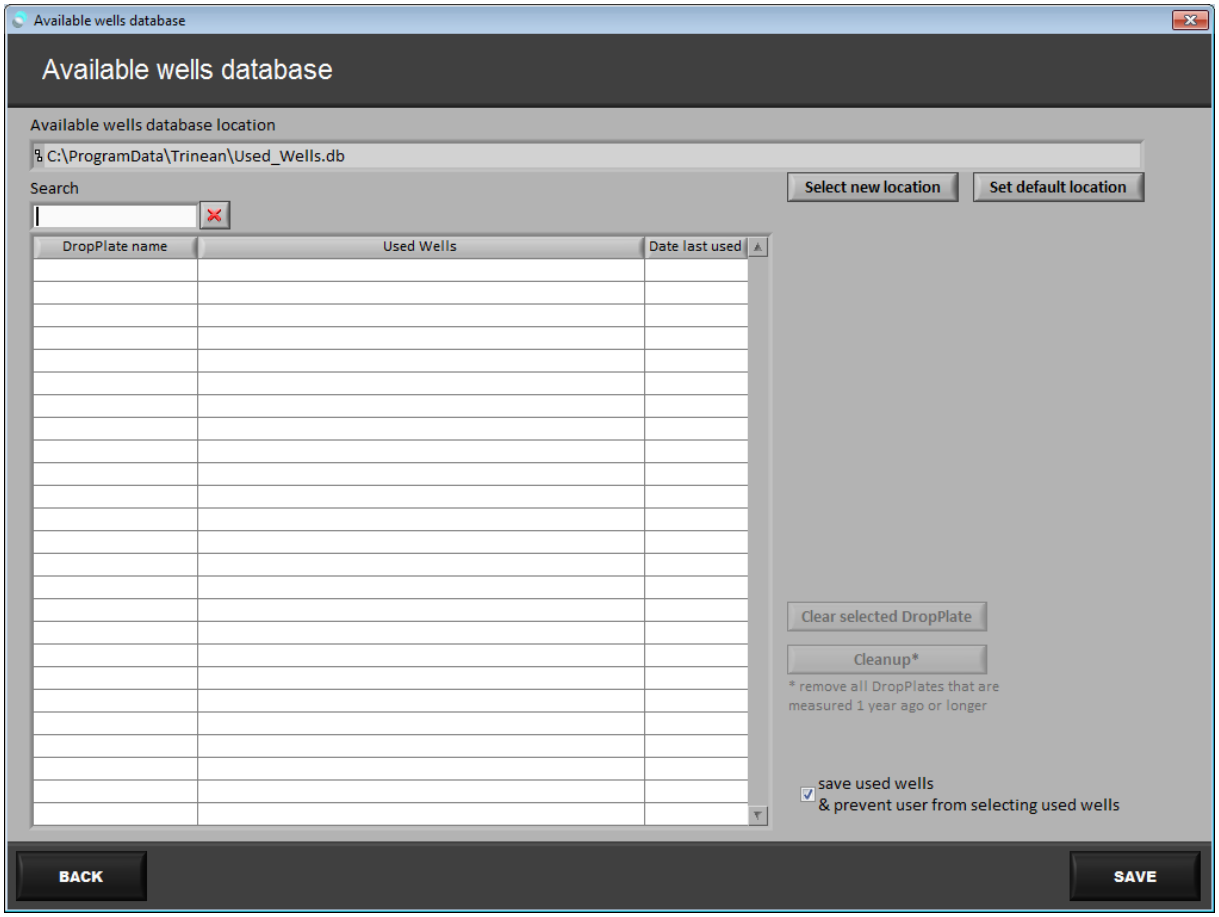
Available well database

Administrator level users have access to the used well database management tools via the settings and tools window.

The user can deactivate this function and change its location. It is recommended to select a network drive for this, so that different PCs can have access to the same available wells database.

It is also possible to:

- Remove entries from the database.
- Cleanup the database
- Search the database



Import and export settings

The DropQuant program stores a number of user settings in .ini files. When installing the DropQuant software on a new PC, the user needs to transfer the settings from the old PC to the new PC. The settings and tool section includes tools to transfer these settings.

The import window also allows importing a disposable database. This in case Trinean updates the disposable database (for example when new types of disposables are launched). As the user cannot edit the disposable database, there is no method to export that information.

