



TBG Q6000

Real-Time PCR System

QPCR960-X00000 / QPCR961-X00000

Operation Manual

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1. Safety Precautions

TBG Q6000 Real-Time PCR System is intended to be used in research laboratories by professional personnel.

This manual covers the use of Q6000 Software in conjunction with TBG Q6000 Real-Time PCR System. It has been designed to give you the information you need to:

- Install TBG Q6000 Real-Time PCR System
- Operate the software
- Adjust the software settings
- Analyze the data

Read the manual in its entirety before using the system. Keep the manual for future reference. The manual is an important part of the system and should be readily available.

For the latest information on products and services, visit our website at:

http:// <http://www.texasbiogene.com/>

In our efforts to produce useful and appropriate documentation, we would appreciate any comments you may have on this user manual for your local TBG representative.



High Temperature Label for Halogen Lamp: The halogen lamp can become very hot after operation. Please be aware of the heated lamp.



Warning Label: Please be aware of the danger of electric shock or other dangers.

2. Q6000 Real-Time PCR System

The Q6000 Real-Time PCR System is a gene quantification and genotyping platform in 96-well block formats. Using Q6000 Software, the system is applicable to absolute quantification, relative quantification, melt curve analysis and allelic discrimination.

2.1 Features

- 6-channel optics enable multiplex detection up to 6 targets per well.
- CCD base detection system for fast and uniform detection.
- Motorized sample plate transportation system for easy and convenient operation.
- Robust and modern outlook design.
- Simple and easy-to-use operation software.
- Complete analysis function including absolute quantification, relative quantification, allelic discrimination and melting curve analysis.

2.2 Product Overview

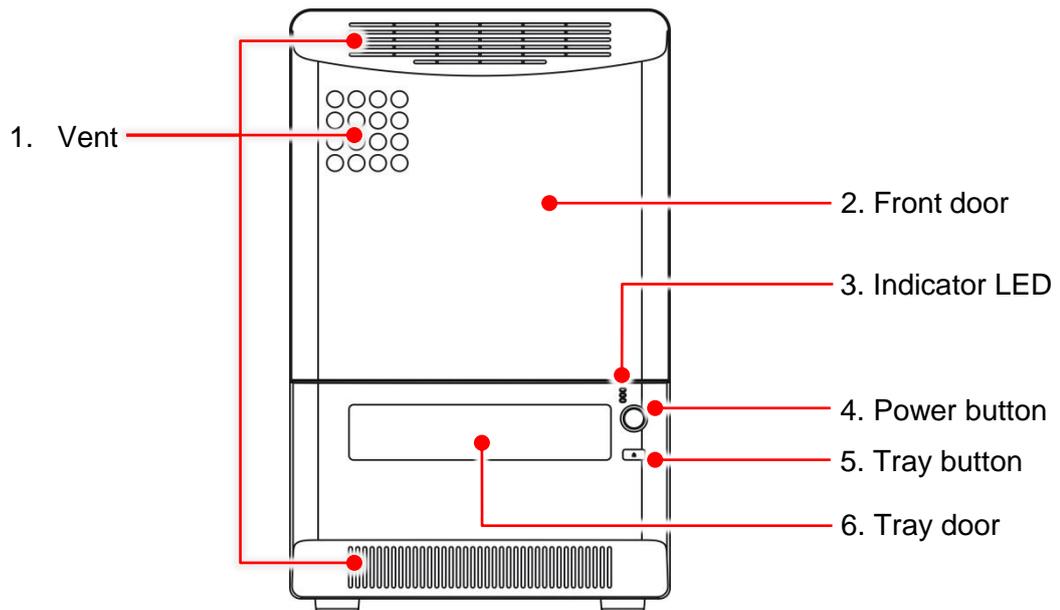


Figure 1. Front view

Table 1. Detailed description for front view

Item	Description
1. Vent	For air intake.
2. Front door	For Lamp replacement (service only)
3. Indicator LED	Indicates the status of the instrument: Yellow: Warm (flash during power on self-test) Green: Ready / In use (turn on after self-test / flash during running) Red: Error (flash when error occurred)
4. Power button	Power On/Off button.
5. Tray button	Tray in/out button
6. Tray door	Sample plate tray door

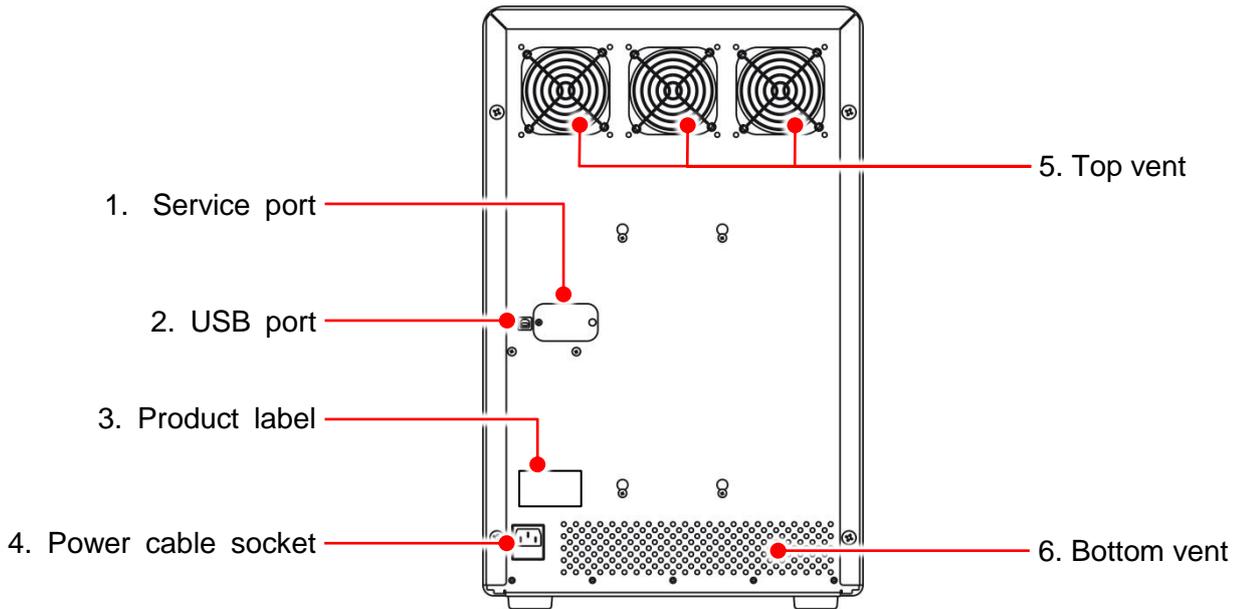


Figure 2. Rear view

Table 2. Detailed description for rear view

Item	Description
1. Service port	Reserved for service
2. USB Port	For PC connection
3. Product label	Indicates the model name, serial number, power specification, and other important information.
4. Power cable socket	Power cable socket and fuse compartment.
5. Top vent	For air output.
6. Bottom vent	For air intake.

3. Getting Started

3.1 Unpacking

Check that the delivery includes the following items:

- Operation manual (x1 pcs)
- Plate carrier (x1 pcs)
- Tungsten-halogen lamp, 75W/12V (spare part, x1 pcs)
- USB cable (x1 pcs)
- Power cord (x1 pcs)
- Q6000 application CD (x1 pcs)
- Fuse 5TT10, 10A/ 125V (spare part, x1 pcs)

Visually inspect the transport package, the instrument and the accessories for any possible damage. If any parts are missing or damaged, contact your local TBG representative.

3.2 Environmental Requirements

When setting up your Q6000 Real-Time PCR System, avoid locations with excessive dust, vibrations, strong magnetic fields, direct sunlight, draft, excessive moisture or large temperature fluctuations. Please refer to table 3 below for the clearance and weight tolerance required for Q6000 installation. Please ensure that there is also enough space for the control laptop PC.

Table 3. Clearance and weight tolerance

Description	Specification	Clearance and tolerance
Width	354mm	600mm
Depth	455mm	600mm
Height	567mm	800mm
Weight	35kg	40kg

The instrument will perform optimally and exhibit the highest reliability within the recommended environmental ranges (Table 3–2). Although the instrument is rated to operate reliably within the environmental ranges, avoid the extremes to best preserve the long-term performance and life span of the instrument.

