

## Operating manual

### qPCRsoft touch

Software for qTOWER<sup>3</sup> G touch/qTOWER<sup>3</sup> touch



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# Contents

<b>1</b>	<b>About this document .....</b>	<b>5</b>
<b>2</b>	<b>Software overview .....</b>	<b>6</b>
2.1	Starting and exiting qPCRsoft touch.....	6
2.1.1	Variant 1 (devices up to delivery year 2018) .....	6
2.1.2	Variant 2 (devices from delivery year 2019) .....	6
2.2	Operating qPCRsoft touch.....	7
2.3	The HOME page.....	8
2.4	Project pages .....	8
2.4.1	The GENERAL project page.....	9
2.4.2	The THERMAL CYCLER project page .....	10
2.4.3	The SCAN project page .....	12
2.4.4	The SAMPLES project page / Layout view .....	14
2.4.5	The MONITORING project page .....	15
<b>3</b>	<b>Templates and projects .....</b>	<b>18</b>
3.1	Creating templates.....	18
3.2	Saving templates.....	18
3.3	Opening templates .....	19
3.4	Saving projects .....	19
3.5	Opening projects.....	20
<b>4</b>	<b>Settings for a real-time PCR experiment.....</b>	<b>21</b>
4.1	General information on the real-time PCR project .....	22
4.2	Programming the PCR protocol.....	22
4.2.1	Creating a 2-step protocol or a 3-step protocol .....	22
4.2.2	Free programming of the PCR protocol.....	24
4.2.3	Programming a DNA melt.....	26
4.2.4	Programming block temperature gradients.....	26
4.2.5	Graphical display and programming of the PCR protocol .....	28
4.3	Programming the parameters for fluorescence measurements.....	29
4.4	Editing samples .....	31
4.4.1	Entering sample properties in the layout.....	31
4.4.2	Displaying sample properties in the table.....	34
4.4.3	Combining of experiments into groups .....	34
4.4.4	Displaying the simple layout.....	36
4.4.5	Exporting and importing the sample layout .....	36
<b>5</b>	<b>Monitoring.....</b>	<b>38</b>
5.1	Starting a qPCR run.....	38
5.2	Monitor display .....	39
5.3	Showing and hiding the data for individual samples .....	40
5.4	Calculating the Ct value.....	41
5.5	Calculating the melting temperature.....	43
<b>6</b>	<b>qPCRsoft touch software settings .....</b>	<b>46</b>
<b>7</b>	<b>Data Management.....</b>	<b>49</b>



# 1 About this document

What is qPCRsoft touch?	<p>The qPCRsoft touch software is used to control the qTOWER<sup>3</sup> touch. The software can be used to prepare and start real-time PCR projects. qPCRsoft touch controls the qPCR run and monitoring of the amplification via fluorescence measuring. Via the inputs in the sample layout, it is possible to assign to the samples information regarding sample type, dyes and GOIs (genes of interest).</p> <p>After completion of the amplification or melting, Ct values and melting temperatures can be determined in a quick overview. For further evaluation, the data can be imported into the qPCRsoft PC software, without the need to convert the data format.</p>
Software version	This manual describes version 3.3.8.0 of qPCRsoft touch.
Requirements on the user	<p>This manual is intended for qualified users who are knowledgeable about real-time PCR analysis. The manual confines itself to describing the functionality of qPCRsoft touch. Basic knowledge about how to use a tablet PC is also required.</p> <p>For safe operation of the qTOWER<sup>3</sup> touch it is also required that the instructions for use "qTOWER<sup>3</sup> touch / qTOWER<sup>3</sup> G touch" and "qPCRsoft - Software for real-time PCR-Thermocycler" have been read and understood.</p>
Notes on this manual	<p>This manual uses the following system and typographical conventions:</p> <ul style="list-style-type: none"><li>■ Program commands and options are indicated by small caps.</li><li>■ Buttons are enclosed in square brackets.</li><li>■ Menu items with submenus are concatenated using arrows, e.g. Templates TEMPLATES ► NEW TEMPLATES.</li></ul>

## 2 Software overview

### 2.1 Starting and exiting qPCRsoft touch

The qTOWER<sup>3</sup> touch uses two different tablet PCs, which behave differently when switched on and off:

- Variant 1 (devices up to delivery year 2018): A flap is visible on the right side of the device, at the upper end of which the power button is located.
- Variant 2 (devices from delivery year 2019): Only one USB port is visible on the right side of the device.

#### 2.1.1 Variant 1 (devices up to delivery year 2018)



##### Notice

It is recommended to switch on the qTOWER<sup>3</sup> touch before the tablet is started up. This makes sure that the tablet is provided with operating voltage via the qTOWER<sup>3</sup> touch, allowing it to start up even if its battery is empty.

To starting qPCRsoft touch

1. Switch on the qTOWER<sup>3</sup> touch using the mains switch on the back of the device.
2. Switch on the tablet using the on/off switch on the right side of the device. To do so, hold the button pressed for 5 s.
  - ✓ qPCRsoft touch starts automatically. The qTOWER<sup>3</sup> touch is ready for operation. The green status LED lights up.

To exiting qPCRsoft touch

The tablet may be shut down without exiting qPCRsoft touch:

- Switch off the tablet using the on/off switch on the right side of the device. To do so, hold the button pressed for 5 s.

It is possible to exit qPCRsoft touch in order to access the tablet's operating system.

1. On the HOME page, tap on OPTIONS.
2. Tap on .
  - ✓ qPCRsoft touch shuts down. The user interface of the operating system appears.

#### 2.1.2 Variant 2 (devices from delivery year 2019)

Switching on the qTOWER<sup>3</sup> touch

- Switch on the qTOWER<sup>3</sup> touch at the mains switch.
  - ✓ The integrated tablet is switched on and the qPCRsoft touch software starts automatically.

Switching off the qTOWER<sup>3</sup> touch

- Tap on the HOME page on . This turns off the tablet.
- Then switch off the qTOWER<sup>3</sup> touch at the mains switch.

## 2.2 Operating qPCRsoft touch

Frequently used icons

The following icons/functions frequently occur in the software:

Icon	Function
	Access Help
	Confirm changes/input/selection
	Reject changes/input/selection
	Return to the HOME page

To activate a function

Within the software, a function is activated by tapping on it. Where the user is required to enter information in an input field, an alphanumerical or a numerical keypad opens, depending on the expected type of input.

Keypads

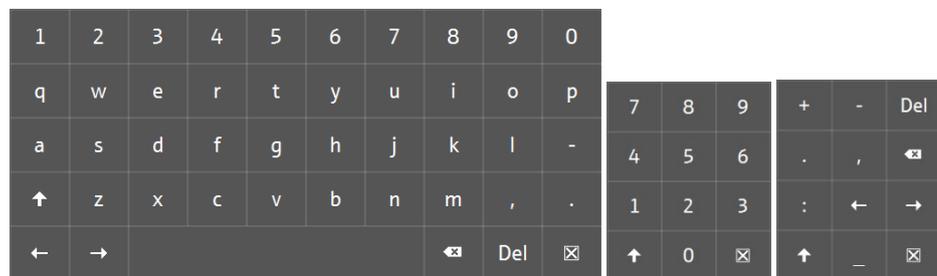


Fig. 1 Alphanumerical and numerical keypads

The alphanumerical keypad appears if text is to be entered. The numerical keypad appears if numerical values are to be entered. The keypads have the following function keys:

Icon	Description
	Alphanumerical: Switch to upper case Numerical Switch to arithmetic operators
	Move cursor
	Delete the character to the left of the cursor
	Delete the character to the right of the cursor
	Conclude entry, hide keypad

Using help

Quick help is available for each software page, providing information on all available parameters, options and icons on that page.

1. On the screen, tap on .
  - ✓ Help is displayed.
2. To return to the program, tap anywhere on the tablet screen.

## 2.3 The HOME page

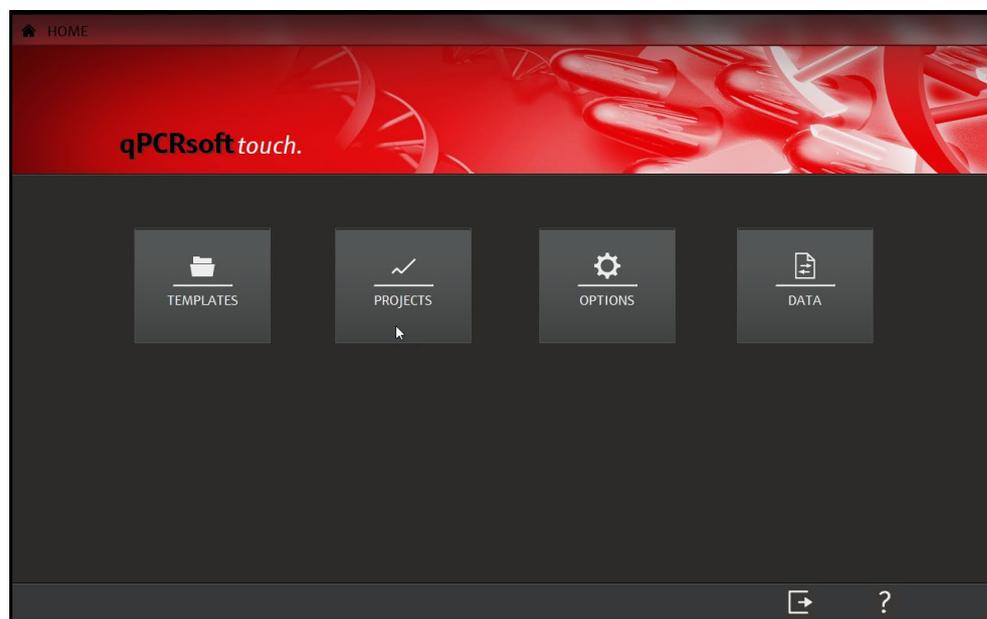


Fig.2 The HOME page

Menus

The HOME page appears after qPCRsoft touch is started. The menus on this page are the starting points for all basic functions of qPCRsoft touch:

MENU	Functions
TEMPLATES	Create new templates for projects Activate a stored template for the qPCR run Start and evaluate a qPCR run
PROJECTS	Open and edit a stored project
OPTIONS	Make general device settings for the qTOWER <sup>3</sup> touch Exit qPCRsoft touch
DATA	Exchange projects, templates and files (*.CSV, *.XLS) between tablet PC and another PC via a USB stick Copy, move or delete files

Icons

Icons	Funktion
	Open help
	Exit qPCRsoft touch and switch off the tablet The qTOWER <sup>3</sup> touch must then be switched off at the mains switch.

## 2.4 Project pages

In qPCRsoft touch, most of the work is done in the Template/Project area. This is where the PCR protocol is entered, fluorescence measurements are specified, qPCR runs are started and experiments are evaluated.

Main elements of the  
Template/Project area

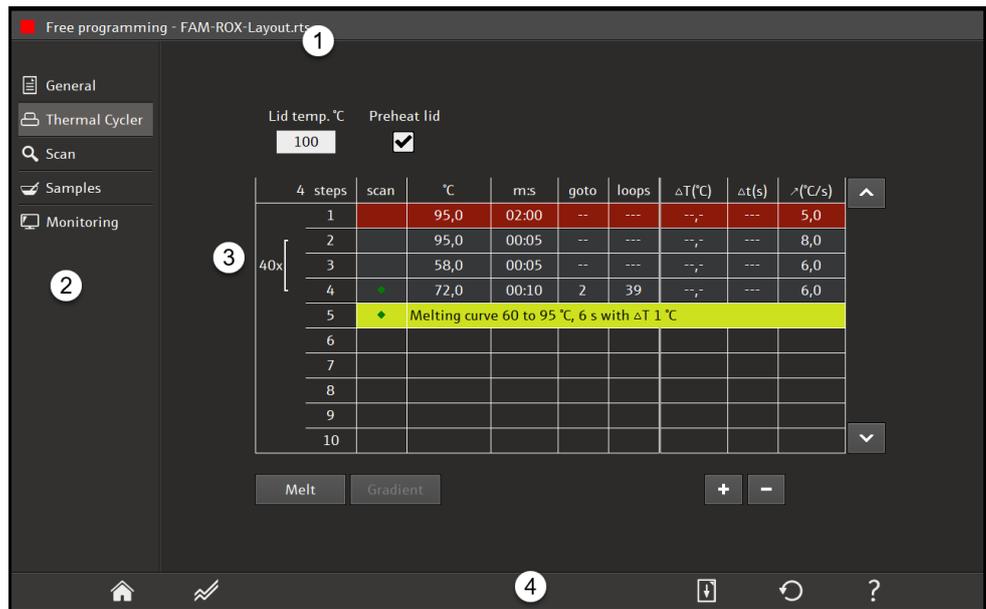


Fig.3 The Template/Project area

The Template/Project area contains the following elements:

No.	Element	Description
1	Header	Information on the name of the page, the name of the template or project file and the logged-on user
2	Quick access	Jump to the first project page of the selected topic
3	Project page	Display the selected topic
4	Icon bar	Function icons The functions and icons vary depending on the content of the current page.