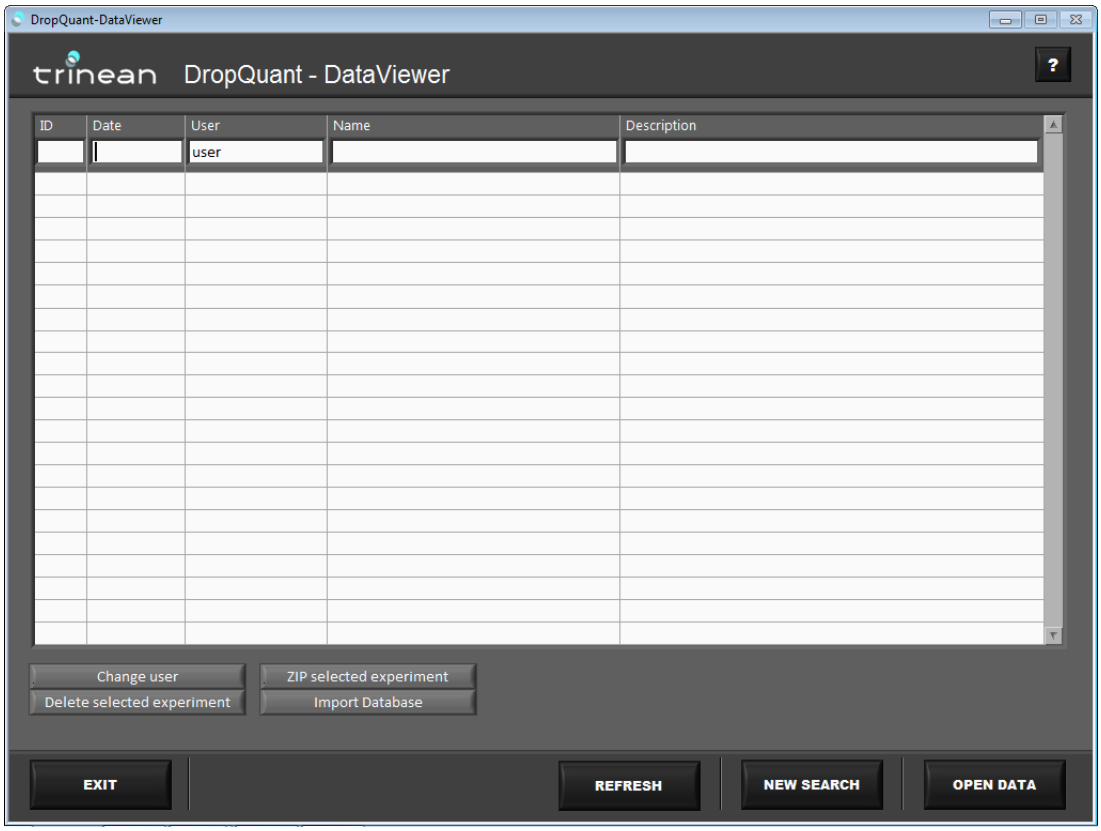
The edit formats settings window

The use of formats is not available for all DropQuant users. Each user defines his formats and these are by default not visible to other DropQuant users. However, the administrator can make one or more of his formats visible to all DropQuant users.

License

Some software extensions to the DropQuant software require a USB dongle and activation code. The dongle and code are shipped when ordering the software extensions, such as the API of cDrop software. DropQuant stores the activation code information so that the user doesn't need to input the activation code each time the software add-on is started (inserting the dongle is always required). The DropQuant can store multiple activation codes, and auto-detects if one of the activation codes matches the dongle(s) inserted.

Data viewer extra functions



Change user

Set a different user for the selected measurements.

This is available for all measurements owned by user levels guest, user and administrator. If for example the measurements are done by a service engineer, then the administrator is not able to change the owner of these measurements.

Delete selected experiment

Deletes the raw data of the experiments on the disk, and removes the entries from the database.

This completely removes the experiments. There is no way to recover the data afterwards.

ZIP selected experiment

Zips a selected experiment to a desired location.

Import database

This function imports a set of experiments from a folder. The experiments must be copied to a temporary directory (and unzipped in case these new experiments are zipped).

