

LuminUltra GeneCount[®] Q-8 Q-16 qPCR Non-Touch Screen Version

For Windows[®] - based Software

Version 0.16 – for Beta release

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Introduction

This manual includes installation instructions, operating instructions, maintenance procedures and other technical information for the GeneCount® Q-8/Q-16 qPCR Touch Screen device.

Please read and understand this document before operating the unit. Failure to do so could result in personal injury or damage to equipment. Please consult your local representative if you do not understand the information in this manual, or if you need additional information.

Care has been used to assemble and describe the information in this manual. LuminUltra Technologies Ltd. shall not be liable for typographical errors or other mistakes in this manual. All instructions, pictures and specifications in this manual are based on the latest information that was available at the time of publication.

Our team strives to improve and update existing information based on user feedback. Newest versions and information are available for download from the website my.luminultra.com and from sales@LuminUltra.com.

Note:

Very important information is contained within this manual and it should be carefully read before first use of the instrument. Failure to operate instrument according to the instruction could result in damage or abnormal functioning of the instrument.

Warning

The warning messages indicate caution when completing operation of a certain step. If the instrument is not used in the manner prescribed by the manufacturer, the protection provided may be compromised.

Safety

During operation, maintenance and repair of this instrument, the following basic notes must be observed. In case of failure to follow these measures or the warnings or notes indicated herein, the basic protection provided by the instrument, its safety criteria of design and manufacture, and its predicted use range would be impaired.

LuminUltra Technologies Ltd. shall not be held responsible for any consequences resulting from the user's failure to observe the following requirements.

Grounding Considerations

To avoid an electric shock, the input power cable of the instrument must be properly grounded. This instrument uses a 10A 3-pin grounded plug. It is for use with a grounded power receptacle and is a safety feature. If the plug cannot be inserted into the receptacle the receptacle must be fixed by a qualified electrician to maintain the safety function of the plug and the protection it provides.

Risk of Electric Shock

Operators are not allowed to disassemble instrument protection, replace components, or make internal adjustment without authorization. If necessary, it must be completed by certified professional maintenance personnel. In the event work is required to be performed the device must be disconnected from all power sources.

Power Supply Considerations

Before connecting to the mains and switching the instrument on, make sure the voltage is consistent with the instrument's requirements 110-240V at 16V and 9A. The rated load for the power socket must not be less than the instrument's maximum load of 144W.

Power cord considerations

The instrument is supplied with a power cable, always use the supplied cable when operating the instrument. If the power cable is damaged it should be replaced with a new one of the same specifications. The power supply wire should be treated with care; do not place objects on the wire and do not place the wire in high traffic areas. If the power cord near a hot surface, add protection to prevent the insulation from being damaged.

Plugging and unplugging your device

At insertion and withdrawal of power cable, the back of the plug shall be firmly held with the hand. The plug must be completely and tightly inserted into the socket and must not be removed by pulling the cable.

Placement of instrument

This instrument should not be positioned in a place where it is difficult to cut off the power supply.

When not in use, the power should be switched off. If the instrument is not going to be used for a long time, the power should be switched off, the power plug withdrawn and the instrument covered with soft cloth or plastic film to prevent dust or foreign bodies entering the machine.

Notes during operation

During test, care shall be taken to prevent liquid from dropping onto the instrument. The castoff used in tests, such as consumables, reagent, and so on, should be treated as required, and should not be thrown away or poured down the drain.

During test, if there are hazardous substances, user must be trained before using. Hazardous substances, which has been used, should be handled, and stored according to direction for

use. Please consult and follow all regulations as required by the local authority having jurisdiction. User who operate the instrument, must be trained, and has relevant quantification.

Warning

If any of the following should occur, you should immediately switch off the power supply, withdraw the power plug from the power socket, and contact the supplier to effect a repair: Repairs can only be carried out by suitably qualified engineers.

- Liquid gets inside the instrument.
- The instrument is rained upon or water is spilled over it.
- The instrument works abnormally, generates an abnormal sound, or generates a strange odour.
- The instrument is dropped, or its casing is damaged.
- There is an obvious change in the function of the instrument.

Warning

When operating or performing work on the device, all relevant PPE guidelines should be followed taking special care to protect oneself and others from potentially contagious material.

Warning Signs

- Hot Surface warning Label



Warning

When "HOT SURFACE!" is pasted in the instrument, it means that the metal part (module) near this sign shall not be touched with any part of the body during the operation of the instrument or a period of time immediately after the operation of the program to avoid burns!