The project pages appear after the selecting functions in the TEMPLATE or PROJECT menu at the HOME page. The project pages contain all the parameters needed to carry out a real-time PCR experiment.

### 2.4.1 The GENERAL project page

Entries on this page are optional.

Fig.4 The GENERAL page

## Elements

On the GENERAL page, the following options can be entered for the project:

Field	Description
TITLE	Project designation
AUTHOR	Name of the author
DAY/TIME	Date and time of the creation of the template This information is automatically taken from the tablet's operating system.
REMARKS	Additional information on the project

## Icons

Icon	Function
	Return to the HOME page, entries will be lost
	Show help

## 2.4.2 The THERMAL CYCLER project page

The THERMAL CYCLER page contains all the parameters for the PCR protocol. When a 2-step or 3-step protocol is selected, an entry mask for these experiments is proffered (→ "Creating a 2-step protocol or a 3-step protocol" p. 22). If free programming is selected, any number of temperature steps can be defined.

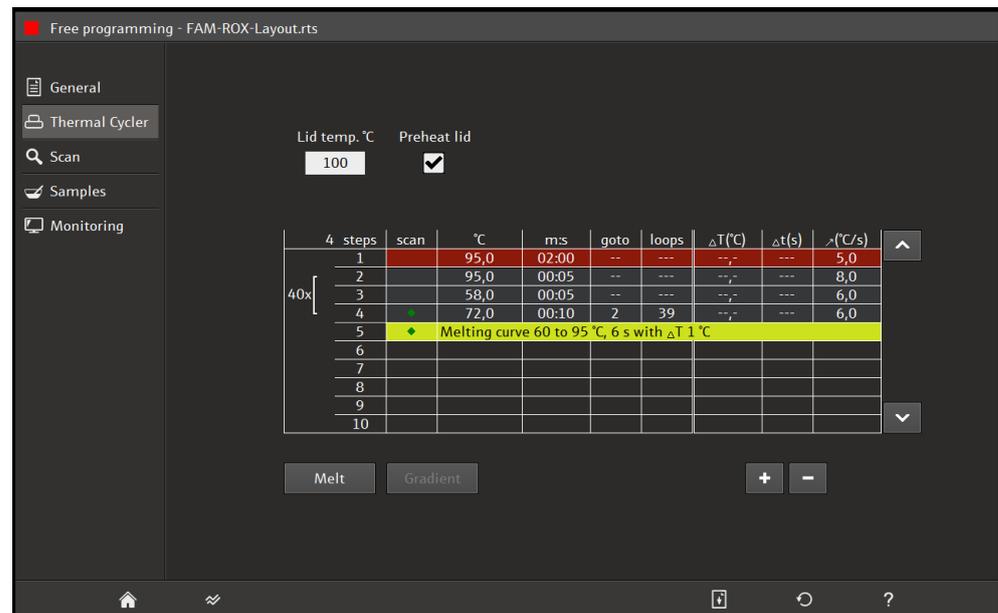


Fig.5 The THERMAL CYCLER page with free programming

## Heated lid

Value	Description
LID TEMP. °C	Lid temperature The lid temperature should be a few degrees above the maximum block temperature to prevent liquids from evaporating from the reaction mixture, causing them to condense at the walls or at the lid of the reaction cups. Input range: 30 to 110 °C

PREHEAT LID	<p>The heated lid is preheated, before the program starts, to the specified temperature.</p> <p>By default, this option is activated. Preheating causes the formation of a homogeneously tempered air cushion in the spaces between the sample cups, resulting in better temperature uniformity between the samples. While the lid is being heated, the block is kept at a constant 25 °C.</p> <p>If this option is deactivated, the PCR software starts while the lid is still being heated.</p>
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## Table area

Column	Description
STEPS	Number of the protocol step (automatically generated)
SCAN	<p>Measure fluorescence</p> <p>The step at which fluorescence is to be determined must be marked with a green diamond.</p>
°C	Target temperature of the step
M:S	Hold time of the step
GOTO/LOOP	Loop with the number of repetitions
ΔT (°C)	Optional increment/decrement for the target temperature within the loop
Δt (s)	Optional increment/decrement for the hold time within the loop
↗(°C/s)	Heating/cooling rate within the step

## Functions

Function	Description
[MELT]	Follow completed product amplification by melting
[GRADIENT]	If the thermocycler supports the gradient function: Specify the temperature gradient for the thermoblock
[+]	Insert an additional step
[-]	Delete the highlighted step

## Icons

Icon	Function
	Return to the HOME page, changes will be discarded
	Switch to the temperature curve display
	Restore the default settings
	Show help

### 2.4.3 The SCAN project page



Fig.6 The SCAN project page

Parameter

Parameter	Description
MEAS.	Dye measurement The fluorescence of the dyes marked with a green diamond (◆) will be measured as part of the qPCR run.
COL.MOD.	Installed color modules Up to 6 different color modules can be installed in the qTOWER <sup>3</sup> touch. The installed color modules are specified in qPCRsoft touch under Options (→ "qPCRsoft touch software settings" p. 46). The figure in brackets indicates the position of the color module on the color module wheel of the qTOWER <sup>3</sup> touch.
DYE	Name of the dye to be measured One dye per color module can be selected.
GAIN	Adjustment of signal intensities The intensity can be adjusted in steps ranging from 0 to 10. The setting should be selected in such a way that the intensity of a positive sample at the end of the PCR run is at least 10,000 The default setting of 5 is ideal for most experiments performed with the qTOWER <sup>3</sup> touch.
PASS. REF.	Reference dye Owing to the LED technology employed by the device it is not necessary to use a passive reference. If you wish to measure a reference dye anyway, place a checkmark in this column.

COLOR COMPENSATION	If several fluorescent dyes are used in one sample (multiplexing), spectral crosstalk of the fluorescence may occur. This spectral crosstalk can be corrected by color compensation. Press the button to select color compensation:
OFF	The default setting for COLOR COMPENSATION is OFF, since color compensation is not necessary for the most common applications (only an active measurement channel or spectrally widely spaced dyes such as FAM and ROX).
STANDARD	With standard color compensation, a compensation matrix is applied to the measurement data, which enables sufficient compensation of crosstalk in all colors with the gain setting "5".
User-specific	User-specific color compensations can be generated by tapping the [...] button (see below) and are then available for selection.

## Icons

Icon	Function
	Return to the HOME page, changes will be discarded
	Specify the scanning range on the PCR plate and the number of repeat measurements
	Show help

## Create user-specific color compensation

A measurement is required for spectral calibration. The dyes, for which color compensation is required, must be activated for the measurement and must be present as individual samples in solution. For example, the probes used in the later PCR experiment can be used for calibration measurements. The dye concentration should be about 0.1  $\mu$ M for the calibration measurements.

1. On the SCAN project page, select the dyes for which the calibration measurements are to be performed.
2. Tap on [...].

The COLOR COMPENSATION page opens with the plate diagram.

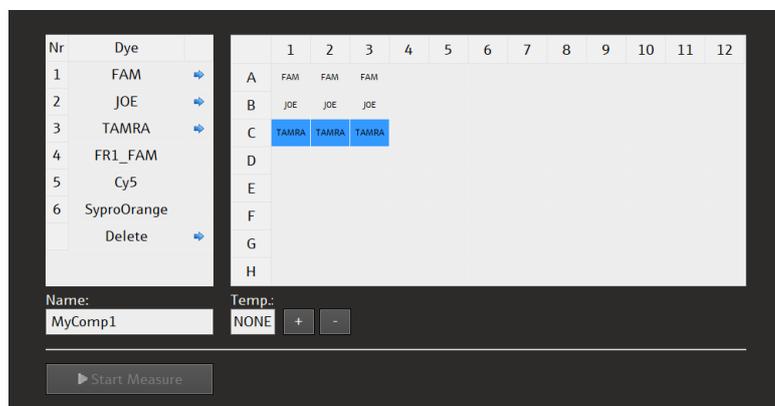


Fig. 7 COLOR COMPENSATION PAGE

3. Mark in the plate scheme for each dye individually the wells in which the calibration samples are located. Press the blue arrow next to the dye to assign this dye to the well.

For an exact calibration measurement, it is advisable to create each dye at least as a triple replica.

4. Enter a name for the new color compensation in the NAME field.
5. If necessary, enter a block temperature under TEMP. or set it with the [+] and [-] keys. No temperature control takes place with the value NONE.
6. Press [▶ START MEASURE] and start the calibration measurement.
  - ✓ The calibration measurement is performed. After successful completion, the new color compensation is available on the SCAN page.

**Note:**

The selection of available dyes cannot be changed in this table. Changes can only be made on the SCAN project page.

## 2.4.4 The SAMPLES project page / Layout view

A sample layout needs to be created in order to be able to use the evaluation function of qPCRsoft touch.

On the SAMPLES page, the sample layout with the sample properties and positions is only displayed as a plate diagram, and it is possible to activate or deactivate samples. The sample layout can only be edited after the pen icon  has been tapped on (→ "Entering sample properties in the layout" p. 31). It can be used to assign properties such as name, gene, type, concentration and dye to each sample. In addition, samples from different experimental preparations can be combined to form groups.

A separate layout can be created for each dye according to the detected genes.



Fig.8 The SAMPLES project page, overview of the plate layout

## Elements / parameters

No.	Element	Description
1	Sample properties	Displays the sample properties entered for the well framed in red If multiple wells are marked, the properties of the sample in the top left position in the frame are displayed.
2	Layout diagram	Overview of the positions occupied in the layout, with brief information on the sample properties The left side of each sample cell is colored corresponding to the sample type and also shows the symbol of the sample type. A checkmark in the color field indicates that the sample is active, i.e. that it is displayed on the Monitoring page and is included in the evaluation. If the layout contains multiple experiments (groups), the background of each cell is colored in the color of the group it belongs to.
3	Dyes	Layout for the selected dye The activated dye is marked with a red bar. The dyes are specified on the SCAN project page.
4	[ACTIVATE] / [DEACTIVATE]	Activate/deactivate samples for display on the Monitoring page and for evaluation To do so, mark the desired well by tapping on it, or mark contiguous wells by wiping. The selected wells are framed in red.

## Icons

Icon	Function
	Tabular view of the sample assignment
	Switch to layout editing
	Combine sample positions to form groups Each group contains one experiment. Groups make it possible to run multiple experiments on a PCR plate and to evaluate them independently of each other.
	Switch between the simple and extended views of the layout
	Load layout from an *.xls file
	Export layout as an *.xls file
	Save template (*.rts file)

## 2.4.5 The MONITORING project page

The MONITORING project page is displayed when saved projects or templates are opened or when templates are created.

After a template has been created or opened, it is possible to start a qPCR run with the settings specified in the template by tapping on the  icon.

Projects are generated after the qPCR run. In addition to the data of the template, they contain the measured fluorescence curves. The fluorescence curves can be evaluated

from the MONITORING page. Since it is not possible to start a qPCR run while the project file is opened, the  icon is no longer displayed.

