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Rotor-Gene AssayManager v1.0
UDT Basic Plug-in User Manual

REF R3

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Sample to Insight

QIAGEN

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Welcome to the Rotor-Gene AssayManager v1.0 UDT Basic Plug-in User Manual.

1.1 Safety Information

User-friendly Rotor-Gene AssayManager™ v1.0 has been specifically developed for use with up to four different Rotor-Gene® Q instruments. Before using Rotor-Gene AssayManager v1.0, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the cycler and to maintain the instrument in a safe condition.

The Rotor-Gene AssayManager v1.0 user manual does not provide detailed information about the Rotor-Gene Q instrument and hardware maintenance. The Rotor-Gene AssayManager v1.0 user manual only describes the functionality of the Rotor-Gene AssayManager v1.0 software in combination with Rotor-Gene instruments.

Note: The terms "Rotor-Gene Q" and "Rotor-Gene Q instrument", used in this manual, apply to all Rotor-Gene Q and Rotor-Gene Q MDx instruments (not available in all countries) unless otherwise specified.

1.2 Introduction

Thank you for choosing Rotor-Gene AssayManager v1.0. We are confident it will become an integral part of your laboratory.

Rotor-Gene AssayManager v1.0 is a software for routine testing in combination with Rotor-Gene Q instruments. Rotor-Gene AssayManager v1.0 is able to read in sample information, set up experiments, control up to four different Rotor-Gene Q cyclers, acquire data from these instruments, automatically analyze results, and create reports.

Rotor-Gene AssayManager v1.0 consists of different components working together. The core application is complemented by different plug-ins that contain assay type specific analysis and visualization of the results. The core application is mandatory for working with Rotor-Gene AssayManager v1.0. Optionally additional plug-ins can be installed. At least one plug-in must be installed. Not all plug-ins may be available in all countries. Refer to ▶ www.qiagen.com/Products/Rotor-GeneAssayManager.aspx to discover our continuously expanding range of plug-ins.

1.2.1 Provided User Manuals

The core application and every available plug-in have their own user manuals with specific information about the functionality of the different Rotor-Gene AssayManager v1.0 components. The user manuals provide a context-sensitive help that can be started by simply pressing the "F1" key.

When installing additional plug-ins, the corresponding user manuals are automatically added to the existing help system. Alternatively the different user manuals can be accessed, read, and printed as *.pdf files.

Rotor-Gene AssayManager v1.0 Core Application user manual	▪ Provides a description of the software. ▪ Describes functions that are the same for the core application and all different plug-ins. ▪ Provides information about troubleshooting.
Rotor-Gene AssayManager v1.0 plug-in user manuals	Provide details on ▪ how to use the assay type specific plug-ins ▪ their functionalities.

1.2.2 About this User Manual

This user manual provides information about Rotor-Gene AssayManager v1.0 UDT Basic Plug-in, version 1.0.x (where x ≥6) in the following sections:

1. Introduction
2. UDT specific tasks and procedures

1.2.3 General Information

Policy Statement

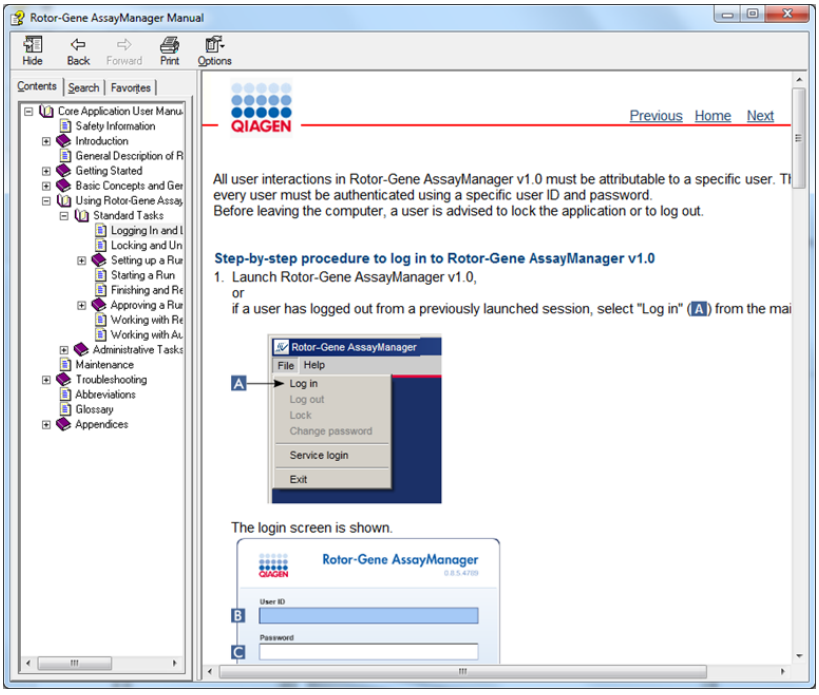
It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time. In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

Version Management

This document is the Rotor-Gene AssayManager v 1.0 UDT Basic Plug-in User Manual, which provides information about the UDT Basic Plug-in, version 1.0.x (where x ≥6).

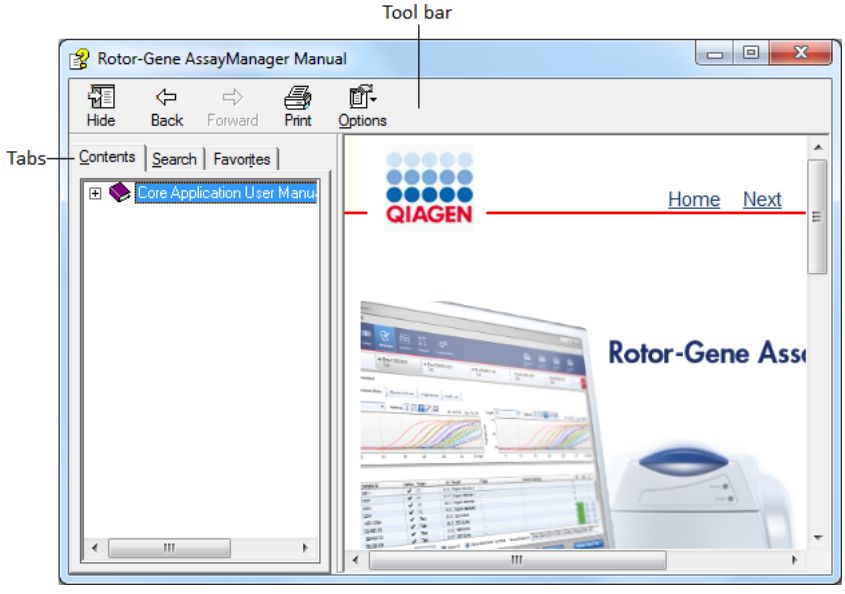
1.2.4 Getting Help

Rotor-Gene AssayManager v1.0 comes with a detailed help system. The help is provided as *.pdf file and as *.chm file (compiled help file). The following image shows the help page corresponding to the login screen as an example:



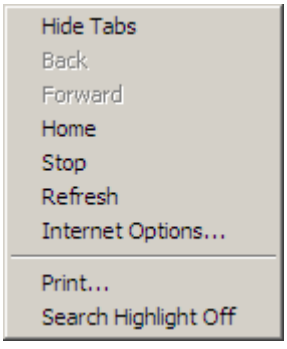
Rotor-Gene AssayManager v1.0 has a context-sensitive help system. After pressing the "F1" key in dialog boxes, a context-sensitive help page is shown.

Using Rotor-Gene AssayManager v1.0 Help



The help file contains two functional areas:
- Tool bar
- Tabs

The tool bar contains the following buttons:

Name	Icon	Description
"Hide" or "Show"		Hides the left-hand side navigation tab. To display the navigation tab again, click "Show". This button appears instead of "Hide".
"Back"		Returns to the previous screen.
"Forward"		Returns to the screen displayed before using the "Back" button.
"Print"		The user has the choice: 1) Print the selected topic. 2) Print the selected heading and all subtopics. Select one option and confirm with "OK" or select "Cancel" to go back.
"Options"		Opens the options menu with the following entries: 

The navigation tab contains the following tabs:

Name	Description
"Contents"	In the "Contents" tab the help content can be browsed by topics.
"Search"	Specific help topics can be found by entering search terms.
"Favorites"	Shortcuts to individual help topics can be added and managed.

1.3 UDT Basic Plug-in Specific Tasks and Procedures

Tasks and procedures specific for the UDT Basic Plug-in are described in this chapter. For a general description refer to the Rotor-Gene AssayManager v1.0 Core Application User Manual.

1.3.1 Approving Samples

The general functionality of the "Approval" environment is described in the core application user manual. Here only the functionality dedicated to the UDT Basic Plug-in is described.

1.3.1.1 Reviewing Assay Data

Step-by-step procedure to review data of a specific assay

After starting the approval process, a screen is opened, split in two main areas: "Plots and information" and "Results". If multiple assays were selected, all the selected assays will be listed in the tab list.

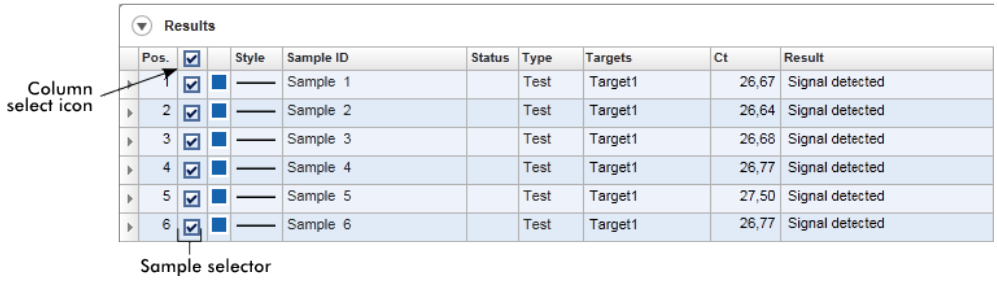
Depending on the assay type, experiment information can be reviewed in six different sub tabs:

- "Raw data"
- "Processed data"
- "Standard curve"
- "Experiment"
- "Assay"
- "Audit trail"

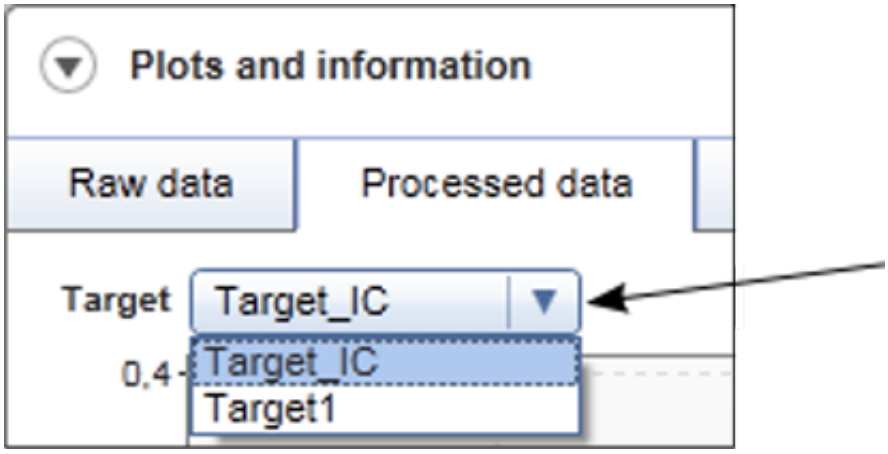
By default the "Experiment" sub tab is opened upon starting the approval process.

Step-by-step procedure to review the amplification plots using the "Raw data" and the "Processed data" sub tab

1. To display only the amplification curves of specific samples:
   a) By default all samples of an assay are selected. Click the "Column select" icon in the header of the results table to deselect all samples.



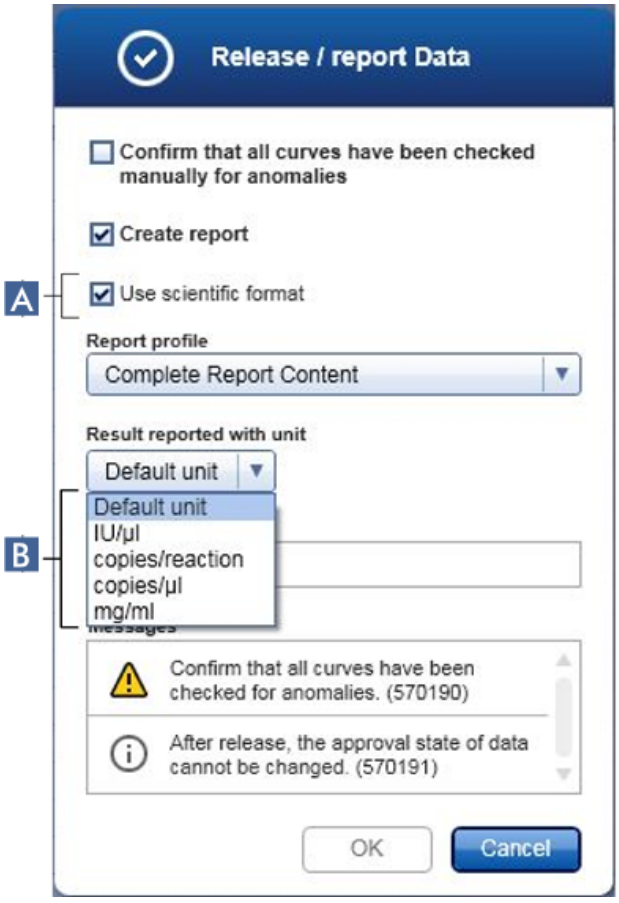
   b) Click the "Sample selector" check box of the samples whose amplification curve should be displayed.
2. Select the target from the "Target" drop-down menu.



3. Review the individual amplification curves.

Scientific Format View

Options to display results in scientific format (A) and to choose the concentration unit in the report overview table (B) are available. If the check box is activated (A), all concentrations in the report are displayed in scientific format.



1.3.1.2 Calculating Sample Concentration

Preconditions

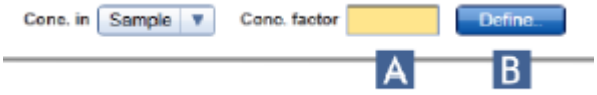
For quantitative assays, Rotor-Gene AssayManager v1.0 displays the concentration in the eluate and in the original sample, based on information given in the assay profile. If the following apply, it is possible to define sample input volume and elution volume in the "Approval" environment:

- The assay is quantitative
- In the assay profile, an Assay Parameter Set is defined, but the sample transfer and initial elution volume are not defined ▶ Creating an Assay Profile
- The work list for the run was generated by importing a QIAsymphony AS result file from an independant QIAsymphony AS run.

Only if these preconditions apply is it possible to provide the information about sample input and initial elution volume in the "Approval" environment. Using this information, Rotor-Gene Assay manager can convert the concentration from the eluate to the concentration in the sample.