Maintenance of Instrument

If there is any stain on the surface of the instrument, it can be cleaned with a lint-free cloth and low strength bleach solution. Heat conducting oil medium is not allowed in the module hole of this instrument.

The drawer should be closed in time after the normal storage and use of the instrument to prevent dust accumulation.

Warning 

- When cleaning the instrument, the power should be turned off.
- The instrument surface should not be cleaned with corrosive cleaning agents.
- The instrument module includes precise optics. Dust, foreign matter and residue should be avoided.

Information to the User

This equipment has been tested and found to comply with the limits for a class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures

1. Reorient or relocate the receiving antenna
2. Increase the separation between the equipment and receiver
3. Connect the equipment into an outlet on a circuit different from that to which the receiver is connected
4. Consult the dealer or an experienced radio/TV technician for help

This equipment has been verified to comply with the limits for a class B computing device, pursuant to FCC Rules. In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and TV reception. The user is cautioned that changes and modifications made to the equipment without the approval of manufacturer could void the user's authority to operate this equipment.

Overview

This is a user's guide for using LuminUltra GeneCount qPCR with Windows[®]-based software.



Parts required

- GeneCount qPCR instrument unit
- AC adapter and power cord
- USB cable
- A Windows[®] - based PC

Key parameters

Capacity (# of wells)	4, 8, 16
Channels (# of Fluorophores per well)	4 (1. FAM/SYBR Green; 2. JOE/HEX/VIC/TET; 3. ROX/Texas Red; 4. CY5/LIZ/Cy5.5)
Multiplex capability	Up to 4 targets per well
Minimum detection threshold	4 copies
Dynamic range	>1.0E9
Signal Interface	USB 2.0, Wi-Fi
Excitation source	High endurance LED
Detector	Ultra-low- light CMOS Bio-imaging chip
Thermal system	Solid-state, Peltier-based
Tube/plate formats	0.2mL, 4 or 8-tube strip
Reaction volume	10uL – 60uL
Filters:	4 sets exchangeable*, **
Excitation Range:	460nm – 670nm
Emission Range	510nm – 720nm
DNA probes supported:	DNA binding dyes (e.g. SybrGreen), hydrolysis probes (e.g. TaqMan probe) and hybridization probes (e.g. FRET probes).
Temperature Uniformity	< +0.2°C
Temperature control resolution	+0.1 °C
Temperature Ramp Rate	6.5°C/s heating; 4°C/s cooling

Size and weight	165mm (L) x 115mm (W) x 75mm (H).
Weight	950g
Power supply	DC 16V, 144W (Adapter provided that accepts 110V/240V AC)

*: Wavelength characteristics can be modified by changing filter modules.

** : We support 1 – 4 channels in different configurations.

Set up the instrument

Step 1

After installing the software (see appendix at end of the document about how to download, decompress and install software from LuminUltra's web site). Connect instrument with Windows[®] - based PC via included USB cable. Power up the instrument using the included AC adapter. In some models, there is a main power switch in the back. Please flip it to the on position.

Figure 1. PDx 16 USB and power connections

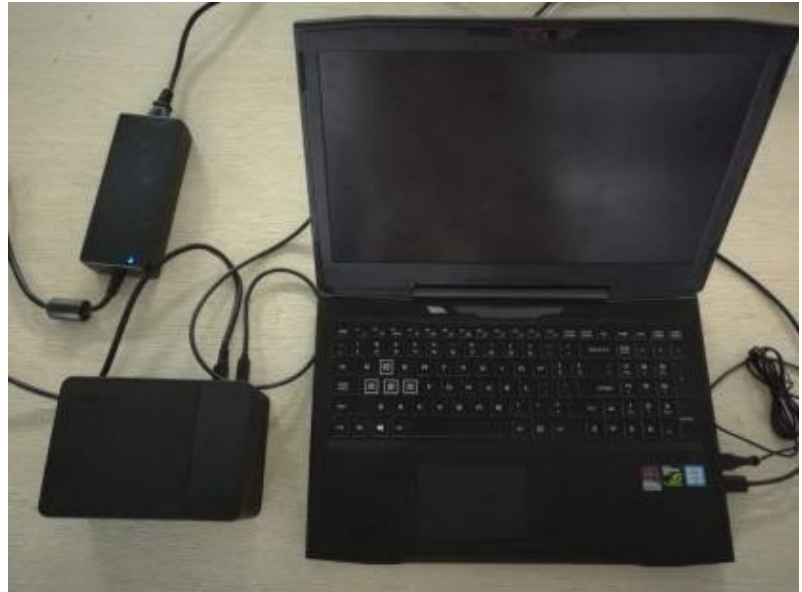


Step 2

Start the software, by launching Anitoo.exe. The software should recognize the instrument HW automatically. There is no need to install a driver as Windows[®] should already have the driver necessary to connect to GeneCount device.²