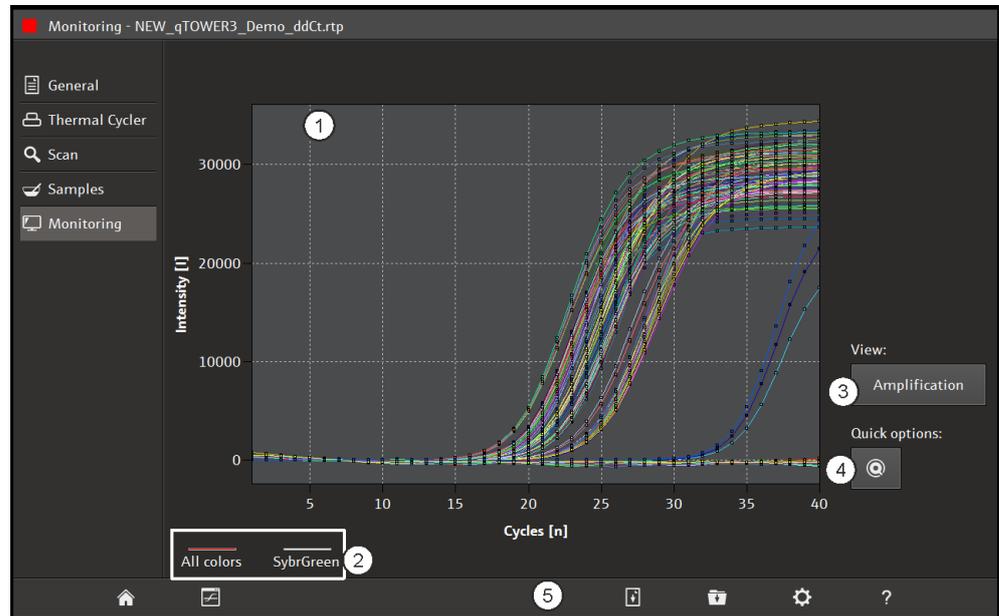


Fig.9 MONITORING page, display of project data

Elements

No.	Element	Description
1	Graph	Display of the amplification or melting curves (data from the fluorescence measurement) The colors for the curves can be defined under OPTIONS (→ "qPCRsoft touch software settings" p. 46).
2	Dyes	Displays the fluorescence intensity for the selected dye (gene) The displayed dye is marked with a red bar.
3	VIEW	List of display options: <ul style="list-style-type: none"> <li>– AMPLIFICATION</li> <li>– RAW DATA (amplification without base line subtraction)</li> <li>– MELTING CURVE</li> </ul>
4		Select base line and scaling Changes made to these parameters are immediately reflected by the graph.
5	Icons	Function buttons

Icons

Icon	Function
	Return to the HOME page, changes will be discarded
	Start the qPCR run
	Interrupt and continue the qPCR run During the interruption, the symbol flashes red. If the symbol is tapped on again, the qPCR run is continued.
	To stop the qPCR run The data recorded up to this point need to be saved and can then be evaluated.

	Save template
	Save project
	Calculate Ct values
	Calculating the melting temperature
	Define options for the calculation and display of fluorescence curves
	Show help

## 3 Templates and projects

Project	<p>qPCRsoft touch saves all experiments in project files. A project contains all the information from the project pages:</p> <ul style="list-style-type: none"><li>▪ Description of the experiment (GENERAL page)</li><li>▪ PCR protocol (THERMOCYCLER page)</li><li>▪ Scan settings of the fluorescence measurement (SCAN page)</li><li>▪ Sample layout (SAMPLES page)</li><li>▪ Measurement results and evaluation of the experiment (MONITORING page)</li></ul> <p>It is not possible to start further qPCR experiments from an open real-time PCR project. It is not possible to change the data or the device settings used for measuring (data integrity). If a further qPCR run is to be started with the same device settings, a template based on the project needs to be created first (→ "Saving templates" p. 18).</p> <p>Projects are saved with the file name extension *.rtp (real-time project file).</p>
Templates	<p>A template contains the information needed to carry out the experiments, i.e., all the information from the project pages, but no measurement data. Stored templates can be used to carry out any number of new qPCR experiments. All the parameters of a template can be edited.</p> <p>Templates are saved with the file name extension *.rts (real-time settings file).</p>

### 3.1 Creating templates

There are two different ways to create a template:

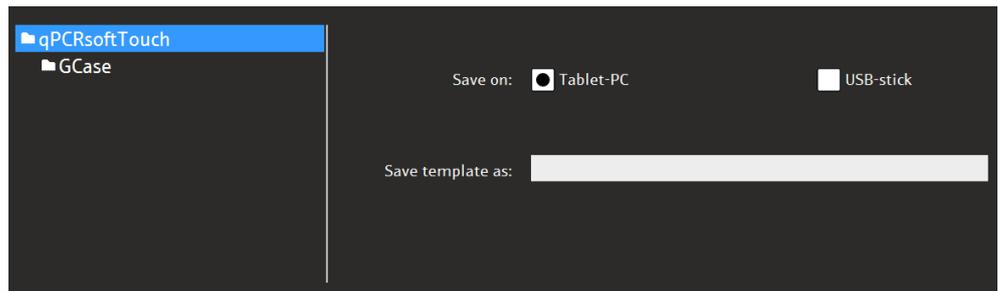
- Create a new template (→ "Settings for a real-time PCR experiment" p. 21)
- Derive a template from a saved project (→ "Saving templates" p. 18)

### 3.2 Saving templates

1. Enter all the parameters on the project pages or in a loaded project, then tap on .
  2. Select the storage medium: TABLET PC or USB STICK.
  3. In the SAVE TEMPLATE AS field, enter the file name.
  4. Tap on .
- ✓ The template is saved and can now be opened using the OPEN TEMPLATE function.

**Note:**

The folder used to save data on the tablet will always be C:\qPCRsoftTouch. Any number of subfolders can be created in this folder using the Data function.



**Fig.10 Saving templates**

Create a template from a project

A project is completed once the measurement data have been stored. Only the evaluation parameters and the sample layout can be varied for further evaluations. If the protocol parameters are to be used for a further experiment, it is possible to generate a template from the project.

1. Open the project (→"Opening projects" p. 20).
2. Tap on  and save the template as described above.
  - ✓ The project parameters are saved as a template.

### 3.3 Opening templates

A saved template can be used to start an experiment

1. Open the HOME page.
2. Select the TEMPLATES ► OPEN TEMPLATES menu item.
3. Select the storage medium: TABLET PC or USB STICK.
4. Mark a template.
5. Tap on .
  - ✓ The template is loaded. The template can be used to start the next experiment, or it can be edited.

Last used template

The last used templates are displayed separately:

1. Open the HOME page.
2. Select the TEMPLATES ► OPEN TEMPLATES ► LAST TEMPLATES menu item.
3. From the menu, select the template.
  - ✓ The template is loaded.

### 3.4 Saving projects

After the measurement has been carried out, the project can be saved.

1. On the MONITORING page, tap on .
2. Select the storage medium: TABLET PC or USB STICK.

3. Enter the file name in the SAVE PROJECT AS field.
4. Tap on .
- ✓ The template is saved and can now be opened using the OPEN TEMPLATE function.

**Note:**

Once the project has been saved, the acquisition of data for this project is complete. The buttons for starting a qPCR run are hidden. As long as the project has not been saved, it is possible to start another qPCR run. If that is done, existing experiment data will be overwritten.

## 3.5 Opening projects

A saved project can be opened and recalculated.

1. Open the HOME page.
2. Select the PROJECTS ► OPEN PROJECT menu item.
3. Select the storage medium: TABLET PC or USB STICK.
4. Mark a project
5. Tap on .
- ✓ The project is loaded. The data of the experiment are displayed on the MONITORING page.

**Last used project**

The last used projects are displayed separately:

1. Open the HOME page.
2. Select the PROJECTS ► LAST PROJECTS menu item.
3. From the menu, select the project.
- ✓ The project is loaded.

## 4 Settings for a real-time PCR experiment

At the start of a real-time PCR experiment, create or load a template.

The template contains the following settings:

- Parameters of the PCR protocol
- Specifications for optical measuring
- Sample layout of the micro plate

Starting the entry

To create a new template, start from the HOME page and select the command sequence **TEMPLATES ▶ NEW TEMPLATES ▶ PCR input form**.

- ✓ The project pages appear.

3 different PCR entry masks are available for PCR protocols:

Option	Description
2-STEP PROTOCOL	PCR protocol with 2 cyclic temperature stages
3-STEP PROTOCOL	PCR protocol with 3 cyclic temperature stages
FREE PROGRAMMING	PCR protocol with a freely definable number of temperature stages

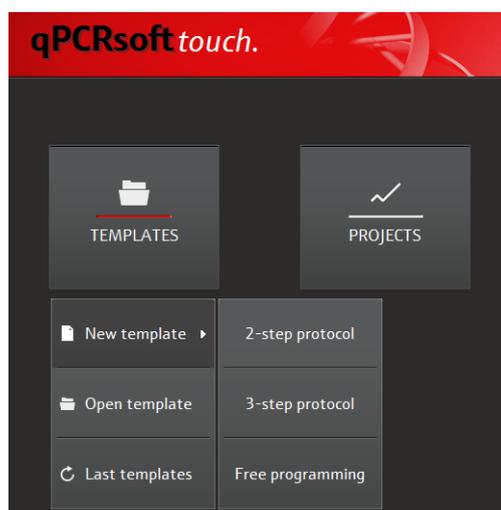


Fig.11 The menu for selecting the PCR entry form

Pages for template creation

After a mask has been selected, the project area for creating the template appears. The entry form for the PCR protocol selected above is displayed on the THERMOCYCLER page.

The function keys linking to the individual project pages (quick access) are located on the left margin of the page:

Function key	Description
[GENERAL]	General information on the project Entries on this page are optional.
[THERMOCYCLER]	Enter the PCR protocol
[SCAN]	Select color modules and dyes for the optical scan

[SAMPLES]	Enter plate layout with sample properties (can also be done after the qPCR run)
[MONITORING]	Start the qPCR run

## 4.1 General information on the real-time PCR project

All entries on the GENERAL project page are optional. The following information on the project can be entered:

Field	Description
TITLE	Project designation
OPERATOR	Name of the user
DAY / TIME	Date and time of the creation of the template This information is automatically taken from the tablet's operating system.
COMMENT	Additional information on the project



The screenshot shows a dark-themed form with the following fields and values:

- Title: [Empty text input field]
- Operator: [Empty text input field]
- Day: 19.08.2016
- Time: 10:00:46
- Comment: [Empty text input field]

Fig.12 The GENERAL project page

## 4.2 Programming the PCR protocol

The PCR protocol is entered into the entry form on the THERMAL CYCLER project page.

### 4.2.1 Creating a 2-step protocol or a 3-step protocol

Entry masks for protocols working with 2 or 3 cyclically repeated temperature stages are already stored in the software and can easily be edited.

- To program a protocol, start from the HOME page, and select the TEMPLATES ► NEW TEMPLATES ► 2-STEP PROTOCOL function or the TEMPLATES ► NEW TEMPLATES ► 3-STEP PROTOCOL function.
  - ✓ The THERMAL CYCLER page with the entry mask for a 2- or 3-step protocol appears.