Step-by-step procedure to define the sample input and initial elution volume

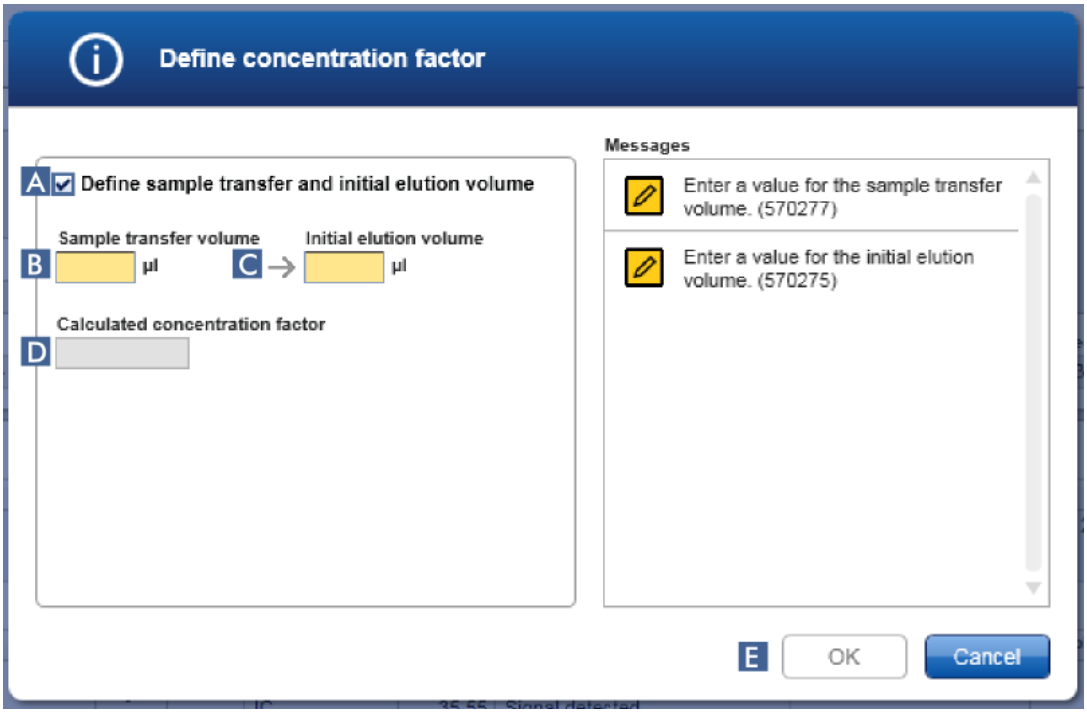
1. If available for the experiment, a field "Conc. factor" (A) and a button "Define.." (B) are shown below the results table.



Note
No concentration at the sample level is displayed until the concentration factor is defined.

Note
The release button is disabled until the concentration factor is defined.

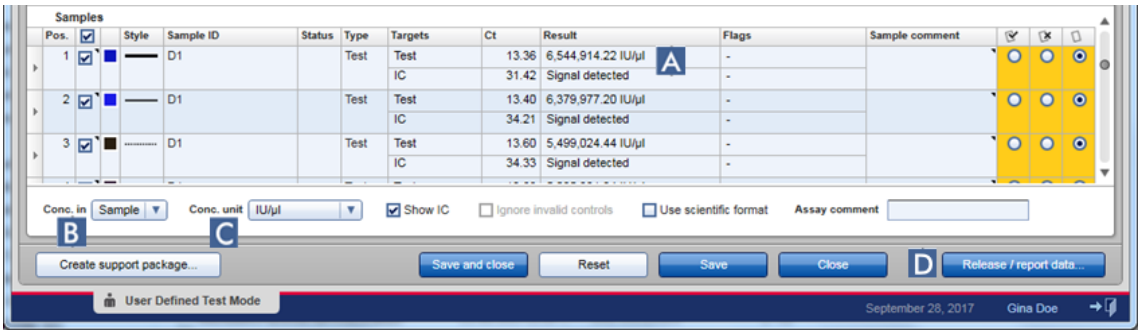
2. Click "Define..". A dialog box opens that enables the concentration factor to be defined.



To define a concentration factor
a) Activate the check box "Define sample transfer and initial elution volume" (A).
b) Enter the sample transfer volume (B).
c) Enter the initial elution volume (C).
d) The calculated concentration factor will be displayed (D).
e) Click "OK" (E).

If no concentration factors are to be defined
a) Deactivate the check box "Define sample transfer and initial elution volume" (A).
b) Click "OK" (E). No concentration at the sample level will be displayed.

3. After the concentration factor has been defined, the following will happen.

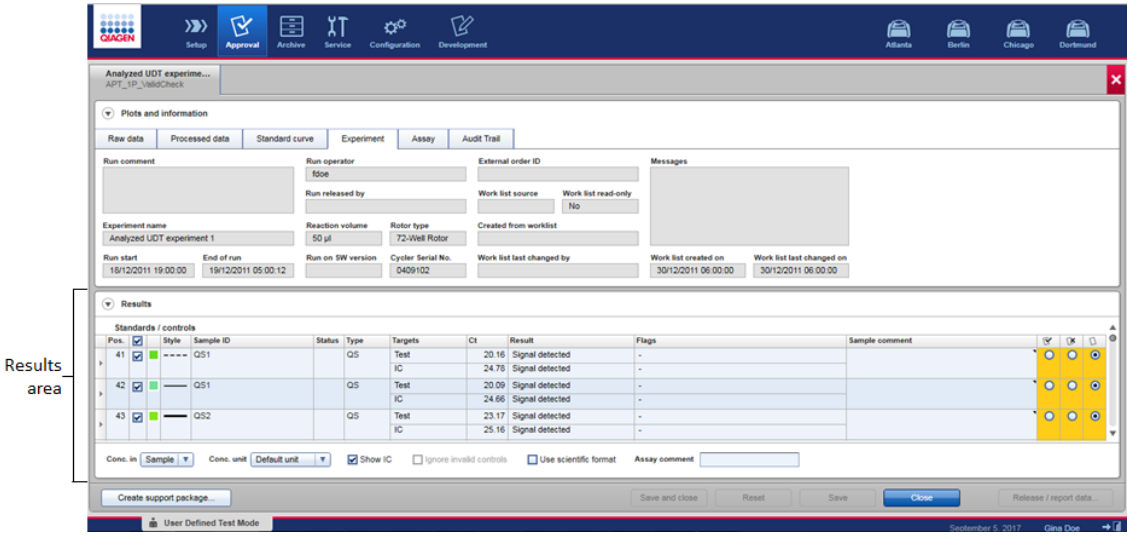


- If "Conc. in sample" (B), is chosen, a quantitative result is displayed (A).
- The concentration factor is displayed (C).
- The button "Release/ report data..." (D) is enabled.
- The defined concentration factor will be noted in the report

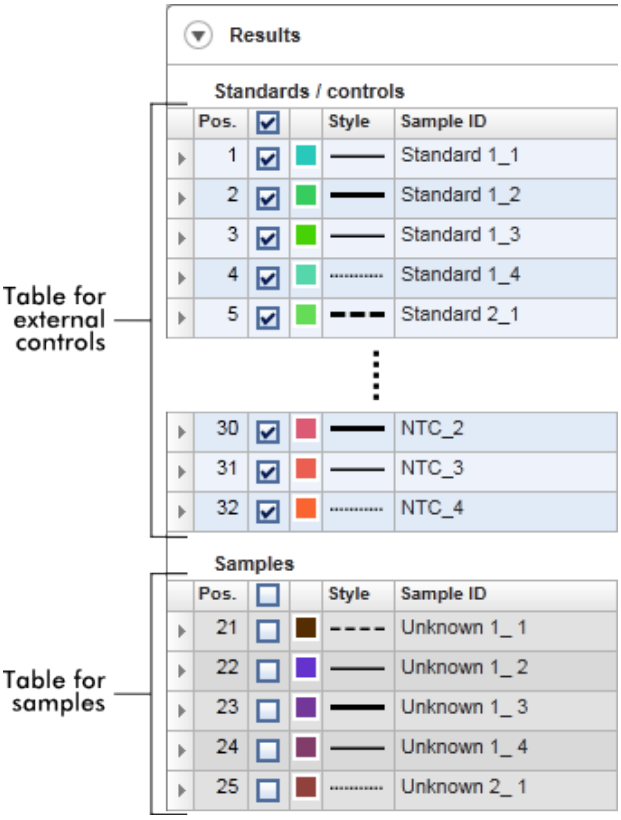
> Note
> After the assay is released, the concentration factor cannot be changed.

1.3.1.3 General Information about Approving Samples

The results of all samples determined by Rotor-Gene AssayManager v1.0 must be approved (accepted or rejected) in the "Results" area of the "Approval" screen.



The "Results" table consists of two tables:
- "Standards / controls"
- "Samples"

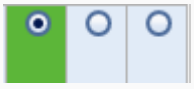


Behavior of the "Results" table
Initially, the approval buttons in the "Samples" table are disabled — only the approval buttons in the "Standards / controls" table are enabled. The external controls must be approved first. After all external controls have been approved, the approval buttons in the "Samples" table are enabled.

The results area contains the "Results" table with the following detailed information about the individual samples.

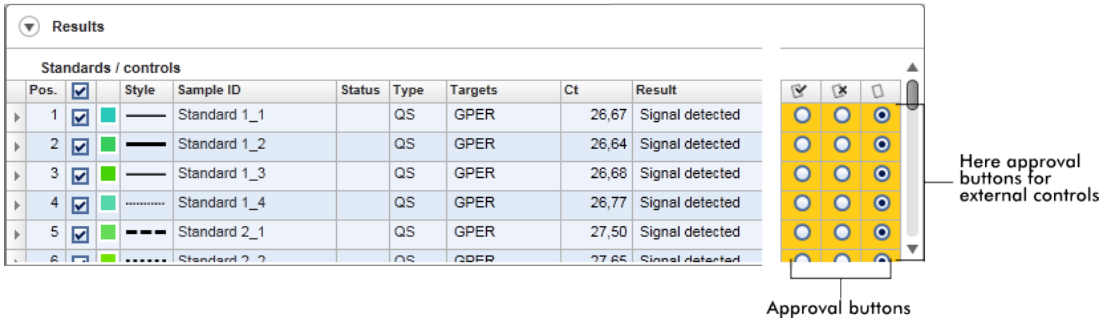
- "Position"
- "Color"
- "Style"
- "Sample ID"
- "Status"
- "Type"
- "Target"
- "C_T"
- "Result"
- "Flags"
- "Sample comment"

Sample results to be approved have three additional approval buttons at the dedicated row end. These buttons are used to interactively accept or reject the sample results. As a visual aid, the background color of the approval bar changes according to the approval state. Initially, all test samples of a finished experiment have the status "Undefined" and are displayed with a yellow background. An "Accepted" sample will change its background color to green. A "Rejected" sample changes its background color to red.

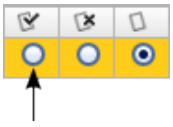
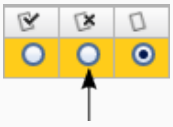
Background color	Status of test sample
	Undefined
	Accepted
	Rejected

Step-by-step procedure to approve samples

1. In the "Results" table, scroll to the sample to be approved. Every sample result to be approved has three radio buttons at the dedicated row end.



2. Either accept or reject the result of a sample.

	Click	Changes to
To **accept** a sample result, click the first button in the row.		
To **reject** a sample result, click the second button in the row.		

Optional: Enter a comment in the "Sample comment" column.

3. Repeat steps 1 and 2 for every sample until all sample results have either been accepted or rejected. To approve several sample results at once highlight the dedicated rows using the row selector  . To highlight adjacent rows, click the first element's row selector, hold down the left mouse button, and move the cursor to last element to be highlighted using the mouse wheel. All rows in between are highlighted. Use the "Control" key to make multiple selections of non-adjacent rows. A right-click in the highlighted rows opens the context menu, which can be used to approve or reject all highlighted sample results at once.

Note

It is also possible to approve sample results only partly and approve the other sample results of an assay later. The button bar provides the following buttons to manage the approval process:

To	Click
▪ Save all changes ▪ Change to "Assay selection" screen	Save and close
▪ Cancel all changes ▪ Revert to the previous saved approval status; amplification plots and result table options are not reset	Reset
▪ Save all changes and remain in this screen	Save
▪ Discard all changes to its previous status ▪ Close this screen and change to "Assay selection" screen	Close

1.3.1.4 Concept of Approval Buttons in UDT Plug-in

Approval of external controls

After clicking "Start Approval" in the assay selection screen the "Approval" screen will be displayed. In the UDT Basic Plug-in, only the rules and parameters defined in "Core Analysis" and "Assay & Sample Analysis" of the "Development" environment can be applied to the raw data. The automatic data scan (AUDAS) method cannot be applied for assay analysis. This means that the amplification curves of external controls, such as quantitation standards, no template controls, positive controls, etc., as well as the amplification curves of the test samples cannot automatically be checked for anomalies by Rotor-Gene AssayManager v1.0.

In the UDT Basic Plug-in, the results of all external controls have to be approved before the results of the test samples. Thus, only the approval buttons for external controls are activated at the beginning of the approval process. The approval buttons for the test samples will be activated as soon as all external controls are approved.

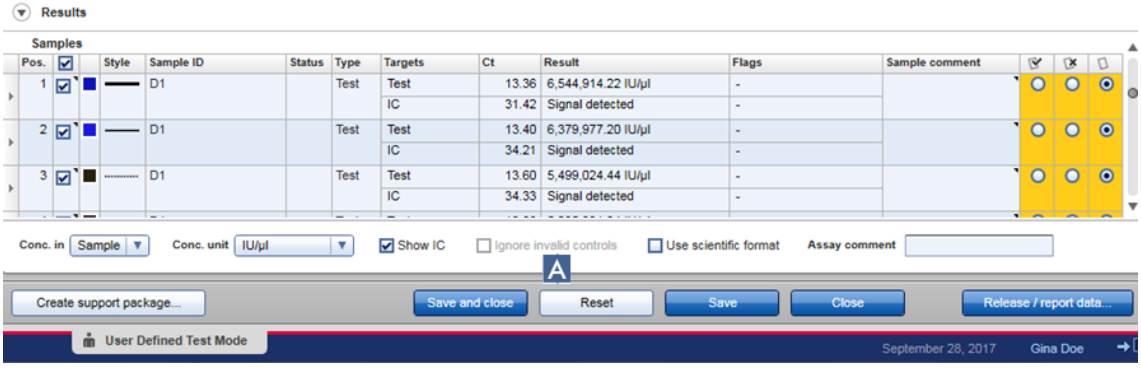
Note

During the approval process in UDT Mode, manually check the shape of the amplification curves for anomalies and reject the result for external controls with abnormal amplification curves.

The following list provides an overview about common anomalies the amplification curves should be checked for:

- Does the amplification curve contain spikes?
- Does the baseline fluorescence contain a strong dip?
- Is the baseline fluorescence abnormally steeply rising, indicating too strong a linear growth?
- Is the baseline fluorescence too wavy?
- Is the amplification curve saturated?
- Does the amplification curve contain any other anomalies?

If one or more of these conditions are fulfilled, the corresponding external control result should be rejected. Thereby, these external controls are excluded from the analysis of the test samples. Options to ignore invalid controls have been added as check boxes (A)



Note

Rejecting one or more external controls may result in the invalidity of the whole assay depending on the rules defined in the "Sample and Assay Analysis" section of the Development Environment.

For amplification curves without any of the mentioned anomalies, the approval buttons should be used to accept or reject the external control result presented by Rotor-Gene AssayManager v1.0. The following table provides an overview about different scenarios:

Rotor-Gene AssayManager v1.0 analysis	Approver accepts the external control result	Expected behavior of the approver
External control result is valid and displayed ("Signal detected", "No signal", or target concentration).	Yes	Click "Accepted".
External control result is invalid justified by at least one corresponding flag.	Yes	Click "Accepted".
External control result is valid and displayed ("Signal detected", "No signal", or target concentration).	No (e.g., the analysis rules defined during assay profile development are not strict enough and an invalid result is not automatically detected by Rotor-Gene AssayManager v1.0)	Click "Rejected".
External control result is invalid justified by at least one corresponding flag.	No (e.g., the result of a generally good-looking external control was set to invalid because of an analysis rule that was set too strict during assay profile development)	Click "Rejected".

Note

A result automatically set to invalid by Rotor-Gene AssayManager v1.0 cannot be converted to a valid result anymore even if the result is rejected.