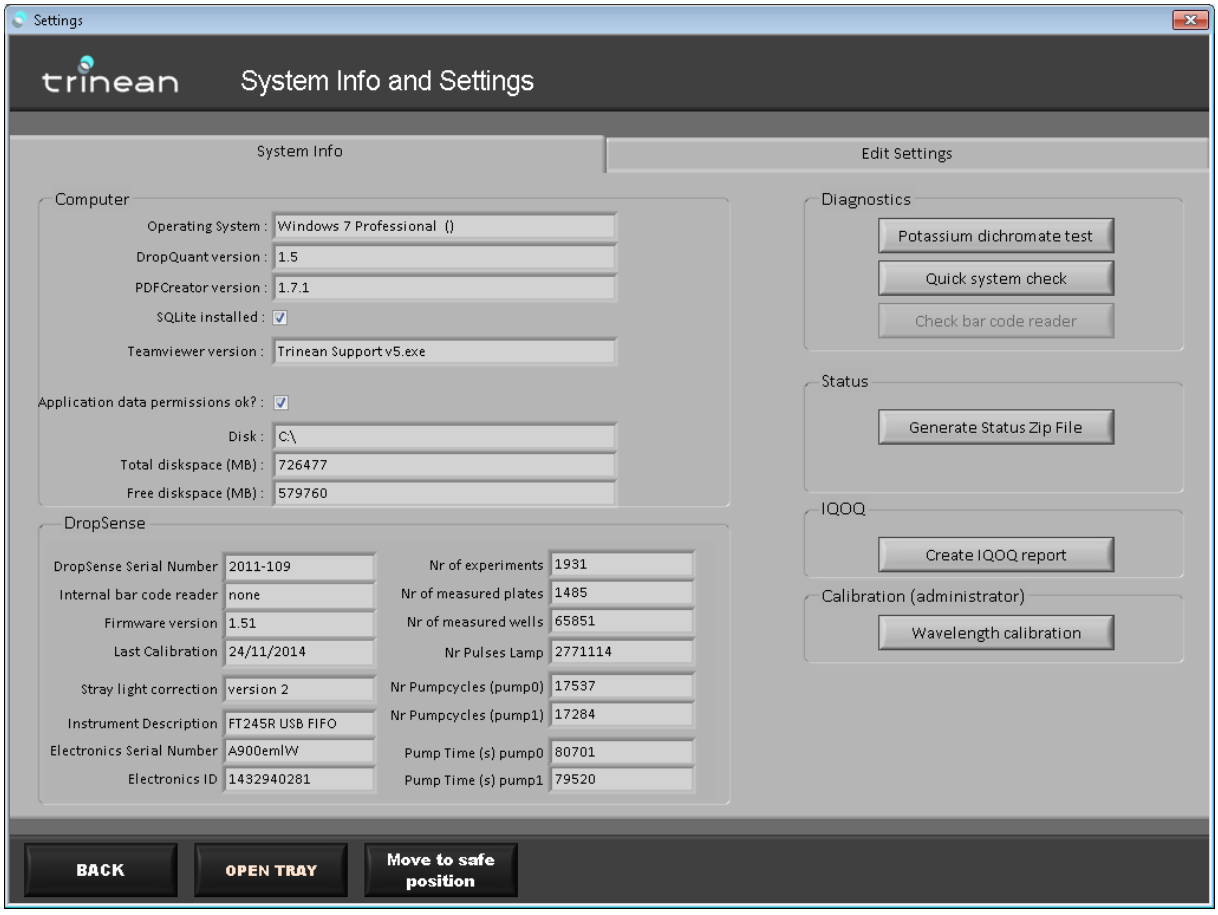
Diagnostic and calibration tools 8

Optical calibration

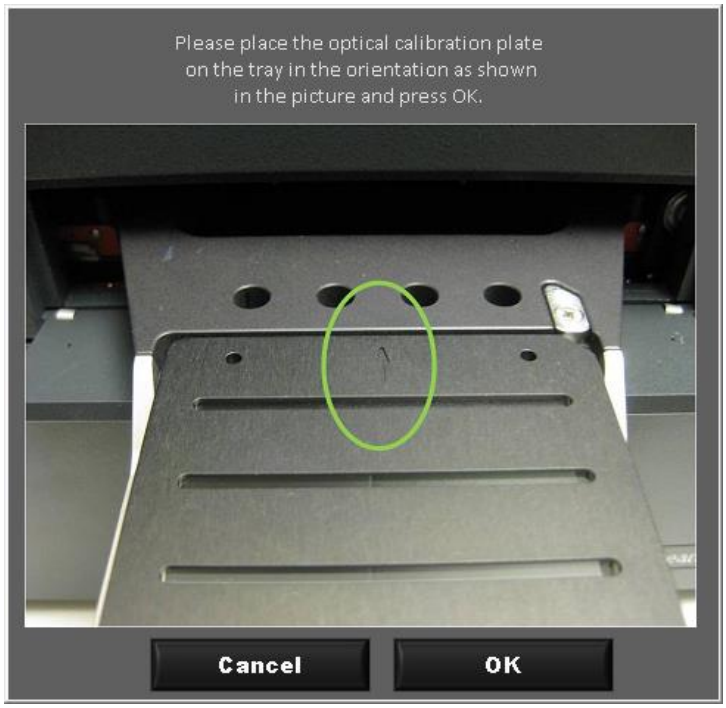
With the new DropQuant v1.5, it is possible for the user, to perform an optical calibration of the DropSense96. For this calibration, an optical calibration plate is required. This calibration plate, can be purchased from your local distributor. This calibration should also be performed, depending on the instrument's usage, at least once a year.