## Functions on the THERMAL CYCLER page

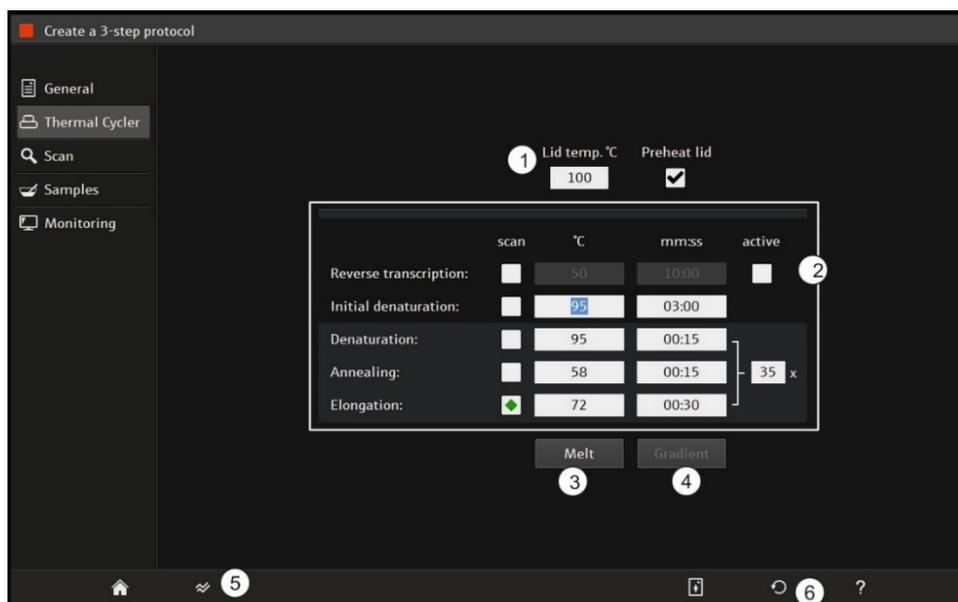


Fig.13 Control elements on the THERMAL CYCLER / CREATE A 3 STEP PROTOCOL project page

No.	Element	Description
1	Heated lid options	In the LID TEMP. field, specify the lid temperature. Adjustable lid temperature: 30 – 110 °C If the PREHEAT LID option is activated, the heated lid is preheated to the specified temperature before the program starts. While the lid is being preheated, the block is kept at a constant 25 °C. If the PREHEAT LID option is deactivated, the PCR program starts while the lid is still being heated.
2	Entry form	Enter the protocol parameters
3	[MELT]	After the qPCR run, record a melting curve (→ "Programming a DNA melt" p. 26)
4	[GRADIENT]	Specify a gradient for the selected temperature step (→ "Programming block temperature gradients" p. 26)
5		Display and edit the temperature curve of the PCR protocol (→ "Graphical display and programming of the PCR protocol" p. 28)
6		Restore the entry form to the default settings

## Enter the protocol

1. If a Reverse Transcription is to be carried out at the beginning of the PCR protocol, tap on the ACTIVE field to place a checkmark.
2. In the SCAN column, activate the step during which the fluorescence measurement is to be carried out. By default, the Scan setting is set to the following step:  
2-step protocol: ANNEALING + ELONGATION  
3-step protocol: ELONGATION
3. In the "°C" column enter the target temperature for each step.
4. In the "mm:ss" column enter the hold time for each step. The software automatically converts the entries to minutes and seconds as follows:  
5 --> 00:05  
30 --> 00:30

300 --> 03:00

310 --> 03:10

5. In the cycle field (the field next to the brace on the right side of the table) enter the total number of repetitions of the temperature cycle.
6. If required, activate a melt (see section "Programming a DNA melt" p. 26).
  - ✓ Entry of the PCR protocol is thus complete.

## 4.2.2 Free programming of the PCR protocol

By contrast to the entry masks for 2-step and 3-step protocols with fixed ramp rates and without increment/decrement function, PCR protocols can be freely programmed with respect to all parameters.

- To program a freely defined protocol, start from the Home page and select the TEMPLATES ► NEW TEMPLATES ► FREE PROGRAMMING functions.
  - ✓ The FREE PROGRAMMING / THERMAL CYCLER page appears.

Insert/delete temperature steps

- To append a step to a protocol:
  - Tap on the next empty table row below the protocol.
  - ✓ The step will be appended to the existing protocol.
- To insert an additional step into the protocol:
  - Mark a step (by tapping on the STEP cell of the step) and then tap on [+].
  - ✓ The step will be inserted in the protocol above the marked step.
- To delete a step:
  - Mark the step and tap on [-].
  - ✓ The marked step will be deleted.

Enter the target temperature, hold time and heating/cooling rates

- In the "°C" column, enter the target temperature for the step in °C.
- In the "m:s" column, enter the hold time. The software automatically converts the entries to minutes and seconds as follows:
  - 5 --> 00:05
  - 30 --> 00:30
  - 300 --> 03:00
  - 310 --> 03:10.
- In the "↗(°C/s)" column, enter the ramp rates in °C/s as positive values.

4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	λ(°C/s)
40x	1	95,0	02:00	--	---	--,-	---	5,0
	2	95,0	00:05	--	---	--,-	---	8,0
	3	58,0	00:05	--	---	--,-	---	6,0
	4	72,0	00:10	2	39	--,-	---	6,0
	5	♦ Melting curve 60 to 95 °C, 6 s with ΔT 1 °C						
6								
7								
8								
9								
10								

Fig.14 Entry of the target temperature, hold times and heating/cooling rates into the PCR protocol

Program a loop

Cycles repeating a number of consecutive temperature steps are referred to as loops. A loop is defined by a target step for the return ("GOTO") and the number of repetitions ("LOOPS"):

1. In the GOTO column of the last step of the loop-to-be, enter the number of the target step.
2. In the LOOPS column of the same step, enter the number of REPETITIONS.
  - ✓ The programmed loop is represented by a brace. The total number of loops indicated next to the brace is the number of programmed repetitions plus 1, since the sequence of steps was already processed once before the loop was reached.

4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	λ(°C/s)
40x	1	95,0	02:00	--	---	--,-	---	5,0
	2	95,0	00:05	--	---	--,-	---	8,0
	3	58,0	00:05	--	---	--,-	---	6,0
	4	72,0	00:10	2	39	--,-	---	6,0
	5							

Fig.15 Programming of a loop

Program a fluorescence measurement

- Tap on the SCAN column of the step during which the fluorescence measurement is to be carried out.
  - ✓ The step is marked with a green diamond. The fluorescence measurement can only be carried out at a temperature step.
  - ✓ **Note:** During melting, an optical measurement is carried out at each temperature step (see section "Programming a DNA melt" p. 26).

4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	λ(°C/s)
40x	1	95,0	02:00	--	---	--,-	---	5,0
	2	95,0	00:05	--	---	--,-	---	8,0
	3	58,0	00:05	--	---	--,-	---	6,0
	4	72,0	00:10	2	39	--,-	---	6,0
	5							

Fig.16 Programming of a fluorescence measurement

Programming increments/decrements for temperatures and hold times

Increments/decrements can be used to change temperatures or hold times by a specific amount from one cycle to the next within a loop. Use the "-" sign to specify a decrement, which means that the temperature or hold time is step by step reduced by the specified amount. No sign or a "+" indicates an increment, which means that the parameter is step by step increased by the specified amount.

- Incremental change of the target temperature:  
Enter the increment in the  $\Delta T(^{\circ}\text{C})$  column.
- Incremental change of the hold time:  
Enter the increment in the  $\Delta t(\text{s})$  column.

4 steps	scan	$^{\circ}\text{C}$	m:s	goto	loops	$\Delta T(^{\circ}\text{C})$	$\Delta t(\text{s})$	$\lambda(^{\circ}\text{C}/\text{s})$	▲
1		95,0	02:00	--	---	---	---	5,0	
2		95,0	00:05	--	---	---	---	8,0	
3		58,0	00:05	--	---	---	---	6,0	
4	●	72,0	00:10	2	39	-1,0	5	6,0	
5									

Fig.17 Programming of an increment/decrement

### 4.2.3 Programming a DNA melt

For experiments with intercalating dyes it is recommended to check the specificity of the products by measuring a melting curve. To that end, a corresponding step should be programmed at the end of the PCR protocol:

1. On the THERMOCYCLER page, tap on [MELT].
2. On the CONFIGURE MELTING CURVE page activate the DNA melt.
3. Edit the parameters for the DNA melt.
  - ✓ The DNA melt will be appended to the PCR protocol. During melting, fluorescence measuring is automatically activated.

The following parameters can be edited:

	Start $^{\circ}\text{C}$	End $^{\circ}\text{C}$	Increment $^{\circ}\text{C}$	Equilibration sec	Heating rate $^{\circ}\text{C}/\text{sec}$
<input checked="" type="checkbox"/> active	60	95	1	6	5

Fig.18 The CONFIGURE MELTING CURVE page

Melt parameters

Parameter	Description
ACTIVE	If activated, the DNA melt will be appended to the PCR protocol. If deactivated, the DNA melt is removed from the PCR protocol. However, the parameters will be retained.
START $^{\circ}\text{C}$	Start temperature of the melt
END $^{\circ}\text{C}$	End temperature of the melt
INCREMENT $^{\circ}\text{C}$	The temperature difference between two temperature steps at each of which a fluorescence measurement is carried out
EQUILIBRATION SEC	Period for equilibration of the sample before the fluorescence measurement is carried out
HEATING RATE $^{\circ}\text{C}$	Enter the rate at which the temperature is to be increased

### 4.2.4 Programming block temperature gradients

The qTOWER<sup>3</sup> G touch allows programming of gradients over the whole temperature range of the thermal block between 4  $^{\circ}\text{C}$  and 99  $^{\circ}\text{C}$ .

The parameters can be entered in two ways:

- Gradient via margin columns  
The temperatures for columns 1 and 12 are specified. The temperature steps between the columns are automatically determined.
- Linear Gradient  
After entry of the temperature for column 6 and of the increment, the temperature is decreased column by column towards column 1, and increased towards column 12.

Gradient via margin columns

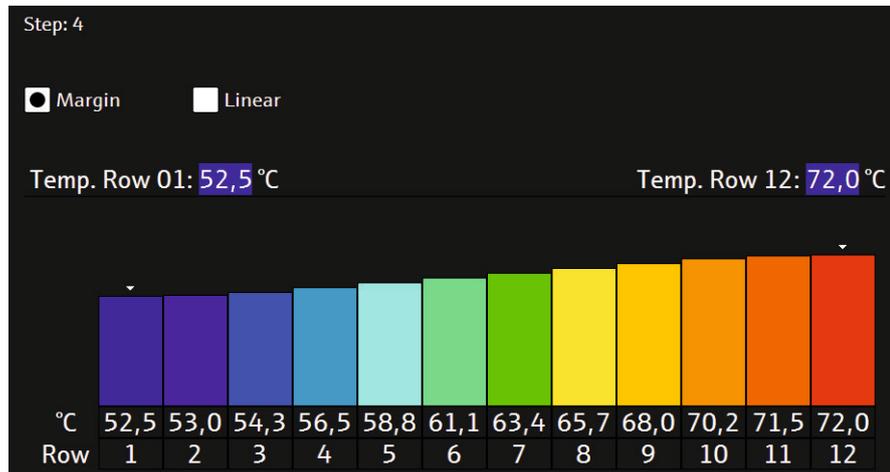


Fig.19 Configuring of the gradient via the margin column temperatures

1. Mark the temperature step in the PCR protocol.
2. Tap on [GRADIENT].
3. Activate the MARGIN option.
4. In the TEMP.ROW 01 and TEMP.ROW 12 fields enter the respective margin temperatures.

Alternatively, tap on the arrows above the ROW 1 and ROW 12 bars and drag the bars upwards (higher temperature) or downwards (lower temperature).

5. Tap on  to confirm the parameters.
  - ✓ In the PCR protocol, the gradient is identified by the two temperature values separated by a dash. In this way, the gradient can also be entered directly into the table of the PCR protocol.

4	steps	scan	°C	m's	goto	loops	$\Delta T(^{\circ}C)$	$\Delta t(s)$	$\lambda(^{\circ}C/s)$	
40x	1		95,0	02:00	--	---	--	---	5,0	▲
	2		95,0	00:05	--	---	--	---	8,0	
	3		58,0	00:05	--	---	--	---	6,0	
	4		52,5-72,0	00:10	2	39	--	---	6,0	
	5									

Fig.20 Gradient in the PCR protocol

Linear gradient

1. Mark the temperature step in the PCR protocol.
2. Tap on [Gradient].
3. Activate the Linear option.
4. Under TEMPERATURE, enter the temperature for column 6, and under INCREMENT, enter the temperature increment.

Alternatively, tap on the arrow above the bar for column 6 and drag the bar upwards or downwards. This varies the temperature.

5. Tap on  to confirm the parameters.

✓ The gradient will be entered into the PCR protocol.