For approval of quantitative assays, the standard curve is not displayed until all external controls were approved with either the status "Accepted" or "Rejected". After approval of all external controls, the standard curve and its dedicated parameters, such as the efficiency, are calculated and displayed in the "Standard curve" sub tab. Based on the standard curve the resulting target concentrations in the test samples are calculated and displayed in the sample results area.

Note

If a valid quantification standard is rejected, the standard curve will be re-calculated without the rejected quantification standrad. All samples will then be analyzed according to the re-calculated standard curve.

Approval of test sample results

After approval of the external controls, the results of the test samples are automatically analyzed and set by Rotor-Gene AssayManager v1.0. The results have to be approved and released by the user logged in with the role of approver.

Note

During the approval process with the UDT Basic Plug-in in UDT Mode, manually check the shape of the amplification curves for anomalies and reject the result for samples with abnormal amplification curves.

The following list provides an overview about common anomalies the amplification curves should be checked for:

- Does the amplification curve contain spikes?
- Does the baseline fluorescence contain a strong dip?
- Is the baseline fluorescence abnormally steeply rising, indicating too strong a linear growth?
- Is the baseline fluorescence too wavy?
- Is the amplification curve saturated?
- Does the amplification curve contain any other anomalies?

If one or more of these conditions are fulfilled, the corresponding test sample result should be rejected.

For amplification curves without any of the mentioned anomalies the approval buttons should be used to accept or reject the sample result presented by Rotor-Gene AssayManager v1.0. The following table provides an overview about different scenarios:

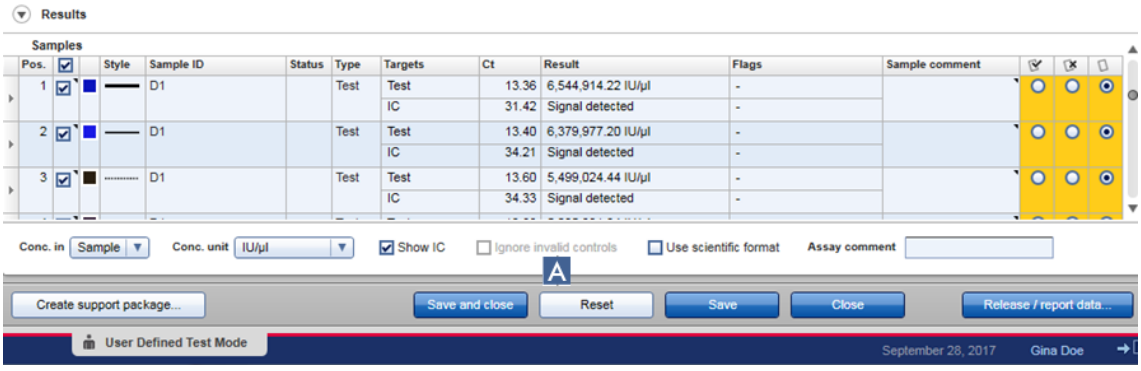
Rotor-Gene AssayManager v1.0 analysis	Approver accepts the test sample result	Expected behavior of the approver
Sample result is valid and displayed ("Signal detected", "No signal", or target concentration).	Yes	Click "Accepted".
Sample result is invalid justified by at least one corresponding flag.	Yes	Click "Accepted" and re-test the sample.
Sample result is valid and displayed ("Signal detected", "No signal", or target concentration).	No (e.g., the analysis rules defined during assay profile development are not strict enough and an invalid result is not automatically detected by Rotor-Gene AssayManager v1.0)	Click "Rejected" and re-test the sample.
Sample result is invalid justified by at least one corresponding flag.	No (e.g., the result of a generally good-looking test sample was set to invalid because of an analysis rule that was set too strict during assay profile development)	Click "Rejected" and re-test the sample.

Note

A result automatically set to invalid by Rotor-Gene AssayManager v1.0 cannot be converted to a valid result anymore even if the result is rejected.

Ignoring Invalid Controls

Rotor-Gene AssayManager v1.0 UDT Basic Plug-in software lets you ignore invalid controls in the “Approval” environment. To do this, click on the check box “Ignore invalid controls” (A), and sample results are not invalidated.



When the check box is activated, the approver has to confirm the message in the 'ignore invalid controls' dialogue box

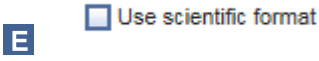
After the message is confirmed, valid results for test samples are reported. The report contains the sentence "Invalid controls were overruled by the approver to enforce assay validity"

Assay Information

Assay Profile:	APT_1P_ValidCheck_ignore_invalid_controls_UDT (Version 2.3.1)
Assay Kit:	Material number: 0937055 (deviating from assay profile), Lot number: 1234, Expiry date: 8/5/2015 (not expired)
Assay status:	Successful (Invalid controls were overruled by approver to enforce assay validity)

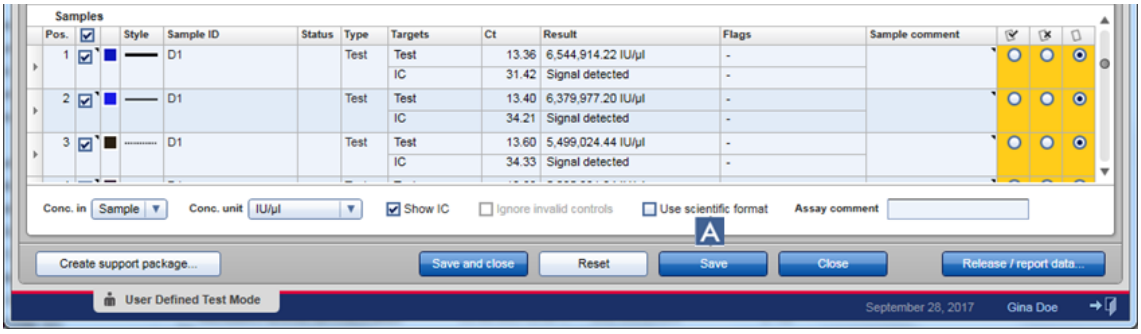
"Results" table options

Option	Explanation
A Conc. in Eluate	Depending on the selection in this drop-down menu the detected concentration will automatically be calculated for the eluate or the original sample material before sample preparation. This function is only available for quantitative assays with a concentration factor defined in the assay profile or when a concentration factor has been defined in the "Approval" environment (▶ Calculating sample concentration.
B Conc. unit Default Unit	If several concentration units are defined in the assay profile, this menu is populated with the default concentration unit and alternative concentration units. The desired concentration unit can be selected from this drop-down menu.
C Show IC	By default, this check box is activated if an assay contains a target of type IC. Deactivate the check box to hide the IC information (target name, C_T value, result, and result flag) from the "Results" table.
D Ignore invalid controls	This check box is deactivated and unchecked by default. The "Ignore invalid controls" check box can be activated by checking the check box "Enable to set assay to valid (UDT Mode)" in the "Settings" tab of the "Configuration" environment. The "Ignore invalid controls" has the following functionality: ▪ If an assay in UDT mode is invalid, it can manually set to be valid by checking the "Ignore invalid controls" check box. By using this functionality, individual external controls that were evaluated as invalid by Rotor-Gene AssayManager v1.0 are excluded from the analysis. The test sample results are set to valid. Invalid quantitation standards will be

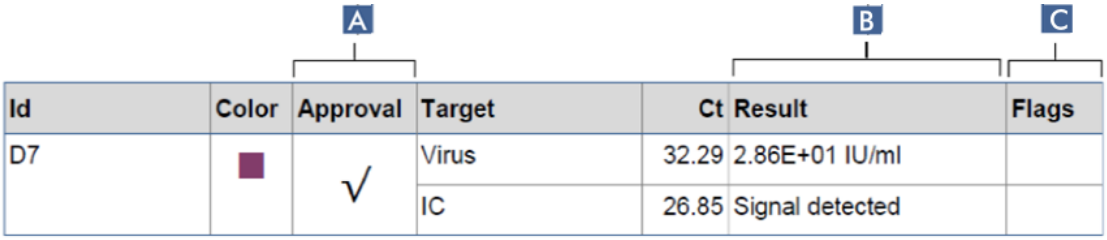
	excluded from standard curve calculation. If the "Ignore invalid controls" check box is used for assay approval, this will be mentioned in the result report
E 	If this check box is activated, the concentrations in the result column of the results report are displayed in scientific format
F 	Text field to enter a comment about the assay. Comment must not exceed 256 characters. After the first sample has been released, the comment cannot be changed anymore.

Scientific format view

To display quantitative results, Rotor-Gene AssayManager v1.0 UDT Basic Plug-in software lets the user choose between scientific format and decimal format in the “Approval” environment and in the report. The approval screen contains a check box “Use scientific format” in the results area below the results table (A). If the check box is activated, the concentrations in the result column of the results report are displayed in scientific format (e.g., 222,732.63 IU/ml would be displayed as 2.23E+05 IU/ml).



Columns in the "Test Results - Overview" report display the approval status for each sample and control (A), the result in concentration unit and scientific format (B) and if flags are assigned to a target (C)



Id	Color	Approval	Target	Ct	Result	Flags
D7	■	√	Virus	32.29	2.86E+01 IU/ml	
			IC	26.85	Signal detected	

All concentrations given in this table are concentrations in the **eluate**

! This target has flags

√ Accepted

x Rejected

1.3.1.5 Target Results

Rotor-Gene AssayManager v1.0 determines the result of a target by combining all relevant analysis results according to normalization options and sample and assay rules defined in the corresponding assay profile. The target result can either be “Signal detected”, “No signal”, the calculated target concentration combined with the selected unit, or “INVALID”.

1. The target gets the result “Signal detected” if a C_T value is detected and the assay is not quantitative. Even quantitative targets may get the result "Signal detected" in case the corresponding standard curve could not be calculated.

2. The target gets the result “No signal” if no C_T value is detected.

3. The target gets a concentration value as result if a C_T value is detected, the assay is quantitative, and the target quantification was successful. The concentration is automatically calculated for the selected concentration unit.

4. The target result is set to “INVALID” if one or more sample flags are assigned to the sample during analysis by Rotor-Gene AssayManager v1.0 that are defined to set the target result to "INVALID". If the check box "Enable processing of unclear samples" in the configuration settings is deactivated, even results of samples with the upstream flag “Unclear” (e.g., flagged by QIAsymphony AS) are set to “INVALID”.

1.3.1.6 Sample Flags

The following sample flags may be assigned to individual targets during analysis by Rotor-Gene AssayManager v1.0. This is a complete list of all flags that can occur when using the UDT Basic Plug-in. Depending on the settings in a specific assay profile not all flags may be relevant.

The appearance of flags in the Rotor-Gene AssayManager v1.0 is connected either with an invalidation of the corresponding target for a test sample, control, or standard, or the flag is only displayed as "warning" without consequences for the result. The column "behavior" below lists how the Rotor-Gene AssayManager v1.0 reacts to a certain flag. For the flag type "Variable", the behavior of the Rotor-Gene AssayManager v1.0 depends on the settings in the specific assay profile.

Flag	Behaviour	Description
ABOVE_UPPER_LOQ	Variable	The upper limit of quantification is exceeded. The target concentration is too high. Only a qualitative result is presented.
ASSAY_INVALID	Invalid	Assay is set to invalid because at least one external control is invalid.
BELOW_LOWER_LOQ	Variable	The lower limit of quantification is not reached. The target concentration is too low. Only a qualitative result is presented.
CONCENTRATION_ABOVE_ACCEPTED_RANGE	Variable	The target concentration is higher than the defined cut-off concentration.
CONCENTRATION_BELOW_ACCEPTED_RANGE	Variable	The target concentration is lower than the defined cut-off concentration.
CORRESPONDING_CONTROL_INVALID	Invalid	Target is set to invalid because at least one corresponding external control is invalid.

CORRESPONDING_POSITIVE_CONTROL_TARGET_INVALID	Invalid	The target result is set to invalid because the corresponding positive control is invalid.
CT_ABOVE_ACCEPTED_RANGE	Variable	The detected C_T value is higher than the defined cut-off C_T.
CT_BELOW_ACCEPTED_RANGE	Variable	The detected C_T value is lower than the defined cut-off C_T.
FLUORESCENCE_TOO_LOW	Variable	The fluorescence signal is lower than the defined fluorescence cut-off.
FLUORESCENCE_TOO_STRONG	Variable	The fluorescence signal is higher than the defined fluorescence cut-off.
IC_INVALID	Invalid	An internal control in the same tube is invalid.
IC_NO_SIGNAL	Invalid	No signal is detected for an internal control in the same tube.
INHIBITION_BY_CT	Variable	The defined maximum C_T range between the C_T for the internal control of that sample and the C_T for the internal control of the NTC is exceeded.
INHIBITION_BY_FLUORESCENCE	Variable	The defined maximum fluorescence difference between the internal control fluorescence of the NTC and the internal control fluorescence of that sample for the last cycle is exceeded.

LOW_FLUORESCENCE_CHANGE	Warning	The percentage fluorescence change for this sample relative to the sample tube with the largest fluorescence change is lower than a defined limit. This flag corresponds to the NEG (NTC) flag of the Rotor-Gene software and can appear only if the "NTC threshold outlier removal" function of the Rotor-Gene software was enabled in the imported .qit file. For more details refer to the *Rotor-Gene Q User Manual* .
LOW_REACTION_EFFICIENCY	Warning	The reaction efficiency for this sample has not reached a defined limit. This flag corresponds to the NEG (R.Eff) flag of the Rotor-Gene software and can appear only if the " Reaction Efficiency Threshold outlier removal" function of the Rotor-Gene software was enabled in the imported .qit file. For more details refer to the *Rotor-Gene Q User Manual* .
MAX_CORRELATION_IN_STANDARD_CURVE_EXCEEDED	Variable	Either an upper limit for the R^2 value or an upper limit for the R value is exceeded.
MAX_EFFICIENCY_EXCEEDED	Variable	The upper limit for reaction efficiency is exceeded.
MULTI_THRESHOLD_CROSSING	Invalid	The amplification curve crosses the threshold more than once. An unambiguous C_T cannot be determined. This flag corresponds to the NEG (Multi C_T) flag of the Rotor-Gene software. For

		more details refer to the *Rotor-Gene Q User Manual* .
NO_CT_DETECTED	Variable	No C_T is detected for this target.
NORM_FACTOR_ALTERATION	Warning	Deviation during the normalization procedure. The amplification curve is displayed with a default normalization; results should be manually checked for correctness.
OTHER_IC_INVALID	Invalid	An internal control in another tube is invalid.
OTHER_IC_NO_SIGNAL	Invalid	No signal is detected for an internal control in another tube.
OTHER_TARGET_INVALID	Invalid	A target in another tube is invalid.
OUT_OF_COMPUTATION_RANGE	Invalid	The calculation of the concentration for this sample exceeds the technical limit.
TOO_LESS_CORRELATION_IN_STANDARD_CURVE	Variable	Either a lower limit for the R^2 value or a lower limit for the R value is not reached.
TOO_LESS_EFFICIENCY	Variable	A lower limit for reaction efficiency is not reached.
TOO_MANY_QUANTIFICATION_STANDARDS_ INVALID	Variable	The number of invalid quantification standards exceeds a minimal number required.
UNCERTAIN	Variable	Results from the automatic data scan (AUDAS) are conflicting with results from the core analysis. An unambiguous automatic

		assessment of data validity is not possible.
UNEXPECTED_CT_DETECTED	Variable	A C_T value is detected for a target that should not amplify.
UPSTREAM	Variable	Sample status was set to invalid or unclear by an upstream process (e.g., from QIAsymphony Assay Setup). Note: For “unclear” flags from upstream processes the behavior of Rotor-Gene AssayManager v1.0 is defined in the “Configuration” environment and not in the Assay Profile. “Invalid” flags from upstream processes always result in an invalid corresponding sample in Rotor-Gene AssayManager v1.0.