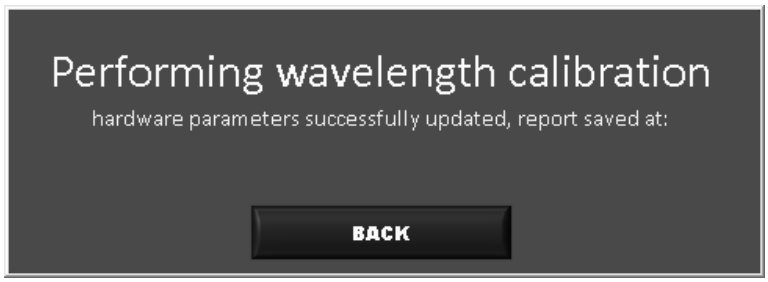
In order to do the wavelength(optical) calibration, please follow the instructions below.

1 In DropQuant, go to System Info and Settings, and press the "Wavelength calibration" button.



2 Follow the onscreen instructions.





Potassium dichromate check

DropQuant software includes a performance validation procedure as an easy check-up of the performances of the DropSense96. The check is based on the measurement of an aqueous potassium dichromate (K2Cr2O7) solution with a verified concentration (Trinean product number 301010).
It is good practice to check the instrument's performance every four months (depending on the amount of samples measured) with a fresh vial of K2Cr2O7.

3. Press "Potassium Dichromate test" button, a new screen pops up with Potassium dichromate automatically entered as "sample material".
4. Enter the DropPlate of choice (DropPlate-D+ or DropPlate-S) and the "target absorbance" of the solution, given by the manufacturer, normalized for a 1mm path length (this information can be found on the vial lable). The DropQuant software will automatically calculate the expected OD values for all the path lengths used in the DropPlate.
5. Load the blank and potassium dichromate solution on a DropPlate as indicated on the screen. Use MQ water for this purpose.

The "report display", the DropQuant software will show the results of the validation procedure. All OD values measured by the 4 optical systems in the DropSense96 are shown.

For every optical system, the mean of all measurements are calculated and compared with the expected OD value of the potassium dichromate solution. Color indications are shown giving a clear overview:

- Green color: indicates that the measurement is within the expected measurement variation. The DropSense96 performs within specifications.
- Red color: indicates that the measurement does not fall within the expected variation. Contact your local distributor for a systems check-up.

A report (txt, xls, .pdf), can be created using the “export” button. This report can be saved for future reference.

In case of red flags

Inaccurate measurements can be caused by:

1. The reference vial being open longer than 30 minutes, leading to abnormal high OD values
2. Time between dispensing and measuring is longer than 2 hours, leading to evaporation
3. Insufficient dispensed volume leading to incorrect filled micro-cuvettes
4. Inappropriate blank
5. Obstruction of the optical path (large particles, finger print, small air bubbles …)
6. The pumps performing out of specifications (see color indications on the thumbnail view)
7. The optical performance of the DropSense96 is out of specifications
8. When red values are shown, repeat the validation test with the opened vial (if repeat can be done within 30 minutes from the test), or with a fresh vial. If the result is consistent with the first test, please contact your local service engineer.

Quick Check

This tool performs a quick check of the spectrophotometer module and the pump module in the DropSense96. This quick check starts automatically when activated and takes about 30 seconds. The Quick system check consists of:

1. Spectrophotometer check – the lamp spectrum is measured and compared to the lamp spectrum at calibration. The report summarizes the measured amplitude and position of the wavelength peaks, and gives a warning if these parameters deviate too much from the calibration values. This indicates that the lamp or spectrophotometer may need service.
2. Pump system check – the output pressure of the pumps is measured using the internal pressure sensors, and compared to the values at the calibration. This allows checking for degradation of the pumps.
3. Motor check – the function of the motors and limit switches is checked.
4. The operation of the internal background light sensor and the internal temperature sensor are checked.

After the check, a pop-up window shows the most important results. An Excel report can be created using the “create report” button. This report can be saved for future reference.

Upon instrument start-up or account log-in, the DropSense96 automatically performs diagnostic checks to ensure that it is functioning within specifications. When one of the parameters is out

of specification a warning screen appears. Please contact your local distributor for a DropSense96 recalibration.

Generate status zip file

This tool allows the user, to zip and save a detailed hardware report. Please keep in mind that this report despite useful, should always be complemented with experiment files.