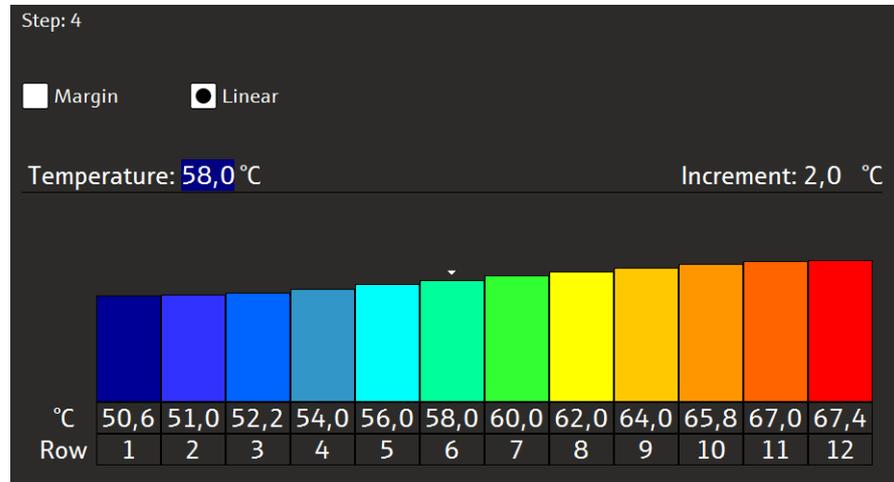


Fig.21 Configuring of a linear gradient

### 4.2.5 Graphical display and programming of the PCR protocol

To get to the schematic representation of the temperature profile, on the THERMOCYCLER page tap on .

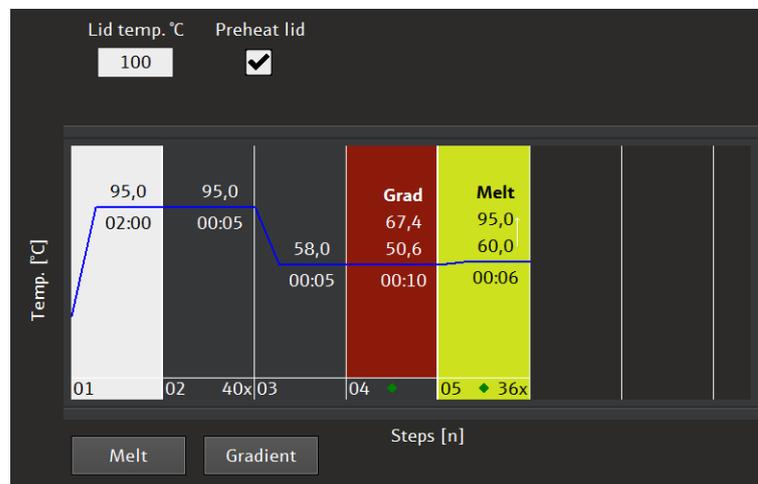


Fig.22 Schematic representation of the PCR protocol

Graph elements

The graph shows the target temperatures of the steps.

Functions	Description
blue curve	temperature curve
white steps	temperature steps that are processed only once

gray steps	temperature steps in a loop The number of repetitions is displayed at the foot of the 1st temperature step of the loop (above: 40 x).
yellow step	DNA melt
red step	marked temperature step This step is currently being edited.
figure above the temperature curve	target temperature of the step
figure below the temperature curve	hold time for the target temperature
	return to the tabular representation of the PCR protocol Any changes made to the PCR protocol will be effective.

#### Editing the PCR protocol

The schematic representation allows quick adjustment of some of the PCR protocol parameters:

- To edit the target temperature, the hold time or the number of repetitions in the loop, tap on the corresponding field and enter the new value.
- The temperature value for each step can also be modified using a finger. To do so, tap on the corresponding curve section and drag the curve upwards or downwards.

#### Note:

New temperature steps can be added only in the table view of the PCR protocol.

## 4.3 Programming the parameters for fluorescence measurements

In real-time PCR, the product accumulation is determined by measuring the increase in fluorescence. The following parameters must be defined:

- Dyes to be measured
- Temperature step of the protocol where a measurement takes place (→ "Free programming of the PCR protocol p. 24").
- Area on the PCR plate that is to be scanned

The dyes to be measured are defined on the SCAN project page.

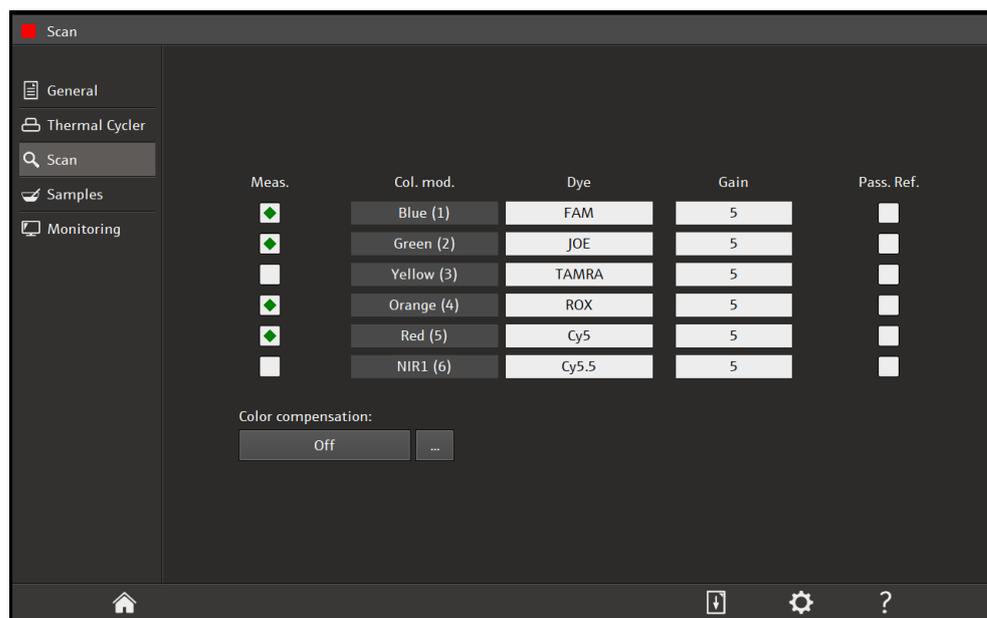


Fig.23 The SCAN project page

#### Configuring of parameters

1. Activate fluorescence measuring for the required color module:  
Tap on the corresponding field of the MEAS. column next to the color module. Activated color measurements are marked with a green diamond.
 

**Note:**  
The number of dyes to be measured does not affect the fluorescence measuring time.
2. Select the dyes:  
Tap on the DYE field and select the dye from the list.
3. Optionally, adjust the GAIN amplification level.
4. If a reference dye is to be used, activate the PASS. REF. option of the color module.
  - ✓ The basic parameters for fluorescence measuring are thus defined.

#### Color compensation

You can correct spectral crosstalk using the color compensation (→ "The Scan project page" p. 12).

#### Manual specification of the scan range

The scan range can be specified based on the plate layout in the sample table (default) or manually. For the thermocycler, the scan range is always defined by column. It must always consist of contiguous columns.

1. Tap on the .
2. Using the EDIT SCAN RANGE slider, set the start and end columns of the range that is to be scanned, or enter the column numbers directly into the input fields.

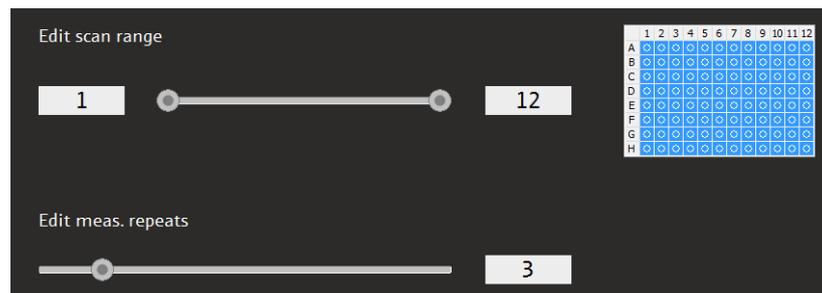


Fig.24 Specifying scan range and repeat measurements

Specification of the number of repeat measurements

The number of repeat measurements for fluorescence measuring can be adjusted. In the case of weak fluorescences, a higher number of repeat measurements may improve the signal-to-noise ratio, but it also extends the measuring time.

1. Tap on .
2. Using the EDIT MEAS. REPEATS slider, select the number, or enter the figure directly into the input field.

Default value: 3

## 4.4 Editing samples

The sample layout is necessary for the evaluation of the experiments and describes the assignment in the sample block. Each well in the block can be assigned a sample type, the genes to be analyzed and the concentrations (for standards). The sample layout does not need to be available at the start of the qPCR run but can also be entered or edited after the qPCR run.

### 4.4.1 Entering sample properties in the layout

To open the EDIT LAYOUT page, on the SAMPLES project page tap on .

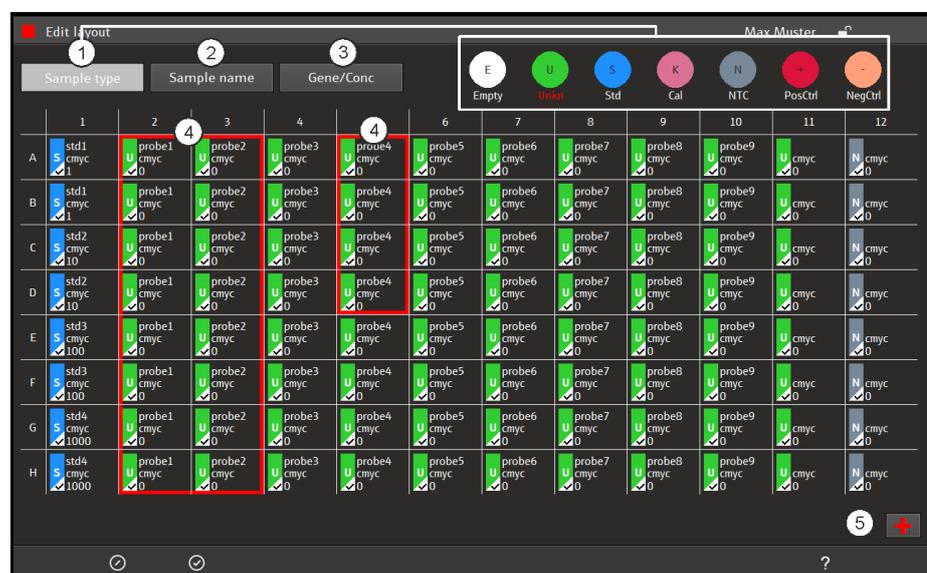


Fig.25 The EDIT LAYOUT page for entering sample properties

Elements of the EDIT  
LAYOUT page

No.	Element	Description
1	[SAMPLE TYPE]	Select the sample type
2	[SAMPLE NAME]	Enter the sample name
3	[GENE/CONC.]	Enter the gene and concentration/unit (for sample standards)
4	marked ranges	The sample properties are assigned to the marked areas. It is possible to mark individual samples or sample ranges.
5	[+]	Activates the "Add" mode that enables marking of multiple, non-contiguous sample ranges. "Add" is active if the plus symbol of the button is displayed in red.

Sample types in  
qPCRsoft touch

The following sample types can be assigned:

Sample type	Icon	Definition
EMPTY		Empty position on the PCR plate/in the layout No evaluation is carried out for empty positions.
UNKN		Sample with unknown concentration or dilution (measuring sample)
STD		Sample with known concentration or dilution
CAL		Sample whose target gene expression level is set as 1
NO TEMPLATE CONTROL (NTC)		Complete reaction preparation without matrix strand
POSCTRL		Positive control preparation for which a reaction product is expected
NEGCTRL		Negative control preparation for which no reaction product is expected

Marking of samples in the  
layout

Samples need to be marked before properties can be entered:

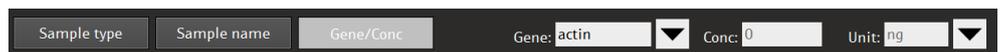
- Mark individual samples by tapping on them.
- To mark multiple samples in contiguous fields, run your finger diagonally or horizontally/vertically across the range.
- To mark entire rows or columns, tap on the row or column designators (A – H or 1 – 12).
- To mark the entire layout, tap on the upper left table field (between 1 and A).
- To mark non-contiguous ranges, tap on the [+]; the "+" turns red. Then mark the ranges one after the other.
  - ✓ Marked samples or ranges in the layout are framed in red.