Table 4. Operating and storage conditions

Environmental condition	Operation	Storage
Ambient temperature	+15~+30°C	-10~+60°C
Ambient relative humidity	below 85%	below 85%

3.3 Electric Power Requirements

110/230V, 50/60Hz, 1500W

3.4 Power On/Off

Press the power on/off button to power on the Q6000 system. The blue LED indicator on the power button will be turned on and all three indicator LEDs will flash once, then the Q6000 will start to perform the power on self-test. During the self-test, the yellow warm up LED indicator will flash continuously. It will take 30 sec. to finish the self-test. After the self-test, the Q6000 will beep twice, the yellow warm up LED indicator will be turned off and the green ready/in use LED indicator will then be turned on.

To power off the Q6000 system, press and hold the power on/off button for 3 sec.

3.5 Front Door (for Service Engineer Only)

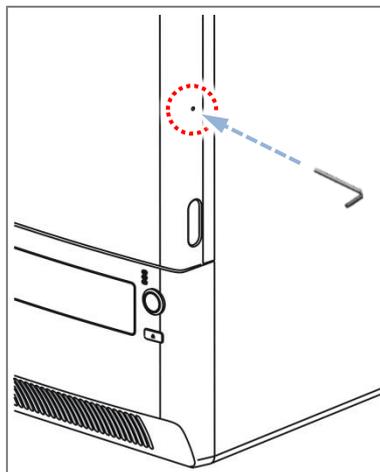


Figure 3. Front door unlock hole

To unlock and open the front door, insert a 2 mm Allen wrench, small screwdriver or other similar tool into the small hole on the right side of the front door and push the tool in until the latch releases and pops the door open.

To close the door, remove the tool and push the door closed until the latch locks the door.

3.6 Tray In/Out

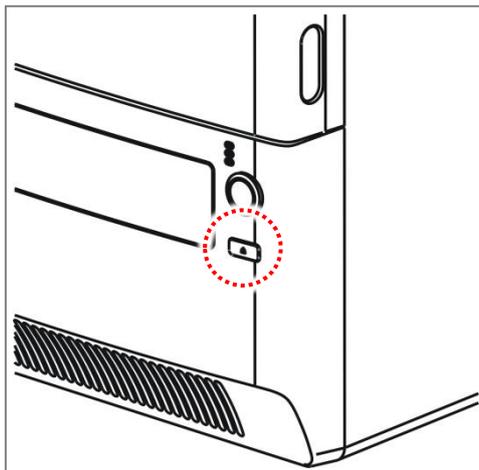


Figure 4. Tray in/out button

Press the tray in/out button to eject or retrieve the plate carrier tray. Please note that the tray can only be ejected or retrieved when the system is in ready or paused status. You can also use the Q6000 software to eject or retrieve the tray if the system is connected to the control lap-top PC.

3.7 Loading the Reaction Vessel

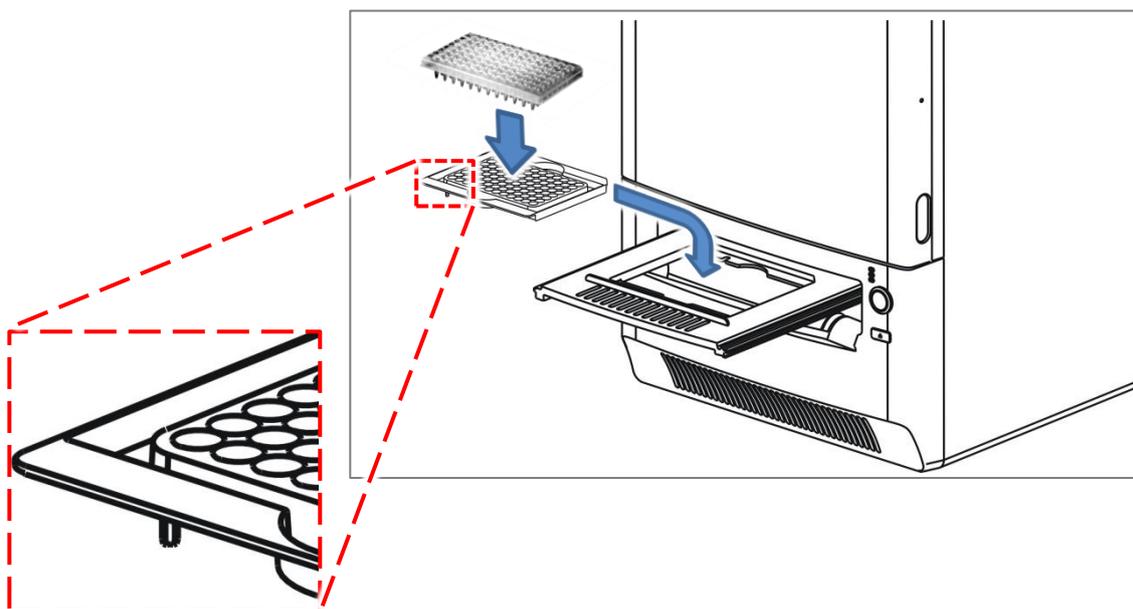


Figure 5. Alignment key of the plate carrier

The Q6000 system is compatible with the 200 μ l regular profile skirted/non-skirted PCR plate. The plate can be put on the plate carrier and then loaded to the plate carrier tray. Note that there are two alignment keys underneath the plate carrier, make sure that the keys are on the upper and lower left corner when loading.

4. User Interface

4.1 Starting Q6000 Software

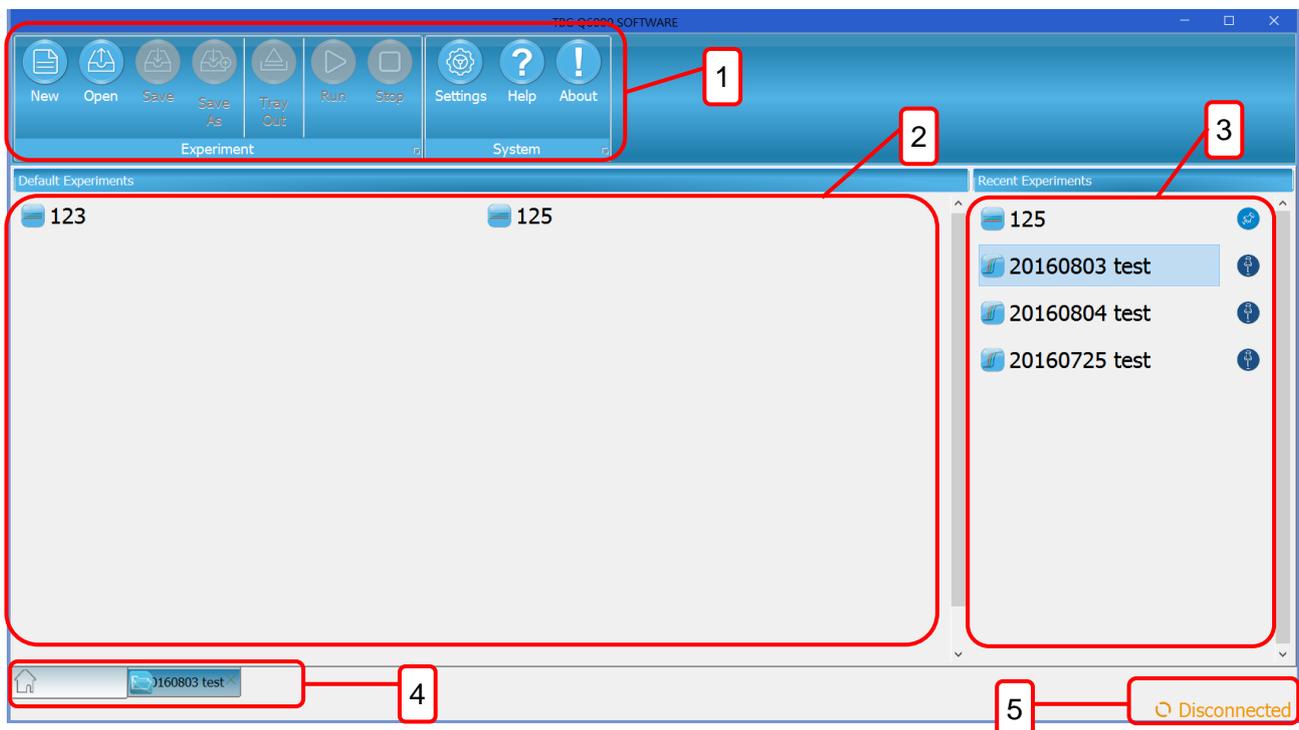
Launch **Q6000** Software from the Start menu:

Start > All Programs > TBG Q6000 Real-time PCR system > **Q6000 Software 1.0**, or by

double-clicking the desktop icon .