Step 3

Open the instrument lid and place the test tubes filled with sample and reagent mix. We support 0.2ml standard qPCR tubes. Make sure the top lid of the tube is transparent and flat. Close the instrument lid when done. For more information about the reaction tubes selection for GeneCount qPCR instrument, please see Appendix A.



¹ We required Windows[®] 10 with Office 2010 or later installed to support print report function.

² We use the USB HID device interface for GeneCount. This is the same USB HID for keyboard and mouse

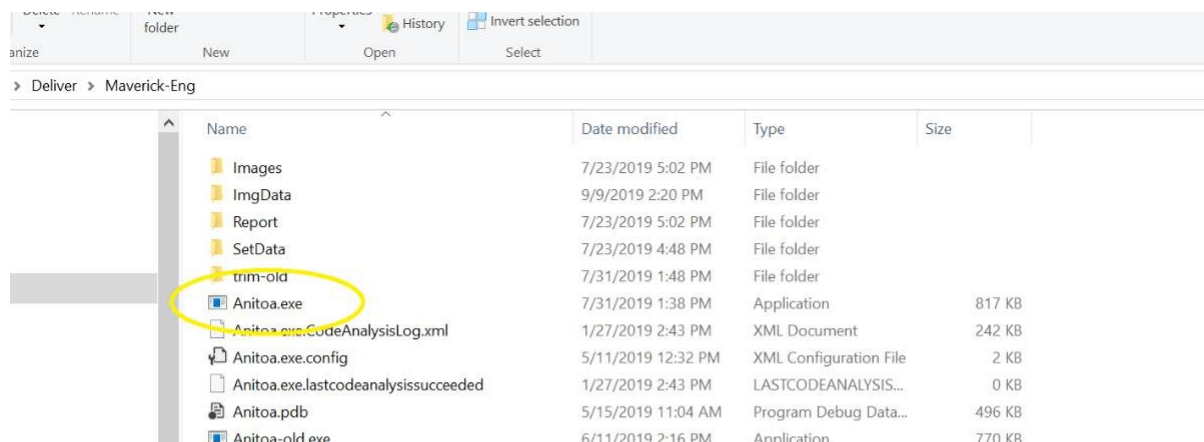
Step by step software guide

Install the software

The Beta version of the software is delivered as either a folder or one compressed file, with the name "Anitoo-Eng" for the English version.

If the file is one compressed file, please decompress it first. If you do not have a compress/decompress utility installed on your computer, go get one at: <http://www.7-zip.org>. It is free.

Once the file is decompressed, just copy the folder to any directory in your PC. The folder should look like this, see below, when opened. The executable file is the file "Anitoo.exe".



Launching the software

When the GeneCount PC software is launched, after a brief delay for device initialization, the front page will display as shown in Figure 1.