Editing samples

Samples with identical sample properties (sample name, sample type, same gene-dye-assignment) are regarded as replicates. The individual values of these samples are averaged and their mean value is used for the remaining calculations.

With a singleplex assay, samples can have the same sample name and sample type but may differ with regard to the gene-dye-assignments. These samples are identified as associated samples due to the same name. The evaluation, however, is performed individually.

1. On the SAMPLE project page, first activate the dye for which the layout is to be entered (→ "The Samples project page / Layout view" p. 14).
2. Tap on . The EDIT LAYOUT page appears.
3. Mark samples in the layout that have the same properties.
4. Assign a sample type:
  - Tap on [SAMPLE TYPE] and then tap on the symbol area for the sample type.
5. Enter the sample name:
  - Tap on [SAMPLE NAME] and enter the designation into the field.
6. Enter gene and concentration:
  - Tap on [GENE/CONC.].
  - Select the desired gene from the GENES list or enter a new gene designation into the field.
  - For standards: Enter the concentration into the CONC field, and select the concentration unit from the UNIT list.
- ✓ The properties were assigned to the marked samples.
7. Edit all further samples in the same way.
8. Use  to confirm the entries and return to the LAYOUT page. If required, activate the next dye and repeat the layout editing process.

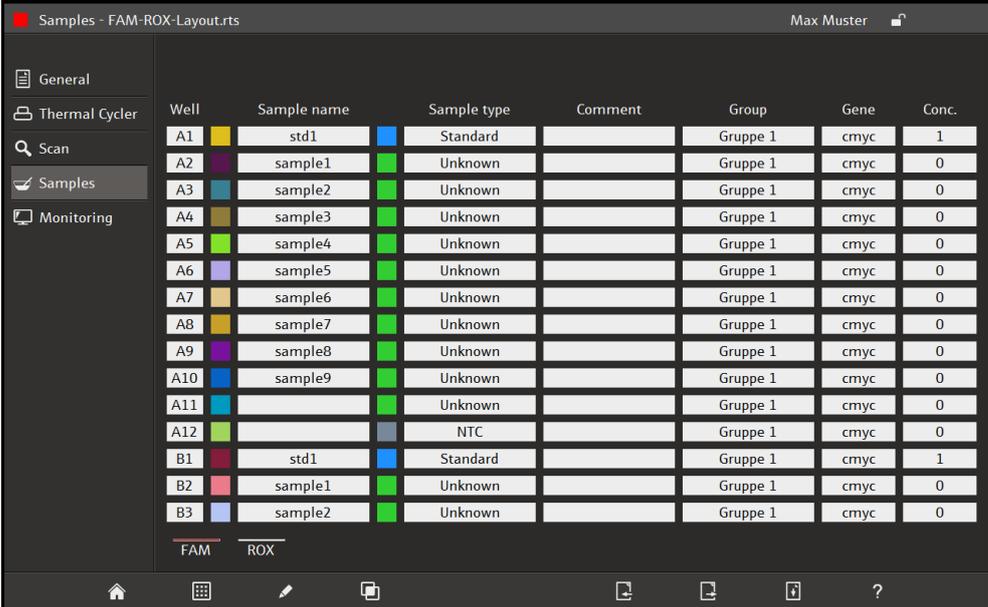


Sample type	Sample name	Gene/Conc	Gene: actin ▼	Conc: 0	Unit: ng ▼
-------------	-------------	-----------	---------------	---------	------------

**Fig.26** Input fields/lists for gene, concentration and concentration unit

## 4.4.2 Displaying sample properties in the table

The sample assignment/properties of a block can be displayed in tabular form.



Well	Sample name	Sample type	Comment	Group	Gene	Conc.
A1	std1	Standard		Gruppe 1	cmyc	1
A2	sample1	Unknown		Gruppe 1	cmyc	0
A3	sample2	Unknown		Gruppe 1	cmyc	0
A4	sample3	Unknown		Gruppe 1	cmyc	0
A5	sample4	Unknown		Gruppe 1	cmyc	0
A6	sample5	Unknown		Gruppe 1	cmyc	0
A7	sample6	Unknown		Gruppe 1	cmyc	0
A8	sample7	Unknown		Gruppe 1	cmyc	0
A9	sample8	Unknown		Gruppe 1	cmyc	0
A10	sample9	Unknown		Gruppe 1	cmyc	0
A11		Unknown		Gruppe 1	cmyc	0
A12		NTC		Gruppe 1	cmyc	0
B1	std1	Standard		Gruppe 1	cmyc	1
B2	sample1	Unknown		Gruppe 1	cmyc	0
B3	sample2	Unknown		Gruppe 1	cmyc	0

Fig.27 Sample layout in the tabular format

To display the sample table

1. On the SAMPLE project page, tap on .
  - ✓ The sample table is displayed.
2. You can scroll through the table by wiping upwards and downwards in the table.
3. Tap on  to return to the layout view.

## 4.4.3 Combining of experiments into groups

During a qPCR run, multiple experiments can run simultaneously in the sample block. A group combines samples that belong to the same experiment. All the reaction preparations in a group are evaluated together. A maximum of 12 groups can be defined.

To open the EDIT GROUPS page, on the SAMPLES page tap on .



Fig.28 The EDIT GROUPS page for combining experiments in groups

Elements of the EDITS GROUPS page

No.	Element	Description
1	Group buttons	Assign marked ranges to a group.
2	Layout diagram	Samples belonging to a group are identified by the group color and by the group number.
3	[+]	Button for simultaneous marking of samples

Combining of experiments into groups

By default, all samples in the layout are assigned to group 1.

1. On the SAMPLE page, tap on . The EDIT GROUPS page appears.
2. In the layout, mark all the samples belonging to the same experiment (see section "Entering sample properties in the layout" p. 31).
3. Tap on the group icon.
  - ✓ The group color and the group number are assigned to the marked samples.
4. If desired, change the name of the group by tapping on the white input field below the group icon.
5. Edit all further samples in the same way.
6. Use  to confirm the entries and return to the LAYOUT page.
  - ✓ The background of the samples is displayed in the group color.

#### 4.4.4 Displaying the simple layout

To switch to the simply layout view, on the SAMPLE project page, tap on .

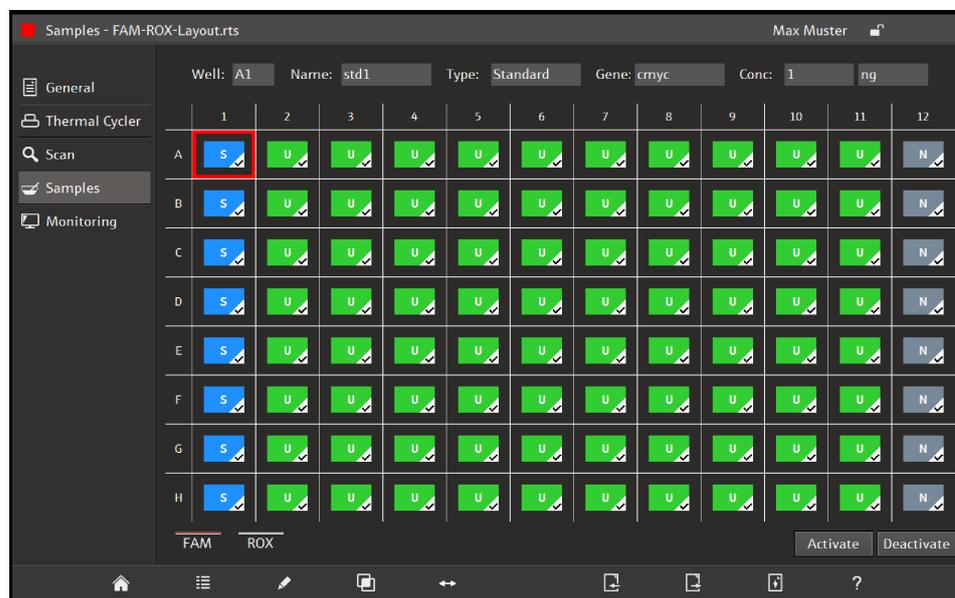


Fig.29 The simple sample layout

The following information is displayed:

- Sample type
- Activation status for measurement and evaluation
- Sample properties of the marked sample

#### 4.4.5 Exporting and importing the sample layout

The layout can be exported or imported as an Excel file (\*.xls). If you wish to create your own layouts in Excel, you should first export a layout to be used as a template.

Exporting a sample layout

1. On the SAMPLES project page, tap on .
2. Select the storage location: Tablet PC or USB stick.
3. In the SAVE LAYOUT AS field, enter the file name.
4. Tap on .
  - ✓ The layout data are exported and can be edited in Excel.

**Note:**

The folder used to save data on the tablet will always be C:\qPCRsoftTouch. Any number of subfolders can be created in this folder using the Data function.

Importing a sample layout

1. On the SAMPLE project page, tap on .
2. Select the storage location: Tablet PC or USB stick.

3. Select a file and tap on .

  - ✓ The layout data are imported.

## 5 Monitoring

All the functions required for starting and monitoring a real-time PCR experiment run can be found on the MONITORING page (→ "The Monitoring project page" p. 15).

### 5.1 Starting a qPCR run

Starting preconditions	<p>A template containing the following information is required to start a PCR run:</p> <ul style="list-style-type: none"><li>▪ PCR protocol sequence (→ "Programming the PCR protocol" p. 22)</li><li>▪ Parameters for fluorescence measuring (→ "Programming the parameters for fluorescence measurements" p.29)</li></ul> <p>The sample layout information can also be entered at a later time.</p>
To start the qPCR run	<ol style="list-style-type: none"><li>1. Insert the sample plates or sample tubes into the block of the qTOWER<sup>3</sup> touch and close the heated lid (see also the operating instructions for "qTOWER<sup>3</sup> touch").</li><li>2. Create or open the template for the project.</li><li>3. Go to the MONITORING page.</li><li>4. Tap on .</li></ol> <p>✓ The qPCR run starts. The progress is displayed on the MONITORING page.</p>
To interrupt the qPCR run	<p>A qPCR run can be interrupted and continued later.</p> <ol style="list-style-type: none"><li>1. On the MONITORING page, tap on .</li></ol> <p>✓ The qPCR run is interrupted at the current step. The Pause button flashes red.</p> <ol style="list-style-type: none"><li>2. Tap on the flashing Pause button again.</li></ol> <p>✓ The qPCR run is continued.</p>
To stop the qPCR run	<ul style="list-style-type: none"><li>▪ On the MONITORING page, tap on .</li></ul> <p>✓ The qPCR run stops and cannot be continued.</p>

## 5.2 Monitor display

During the qPCR run, the fluorescence curves (intensity of the fluorescence as a function of the cycle number) are recorded in the diagram on the MONITORING page.

Amplification curves

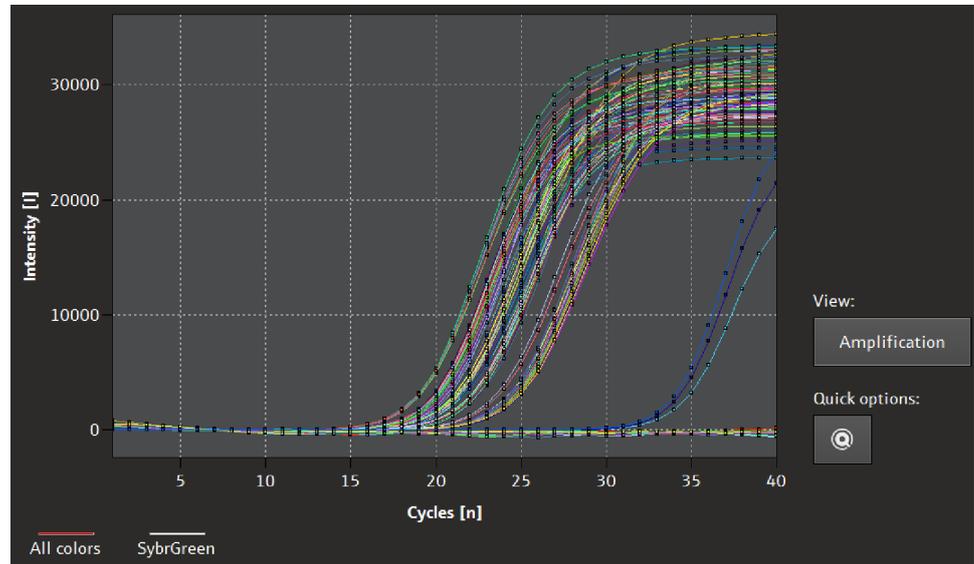


Fig.30 Amplification curves

Via the selection of the dyes in the bottom left corner, the user can choose between the overlaid representation of the measuring results with all dyes and the display of the individual dyes.