- Core Analysis
- Assay and Sample Analysis

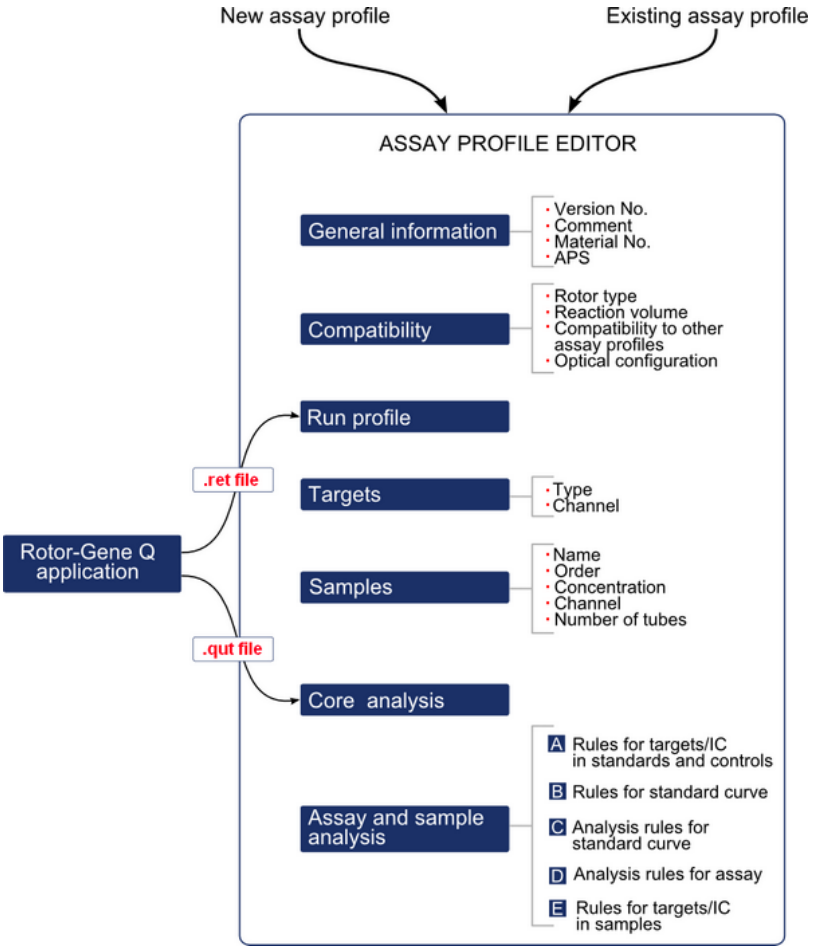
1.3.2 Development Environment

The "Development" environment of the UDT Basic Plug-in allows the user to design their own assay profiles. The corresponding assays should have been previously optimized using the standard Rotor-Gene Software. Rotor-Gene experiment and quantitation analysis template files from the Rotor-Gene Software can be imported in Rotor-Gene AssayManager and completed to an assay profile.

1.3.2.1 General Work Flow Assay Profile Development

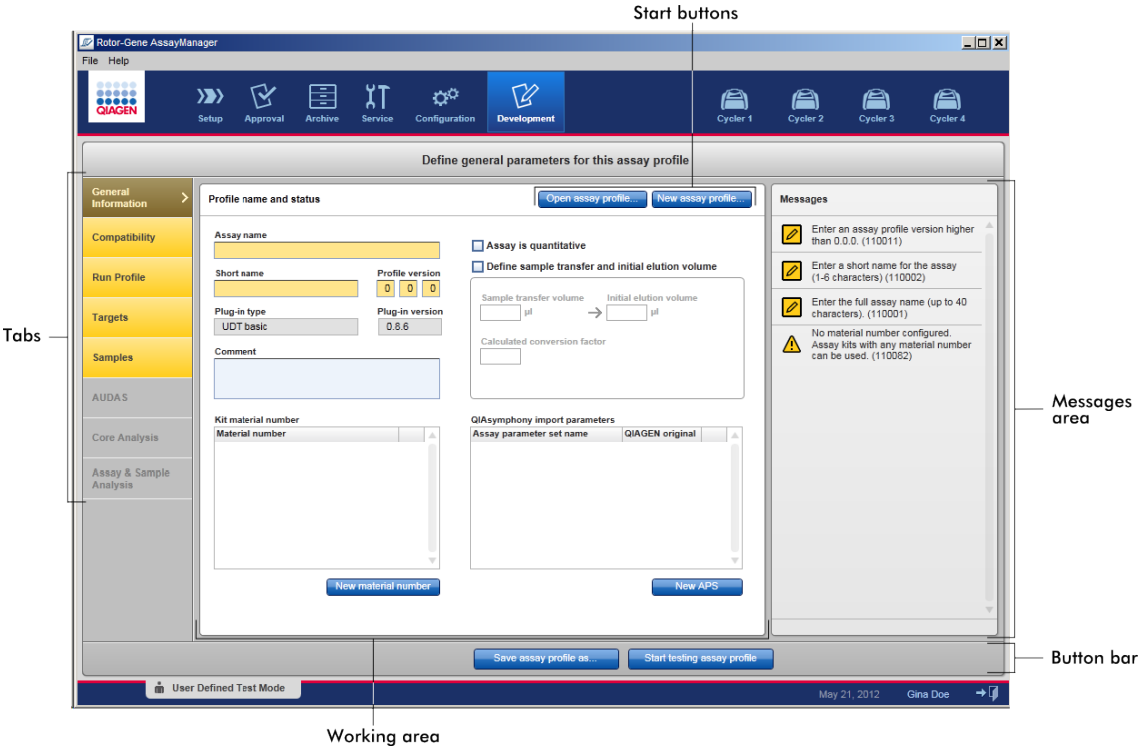
An assay profile can be created either by modifying an existing assay profile or creating a new one. The general work flow in the Assay profile editor comprises eight steps that are subdivided in eight tabs. The assay developer enters necessary information in every step except for the "Run profile" and "Core analysis". Here, the necessary information is

imported from the Rotor-Gene Q Software using *.ret (Rotor-Gene experiment template) and *.qut (quantitation analysis template) files.
The assay profile can be saved and imported to the Rotor-Gene AssayManager database after all information is entered and no errors exist.



1.3.2.2 General GUI Description

The "Development" environment contains the following elements:

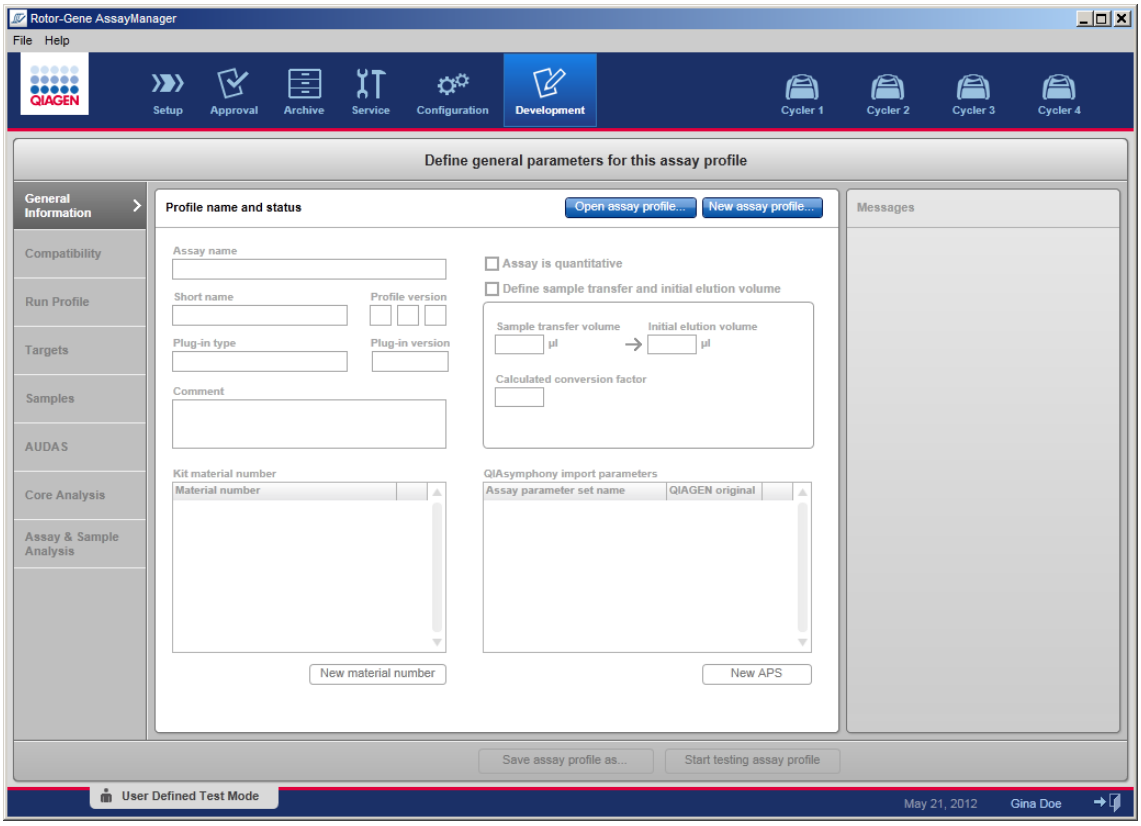


- Start buttons
- Tabs
- "Messages" area
- Working area
- Button bar

Start buttons

The start buttons are used to start working with the assay profile development.

When a user changes to the "Development" environment, only the two start buttons are enabled:



An assay profile can be customized either by creating a new assay profile (button "New assay profile...") or opening and modifying an existing assay profile (button "Open assay profile...").

Tabs

The whole process of creating/modifying an assay profile is divided into eight different tabs:

- "General Information"
- "Compatibility"
- "Run Profile"
- "Targets"
- "Samples"
- "AUDAS"
- "Core Analysis"
- "Assay & Sample Analysis"

Working area

The content and layout of the working area depends on the active tab.

"Messages" area

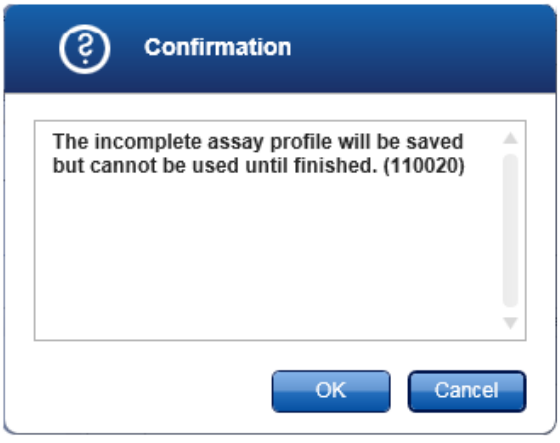
The messages area contains all warnings, errors, and information related to the current step.

Button bar

The button bar at the bottom of the screen is available as soon as assay name, short name, and profile version are defined in the "General Information" sub tab. The button bar contains two buttons to save the assay profile and to test the assay profile once it is ready.

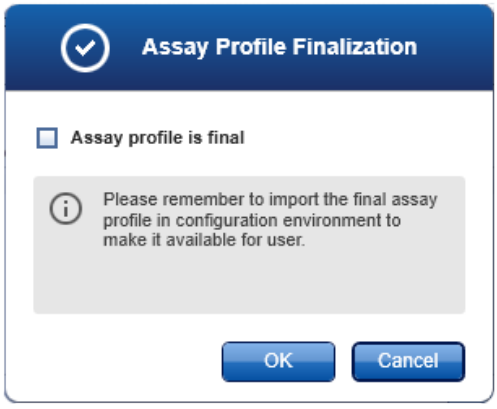
	Description
A	Save the assay profile.

- If this button is clicked before assay profile development is finished and all mandatory data are entered, the following message is displayed:



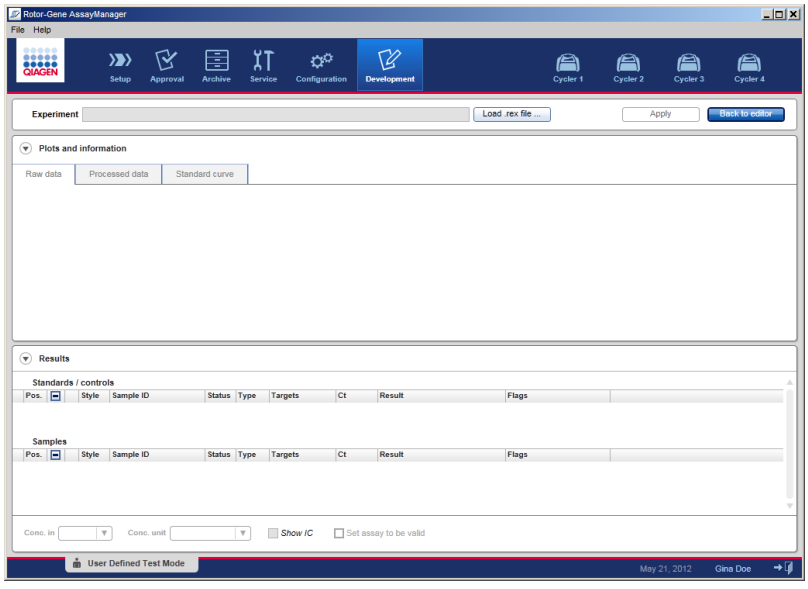
Missing data have to be entered in the yellow marked tabs before the assay profile can be used.

- If all mandatory data are entered, clicking the "Save assay profile as..." button opens the following dialog:



  The user has to activate the "Assay profile is final" check box. Only assay profiles with this option activated can be imported in the "Configuration" environment for subsequent usage.

B Test the developed assay profile and perform a virtual analysis of a prior finished PCR experiment. Using this button opens a screen with the possibility of uploading a *.rex file from an experiment performed with the Rotor-Gene Software or even Rotor-Gene AssayManager.



For further details and a step-by-step procedure, see Testing an assay profile

1.3.2.3 Using the Development Environment

The "Development" environment is used to create a new assay profile either starting from scratch or modifying an existing assay profile. Both alternatives have the same work flow — except that modifying an existing assay profile has a different starting point: an existing assay profile must be opened.
The created or modified assay profile can be tested in a final step.