4.2 Navigating the software

Launching **Q6000** Software opens the application's main window with the **Home** view.



The main application window contains the following elements:

1. Action panel

View-specific function buttons.

2. Default folder

Display the experiments in the default folder. Double click to open an experiment.

3. Recent experiments

A user-definable list of recently used experiments. Double-click an experiment to load it and make it active. To keep an experiment on the list, click the pin icon next to the experiment.

4. Navigation bar

Tabs for selecting the view. Only the currently selectable tabs are shown.

5. Instrument status

Display the status of the connected Q6000 instrument.

When you launch **Q6000** Software, only the **Home** view is available. After you create a new experiment or re-open an existing experiment, the specific **Experiment** views are available. The window contents depend on the selected view. Buttons and controls are active only when they can be used.

4.3 View Selection

To view and active an opened experiment, select the desired experiment tab with the navigation bars at the bottom of the main window.

4.4 Home view

The **Home** view opens when you launch **Q6000** Software. In the **Home** view, you can create new experiments, open existing experiments, and save newly created and modified experiments.

When you open **Q6000** Software without active experiments, only the **Home** view is available with the Experiment and System action panels.



Table 3–2. Home view action buttons

Icon	Function	Description
	New	Create a new experiment.
	Open	Open an existing experiment.
	Save	Save the current experiment.
	Save as	Save the current experiment with a new name.
	Tray In/Out	Move the plate holder tray in/out.
	Run/Resume/Pause	Start, Resume or Pause the current experiment.
	Stop	Stop the the running experiment.
	Setting	Open Q6000 Software settings.

	Help	Open Q6000 Software help.
	About	Display Q6000 software information.

4.5 Instrument Status

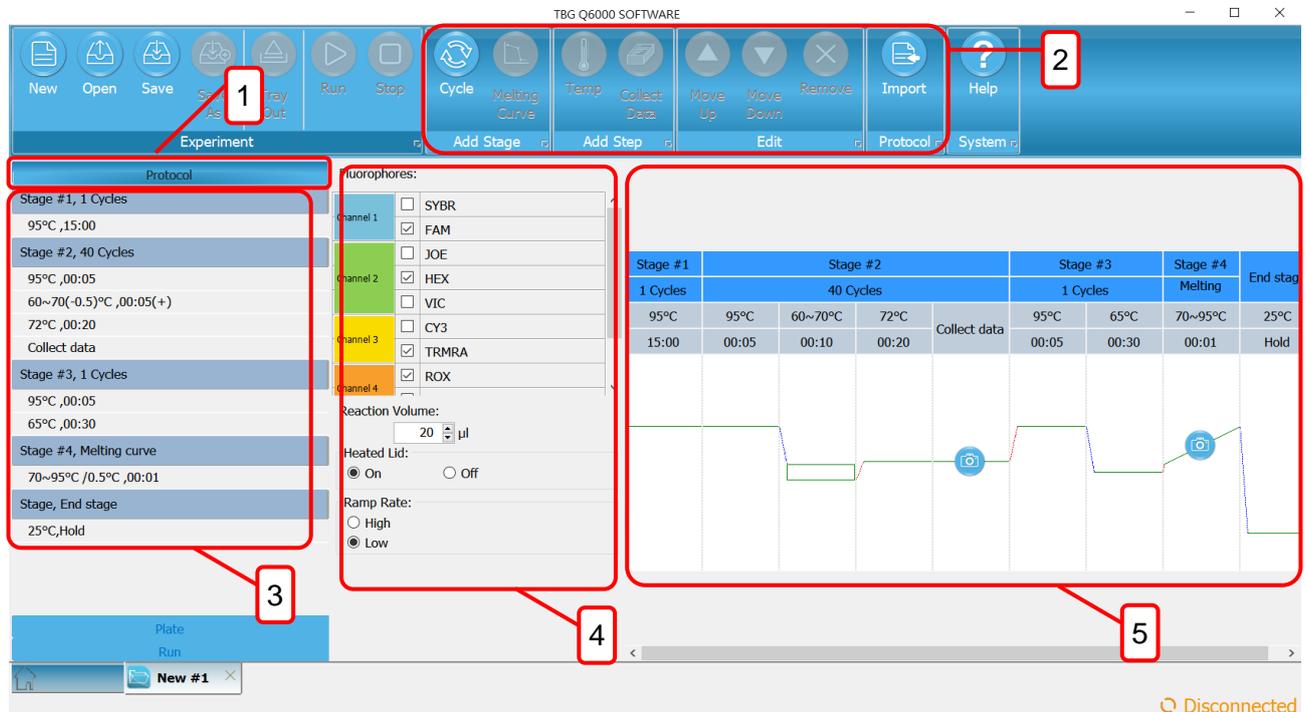
This indicates the current instrument status. See the following table for the detail.

Table 3–2. Instrument status

Icon	Status	Description
	Connected	The Q6000 instrument is connected and ready
	Disconnected	The Q6000 instrument is not connected
	Running	The experiment is running
	Paused	The running experiment is paused
	Tray in	The tray is retracting
	Tray out	The tray is ejecting
	Error: XXX	Error(s) detected

5. Protocols

A protocol is a sequence of thermal cycling and data collection instructions given to a connected instrument. In the **Protocol** view, you can create and edit the protocol of the current experiment.



The Protocol view contains the following main fields:

1. **Protocol view**
Display protocol editing view
2. **Action panel**
Action buttons for adding, removing and reordering steps, importing protocols, and running the experiment.
3. **Protocol steps**
Step sequence tree for viewing and selecting steps for editing. Click a step to view and edit its properties.
4. **Step properties**
Properties of the selected protocol step.
5. **Protocol graph**
Protocol temperature graph with the run-time estimate.

5.1 Creating a New Protocol

You can create a new protocol by editing the default protocol template or the protocol in an existing experiment. To open an existing protocol for editing, import a protocol by clicking the **Import** button in the **Protocol** view.



Edit the protocol by first selecting a protocol step and then using the buttons in the **Stages**, **Steps** and **Edit** action panels.

Table 4–4. Protocol editing commands

Icon	Function	Description
	Cycle	Add a cycle stage, which can include temperature and data collection steps. Select the cycle stage and click the Temp or Collect Data button to add them to the cycle.
	Melting Curve	Add a melting curve stage, for which you can set the start and end temperatures, as well as advanced properties.
	Temperature	Add a temperature step, for which you can define the temperature and time, as well as set an infinite hold and define advanced properties.
	Collect Data	Add data collection step to a stage.
	Move Up/Down	Move the selected stage/step upwards or downwards within the protocol.
	Remove	Remove the selected stage/step from the protocol.

Click the **Cycle** or **Melt Curve** button to add the corresponding stage to the protocol. The new stage is added below the selected stage.