Under VIEW, the display of AMPLIFICATION, RAW DATA and MELTING CURVE is available.

The assignment of curve colors is made in qPCRsoft touch under Options (→ "qPCRsoft touch software settings" p. 46).

Changing of display options

The graphical representation can be adjusted during or after the qPCR run.

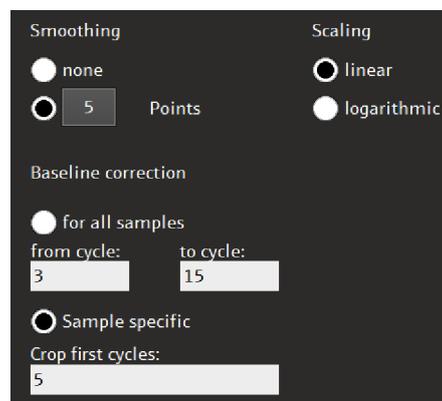


Fig.31 Monitoring options

Parameter	Description
SMOOTHING	Smoothing of the measurement data: NONE, 2 – 12 points
SCALING	Scaling of the intensity axis: LINEAR OR LOGARITHMIC

BASELINE CORRECTION	FOR ALL SAMPLES:	Determine the baseline for each sample in the same range Enter the lower and upper range limits in the FROM CYCLE and TO CYCLE fields.
	SAMPLE SPECIFIC	This correction is recommended for samples with different Ct values. Enter the lower range limit in the CROP FIRST CYCLES field. The upper range limit is determined separately for each sample by an algorithm.

1. On the MONITORING page, tap on .
2. Edit the parameters. Tap on .
  - ✓ After the OPTIONS MONITORING page is closed, the graph will be refreshed.

#### Quick options

The parameters for base line and scaling can be varied. The type of base line correction, however, can only be selected on the OPTIONS MONITORING page (see above).

1. On the Monitoring page, tap on QUICK OPTIONS .
2. Adjust the scaling of the intensity axis or the base line parameters.
  - ✓ The changes will immediately take effect in the graph

## 5.3 Showing and hiding the data for individual samples

The display of the fluorescence curves and of the evaluations on the MONITORING page is controlled via the activation/deactivation of the samples in the SAMPLE LAYOUT.

1. Go to the SAMPLES page.
2. Mark individual samples or samples in contiguous ranges (→ "Entering sample properties in the layout" p. 31).
3. Tap on [ACTIVATE] or on [DEACTIVATE].
4. The process can be repeated until all samples are activated or deactivated.
  - ✓ Activated samples are marked in the layout by a checkmark. Only these samples are displayed on the MONITORING page and included in the calculation.

## 5.4 Calculating the Ct value

Elements of the Calculate Ct page

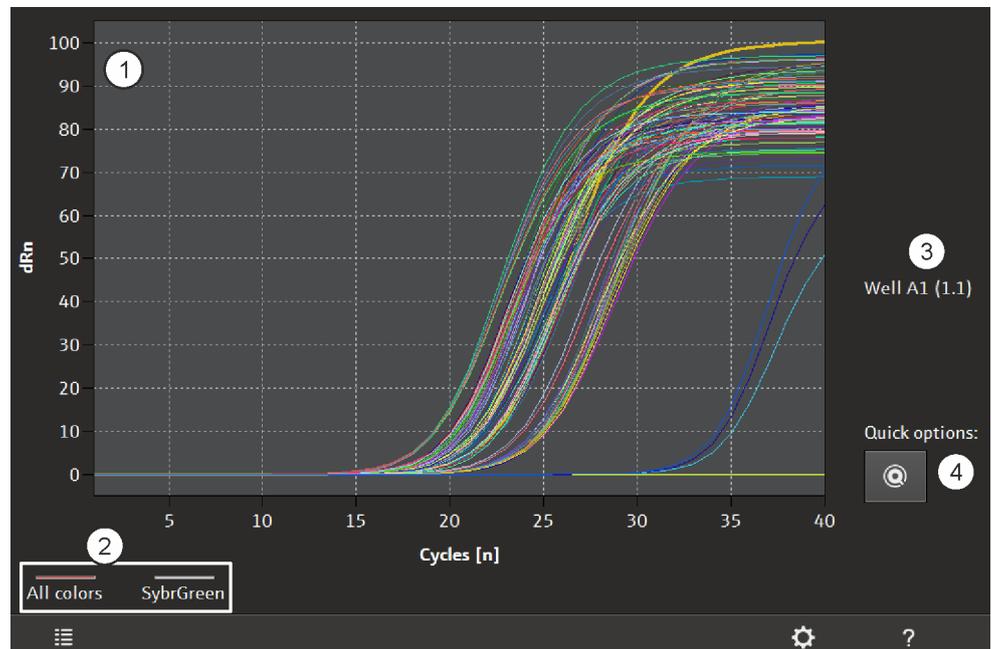


Fig.32 The CALCULATE CT page

No.	Element	Description
1	Graph	Display of the amplification curves
2	Dyes	Select the target gene/dye combination for the display The selected dye is marked with a red bar.
3	WELL	Selected well The selected well is marked in the graph with a thicker line. The selection is made by tapping on it in the graph or by marking it in the table (see below).
4	QUICK OPTIONS	Selecting baseline correction and scaling of the intensity axis

The following icons are activated on the CALCULATE CT page:

Icon	Description
	Options for Ct calculation
	Ct values are output into a table

Options for Ct values

After selecting , the following options can be specified for the Ct values:

Option	Description
SMOOTHING	Number of smoothing points for the amplification curves: NONE, 2 - 12
SCALING	Select scaling of the dRn intensity axis: LINEAR or LOGARITHMIC
BASELINE CORRECTION	Select the type of baseline correction: FOR ALL SAMPLES or SAMPLE SPECIFIC (see section "Monitor display" p. 39)

FILTER	Valid real-time PCR amplification curves are smoothed by the filter to a slight, medium or high degree, depending on the selected option.
CANCEL NOISE	If noise reduction is activated, fluorescence curves that are not recognized by the software as valid real-time PCR amplification curves are represented as zero lines. This makes sure that Ct values are not also calculated for invalid real-time PCR amplification curves.
AUTO THRESHOLD	Activated: Recalculate the threshold when settings were changed Deactivated: Maintain the previous Threshold

To calculate the Ct value

1. On the MONITORING page under VIEW, select the AMPLIFICATION or RAW DATA option.
2. Tap on .
3. Optionally: Tap on  and edit the options for the calculation of Ct values.
4. Tap on .
  - ✓ The Ct values of the individual samples are output into the results table.

**Note:**  will take you back to the graph or via quick access back to the Monitoring page.

Well	Sample name	Sample type	Dye	Gene	Ct	Mean Ct
A1	1.1	Calibrator	SybrGreen	actin	20,16	20,28
A2	1.1	Calibrator	SybrGreen	actin	20,41	20,28
A3	1.1	Calibrator	SybrGreen	actin	20,28	20,28
A4	1.1	Calibrator	SybrGreen	GCase	19,12	19,03
A5	1.1	Calibrator	SybrGreen	GCase	19,13	19,03
A6	1.1	Calibrator	SybrGreen	GCase	18,85	19,03
A7	Cal_1cop_a	Calibrator	SybrGreen	actin	19,28	19,32
A8	Cal_1cop_a	Calibrator	SybrGreen	actin	19,23	19,32
A9	Cal_1cop_a	Calibrator	SybrGreen	actin	19,45	19,32
A10	Cal_1cop_a	Calibrator	SybrGreen	GCase	19,57	19,53

**Fig.33** Table of Ct results

The results table contains the following information:

Column	Meaning
WELL	Position of the sample
Color field	Graph color Each well is assigned a color that is used to represent the corresponding fluorescence curve. The color scheme is selected in qPCRsoft touch under OPTIONS (see section "qPCRsoft touch software settings" p. 46).
SAMPLE NAME	Name of the sample
SAMPLE TYPE	Type of the sample
DYE	Dye
GENE	Name of the gene analyzed in the sample
CT	Ct value of the sample

MEAN Ct	Mean Ct value of replicates
---------	-----------------------------

### Exporting of results

The results can be exported into the following formats:

- Excel table (\*.xls)
- CSV file (\*.csv)

1. Below the results table, tap on 
2. Select the export format
3. Enter the file name into the field.
4. Tap on .

✓ The result data are exported and stored in the selected folder of the tablet.

## 5.5 Calculating the melting temperature

### Elements of the Melting curve page

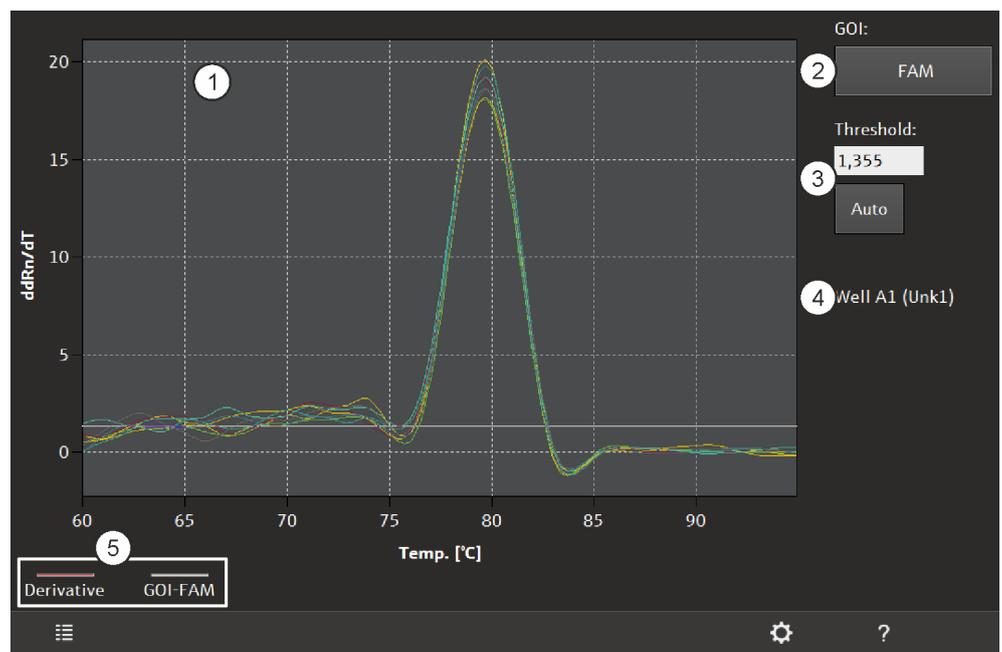


Fig.34 The MELTING CURVE page

No.	Element	Description
1	Graph	Representation of the melting curve or of its derivatives - $dRn/dT$
2	GOI	(Gene of Interest) Select the target gene/dye combination For purposes of melting curve analysis, the dye selected for the target gene must generally be an intercalating dye.