Tasks assigned to the "Development" environment:
- Creating an assay profile
- Modifying an assay profile
- Testing an assay profile

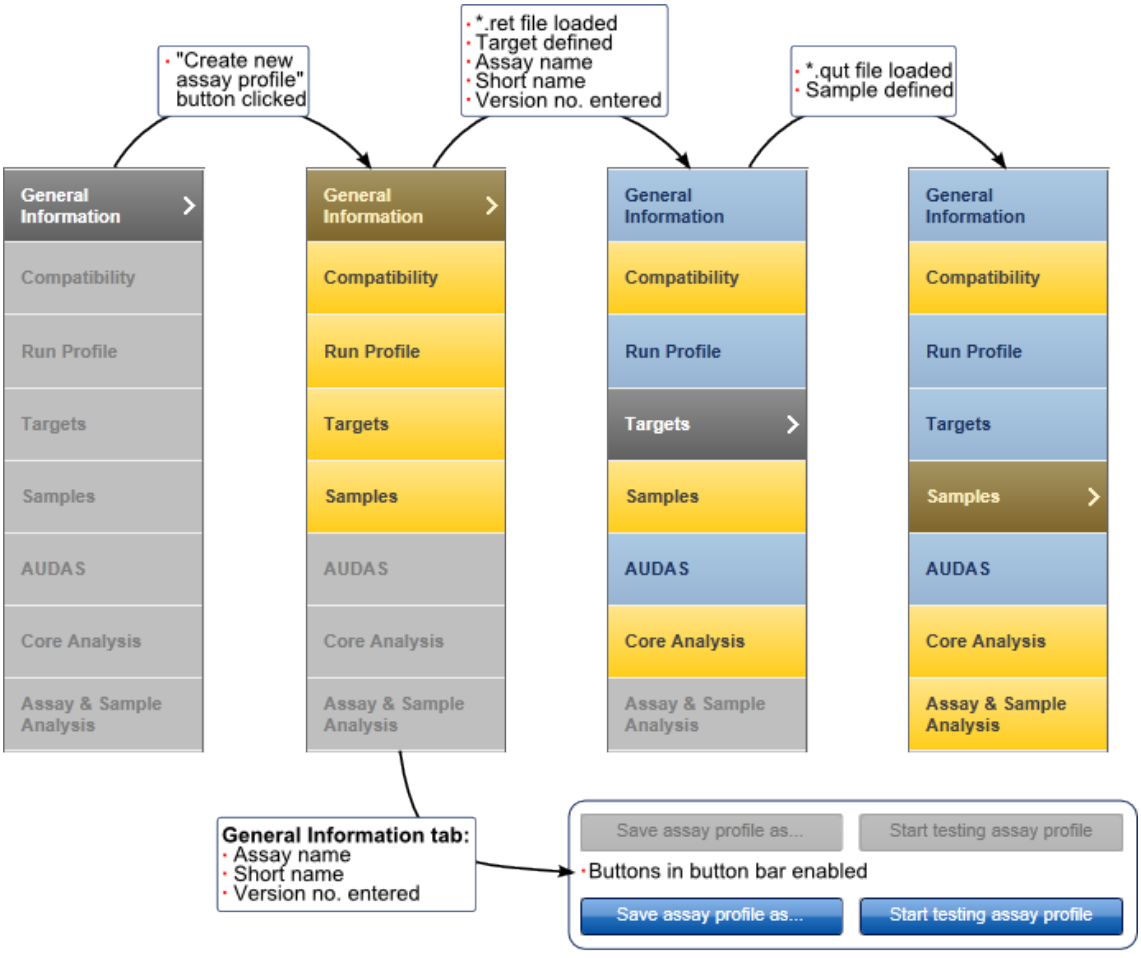
To accomplish the first two tasks, additional files from the Rotor-Gene application are necessary. These tasks are described in two separate topics:
- Creating a *.qut file
- Creating a *.ret file

Creating an Assay Profile

The steps to create an assay profile are localized in the "Development" environment.

Behavior of the "Development" environment

When a new assay profile is created, the first five tabs are activated and colored yellow. The buttons "Save assay profiles as..." and "Start testing assay profile" in the button bar are initially disabled. These buttons are enabled if valid values in the mandatory fields of the "General Information" tab are entered. This makes it possible to save an assay profile and continue working on it at a later time. The buttons for creating new targets and samples in the "Targets" and "Samples" tabs are disabled initially and enabled if a *.ret file is loaded in the "Run Profile" tab. After a target is defined the "AUDAS" and the "Core Analysis" tabs are enabled. The "Assay & Sample Analysis" tab is enabled when a sample is defined in the "Samples" tab.

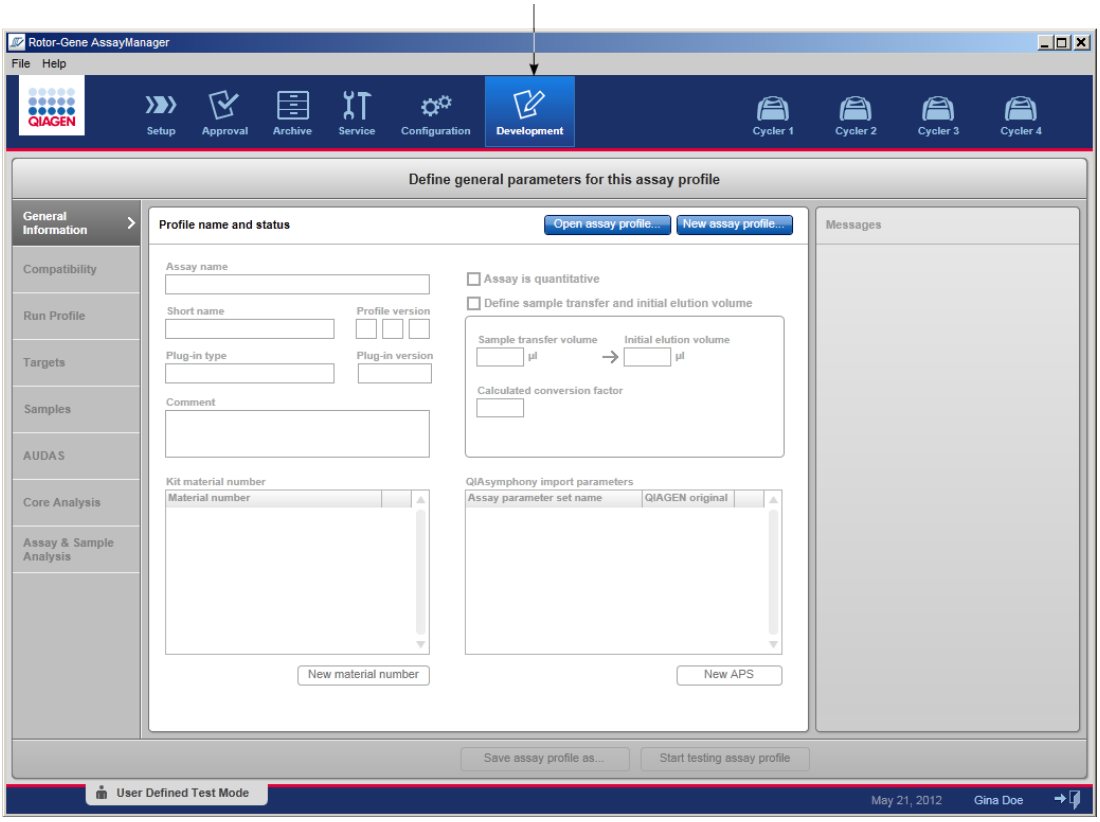


Step-by-step procedure to create an assay profile

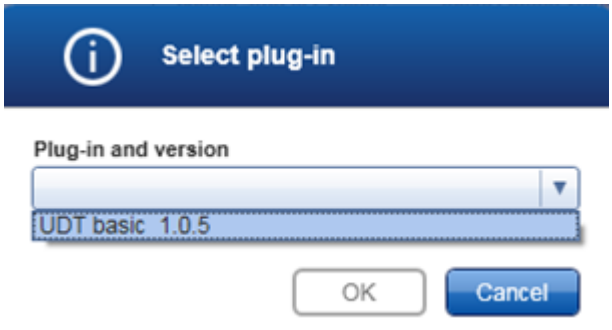
Precondition: At least one *.qut file and a *.ret file are necessary in the "Run profile" and "Core Analysis" steps. These files have to be created with the Rotor-Gene software. Details can be found here:

- Creating a *.qut file
- Creating a *.ret file

1. Click the "Development" icon to change to the "Development" environment.

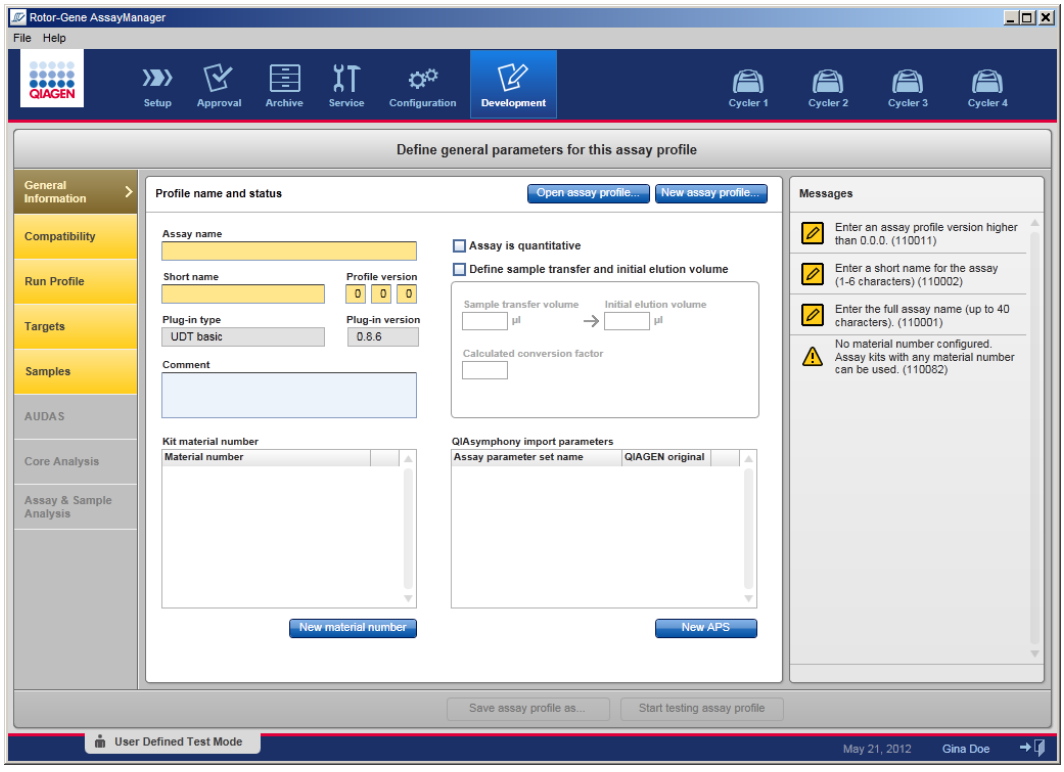


2. The "Development" environment opens. In this initial state only the two buttons, "Open assay profile..." and "New assay profile..." are enabled. All other elements are disabled.
3. Click "New assay profile...".
4. The "Select plug-in" dialog is displayed.



5. Select the "UDT basic" entry from the "Plug-in and version" drop-down list.

6. Click "OK".
7. The dialog closes. The first five tabs are enabled. The tabs are colored yellow to indicate that mandatory entries are missing. The "General Information" tab is active; the fields "Assay name", "Short name", and "Profile version" are also colored yellow. The "Messages" area shows the corresponding messages.

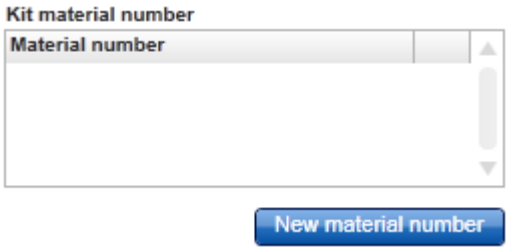


8. Enter an assay profile name in the "Assay name" field with up to 40 characters.
9. Enter a short name in the "Short name" field with up to 6 characters.
10. Enter the assay profile version.

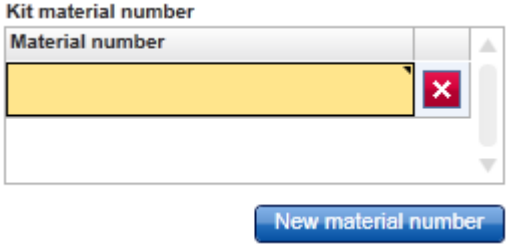
11. Optional steps in the "General Information" tab:

- Enter a comment
  Enter a comment specific for this assay profile in the "Comment" field.

- Define a kit material number
  The user can define kit material numbers for assay kits that must be used in combination with the assay profile. The material number entered during work list setup or transferred from QIAsymphony AS result file must match the material number entered here. Otherwise the run cannot be started.
  a) Click "New material number".



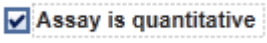
A new material number row is inserted and colored in yellow.



b) Enter a material number.
The new material number is displayed in the "Kit material number" table.
Repeat steps a–b for additional material numbers.

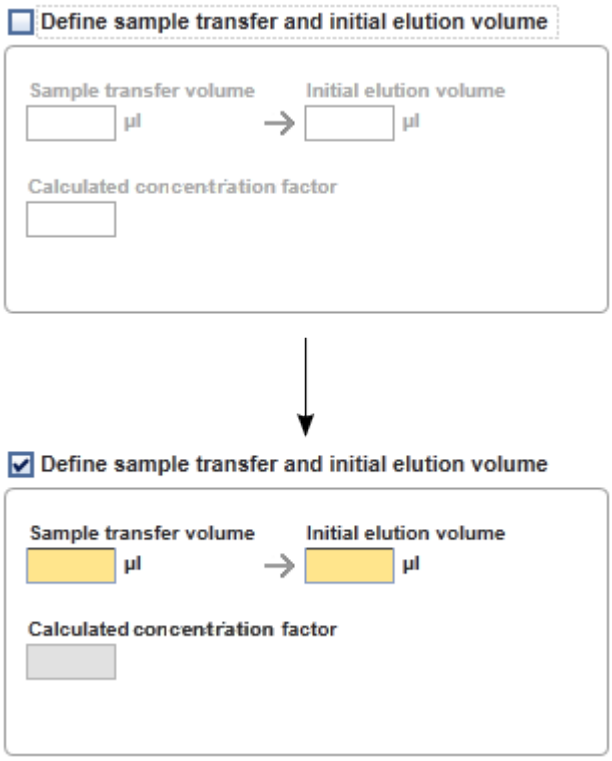
Note: Click the icon to remove a material number.

- Define an assay profile as quantitative
  Activate the check box "Assay is quantitative" to define the assay as being quantitative. In this case at least one quantitative target must be added.



> Note
> If the assay does not contain quantitation standards, the check box must be unchecked.

- Define sample transfer and initial volume
  Activate the check box "Define sample transfer and initial elution volume" to enable automatic target concentration calculation for the original sample material.

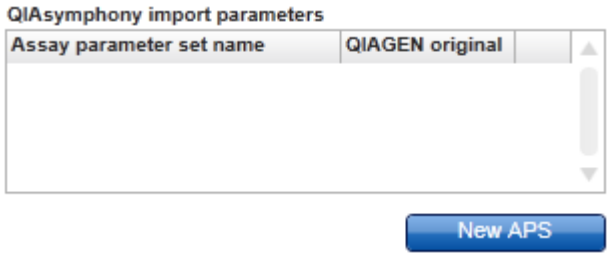


a) Activate the "Define sample transfer and initial volume" check box.
   The "Sample transfer volume" and "Initial elution volume" fields are enabled and colored yellow.
b) Enter the sample volume that is transferred to the nucleic acid purification process in the "Sample transfer volume" field.
c) Enter the volume that is initially used for elution in the "Initial elution volume" field.
   The resulting concentration factor will automatically be calculated by Rotor-Gene AssayManager in the "Calculated concentration factor" field.

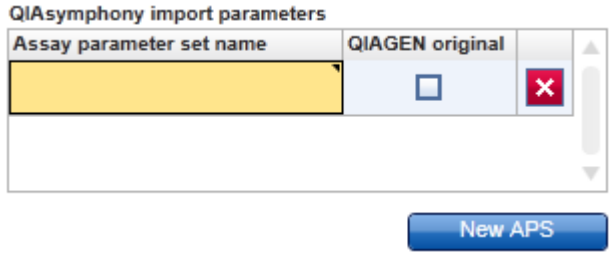
If this information is not entered, only the target concentration in the eluate can be calculated by Rotor-Gene AssayManager.