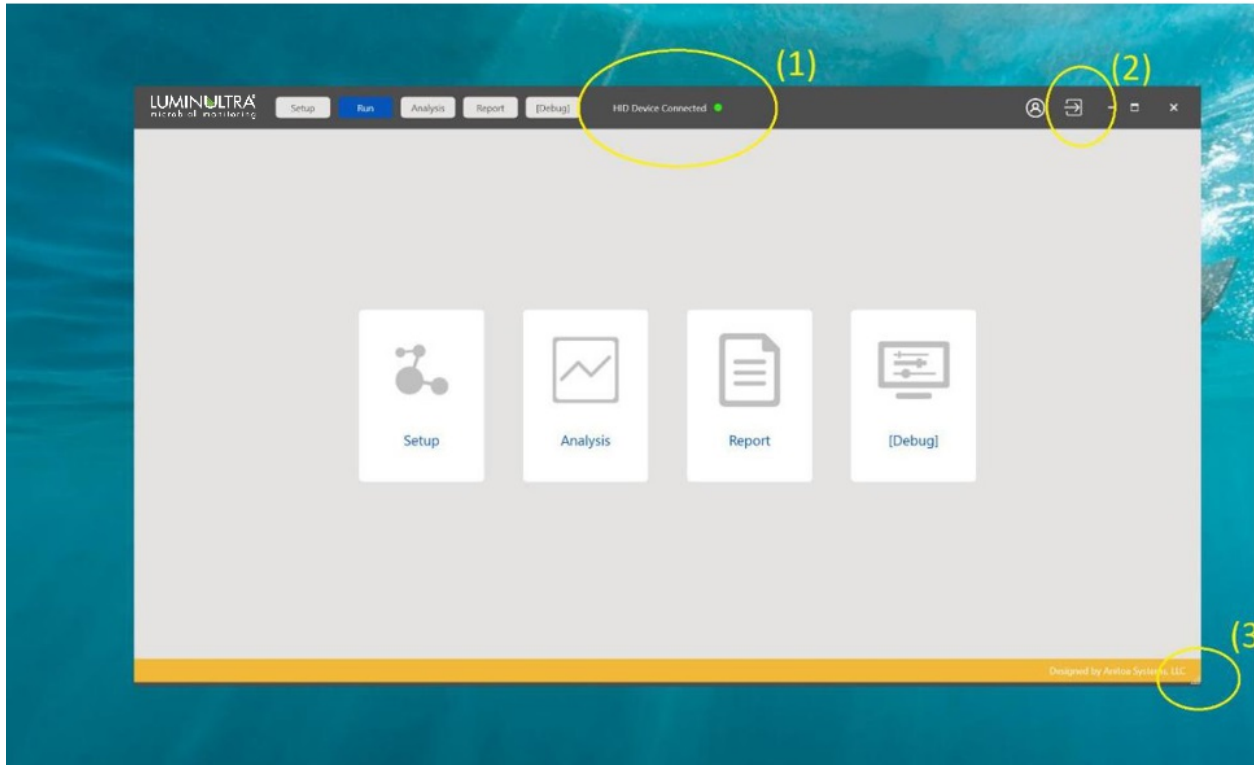


Figure 1 GeneCount software front page

Several notable features are marked in Figure 1. Marker (1) is an indicator of whether the GeneCount USB device is found. If found, a message “HID Device Connected” is shown along with a green dot. We call it HID, because the GeneCount instrument uses USB HID device class.

The home button

Marker (2) is the home button. At any time of running this software, click this button to return to this front page.

Resizing the window

Marker (3) is the place to drag to resize the GeneCount software windows.

The setup page – name setup

Click on the “Setup” button will take us to the setup page (Figure 2). Here we can set up the experiment. Here we can name the experiment and all the samples in the wells. We can also name and select the fluorescence channels for qPCR fluorescence reading.

A default date-coded experiment name is given on this page when we first enter. Feel free to change it to something you choose.

Samples and fluorescence channels set up

The software automatically detects the number of reaction wells the connected instrument can support. Each reaction well can be associated with a sample. In this page, the user can enter a name for each sample. If a sample well name field is left empty (white spaces), it is considered that the sample does not exist for that well.

It is important to select the fluorescence channels (Marker (2)) we need to use for the experiment. The selection of the channels depends on the type of fluorescence dyes used in the assay design. We can also give a name for each selected channel