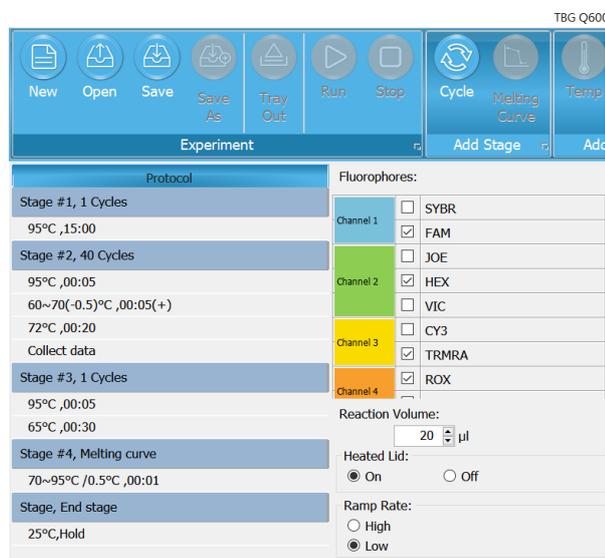
Click the **Temp**, **Collect Data** button to add the temperature step or data collection to the stage. The new step is added below the selected step in the stage. If you select the stage header, the new step is added as the first step. The melting curve step contains data collection by default.

The protocol is executed as a sequence from top to bottom, that is, the first step is at the top and the last is at the bottom. You can change the execution order of the stages/steps in a protocol by selecting a stage/step and clicking the **Move Up** or **Move Down** buttons.

Delete stages/steps from the protocol by highlighting them and clicking the **Remove** button.

5.2 Protocol properties

Select the Protocol header in the protocol sequence to view the Protocol Properties and define the Fluorophores and Reaction Volume used.



5.2.1 Fluorophores

All fluorophores are selected by default in a new experiment. Click a fluorophore to disable or enable it. You can define the colors used to designate the fluorophores in Settings > Fluorophores.

We recommend that you select only the fluorophores that are required for the experiment.

Note:

Only the selected fluorophores and corresponding channels are measured and available in the layout. Unmeasured channels cannot be added after an experiment has been run.

5.2.2 Reaction volume

Enter the reaction volume. The range is 10–100 µl.

5.2.3 Heated Lid

Select On to enable or Off to disable the lid heater.

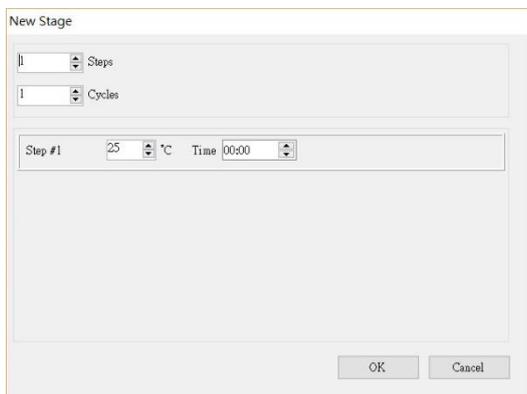
5.2.4 Ramp Rate

Set the default block heating/cooling rate for the protocol. Individual ramp rate settings override this default setting. See “Temperature Step” on page 25.

5.3 Cycle stage

Add a cycle stage to a protocol by clicking the Cycle button. The new stage window pops up. Select the numbers of temperature steps you want to include in this new stage and the cycle

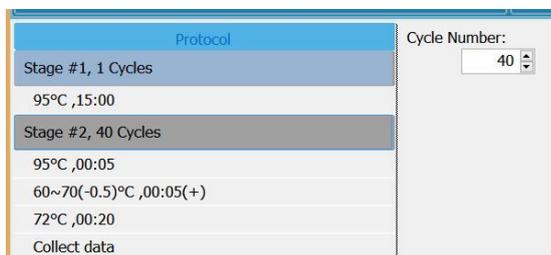
numbers for the stage. Set the temperature and the time for each step and click OK. The new stage is added below the currently selected stage. If the protocol header is selected, the new stage is added as the first stage in the protocol.



A cycle stage can contain one or several temperature steps with or without data acquisition.

Extra temperature or collect data steps can be added to a cycle stage afterwards. You can also re-arrange the order of the stage by selecting it and clicking the move up or move down icon. When you move a cycle stage, all of its contents move with it.

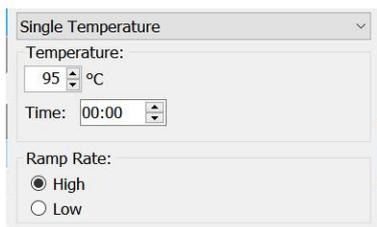
Use the Cycle Number to set the number of cycles in the selected step.



5.3.1 Temperature step

Add a temperature step to a stage by clicking the Temperature button. The new temperature step is added below the currently selected step. If the stage header is selected, the new step is added as the first step in the stage.

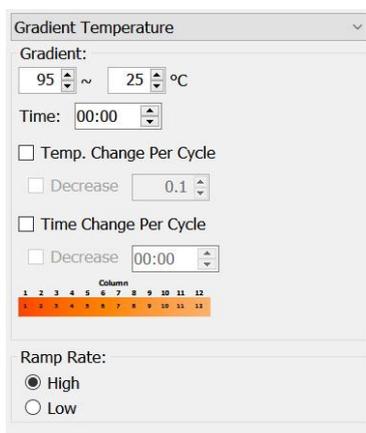
After adding a temperature step,



1. Define the target temperature. The temperature range is 25.0–99.9°C.
2. Set the step duration using the Time control. The duration range is 00:00–59:59 (mm:ss)

3. you can also specify the ramp rate for the specific step.

In addition to the basic settings, you can configure the following temperature step properties by selecting the Gradient Temperature.



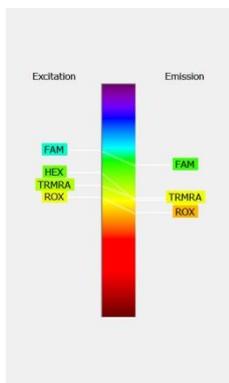
Gradient allow you to set the gradient temperature range for the step. An estimated temperature range for each column is displayed.

Temperature Change Per Cycle allows you to define a fixed temperature increment per cycle until a temperature range limit is reached for a temperature step within a cycle stage. If you want the step temperature to decrease per cycle, check the Decrement box.

Time Change Per Cycle allows you to define a fixed time increment per cycle until a time range limit is reached for a temperature step within a cycle stage. To decrease time, check the Decrement box.

5.3.2 Data collection step

Data collection step can be added after an individual or cycled temperature step. A melting curve step contains data collection by default.



Select the Data collection step to view its properties, which present the channel and fluorophore mapping and the channels that have been selected for measurement.

5.3.3 Channel wavelengths

The detection channel wavelength ranges of the Q6000 system are presented below.

Table 4–5. Detection channel wavelength ranges

Channel	Excitation wavelengths	Emission wavelengths	Fluorophore
1	450-490nm	505-535nm	FAM, SYBR Green
2	510-530nm	545-570nm	HEX, VIC
3	540-560nm	575-600nm	TAMRA
4	565-595nm	610-645nm	ROX
5	625-645nm	660-685nm	Cy5
6	665-695nm	705-735nm	Cy5.5

5.4 Melt Curve stage

A melting curve stage is included in any new protocol by default. If you don't want to include the melting curve stage, you can delete it. Add a melt curve stage to a protocol by clicking the Melt Curve button. The new melt curve stage is added under the currently selected stage.

Note:

There can be only one melt curve stage in a protocol.

Start Temperature: °C

Stop Temperature: °C

ΔT: °C

Hold Time:

Use the Start Temperature and End Temperature controls to set the beginning and end temperatures of the melt.

Hold Time defines the duration of one step, and can be set to between **00:00 and 59:59** (mm:ss).

ΔT defines the increase in temperature between steps, and can be set to between **0.1–5.00°C**.

Note:

The minimum actual hold time depends on the number of channels measured and may be as high as 13 seconds even when the Hold Time setting is set to 00:00.

5.5 Importing protocols

You can import protocols from the existing experiments. This allows you to handle protocols separately from experiments.

To import a protocol file, click the Import button in the Protocol action panel of the Protocol view. Browse to the file and click Open.

6. Plate Layout

Plate layout refers to the location of samples in the plate wells. The layout contains, for each plate well, information on the fluorophore, target, sample type, and quantity of a standard as well as descriptive information.

You can create and edit the layout in the Plate Layout view.