- Define an assay parameter set (APS)
  When using the QIAsymphony for nucleic acid purification and assay setup, the sample and process information can be transferred to Rotor-Gene AssayManager. To connect the QIAsymphony information with the correct assay profile, click "New APS" to enter the dedicated assay parameter set name. The APS name in the assay profile has to match the APS name in the QIAsymphony AS result file exactly, otherwise an import of the result file into Rotor-Gene AssayManager is not possible.

a) Click "New APS".



A new APS row is inserted and colored in yellow.



b) Enter an APS name.

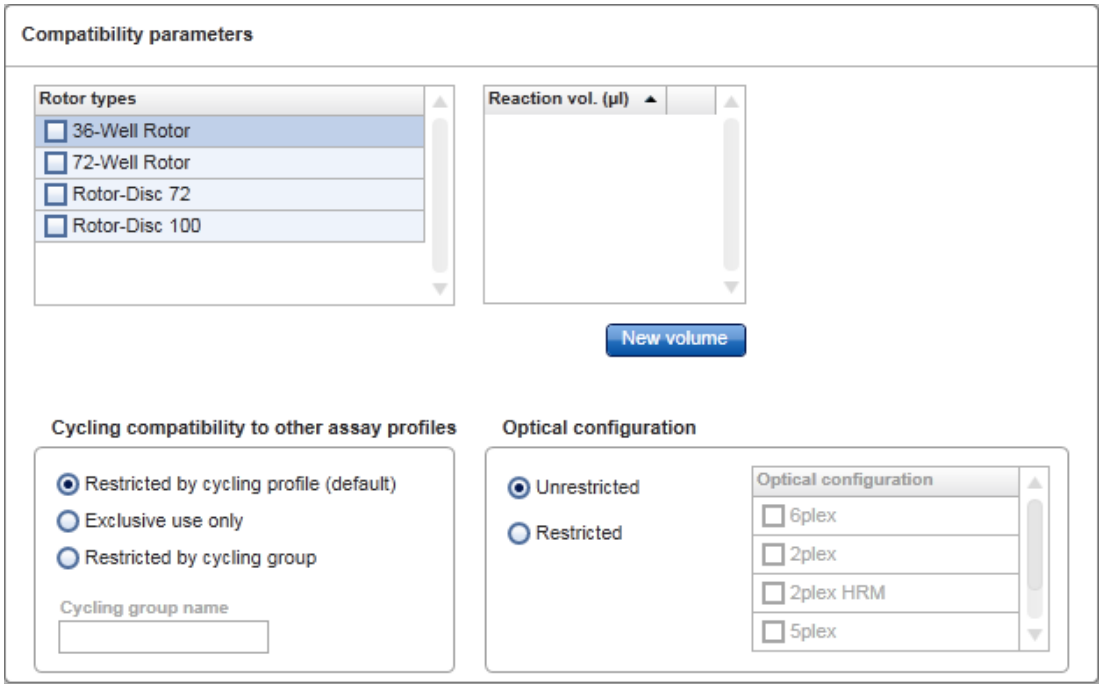
The new APS name is displayed in the QIAsymphony import parameters table.

c) Activate the "QIAGEN original" check box if the assay parameter set is originally from QIAGEN. Deactivate it if not.

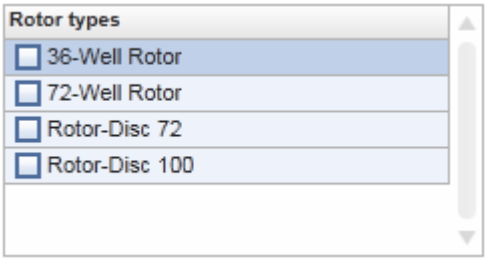
Repeat steps a–c for additional APS names.

Note: Click the icon to remove an APS name.

12. Change to the "Compatibility" tab to set compatibility parameters of the assay profile. The features of this dialog allow you to restrict your assay compatibility to only those rotors, volumes, or instrument types you have tested in your assay validation.



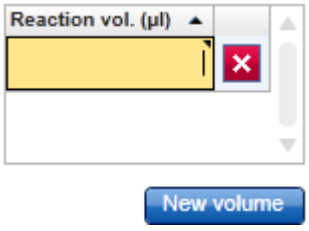
- Define rotor type compatibility



Activate the check boxes of the rotor types with which the assay profile will be compatible. Multiple activations are possible.

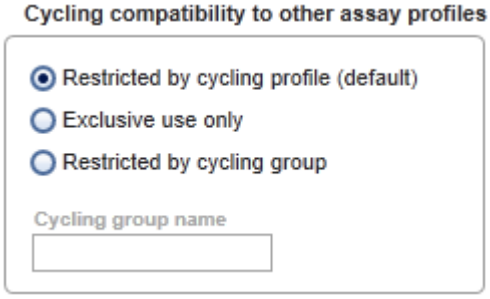
- Define reaction volume
  a) Click "New volume".

A new reaction volume row is inserted and colored in yellow.



b) Enter a reaction volume. When a decimal separator has to be entered, the language configuration of your computer system defines if the decimal separator must be comma or period. On a German system, for example, the comma (25,5 µl) must be used for decimals. On an American system the period (25.5 µl) must be used for decimals.
The new reaction volume is displayed in the "Reaction vol." table.
Repeat steps a) and b) to add additional reaction volumes.

- Define cycling compatibility conditions to other assay profiles
  In the "Cycling compatibility to other assay profiles" area three options are available:

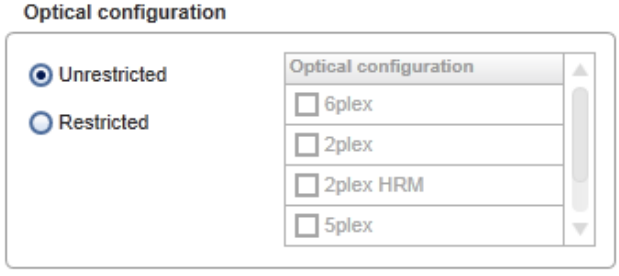


- "Restricted by cycling profile (default)" — Assay profiles sharing the same temperature cycling conditions can be applied in parallel on the same rotor.
- "Exclusive use only" — Assay profile cannot be combined with other assay profiles even if exactly the same cycling conditions apply.

- "Restricted by cycling group" — The assay profile can be applied with other assay profiles sharing the same cycling group. When using this option, a cycling group name must be entered.

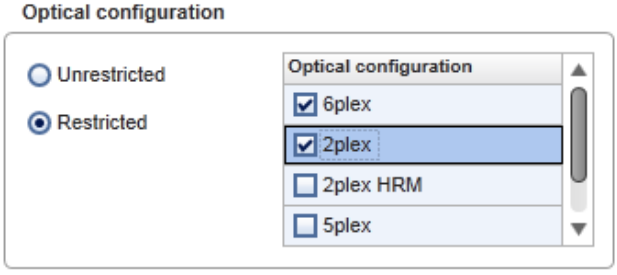
  This name must match the cycling group name of other assay profiles that should be compatible. Assay profiles sharing the same cycling group have to share the same temperature cycling conditions.

- Define optical configuration compatibility parameters
  Define whether the assay profile can be applied on Rotor-Gene Q instruments with any optical configuration, or restrict the optical configuration by selecting an appropriate optical configuration option.



"Unrestricted" means the assay profile can be applied to any technically compatible Rotor-Gene Q instrument.
"Restricted" means the assay profile can only be applied to a Rotor-Gene Q instrument with optical configurations defined in the following step.

Activate the check box of the optical configuration that the assay profile shall be restricted to. Selecting multiple optical configurations is possible.

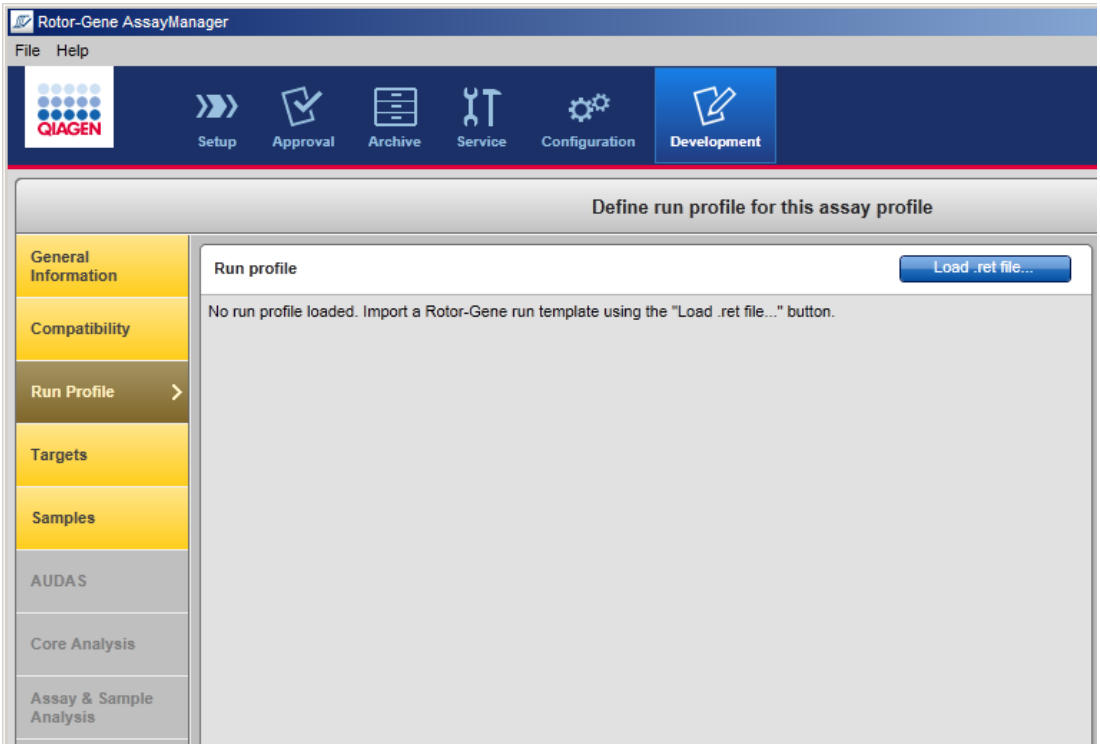


For details about the optical configuration of the Rotor-Gene Q instrument, refer to the *Rotor-Gene Q User Manual* .

Note

Assay profiles can never be applied to Rotor-Gene Q instruments with fewer acquisition channels than required by the assay profile. This is prevented by Rotor-Gene AssayManager. The "Optical configuration" area is used to set additional compatibility rules by the assay profile developer, for example, the assay profile should only be applicable to 5plex HRM® instruments even if it is also technically compatible with a 2plex or 2plex HRM instrument.

13. Change to the "Run Profile" tab to load a *.ret file.

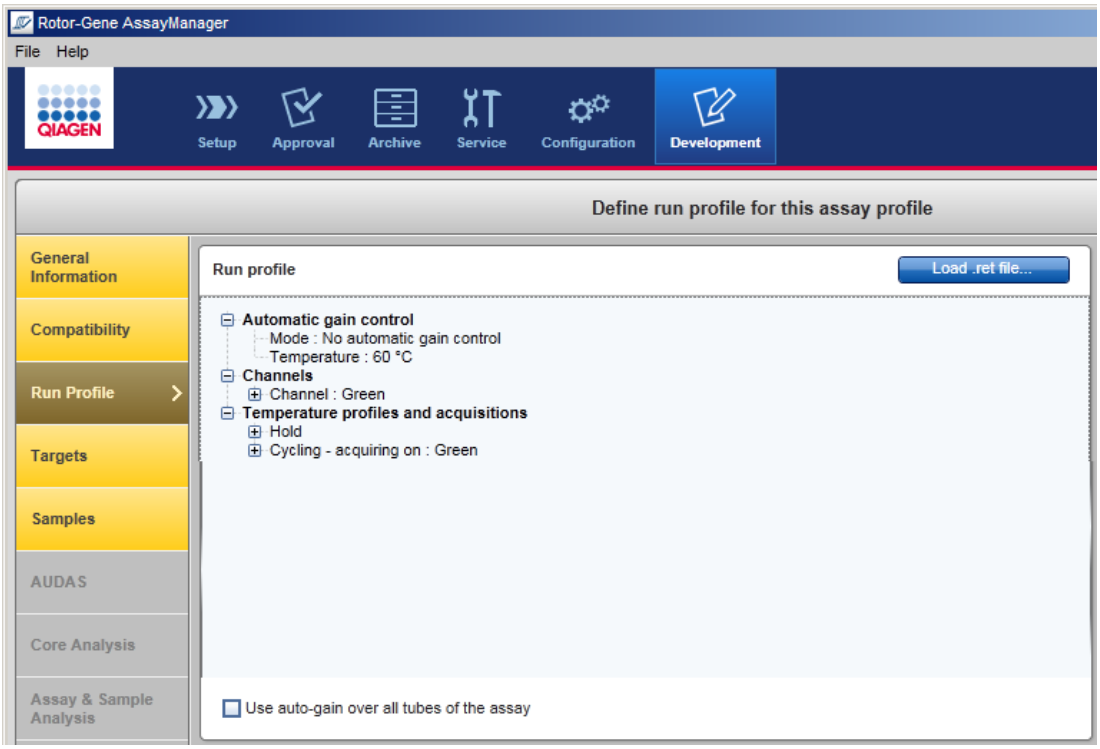


14. Click "Load *.ret file".

    The file selection dialog opens.

15. Browse the directory containing the *.ret file, select it, and click "OK".

16. The *.ret file is loaded and run profile parameters are shown:



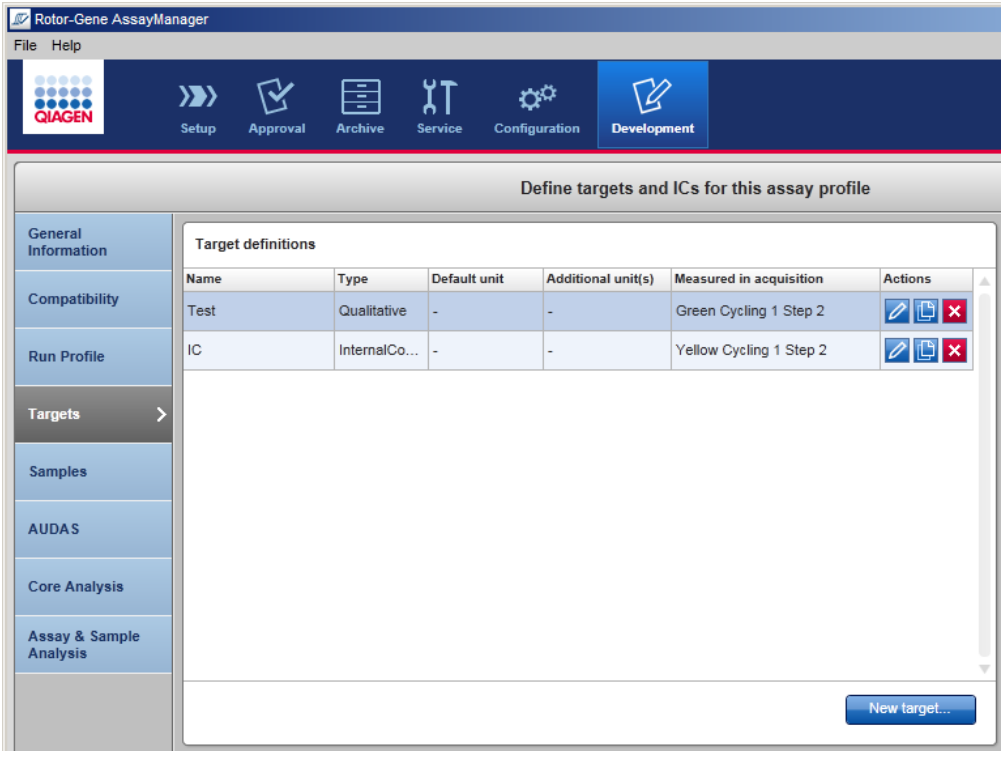
The run profile is divided into three sections:

- "Automatic gain control"
- "Channels"
- "Temperature profiles and acquisitions"

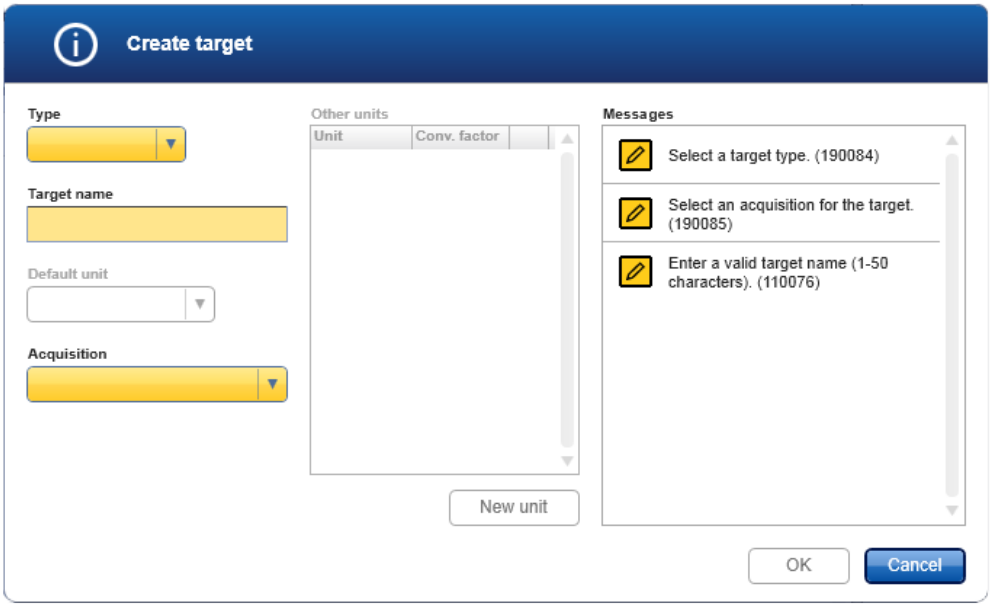
> Note
> The run settings cannot be altered using Rotor-Gene AssayManager.