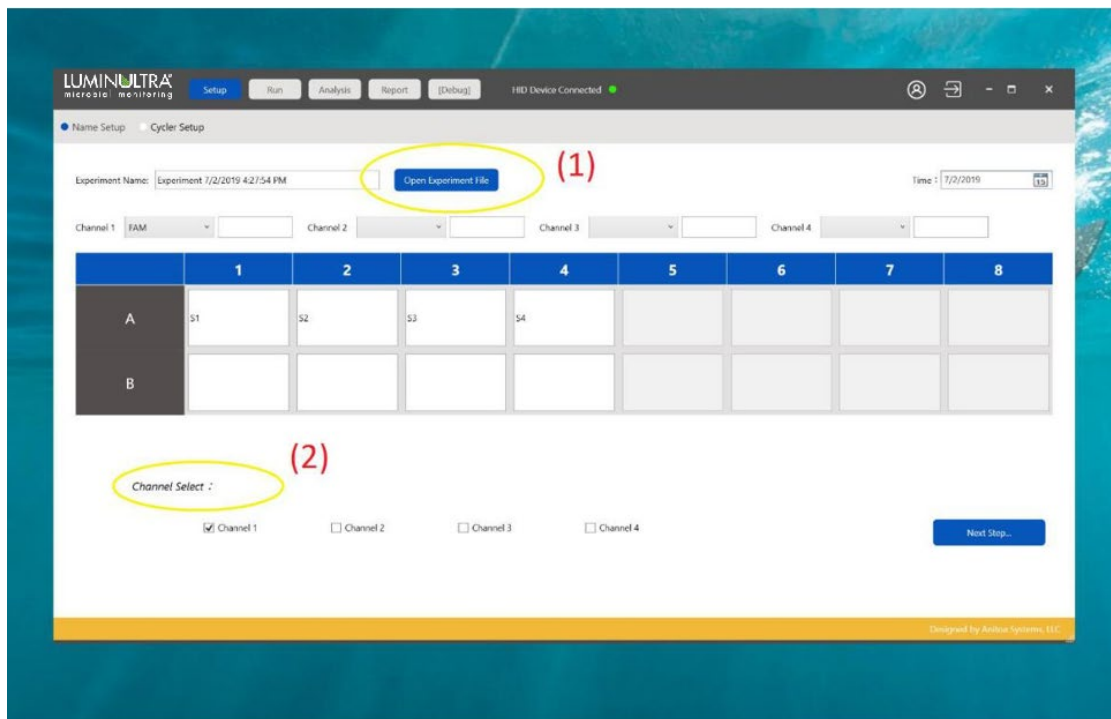


Figure 2 The setup page – name setup

Open existing experiment for template file

We can also load an existing experiment file or template file by clicking on the “Open experiment file” button (Marker (1)).

When this step is done, we can click on the “Next step” button to continue setting the thermal cycling program

The setup page – cycler setup

Here we can setup the thermal cycler program. We could choose the type and number of thermal periods and steps, assign time period and temperatures. We could have up to two Pre-Denature periods. The main thermal cycling period can have 2, 3, or 4 steps. We can also define at which step the fluorescence capture is done. Lastly, we have a hold period whose temperature and time period are configurable.

A cycler program can have several periods; and each period can have one or more steps. For example, we could have pre-denature period, cycle period and hold period. In the cycle period, we can have many repeated cycles consists of denature, annealing, and extension steps.