Troubleshooting 9

Solving an issue

In case of issues with the instrument that are not included on our FAQ list, please contact first your local distributor.
In order to have this process running smoothly, please follow these steps:

1. Export and zip the experiments when the issue started to happen. Include also at least one experiment right before the issue appeared for the first time.
2. In case of startup issues, please generate a status zip file.
3. Write in detail what you were supposed to see on that experiment. Some of the issues might be related to a specific assay and not to hardware.

On our website you can find on the support section a checklist to be sent to the distributor in case of issues.

FAQ list

Please bear in mind that this list is being constantly updated. For a full FAQ list, please consult our website.

1. The disposables expiry date is overtime.
2. What if too much sample is dispensed?
3. Can I re-use a disposable?
4. Can I install software on iOS, Linux, others ?
5. What are operating conditions: temperature, humidity?
6. Can TRINEAN provide a qualified and documented System?
7. What software add-ons are dongle protected?
8. Can I use my dongle after an upgrade of the software add-on program?
9. What if I lose the activation code?
10. What if I lose the dongle?
11. Can I copy the dongle?
12. I changed measurement database but I cannot see the measurements.
13. I cannot open / import a DropFile
14. Can I correct errors in definition of samples after the measurement?
15. Spectrum looks skewed / part of the spectrum is negative

1 The disposables expiry date is overtime.

The expiry date is 12 months (increased from 6 to 12 months in Q2 2011). It is not recommended to use the disposables after the expiry date. The customer must use the disposable before the end of the month indicated on the expiry date.

2 What if too much sample is dispensed?

	DP-S	DP-D+
Maximal sample quantity	2.2uL	2.5uL

There are 2 effects if too much sample is dispensed:

- the excessive sample volume remains in the input well, resulting in evaporation and a change in concentration
- there is a risk that in some wells the measurement reservoir gets overfilled during pipetting. This overfilling results in bad measurements. The probability of overloading is quite small (max few wells per 96-well plate), but too large to guarantee error-free operation of plates when loaded with too much sample volume.

3 Can I re-use a disposable?

No, it is not possible to re-use a disposable: the risk of cross-sample contamination increases and the self-loading properties disappear, resulting in low-yield of the sample pumping process.
It is neither possible to do a second measurement on the disposable with the same samples. The measurement algorithm assumes the sample is in the meander and the measurement reservoir is empty at the start of the measurement, and compares the optical transmission of the filled reservoir with the transmission of the empty reservoir. This process fails if the reservoirs are already filled at the beginning of the measurement process.

4 Can I install software on iOS, Linux, others ?

No, software runs on Windows systems only.

5 What are operating conditions: temperature, humidity?

Operating temperature range is 15 – 35deg C (~room temperature) (this is 59-95 deg F).
Humidity range is 10% - 80%, no condensation.
The shipment weight of an instrument is about 30kg.
Power consumption: the external power supply is a 50W type (24V, 2.1A).

6 Can TRINEAN provide a qualified and documented System?

Trinean has an add-on software product which automates the IQOQ procedure as much as possible. It guides the user through the validation process and creates a report.
The software allows validation of the system for next applications:

- Qualify the installed DropSense instrument and DropQuant software as generic UV/vis spectrometer system for microliter sample quantification.
- Qualify the installed DropSense instrument with internal barcode scanner and DropQuant software as generic UV/vis spectrometer system for microliter sample quantification.

The validation process includes:

- Check installation of instrument and software
- Check self-test of instrument
- Check OD performance using potassium dichromate test

- Check of the pump profiles is part of the OD performance test.

For more information regarding system requirements and purchase details, please contact sales support at sales@trinean.com

7 What software add-ons are dongle protected?

The list of modules is:

- cDrop
- DropControl
- Service

8 Can I use my dongle after an upgrade of the software add-on program?

You can upgrade the software add-on program to newer versions, and still use the old dongle and activation code: you will have access to all functions that are allowed by the dongle.

9 What if I lose the activation code?

You can always ask Trinean a copy of the activation code.

10 What if I lose the dongle?

The dongle is unique: there are no copies available. This means you have to buy a new dongle.