17. Activate the check box "Use auto-gain over all tubes of the assay" at the bottom of the screen to apply the auto-gain optimization to all reserved rotor positions, not only on the one rotor position defined during run setup in the Rotor-Gene software. If "Use auto-gain over all tubes of the assay" is checked, the median fluorescence measured in all tubes of the assay is used to optimize the gain setting. This option applies to all different acquisition channels and steps defined in that assay profile.

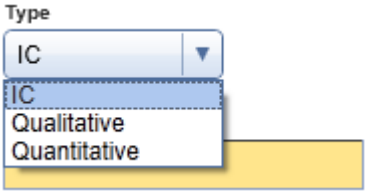
18. Change to the "Targets" tab to define the targets.



19. Click "New target..." to define the targets for the assay profile. The following dialog box opens:



20. Select a target type from the "Type" drop-down list.

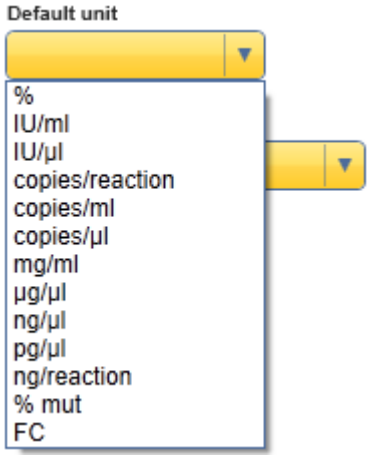


Note

In the "General information" tab, the assay profile was either set to be quantitative or not. Therefore, the available target types will differ in the "Targets" step:

- If the assay profile is quantitative: IC, Qualitative, and Quantitative can be selected.
- If the assay profile is not quantitative: IC and Qualitative can be selected.

21. Enter a target name in the "Target name" field with up to 50 characters.
22. For quantitative targets select the default concentration unit from the "Default unit" drop-down list.

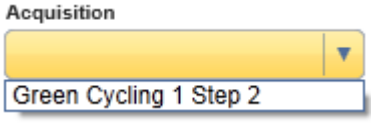


Note

This drop-down list is only activated for targets from type "Quantitative".

23. In the "Acquisition" drop-down list all acquisition steps of the PCR cycling are listed that are defined by the *.ret file loaded in the previous tab. The different acquisition steps can be identified by the acquisition channel (e.g., *Green* , *Yellow* , etc.) and the cycling step in which the acquisition is performed during the PCR cycling (e.g.,

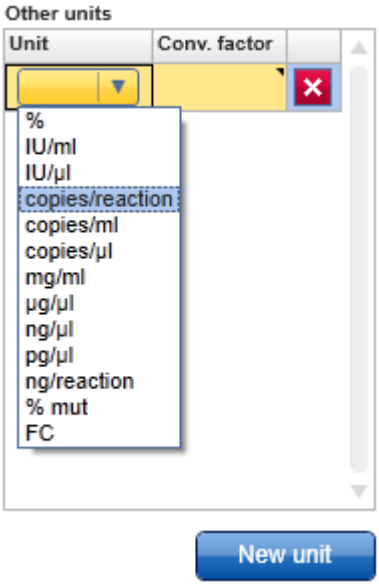
Cycling 1 Step 2). Select the acquisition step for the particular target from the drop-down list.



Note

The available acquisition options depend on the *.ret file loaded in the "Run Profile" tab.

24. Click "New unit" to assign additional concentration units besides the default unit for the target. A drop-down list will appear.



Note

This drop-down list is only available for targets with the type "Quantitative".

25. Select an additional unit and enter a factor to convert the target concentration from the default unit to the selected additional unit.

Note

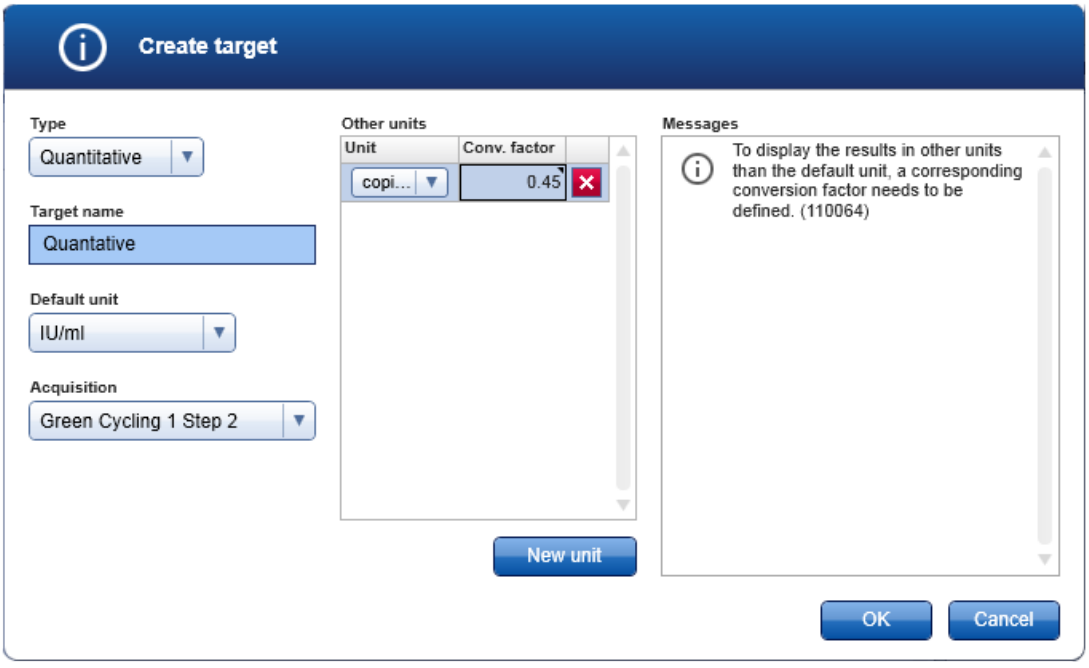
Multiple additional units can be defined by clicking "New unit" several times.

Example:
Default unit: IU/ml

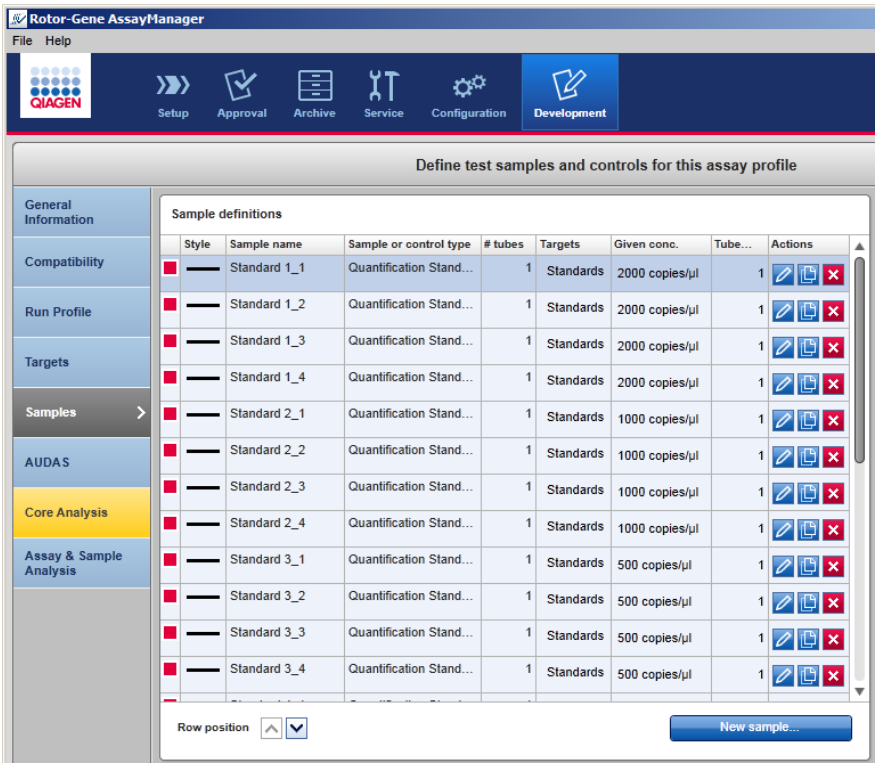
Other unit: copies/ml

1 IU/ml corresponds to 0.45 copies/ml for detection of the selected target.

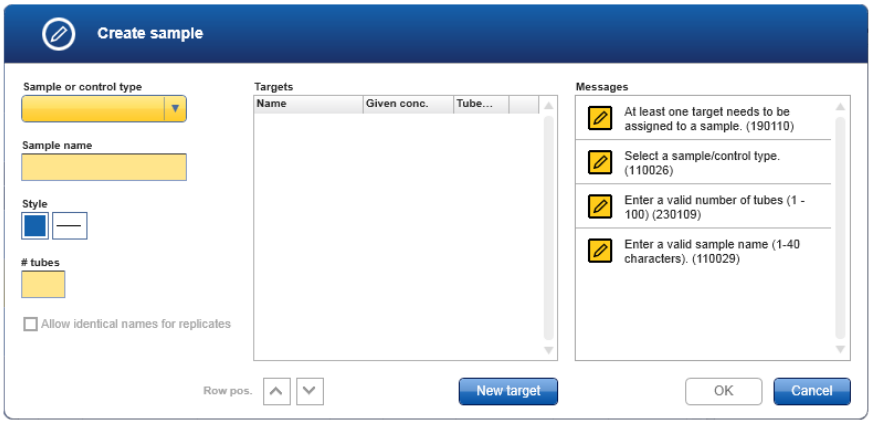
Enter *0.45* as conversion factor.



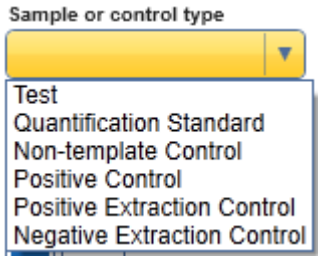
26. Repeat steps 19–25 for all other targets.
27. Change to the "Samples" tab. Here, the arrangement of the different samples and controls on the rotor can be configured.



28.Click "New sample" to create a new sample profile. The following dialog box opens:

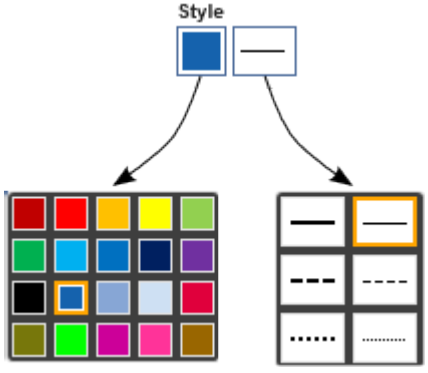


29. Select a sample or control type from the drop-down list. The following items are available:



> Note
> The control type "Quantification Standard" is only available for quantitative assays.

30. Enter a sample name into the "Sample name" field with up to 40 characters.
31. Click the color or line style button and select a color or line style for the amplification curve of the sample:



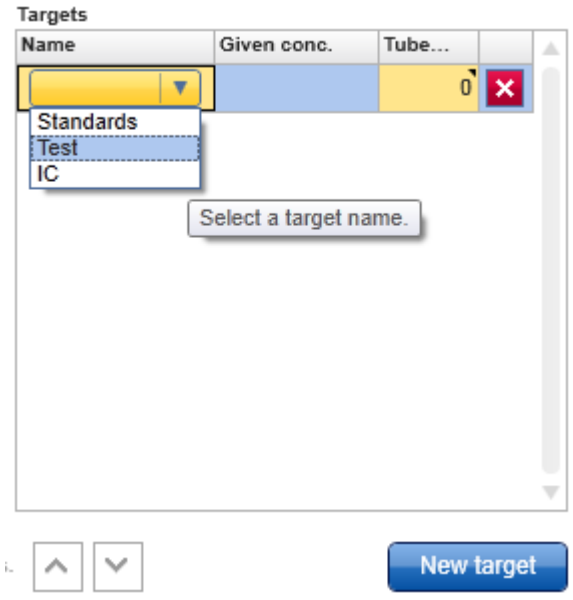
32. Define the number of rotor positions. The specific sample will be positioned and analyzed for different targets in as many rotor positions as entered in the "# tubes" field.

    Examples

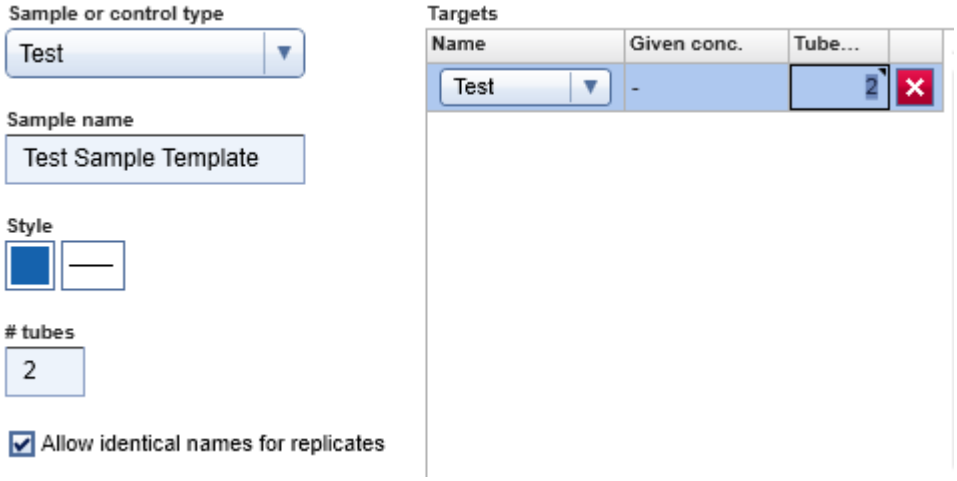
    a) If one specific sample will be analyzed in one rotor position for target x and in two other rotor position for target y and z, enter a value of *3*.
    b) If the sample will analyzed for multiple targets in the same rotor position (multiplex PCR), enter a value of *1*.
    c) Also a multiplex PCR with, for example, three targets in one tube and two targets in another tube, can be configured. In that case enter a number of *2* in "Tube position".