The **Plate Layout** view contains the following fields:

1. **Plate view**
Display the plate layout setting view
2. **Action panel**
Action buttons for entering well content and sample properties using the *Pipette* and *Select* modes, creating replicates and sample groups and importing and exporting layout files
3. **Editing panel**
This panel is used to select well contents and replicate data
4. **Property panel**
This panel is used to edit sample properties and fill quantity series
5. **Plate layout**
Graphical representation of the contents of each well

The layout can be empty when running a protocol. An edited layout can also be modified during or after protocol execution.

6.1 Creating a layout

You can create a new plate layout from a blank template or by editing an existing layout. To edit an existing plate layout, import the layout from a protocol file using the **Import** button in the *Plate Layout* action panel.

To create a plate layout:

1. Select the appropriate fluorophores from the *Fluorophore* list in the *Editing panel* by clicking them.

Note:

The *Fluorophore* list contains only those fluorophores that have been selected in *Protocol Properties* in the **Protocol** view.

Remove fluorophores by un-check them from the *Fluorophore* list.

2. Define the sample type for each fluorophore by selecting the appropriate sample type in the *Sample Type* drop down list. See “Sample type” on page 32.
3. If required, define the replicate layout by entering the number of columns and rows.
4. Select well or wells on the graphical plate layout. The selected wells are filled. You can select a row or a column by clicking the appropriate heading and the entire plate by clicking the button in the top left-hand corner of the plate. If you have entered a grid pattern into the *Replicates* panel, mimic that pattern when painting the target wells. You can generate several replicate sets by painting over multiples of the replicate grid area. Once you have filled the desired wells, click the Assign to Wells icon in the Plate Layout action panel.
5. Select the appropriate wells in the layout and enter their properties in the *Property panel*.

You can enter the sample name and choose the target. With standards, you can enter the quantity and with AD standards, you can enter the standard value. If necessary, add targets in the *Target gene* field by selecting New in the target gene name drop down list, entering the designation and press enter. Within one well you can associate one target per fluorophore. Remove a target by selecting it in the target gene name drop down list and clicking the **X** icon.

6. Add any relevant descriptive sample information in the *Description* field. Repeat these steps to enter the layout data.

6.2 Sample type

The sample types are designated by a color code and an abbreviation in a circle. The following sample types are available:

Table 5–6. Sample types

Icon	Sample type	Description
	Unknown	Unknown sample.
	Standard	Sample with known concentrations.
	NTC	No Template Control. A control sample.
	Positive	Positive amplification control.
	Negative	Negative target control.
	Reference	Endogenous reference gene or housekeeping gene.
	AD	Allelic discrimination standard

The sample type icon is displayed on top of the fluorophore bar in the layout wells.

If you selected *AD Standard* as the sample type, enter the *AD result* in the *Property panel*.

If you selected *Standard* as the sample type, enter the standard value in the *Property panel*. You can create a standard series by checking the Dilution Series box.

6.3 Dilution series

Select the filling direction, the initial quantity, the mathematical operator for creating the series and the factor, then click on the SUBMIT icon

6.4 Grouping samples

You can combine several wells into sample groups if necessary. Each sample group has individual analysis options. Individual sample groups are added to the *Analysis tree* as separate items.

The created sample groups are analyzed independently of each other even when sample groups overlap.

To group samples:

1. Select the appropriate wells in the plate layout.
2. Click the **Assign To Group** button in the *Group* action panel and select the group name from the list, or select **New...** and enter the group name. Click **Ok** after you have entered the group names, then select the new group.

You can remove wells from a group by selecting the wells and clicking the **Remove from Group** button.

You can edit sample group names and delete sample groups in the **Assign To Group** drop down list.

6.5 Importing and exporting layouts

You can import and export plate layouts as separate files with the .csv file extension. This allows you to handle layouts separately from experiments. **Note that you cannot import layouts directly from an experiment.**

To import a plate layout, click the **Import** button in the *Plate Layout* action panel of the **Plate Layout** view. Browse to the file and click **Open**.

To export a plate layout, click the **Export** button in the *Plate Layout* action panel of the **Plate Layout** view. Browse to the desired file location, name the file and click **Save**.

7. Executing an Experiment

An experiment can be executed as long as you have a defined protocol with the appropriate steps. A protocol can be run with an empty plate layout.

The following procedure assumes that you have a Q6000 instrument already powered up and connected.

To execute an experiment:

1. Open the Q6000 tray and insert the reaction vessels.
2. Close the tray and click the **Run** button in the **Home** or **Protocol** view to initialize protocol execution.
3. If you have not saved the experiment, enter a name for the experiment file to be created and click **Save**.

When protocol execution is started, the **Run** view opens.

7.1 Executing an existing experiment

You can rerun a previously generated experiment file, for example, with a different set of samples layout.

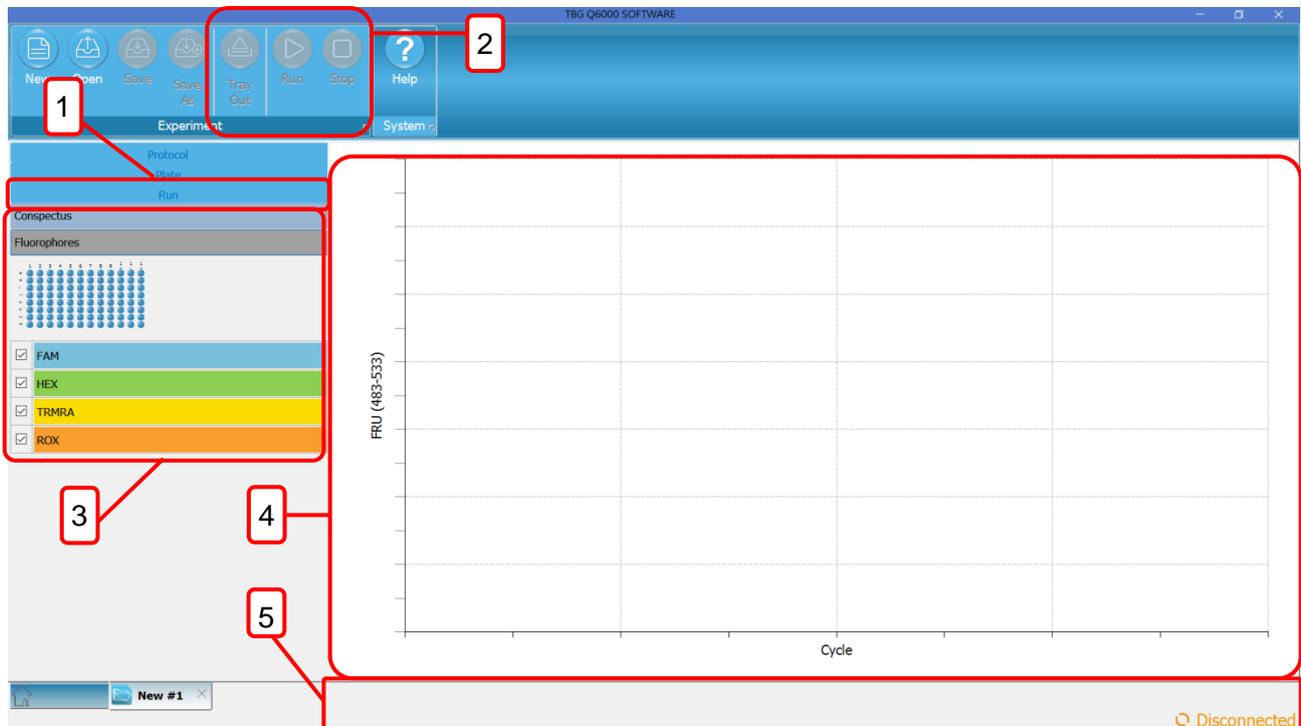
To rerun a previously generated experiment file, create a new experiment and import the protocol.

You can create a new plate layout or import a plate layout from an existing experiment file.

After editing, you can execute the experiment.

8. Monitoring a Run

The execution of an experiment can be monitored in the **Run** view. This view is active only when an experiment is being executed.