3	THRESHOLD	<p>Only for the DERIVATIVE view: Select the threshold value</p> <p>Only curves with a maximum <math>dRn/dT</math> that is greater than the threshold are evaluated.</p> <p>Determine the threshold automatically: Tap on [AUTO].</p> <p>Enter the threshold manually: Enter the value in the input field or move the horizontal threshold line in the graph using your finger.</p>
4	WELL	<p>Selected well</p> <p>The selected well is marked in the graph with a thicker line. The selection is made by tapping on it in the graph or by marking it in the table (see below).</p>
5	DERIVATIVE / GOI	<p>Show the melting curves or their derivative - <math>dRn/dT</math></p> <p>The selected view is marked with a red bar.</p>

The following icons are activated on the MELTING CURVES page:

Icon	Description
	Options for melting curve analysis
	Output melting temperatures into a table

Options for melting curves

Option	Description
SMOOTHING	Number of smoothing points for the melting curves: NONE, 2 - 12
FLIP CURVE	Invert melting curves that show increasing fluorescence during the melting process (e.g. in protein analysis)
SCALING	<p>Specify the starting point for the calculation of the melting curves</p> <p>ALL CURVES START AT 100 %</p> <p>The melting curves are normalized in such a way that their initial fluorescence at start temperature is 100%. As a result, the peaks in the derivative have approximately the same height, but oscillations of the derivative curve may occur in the case of weak samples.</p> <p>MAXIMUM INITIAL FLUORESCENCE = 100 % (default)</p> <p>The initial fluorescence of the most intensive sample is normalized to 100 % at start temperature, and all other samples relative to that.</p>

To calculate the  $T_m$

1. On the MONITORING page under VIEW, select the MELTING CURVE option.
2. Tap on .
3. In the GOI list select the dye (the gene).
4. Change to the DEVIATION view and specify the threshold value to distinguish between significant and insignificant peaks.
5. Tap on .
  - ✓ The melting temperature values of the individual samples are output into the results table.

**Note:**  will take you back to the graph or via quick access back to the Monitoring page.

Table of melting temperatures

Well	Sample name	Sample type	T <sub>m</sub>	Mean T <sub>m</sub>	Std.Dev. Mean T <sub>m</sub>
A1	Unk1	Unknown	79,7	79,7	0
B1	Unk2	Unknown	79,6	79,6	0
C1	Unk3	Unknown	79,6	79,6	0
D1	Unk4	Unknown	79,7	79,7	0
E1	Unk5	Unknown	79,7	79,7	0
F1	Unk6	Unknown	79,7	79,7	0
G1	Unk7	Unknown	79,7	79,7	0
H1	Unk8	Unknown	79,7	79,7	0

**Fig.35** Table of melting temperature results

The results table shows the following information:

Column	Meaning
WELL	Position of the sample
Color field	Graph color Each well is assigned a color that is used to represent the corresponding fluorescence curve. The color scheme is selected in qPCRsoft touch under OPTIONS (see section "qPCRsoft touch software settings" p. 46).
SAMPLE NAME	Name of the sample
SAMPLE TYPE	Type of the sample
T <sub>M</sub>	Melting temperature of the sample
MEAN T <sub>M</sub>	Mean melting temperature of replicates
STD.DEV.MEAN T <sub>M</sub>	Standard deviation of the mean melting temperature of replicates

Exporting of results

The results of the melting temperature calculation can be exported in the same way as the Ct values (see "Calculating the Ct value" p. 41).

## 6 qPCRsoft touch software settings

Under [OPTIONS] on the HOME page, you can specify basic settings for the qPCRsoft touch software. You can also exit qPCRsoft touch from this page.

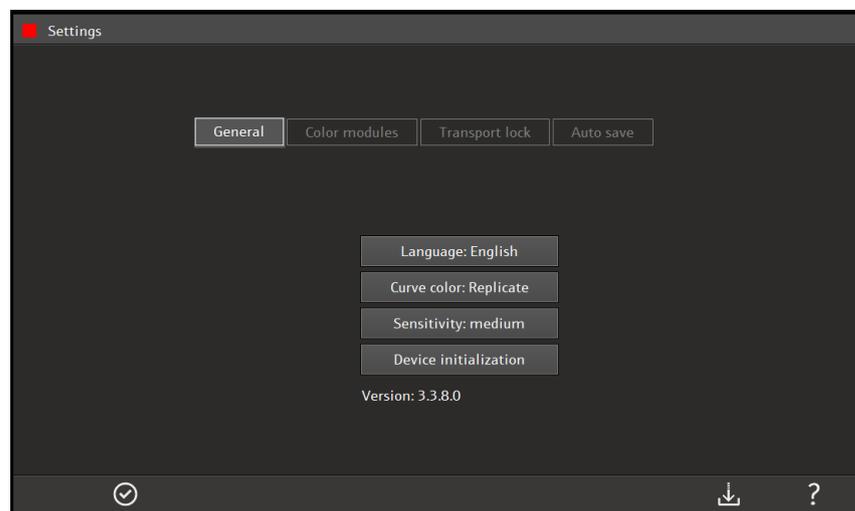


Fig.36 SETTINGS page with basic software settings and software version number information

Icon buttons

Icon	Function
	Accept changes and exit page SETTINGS
	Update qPCRsoft touch

Setting general program options

For setting the program options, tap on [GENERAL]. The following functions can be defined:

Function	Options	Description
[LANGUAGE]	GERMAN ENGLISH	Select the language of the user interface.
[CURVE COLOR]	SAMPLE TYPE REPLICATE WELL	Fluorescence curves can be displayed grouped by color according to one of the three options.
[SENSITIVITY]	HIGH MEDIUM LOW	Set the basic sensitivity of the detection system. This setting affects all dyes and should only be changed if particularly weak or intensive samples are to be measured. The default setting is medium.
[DEVICE INITIALIZATION]		If the qTOWER <sup>3</sup> touch no longer responds to inputs, the device can be reset here.
VERSION		Number of software version

Setting color modules

The positions of the color modules used in the device are set under [COLOR MODULES].

1. Enable the EDIT POSITION option.

2. Tap on the corresponding position button and select the module from the list. Set the NONE option for unoccupied positions.

✓ The settings are applied when leaving the OPTIONS page.

#### Installing the transport lock

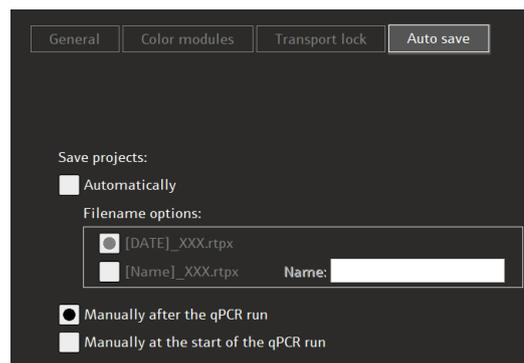
Before the device is shipped, e.g. for service purposes, the transport lock must be installed.

- On the SETTINGS page, tap [TRANSPORT LOCK].
- Insert the red backup plate or an empty PCR plate into the block and tap [SET]. Then switch off the unit.
- The transport lock is released automatically when the unit is switched on or manually by tapping on [RELEASE].

#### Saving projects

With [AUTO SAVE] you can set the parameters for saving projects:

Option	Description
AUTOMATICALLY	Projects will be automatically saved. DATE                      The file name is generated from the date and a consecutively number. Name                      The file name is generated from a freely editable name (enter in the input field) and a consecutively number.
MANUALLY AFTER THE qPCR RUN	After the qPCR run, the SAVE PROJECT page opens for entering file name for the project.
MANUALLY AT THE START OF THE qPCR RUN	After the start of the qPCR run the SAVE PROJECT page opens. The qPCR run does not start until the file name has been enters and confirmed.



**Fig. 37** Options for saving projects

#### Software Update

Depending on the device variant (→ "Starting and exiting qPCRsoft touch" p. 6), different steps may be necessary:

1. Transfer the setup files to a USB stick.  
 For device variant 2: The setup file must be located in the root directory of the USB stick, not in a subfolder.
3. Connect the USB stick to the USB port of the device.
4. Variant 1 (up to delivery year 2018):

- 
- Tap  on the OPTIONS page.  
The tablet's operating system (Windows 8.1) opens.
  - Start the setup file using Windows Explorer.
5. Variant 2 (from delivery year 2019):
- Tap  on the OPTIONS page.  
qPCRsoft touch is terminated and the software update is started automatically.  
The update is complete when the corresponding message appears.
6. Switch qTOWER<sup>3</sup> touch off and on again to start the updated software.

## 7 Data Management

qPCRsoft touch has data management features that enable, among other things, exchanging of data between tablet and USB stick.

### Data formats

The following data formats are available in qPCRsoft:

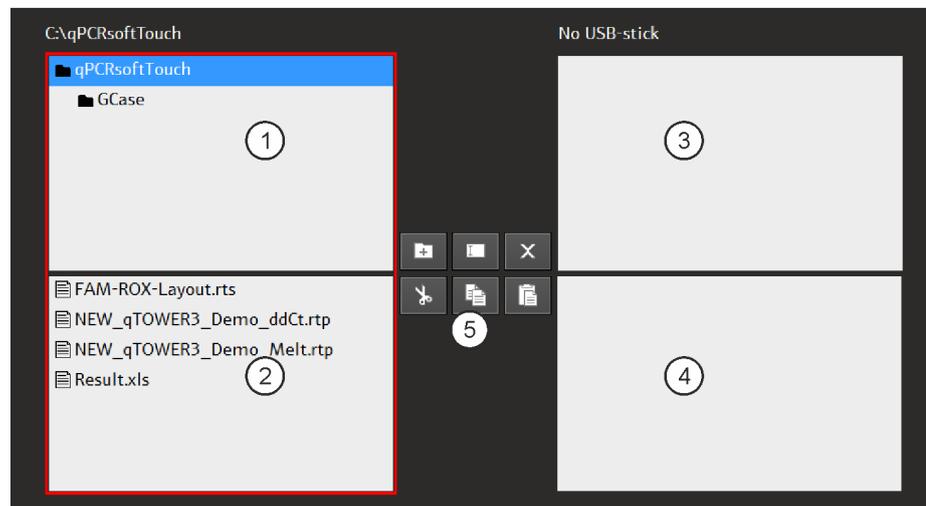
Extension	File type
*.rtp / *.rtpx	Project file with real-time PCR data
*.rts / .rtsx	Template for real-time PCR experiment
*.xls	Excel file: <ul style="list-style-type: none"> <li>▪ Results file of a Ct value or melting temperature calculation</li> <li>▪ File with sample layout</li> </ul>
*.csv	▪ Results file of a Ct value or melting temperature calculation;

### Note

The folder used to save data on the tablet will always be C:\qPCRsoftTouch. Any number of subfolders can be created in this folder using the Data function.

### Opening the data management

- On the HOME page, tap on DATA.
  - ✓ The MANAGE DATA page appears.



**Fig.38** The MANAGE DATA page

### Elements

The MANAGE DATA page contains 4 overviews of the folders and files on the tablet and on the USB stick:

No.	Function
1	Folders on the tablet including user-created subfolders
2	Files in the selected tablet folder
3	Folders on the USB stick
4	Files in the selected folder on the USB stick
5	Data management icons

## Icons

Icon	Function
	Create a new folder
	Rename folder/file
	Delete a folder/file
	Cut folder/file Folder/file is copied to the clipboard and removed from its original location
	Copy folder/file to the clipboard
	Paste folder/file from the clipboard into a selected folder

## Exchange folders/files between tablet and USB stick

Files are exchanged between a folder and the USB stick in the following way:

1. Tap on the file that is to be transferred.
  - ✓ The marked file is identified by a blue bar.
2. Use  to copy it to the clipboard, or  to cut it.
3. Tap on the target folder.
4. Use  to paste the file into the target folder.
  - ✓ The file has thus been transferred. To transfer folders, proceed in the same way.

## Create a new folder

1. In the folder area of the tablet or of the USB stick, tap on the folder in which a new subfolder is to be created.
2. Tap on .
3. Enter a name for the folder.
4. Tap on .
  - ✓ The new folder is inserted into the folder structure.

## Rename a file/folder

1. Tap on the folder or file.
2. Tap on .
3. Enter the new name.
4. Tap on .
  - ✓ The folder or the file appears under the new name.

## Delete a folder/file

1. Tap on the folder or file.
2. Tap on .
3. A confirmation prompt appears. Tap on  to confirm deletion of the folder/file.
  - ✓ The folder or file is deleted.