11 Can I copy the dongle?

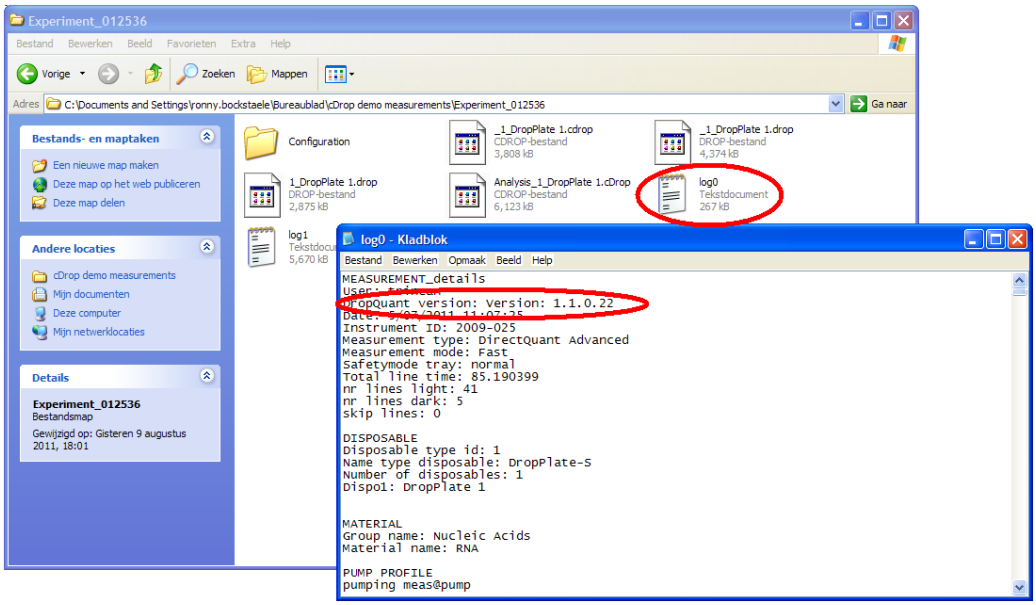
No.

12 I changed measurement database but I cannot see the measurements.

Check if the measurements are done by same user account. If measurements in database are measured by user different from the user currently logged in the DropQuant software, then they will not be visible.
Change to the administrator account to see all measurements defined in the user database of that DropQuant installation. Measurements done by service are never visible by lab manager or standard users. These measurements are visible by service level only. This may happen when doing service interventions, where the measurements are done under username service, thus not visible to other users.

13 I cannot open / import a DropFile

DropQuant (and cDrop) cannot import zipped experiments. The software can only import the experiment directory (typically a directory with name /experiment_1234).
Most probably the version of the DropFile is more recent than the version of the DropQuant software.
You can check the version of the installed DropQuant from the “settings and tools” (login as administrator). You cannot check the version of the DropFile. It is also possible to look up the DropQuant version that was used during the measurements. You can find this by opening the log file which is part of the experiment directory.



14 Can I correct errors in definition of samples after the measurement?

No, this cannot be done in the DropQuant software: it is not possible to edit the experiment definition afterwards (type of plate, type of material, position of the blanks etc).

I have several red labeled results

Reasons for this may be various:

- user errors during pipetting,
- hardware problems on the pumps or manifolds
- viscous samples (use viscous mode!).

Get the measurement, open it, and check the pump profiles in the debug tool.

15 Spectrum looks skewed / part of the spectrum is negative

This occurs when a labeled DNA sample is measured without selecting the label in the experiment definition. Similar situations occur then, for example, potassium dichromate using DNA mode.

Solution is straightforward: set the background to no background, or single wavelength background correction (selecting a wavelength at which sample is not absorbing).

Theory A

DNA/RNA concentration and purity determination

Nucleic acid samples can be checked for concentration and quality using the DropSense96 spectrophotometer. Nucleic acids have an absorption maximum at 260 nm (A260) and the spectrographic reading at this wavelength is the most common method for detecting dsDNA, ssDNA, RNA and oligonucleotides on a solution. The nucleic acid concentration is calculated using the Beer-Lambert law (see below), which predicts a linear change in absorbance with concentration. Using this equation with a 1 cm path length, an A260 reading of 1.0 is equivalent to ~50 ng/µl dsDNA, ~33 ng/µl or ~40 ng/µl single-stranded RNA.

Beer-Lambert Law:
$A=\varepsilon.c.l$

A = absorbance at a particular wavelength
ε = the extinction coefficient
c = concentration of nucleic acid
l = path length of the spectrophotometer cuvette

The absorbance data is archived on the database and displayed on the software screen after every measurement. On the nucleic acid application, the software chooses automatically which path length generates the best absorbance values and normalizes this measurement to a 1.0 cm (10.0 mm) path. These are displayed in the software for further data analysis.