The **Current Status** view contains the following main fields:

- 1. Run view**
Display the Running status view.
- 2. Action panel**
Action buttons for controlling the instrument drawer, pausing resuming and stopping the experiment.
- 3. Display option**
Allow you to select the information you want to monitor. You can toggle between the current protocol step or the sample's fluorescence intensity. You can also select to monitor specific wells or fluorescence channels.
- 4. Display area**
The selected information will be displayed here. They will be updated per cycle.
- 5. Status**
Shows the remaining run time, current cycle number, and a visual and numerical indication of the instrument temperature.

8.1 Adding reagents during a run

You can add reagents to a protocol under execution. The Q6000 instrument will maintain the block temperature specified in the current step when the protocol is paused for adding reagents.

To add reagents:

1. Click the **Pause** button on the action panel to temporarily halt protocol execution
2. Open the instrument tray and add the reagent
3. Close the instrument tray
4. Click the **Resume** button on the action panel to resume protocol execution.

9. Analysis

After the execution of the experiment and the data collected, the **Run** view will be replaced and the **Analysis** view is available. The **Analysis** view is used to perform various analyses on the fluorescence data.



The **Analysis** view contains the following main fields:

- 1. Analysis view**
Display the Data analysis view.
- 2. Action panel**
Analysis view action buttons for adding and removing analyses and exporting data.
- 3. Analysis tree**
A hierarchical description of the selected analyses.
- 4. Well selector**
Well selector for selecting individual wells, well groups or the entire plate for result viewing.
The well selector does not affect the analyses.
- 5. Analysis data**
The data and settings of the selected analysis.

9.1 Run log

When first opened, the **Analysis** view shows the run log for the experiment. This log view presents **instrument details and any errors that have occurred during protocol execution**.

You can access this view by selecting the *Analysis* item in the *Analysis tree*.

9.2 Adding analyses

Depending on the protocol, you can add some or all of the following analyses:

- Absolute quantification
- Relative quantification
- Allelic discrimination
- Melting curve analysis

The analysis tree contains one *RFU-Cycling* item for each data collection step in the protocol and a *Melt Curve Data* item, if the protocol includes a melting step. The *RFU-Cycling* items are numbered consecutively.

By default, each *RFU-Cycling* item includes a *Ct* (cycle of threshold) item and a *RFU-Melting* item includes a *Melting Curve* item. New analyses are added below a *Ct* in each data collection step.

To add a new analysis:

1. Select the appropriate *Ct* item in the analysis tree.
2. Click the desired analysis button in the action panel. Only the currently selectable analyses are available. Select the added analysis item to view its data and settings.

Note:

Some analysis items share settings, for example, absolute quantification shares all of its settings with its parent *Ct* item.

9.3 Analyzing sample groups

If you wish to limit an analysis to a group of samples, you need to create a new *Ct* or *Melt Curve Data* item with a sample group under which you can add the desired analyses. Sample groups are defined in the **Plate Layout** view, see “Grouping samples” on page 33.

9.4 Exporting analyses

Q6000 Software allows you to export analysis data either as a.csv.

Note:

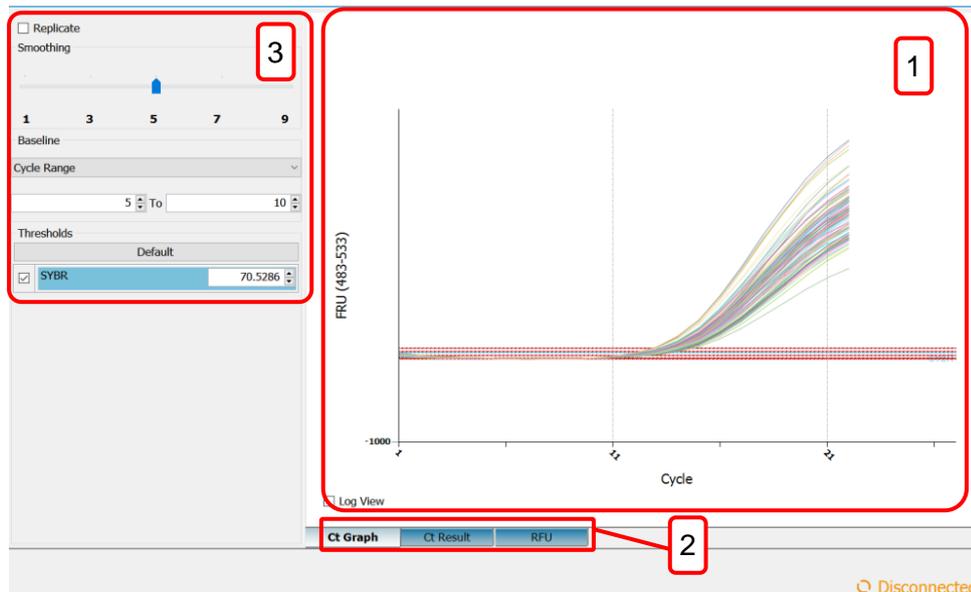
The exported data is from the sample group currently selected in the *Analysis tree*.

To export data t, click the **Export** button in the *Results* action panel of the **Analysis** view. Browse to the desired file location, name the file and click the **Save** button.

10. Ct and Absolute Quantification

The **Cycle of Threshold (Ct)** and **Absolute Quantification** analysis views present the measured fluorescence values, analysis settings, as well as the RFU (Relative Fluorescence Unit) values and results as a table.

In addition, the **Absolute Quantification** analysis view contains the *Standard Curve Graph* (shown below).



The Ct analysis includes:

1. Display area

Display the selected data.

2. Results tab

Select to display the fluorescence graphs of the samples for the selected fluorophores, the Ct results or RFU values for the selected fluorophores as a table.

3. Settings

- For selecting the fluorophores for which the data is shown in the *Amplification Graph* and result fields.
- For viewing results for replicates or all wells.
- For applying curve smoothing.
- For selecting the baselining method.
- For selecting the Ct method and automatic or manual threshold settings.

You can use the *Well Selector* to select the well data to be shown. The *Settings* field provides options for presenting data for replicates or all wells.

10.1 Adding an absolute quantification analysis

To add an absolute quantification analysis:

1. In the **Analysis** view, select the *Ct* item for which the analysis is to be performed. The *Ct* item can encompass all wells or be the *Ct* of a distinct sample group.
2. Click the **Absolute** button in the *Quantification* action panel.

The absolute quantification analysis is presented as an *Absolute Quantification* item in the *Analysis tree*. Select the analysis item to view its data and settings.

10.2 Graphs

The *Ct Graph* presents the fluorescence graphs of the selected samples (for the selected fluorophores). The RFU intensity is presented on the Y axis and the cycles are presented on the X axis. Tick the **Log View** box below the RFU axis to use a logarithmic scale in the *Ct Graph*. This setting can be toggled on or off.

The *Standard Curve Graph* plots the standard sample quantities against the determined *Ct* values for the samples specified as standards in the plate layout. The logarithmic quantities are presented on the Y axis and the *Ct* values on the X axis.

The Standard Curve Graph also shows the standard curve equation, its R2 value and the calculated reaction efficiency.

Note:

When using multiple channels (multiplexing), you can have a separate standard curve for each fluorophore.

10.3 Settings

You can control the settings for *Ct* determination in the *Settings* field.

Tick **Replicate** to view the results based on replicates.

Adjust the *Smoothing* slider to remove unwanted signal noise from the plotted graphs. Move the slider to the right to increase the smoothing effect. Move the slider completely to the left to disable smoothing.

10.4 Baseline

Baselining is required to compare amplification curves with different signal levels and to determine suitable threshold values. Baselining is the basis for determining *Ct*.

The **Baseline** setting has the following method options:

- **None**
No baseline is set for the curves.
This option can be used to look at the raw data and, for example, to find and exclude abnormally behaving samples, as well as to compare reagent, primer and probe batches.