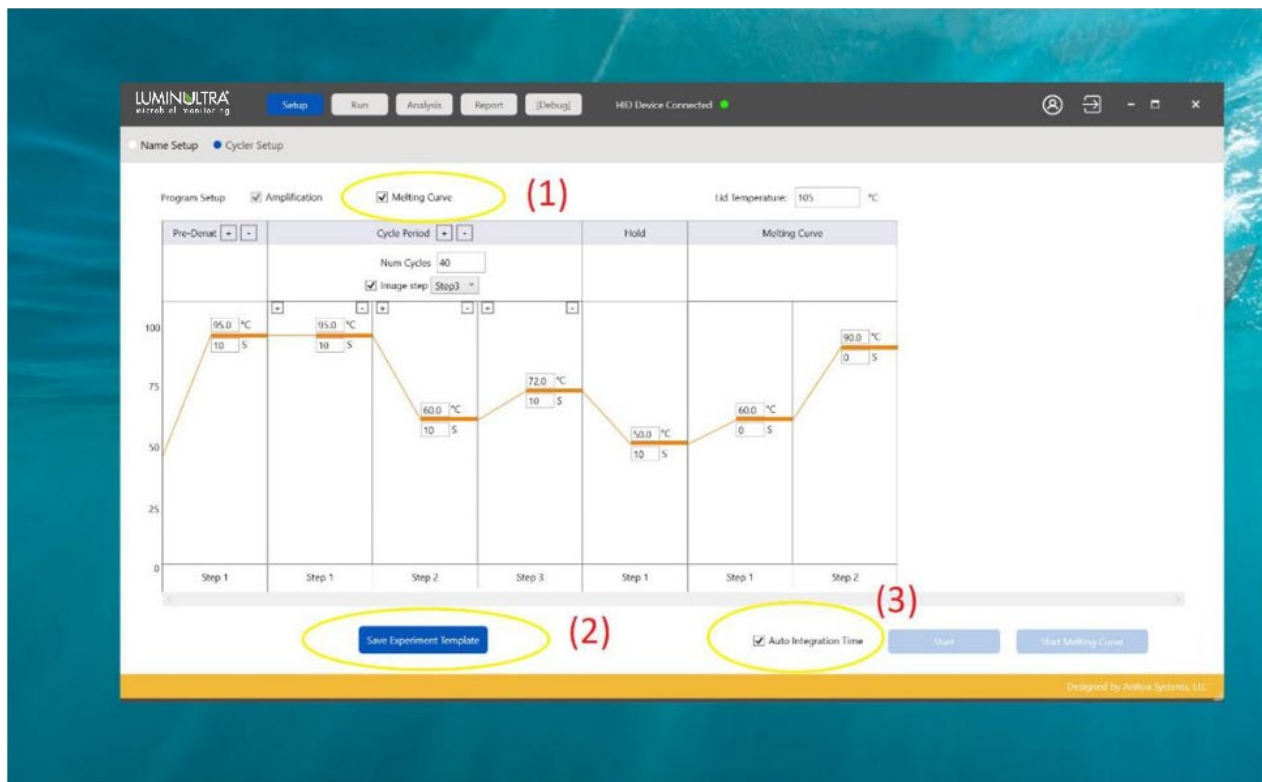


Figure 3 Cycler setup

qPCR amplification thermal cycling program starts when the user clicks on the “Start” button.

Melting curve analysis period

We can also add a Melt Curve (Marker (1)) analysis period at the end of the main thermal cycling program. The Melt curve analysis can be triggered either manually by clicking the start melt analysis button, or automatically by checking “” check box (not done yet).

Hot lid temperature and reaction volume

In this page, we can also set the desired hot lid temperature and reaction volume. The latter (reaction volume setting) is used for data analysis only. It will not affect how the experiment is run.

Saving template

At this step, we can also save the settings to a template file (Marker (2)). So next time when we perform a similar experiment, we can just load the template file instead of manually entering all the information again.

Auto Integration Time

This is an option to let the instrument automatically choose the optical sensor integration time (i.e. Exposure time of the fluorescence camera) according to the fluorescence strength of the assay (Marker (3)). The goal is to adjust the sensitivity of the fluorescence camera so that the signals from the chemistry fall into the appropriate dynamic range of the sensors and yield the best signal to noise ratio (signal quality). We recommend leaving this check box checked.

Running the experiment and monitor status

In the setup page – cycler setup, when we are satisfied with all the settings and loaded the samples, we can start running the experiment by click the “Start...” button.

Before the instrument starts to execute thermal cycling program, it will automatically check to ensure the lid is closed and the main power is applied. If either of these conditions is not met, the software will show popup windows with warnings. We could correct these programs and start again by simply press the “Start...” button again.

When the qPCR cycler starts to run, the software will automatically switch to Run pages. The first Run page will allow us to monitor hot lid and reaction well temperature. We can easily see if the temperature is going through the cycling programs or not.

Running amplification program

When running normal qPCR amplification program, the amplification window will real time monitor the fluorescence signal from the samples. In addition, the Run-Amplification Curve page will also show the current run status and estimated remaining run time

Running melt analysis

When running Melt analysis, the “Run-Melt curve” will show the fluorescence change rate as function of temperature.

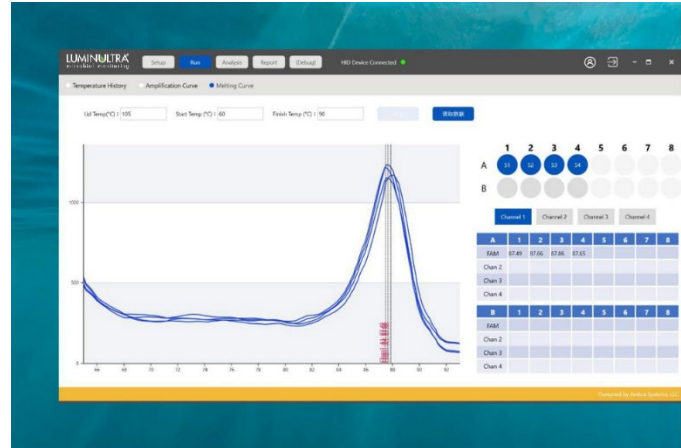


Figure 4. Melt analysis

Analysis

There are two analysis pages: Amplification analysis and Standard curve analysis. The latter is used for determining the quantity of the DNA targets using standard curve analysis method.

Amplification analysis

This will show the amplification curves for all samples. We can select the samples and study, set several parameters to this analysis.

Ct Threshold

The threshold for determining Ct is expressed as the percentage of the saturating level of the amplification levels after normalization. The default Ct threshold is 10%. The bigger this percentage, the more delayed the Ct values are.