DNA/RNA purity determination – Residual cellular contaminants like proteins or compounds used in the DNA/RNA preparation frequently remain present in the DNA solution and often interfere with the measurement at 260 nm leading to incorrect results. Both protein and DNA absorb UV light but have different absorbance curves. The peak of light absorption for DNA is at 260 nm, while proteins absorb strongly at 280 nm, mainly due to tryptophan and tyrosine side chains. Therefore, the purity of a DNA sample can be calculated by examining the ratio of the two absorbance values. A260/A280 values of ~1.7 to 1.8 predict “clean” DNA; good quality RNA will have a 260/280 ratio of ~1.8-2.0. Lower values may be indicative of significant protein, phenol or other aromatic compound contamination.
However, the A260/280 ratio is not always an accurate representation of DNA purity. Other contaminating substances like EDTA and carbohydrates have a low 280 nm absorption but absorb UV light around 230nm. Furthermore, some proteins containing few aromatic residues have little absorbance at 280 nm, while all proteins have a clear absorption peak at 228 nm due to their peptide bonds. This makes the 260/230 ratio often a more constant indicator of the presence of protein in a nucleic acid sample. Therefore, absorbance readings measured both at 230 nm and at 280 nm provide a more accurate estimate of contaminants that may be present in nucleic acid samples. The ratio of the A260/A230 should be ~1.8 or greater since nucleic acids have an absorbance minima at 230 nm.

It is recommended to use the absorbance readings at A260, 280, and 230 and examining both A260/280 and A260/230 ratios for every sample. As a general rule, a 260/280 ratio of ~1.8 and a 260/230 ratio of ~2.0 or greater predict 'clean' DNA; good quality RNA will have a 260/280 ratio of ~1.8-2.0 and an OD ratio 260/230 of ~2.0 or greater.

The 260/280 ratio can differ depending on the spectrophotometer used. It is dependent on both the characteristics of the sample (pH, ionic strength) and the wavelength accuracy of the spectrophotometer used. Since the DNA absorption peak shows a steep slope at 280 nm, a slight shift in wavelength accuracy (+/- 1 nm) can result in ~0.2 change in the 260/280 ratio.

Protein concentration and purity determination

Protein concentration like nucleic acids, can be determined by measuring their UV absorbance at 280nm and calculating the concentration using the extinction coefficient of the protein in the Beer-Lambert equation. This method is suitable to quantify purified proteins.

However, the 280nm absorption of proteins depends on the presence of aromatic amino acids (Trp, Tyr and Phe) and Cys-Cys disulfide bonds. The UV absorption of proteins varies greatly and depends on the protein's particular amino acid concentration. In addition, buffer type, ionic strength and pH affect the UV absorption and even pure protein solutions may have different conformations and modifications. When performing protein A280 concentration measurements, the best approach is to empirically derive the extinction coefficient for the protein of interest, or search for published protein extinction coefficients (examples in the Practical Handbook of Biochemistry and Molecular Biology). Alternatively, if the protein sequence of the protein to be measured is known, the theoretical molar extinction coefficient can be calculated using the equation:

$$\varepsilon = 5500(\#Trp)+1490(\#Tyr)+125(\#Cys)$$

ε = the extinction coefficient
# = number of
Trp = Tryptophan, Tyr = Tyrosines, Cys = Cysteines

A very rough protein concentration can be obtained by making the assumption that the protein sample has an extinction coefficient of 1, so 1 OD = 1 mg/ml protein.

In combination with the DropPlate-D+ consumable, the DropSense96 performs a dual path measurement, with path lengths of 0.7 and 0.1mm to enable quantification of proteins with a wide concentration range without the need for dilution. Using the DropPlate-S with a single micro-cuvette (0.5mm path) on the DropSense96 is ideal for quick analysis of protein samples within a more limited measurement range.

The absorbance data is archived on the database and displayed on the software screen after measurement. In the protein A280 application, the software chooses automatically which path length generates the best absorbance values and normalizes this measurement to a 1.0 cm (10.0 mm) path.

Since the UV absorption of nucleic acids at 280 nm can be as much as 10 times that of a protein, a small percent of nucleic acids in the sample can greatly distort the protein quantification. Therefore, the protein sample purity must be determined using the A260/A280 ratio. An A260/A280 ratio < 1 indicates “pure” protein whereas a higher value indicates nucleic acid contamination.

An alternative method for protein quantification is a colorimetric protein assay, such as a BCA, Bradford, and Lowry assay. These are commonly used for quantification of protein solutions and cell lysates. These types of assays can easily be performed with the DropSense96, using the ‘general UV/vis mode’ in the software. Quick export of the data to excel allows fast generation of a standard curve and concentration determination.

General UV/Vis spectrophotometry

In the general UV-Vis application, the DropSense96, functions as a conventional spectrophotometer. An absorbance scan from 230 to 750 nm of any liquid samples can be analyzed, enabling simple identification of absorption peak heights and positions. An unlimited number of wavelengths can be designated in advance for absorbance monitoring and inclusion in the report.
Note that the values reported are NOT normalized to a 10mm path length, so if comparison is being made to other systems the appropriate factor should be used to convert them.