- **Cycle Range**

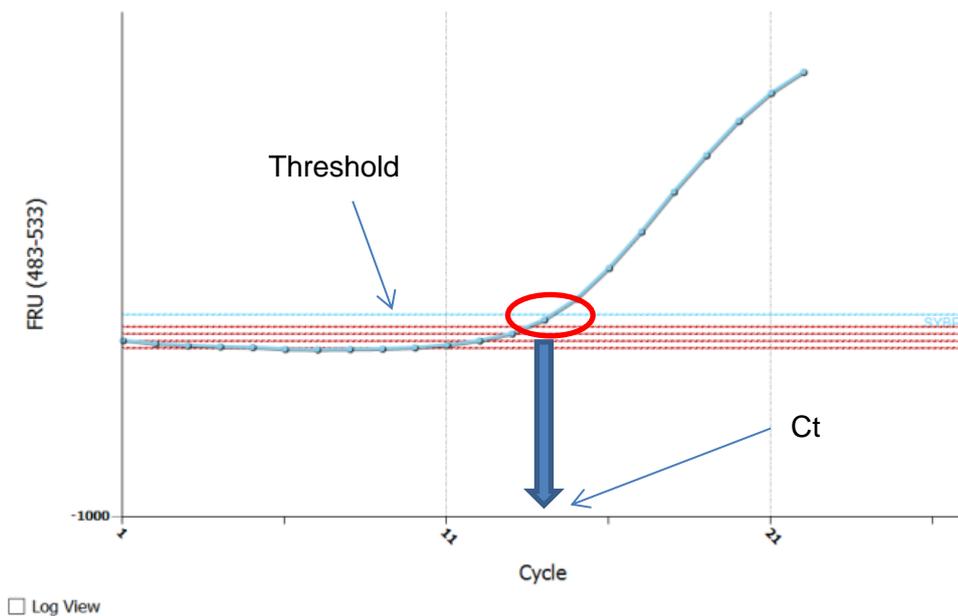
The baseline of each curve is calculated based on a trend prediction algorithm.

The cycles used for baseline trend prediction are set manually. The baseline (and whole curve) is shifted to a horizontal line and the average of the baseline value is set to zero.

10.5 Ct determination

Q6000 Software uses *Threshold* method for calculating the cycle of quantification. The method is discussed in detail below.

In the *Threshold* method for calculating the Cycle of Threshold, a threshold value is set for the fluorescence values.



The threshold is presented as a line in the graph and the intercept point of the amplification graph and the threshold line determines the Ct value. The threshold lines of different channels are presented in different colors. The threshold value can be set automatically or manually.

Clicking the **Default** button uses an algorithm to calculate the default threshold value based on the baseline and signal noise.

You can also adjust the threshold manually by clicking and dragging the threshold line or by entering a numeric value in the threshold field of a fluorophore.

Note:

Whenever you change the baseline method, click the **Default** button to recalculate the default threshold values.

10.6 Importing a standard curve

A standard curve that is created in one experiment can be used in other experiments by using the *Import* function. This feature can be used, for example, in experiments with same reaction setup, reagent batches and protocol, and in which the results are analyzed using identical analysis settings.

To import a standard curve from one experiment (source file) to another experiment (target file):

1. Open both source and target experiment files in Home view.
2. Activate the target file in the Active Experiments list.
3. In the **Analysis** view, click the **Import** button and select the source file.
4. A standard curve graph with text: "Imported from file..." appears on the target experiment's *Standard Curve Graph*.

Note:

In order to verify the amplification efficiency in the target file experiment to match the amplification efficiency of the imported standard curve, a minimum of three standard samples should be included in the target file experiment. Use same analysis settings as in the source file experiment and check for the similarity of the Ct values.

10.7 Results

The *Ct Result* tab shows the results of the data analysis:

- The Well, Fluorophore, Sample Type, Sample Name, Target and *Description* columns show the selected well positions and the sample data entered in the layout.
- The *Ct* column shows the determined Cycle of Threshold.

The *Result* tab for the *Absolute Quantifications* item shows the results of the absolute quantification analysis:

- The *Quantity* column shows the specified quantities of the standards and the calculated quantities for the other samples. Based on the standard curve, the system automatically calculates the quantity for each sample not specified as a standard..

Note:

The standard curve is created separately for each fluorophore and optionally for each sample group.

- The *Omitted* column shows the standard samples removed from the Standard Curve Graph.

The *RFU* tab presents the raw fluorescence data measured for each fluorophore. The data also indicates the cycle number and the temperature for each data collection step.

You can export the data from the *Results* or *RFU* tab for use in other software tools by clicking the **Export** icon in the action panel.

11. Relative Quantification

Relative Quantification is based on determining the ratio between the expression levels of a target molecule and one or more reference molecules in a sample, and to further compare these ratios between the sample and the calibrator. The results of the relative quantification analysis are expressed as relative quantity between the samples.

In Q6000 Software, the ratios are calculated using the $\Delta\Delta Ct$ method, which uses the Ct values.

First, the following is calculated for each sample:

$$Ct_{\text{target}} - Ct_{\text{ref}} = \Delta Ct$$

where *target* is the target molecule and *ref* is the reference molecule.

The subtraction of the Ct_{ref} values normalizes for the variation in the sample material.

If multiple reference genes are used, Ct_{ref} is calculated as a geometrical average of all reference genes: $[Ct_{\text{ref}} = Ct_{\text{ref}1} \times Ct_{\text{ref}2} \times Ct_{\text{ref}3} \times \dots \times n)^{1/n}]$, where n = number of reference genes.

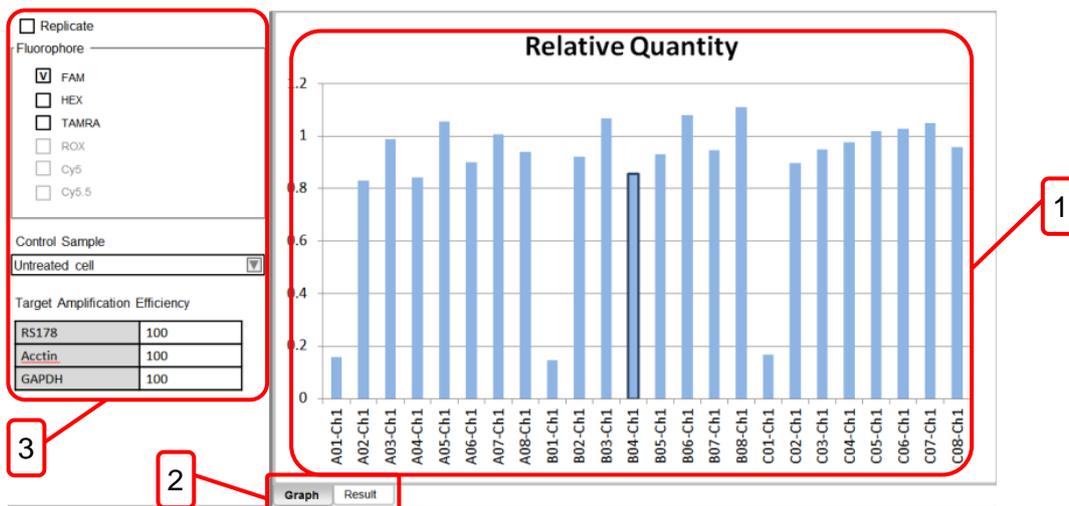
Second, one sample is designated as the calibrator sample. This is usually the sample with the lowest expression (highest Ct) or a control sample.

The ΔCt for the calibrator is then subtracted from the ΔCt of each of the other samples using equation:

$$\Delta Ct_{\text{sample}} - \Delta Ct_{\text{calibrator}} = \Delta\Delta Ct$$

The final calculation converts the $\Delta\Delta Ct$ values to a factor representing the relative quantity, that is, the difference in expression of the target molecule in the sample compared to the expression in the calibrator sample:

$$2^{-\Delta\Delta Ct} = \text{relative quantity}$$



The **Relative Quantification** analysis view includes the following main fields:

1. Display area

Display the selected data or graph.

2. Results tab

The graph presents the results as relative quantities for each sample well or replicate group.

Clicking the result bar for each sample well or replicate group shows the well position/replicate number, fluorophore, sample name, target and result at the top of the graph.

For replicate groups, the sample name and target/reference is shown also on the result bars.

In multiplex assays, each fluorophore is shown in a distinct color.