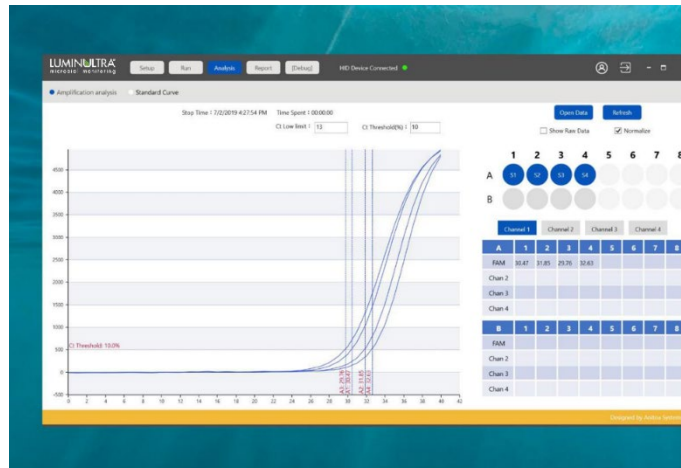


Figure 5. Amplification analysis

Ct Low limit

The minimum value of Ct is set through this parameter. This setting is helpful in determining the base fluorescence value for amplification curve analysis. In most qPCR experiments, the concentration of the analytes prior to amplification is low, such that the Ct value cannot be less than 13 or so.

Normalize

When displaying amplification curve, we usually normalize the fluorescence values. This will allow us to visualize Ct threshold value. This choice will not affect the result of the analysis.

Positive vs negative results

For each sample's DNA target, we get a positive vs negative result. Generally, the negative result will be shown as "Neg" and the positive result will be shown with a Ct value between Ct low limit and maximum cycle number. If we mouse over the Ct value in the data table, we will see some additional parameters that justifies the positive/negative test. These parameters include the saturation fluorescence level, amplification efficiency and fluorescence reading signal to noise ratio.

False positive results

Sometimes a negative result is the result of a "false positive" result. If we mouse over the result, we see that the "Confidence" level of positive is below a threshold, for example, 10%. The confidence threshold is adjusted through advanced setting window.

Further discussion of how to judge positive vs. negative results is given in Appendix B.

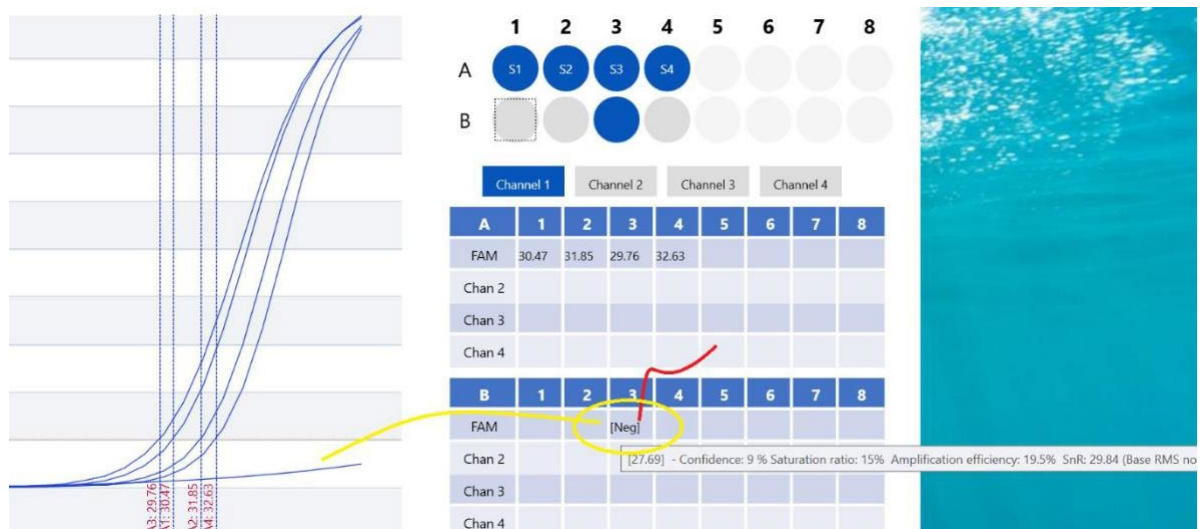


Figure 6. Positive vs Negative analysis

Standard curve analysis

Standard curve analysis is used to determine the absolute concentration of a sample by comparing to other samples with known amount of analyte concentration. We called the samples with known concentration “standards” and the ones with concentration that needs to be determined “unknowns”.