Principle of standard curve measurements

In previous paragraphs, the concentration measurements are based on direct quantification: the OD10mm spectrum is measured and the concentration is calculated from the OD at a certain wavelength, using the concentration factor which is derived from the Beer Lambert law. This requires knowledge on the extinction coefficient of the substances.
The standard curve method offers an alternative approach. The starting point is a set of reference sample with known concentration. The DropSense system measures the absorption spectra, and builds a standard curve: this is the relation between the reference concentration and the measured OD at a certain wavelength. The samples with unknown concentration are also measured, the OD at the reference wavelength is measured, and the standard curve is used to calculate the concentration of the unknown sample.
This method is typically used in case of colorimetric assays, where the OD depends on local factors such as temperature, pH value and so on. These factors are calibrated in the standard curve.
There are 2 methods to define the standard curve:

- The samples used to build the standard curve are part of the experiment.
- A standard curve of a previous measurement is used. The customer can save a standard curve for future use when opening a standard curve experiment in the post-processing window. Once this is saved, it is available for all DropQuant users.

Note using older standard curves in new experiments may lead to inaccuracies in case the old reference samples are not representative for the newer unknown samples. It is therefore recommended to add references in the experiment to check to validity of the standard curve.

The typical process of measuring samples using the standard curves method (where the reference samples are part of the experiment) is:

- The user defines the standard curve method in the experiment definition window.
- The user defines reference samples (with reference concentration value) and unknown samples in the sample definition window.
- The DropSense measures the plate, and thus the optical absorption for each sample.
- The software calculates the standard curve: this is the relation between (measured) OD and (input) reference concentration. The user can select the type of curve (linear, polynomial, etc). The standard curve is shown as OD=f (concentration).
- Based on this standard curve, the concentration of the other (unknown) samples is calculated (from the measured OD value and the constructed standard curve). Thus concentration=f^{-1}(OD).
- In the results window, the standard curve is shown.

The standard curve is an analytical equation, fitted to the measured OD of the reference samples. The user can select from a number of models shown on the table below.

Model name	Standard curve equation	Notes
Proportional	OD = a0*CONC	
Linear	OD = a0+a1*CONC	
Interval	OD = a_i * CONC	Piecewise linear
2nd order polynomial	OD = a0+a1*CONC+a2*CONC^2	
2nd order polynomial through (0,0)	OD = a1*CONC+a2*CONC^2	
Sigmoid	OD = a0 / [1 + exp(-(CONC-a1)/a2)]	
4 parameter curve fit	OD=[(A-D)/(1+{conc/C}^B)]+D	

Maintenance B

The following maintenance procedures and performance checks must be carried out to ensure reliable operation of the DropSense 96.

Cleaning the DropSense 96

Important: Switch the instrument off and disconnect the line power cord from the power outlet before cleaning.

Risk of electric shock [W7]
Do not open any panels on the DropSense 96.

Damage to the instrument [C3]
Do not use solvents, or reagents containing acids, alkalis, or abrasives to clean the DropSense 96.

Damage to the touchscreen and computer [C4]
Do not pour or spray liquids, e.g., cleaning agents, on to the DropSense 96. Use a tissue moistened with water only for cleaning.

We recommend wiping the DropSense 96 with a damp cloth only.
The following disinfectants and detergents are recommended for cleaning the DropSense 96.
Note: If cleaning agents different from those recommended are used, ensure that their compositions are similar to those described below.

General cleaning of the DropSense 96:

- Mild detergents
- 70% ethanol

Maintenance

General instructions

Do not use spray bottles to spray cleaning or disinfectant liquids onto surfaces of the DropSense 96.
If solvents or saline, acidic, or alkaline solutions are spilt on the DropSense 96, wipe the liquid away immediately.
Follow manufacturer's safety instructions for handling cleaning agents.
Follow manufacturer's instructions for soaking time and concentration of the cleaning agents: exposure for longer that the recommended

Glossary X

Sample size requirements – Although the dispensed sample volume is not critical, it is essential that a minimal amount of sample is dispensed for correct filling of the measurement chamber allowing precise measurements. Extensive testing indicates that sample volumes of 2 µl are sufficient to ensure reproducibility. Although this volume range takes a pipetting error into account, it is best to use a precision pipette (e.g. 0.5-5 µl) with precision tips to ensure that the recommended sample quantity is dispensed.

OD – optical absorption measured by the instrument. The OD10mm is the OD calculated as if the path length is 10mm (=the path length of a standard cuvette).

Recommended sample quantity – volume to be dispensed in the input well to guarantee good and high-yield measurements, with the given measurement range and sample residence time. Check the accuracy of the pipette and the tips to avoid the dispensed sample volume exceeds the maximal sample quantity.