Relative quantification results are displayed as a table for each sample well or replicate group, depending on the chosen display settings.

3. Settings

The *Settings* field has options for showing the replicate groups and all sample wells, as well as settings for defining the calibrator sample and selecting the fluorophores to view.

The *Target Amplification Efficiency* allows you to adjust the reaction efficiency used in the calculations.

11.1 Adding a relative quantification analysis

To add a relative quantification analysis:

1. In the **Plate Layout** view, make sure that the following information is entered in the layout data, or enter it at this stage:
 - a. Designate the reference genes with the *Reference Gene* sample type.
 - b. Identify the different genes in the layout using different Targets.

- c. Designate the samples using distinct *Sample Names*.
2. In the **Analysis** view, select the *Ct* item for which the analysis is to be performed. The *Ct* item can encompass all wells or be the *Ct* of a distinct sample group.
3. Click the **Relative** button in the *Quantification* action panel.
The relative quantification analysis is presented by a *Relative Quantification* item in the *Analysis tree*. Select the analysis item to view its data and settings.
4. Select the calibrator sample from the **Calibrator Sample** menu in the *Settings* field. This menu lists the samples that have been given a name in the *Sample Name* field in the layout.

Note:

Before analyzing relative quantification data, make sure that you have valid *Ct* values.

11.2 Amplification efficiency

The *Amplification Efficiency* tab provides the option of entering accurate values for PCR amplification efficiency to provide more accurate relative quantification results. The default value is 100%. The amplification efficiency values can be determined from a standard curve using the absolute quantification analysis and are entered per target and per fluorophore.

11.3 Settings

In the *Settings* field, you can define various view settings for relative quantification.

Tick **Replicate** to view the graph based on replicates and to view the standard deviation of replicates.

Tick **Show All Wells** to force the results table to display all available results regardless of well selection.

Select the **Fluorophores** to view by ticking them. This setting only affects the view and has no effect on the analysis.

Note:

The settings for determining the *Ct* values are described in chapter 10. “*Ct* and Absolute Quantification” on page 27.

11.4 Results

The following information is presented in the results table:

Well – Well designation

Fluorophore – Fluorophore name

Sample Type – Sample type as specified in the layout

Sample Name – Name given to the sample in the layout

Target – Target as specified in the layout

Description – Optional description

Relative Quantity – Relation of the starting quantities of the target and the reference material

Ct – Cycle of Threshold for the sample

Delta Ct – Difference in Ct between the reference gene and the target

Delta Delta Ct – Difference in Ct between the calibrator and the sample

Efficiency – User-entered amplification efficiency used for the analysis

12. Melt Curve

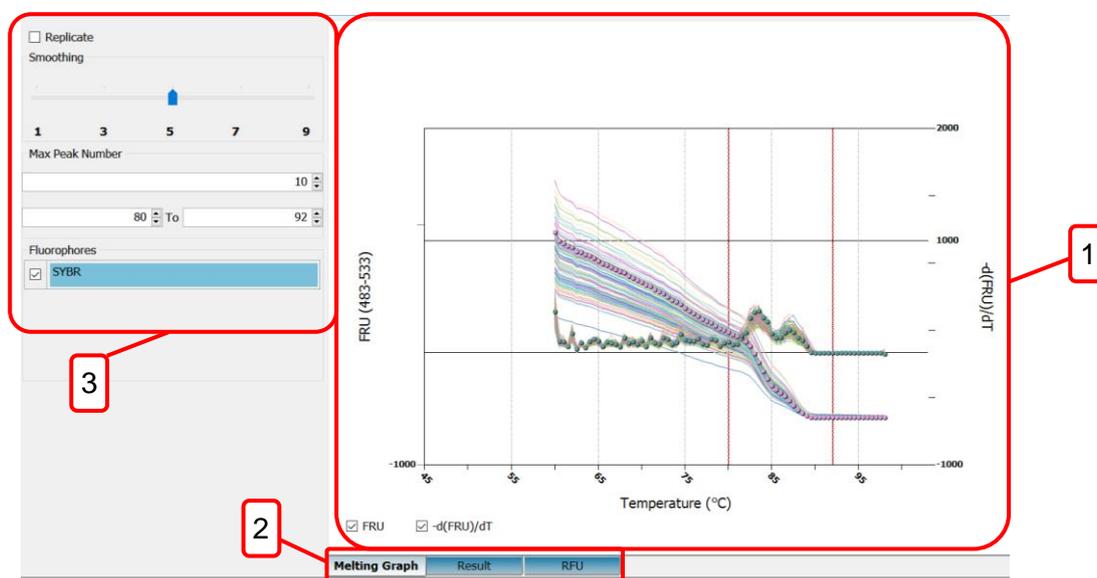
The melt curve is a post-PCR analysis step performed on an amplified product. Typically, the analysis follows the amplification step and it utilizes an interchelating/dsDNA-binding dye, such as SYBR Green I.

The amplified product is subjected to a gradual increase of temperature, from the annealing temperature to about 95°C, while monitoring the fluorescence value every 0.5–1.0°C.

As a result of the temperature increase, the amplified product dissociates, that is, melts to form single-stranded DNA strands. The dsDNA binding dye molecules are released, which leads to a decrease in fluorescence.

The melting temperature, T_m , of the product is defined as the temperature at which half of the dsDNA products have melted. The melting temperature is a characteristic value for the amplification and it is calculated from the melting curve data by plotting the first negative derivative of the fluorescence plot.

The melt curve is used as a qualitative analysis of DNA products, for example, in the identification or characterization of PCR products. In addition, the analysis allows the user to check for unwanted PCR products, such as primer dimers and secondary amplification products and thus provides a tool for assay optimization.



The **Melt Curve** analysis view includes the following main fields:

1. Display area

Display the selected data.

2. Results tab

The graph plots fluorescence values relative to the temperature and/or the first negative derivative of the fluorescence plot.

The results are presented as a table with melt temperature, peak height, peak area and full-width/half-magnitude ratio information for the selected fluorophores. The RFU readings for each sample during the melting are also provided.

3. Settings

- For selecting the fluorophores for which the data is shown.
- For viewing results for replicates or all wells.
- For applying curve smoothing.
- For showing the located peaks on the graph.
- For selecting the number of peaks for which the data is shown in the results table.

12.1 Adding a melt curve analysis

A melt curve analysis that encompasses all layout wells (*Melt Curve*) is automatically added to the *Analysis tree* when the protocol includes a *Melt Curve* step.

12.2 Settings

Select the curves to view by ticking the boxes below the graph:

- Tick **RFU** to display the detected fluorescence. This curve typically decreases in signal intensity as the temperature increases.
- Tick **-d(RFU)/dT** to display the negative first derivative of the RFU curve. The curve presents the change in detected fluorescence relative to the change in temperature. The peak is used to determine the melting point. The derivative curve calculation algorithm automatically corrects for signal intensity decrease that is unrelated to DNA melting.

You can select to view individual fluorophores from the *Fluorophores* field and individual wells from the *Well Selector*.

Adjust the *Smoothing* slider to remove unwanted signal noise from the plotted graphs. Move the slider to the right to increase the smoothing effect. Move the slider completely to the left to disable smoothing.

12.3 Peaks

You can view the located peaks on the derivative curve [$-d(RFU)/dT$] by selecting a channel with the *Show peaks* control.

Use the *Max number of peaks* control to limit the number of detected peaks within the range of **1–10**.

You can also set the temperature range by adjusting the position of the low and high temperature limit bars on the graph.

12.4 Results

The results table presents the calculated melting curve data.

The following information is presented for each peak:

- *Melt Temp* – Detected melting point
- *Peak Height* – Highest value of the peak
- *FW/HM Peak* – Full-width/half-magnitude ratio
- *Area Peak* – Calculated peak area

13. Settings

To access Q6000 Software settings, click the **Settings** button in the **Home** view. In the *Settings* dialog, you can configure general software settings.

In the Settings, you can:

- Set the default folder for Q6000 Software.
- Set the user interface language.
- Set the Lid Pre-heat on or off.

The service tool area is password protected and only authorized service engineers are allow to get access.



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