For such analysis, the “standards” and “unknowns” should be in the same fluorescence channel to ensure they are comparable. Thus standard curve analysis is always performed on one specific channel.³ We have to first the select the specific channel and perform such analysis.

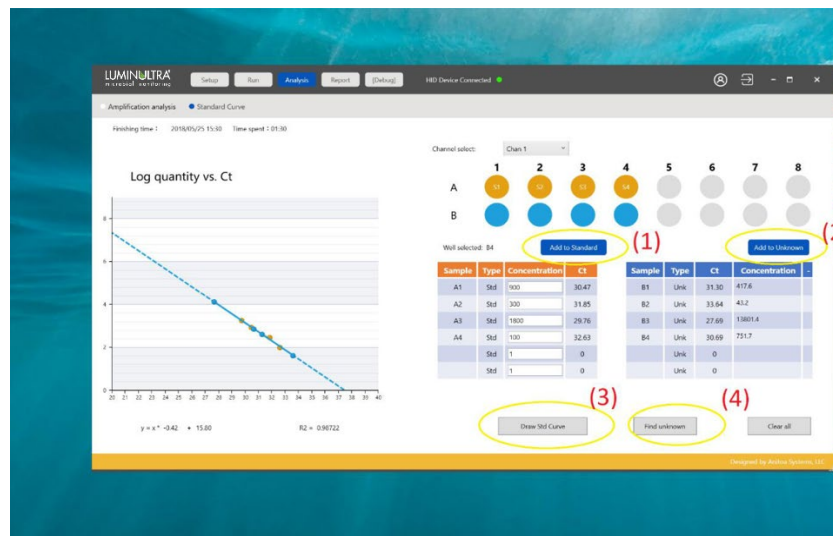


Figure 7. Standard curve analysis page

Steps to perform standard curve analysis:

Step 1: Select the fluorescence channel for such analysis

Step 2: Add standard samples – we can select the samples that are standard and click “Add the standard” button (Marker 1) to add to standard table. We can then assign the concentration numerical values to these standard samples by typing into the table.

Step 3: Draw the curve by clicking “Draw Std Curve” - Marker (2)

Step 4: Add unknown samples to unknown table - Marker (3)

Step 5: Calculate concentration for unknown samples - Marker (4)

³ We usually also make sure no other competing DNA targets and primer/probe sets are present in standard curve analysis samples, unless in very low concentration.

Save experiment & print report

Open report page to print report and save experiment.

The experiment file will contain all the settings and experiment results.

Two report formats will be used for report generation. The excel file report is hand editable. The PDF report is easier to store and use on different platforms.

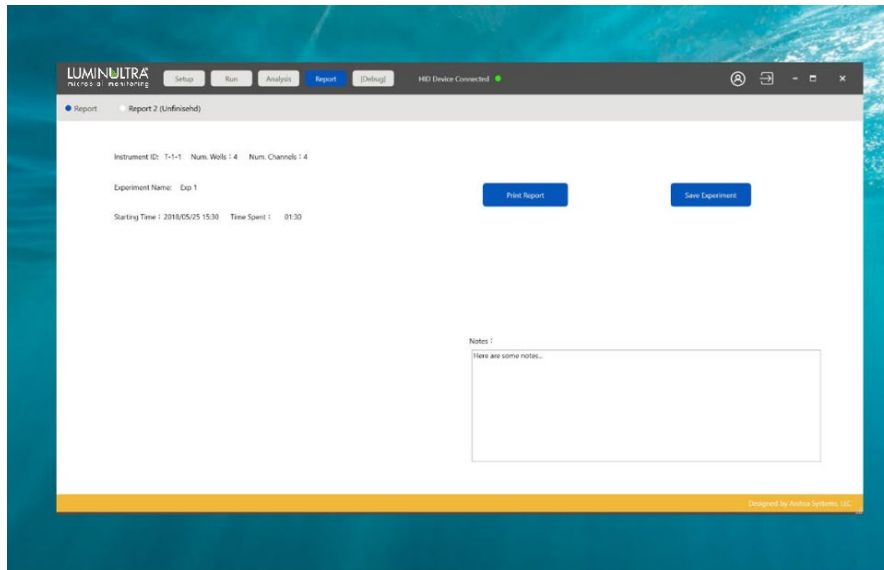


Figure 8. Save and export page

In this page, we can also enter the operator's name and some comments. These will be saved with the experiment file.