33. Click "New target" to assign one or more targets to the sample. The available drop-

down menu items represent the targets defined in the previous tab "Targets".



34. Select a specific target from the drop-down list, and enter the tube position within that sample or control type the target will be analyzed in. The entered value must be between *1* and the specified number of tubes for that sample or control type.



Examples (continuation of examples in step 32)

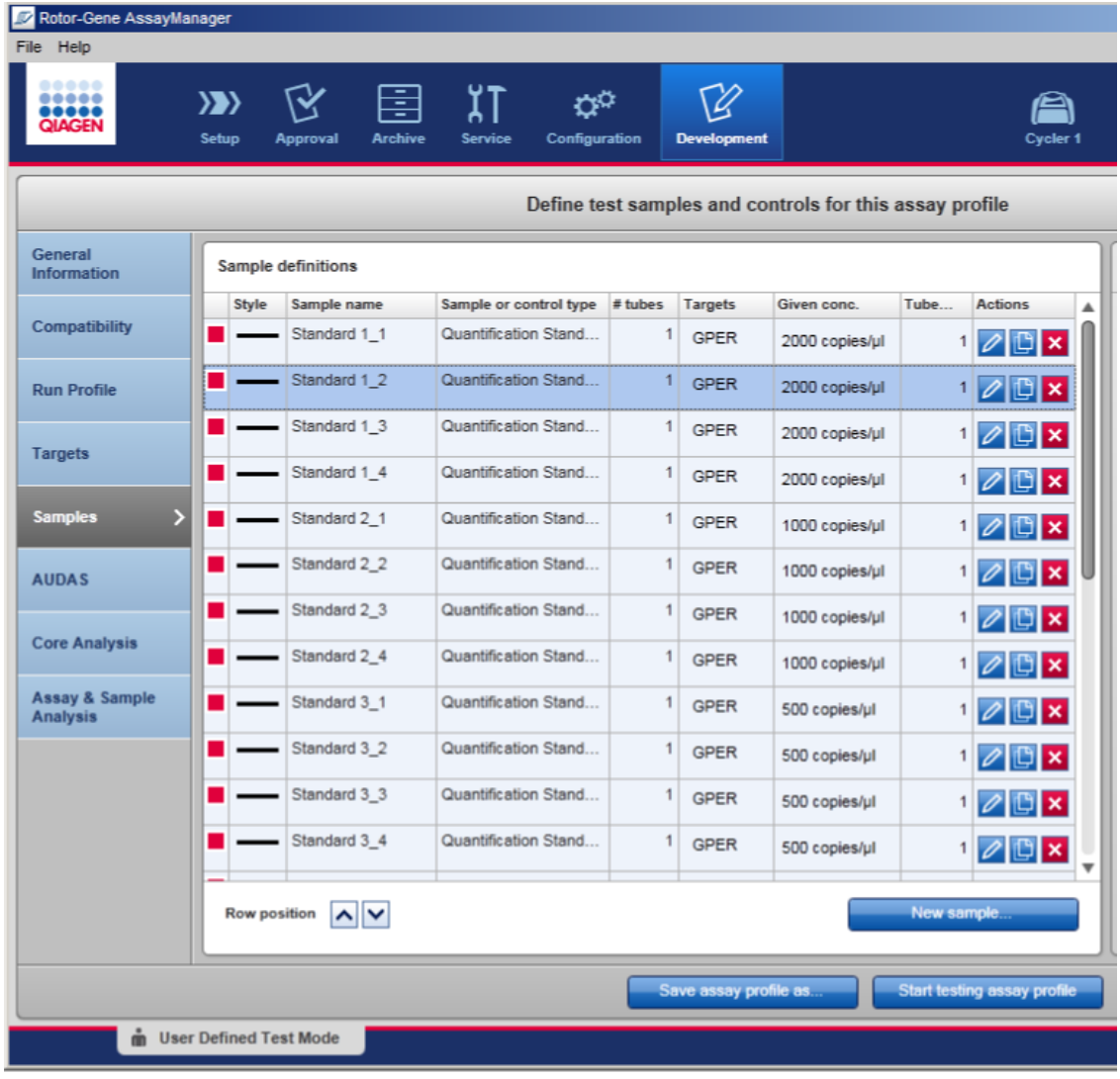
a) If a value of *3* was entered for the # tubes, the tube position for target x would be *1*, for target y it would be *2*, and for target z it would be *3*.

b) For a multiplex PCR all the different targets must be assigned to tube position 1.

c) Assign the first 3 targets to tube position 1 and the other 2 targets to tube position 2.

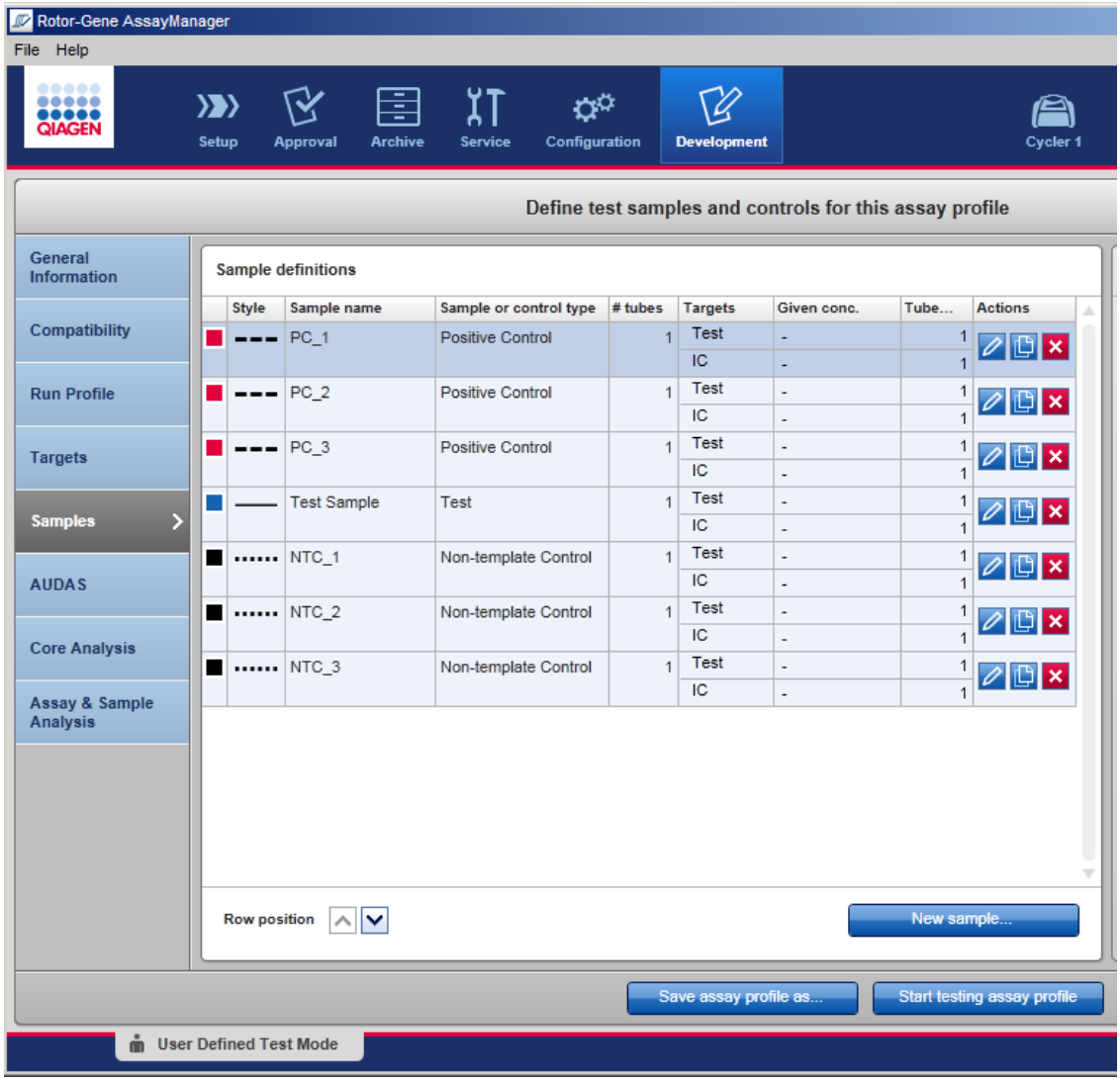
   For samples from type "Quantification Standard" at least one quantitative target defined in the previous tab "Targets" has to be assigned. If a quantitative target is selected from the drop-down list, the given concentration cell is automatically activated.

The concentration of this quantification standard can be entered followed by defining the tube position. If applicable, also several quantitative targets can be assigned to only one quantification standard. In that case the different quantitative targets should be set up in separate tubes to prevent competition or cross talk during amplification.



For all sample and control types not from type "Quantification Standard" the "Given

conc." cell is deactivated.
Multiple targets can be assigned by clicking "New target" several times. Redundant targets can be removed by clicking "Close". The position of the different sample and control types to each other can be adapted by selecting a certain row and using the row selection buttons to move this row in the list up or down.

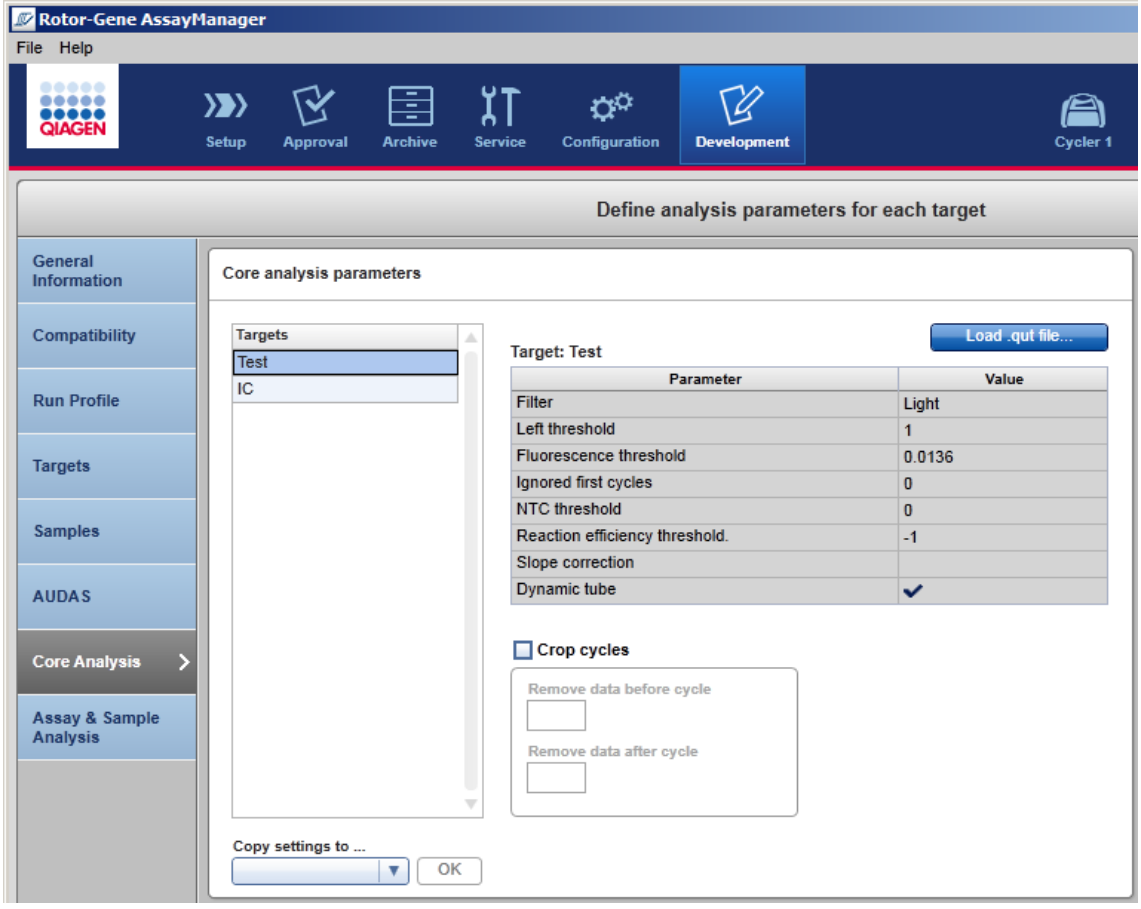


35.Change to the "AUDAS" tab.

> Note
> AUDAS stands for "Automatic Data Scan". This option is not available for the UDT Basic Plug-in. The AUDAS sub tab is thus inactive and must be skipped for creation of an assay profile with the UDT Basic Plug-in.

36. Change to the "Core Analysis" tab.
    The core analysis defines algorithms for normalization of the amplification curves and quantification of the targets. In the "Core Analysis" tab most of the parameter values must be imported from a Rotor-Gene quantification template file. This *.qut file can be generated after analysis of an assay in the standard Rotor-Gene software.
    The procedure how to create *.qut files is described in ▶ Creating a *.qut file with Rotor-Gene application.

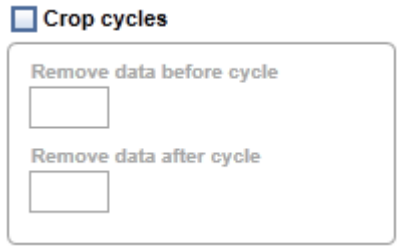
> Note
> For every single acquisition channel an individual *.qut file has to be generated.



37. Select a target from the "Target" table.
38. Click "Load .qut file".
    The file selection dialog is shown.
39. Browse to the directory containing the *.qut file, select it, and click "OK".

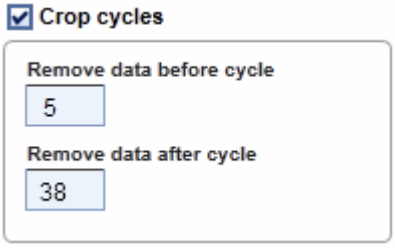
The parameters and values are loaded from the file and displayed at the right of the screen.

40. Repeat steps 37–39 for every single target.
41. Adjust the "Crop cycles" parameters. After successful import of a *.qut file the check box "Crop cycles" will be activated.
    The "Crop cycles" function in Rotor-Gene AssayManager has the same impact on sample analysis as the "Crop cycles" function in the standard Rotor-Gene software. If this function was used for sample analysis in the Rotor-Gene software for that assay, it should also be used in Rotor-Gene AssayManager. The values for the "Crop cycles" function will not be imported via the *.qut file, therefore the additional editing is necessary.



If needed, check the check box to define the number of cycles that should be removed from the start and the end of the cycling for analysis. This is useful if larger deviations from a flat baseline are observed in the initial or end cycles, which can occur when using certain chemistries.

After checking the "Crop cycles" check box, the input boxes "Remove data before cycle" and "Remove data after cycle" will be activated. Enter the respective cycle values into these boxes.



> Note
> The value for "Remove data after cycle" must be higher than the value of "Remove data before cycle". At least seven cycles must be left for data analysis.

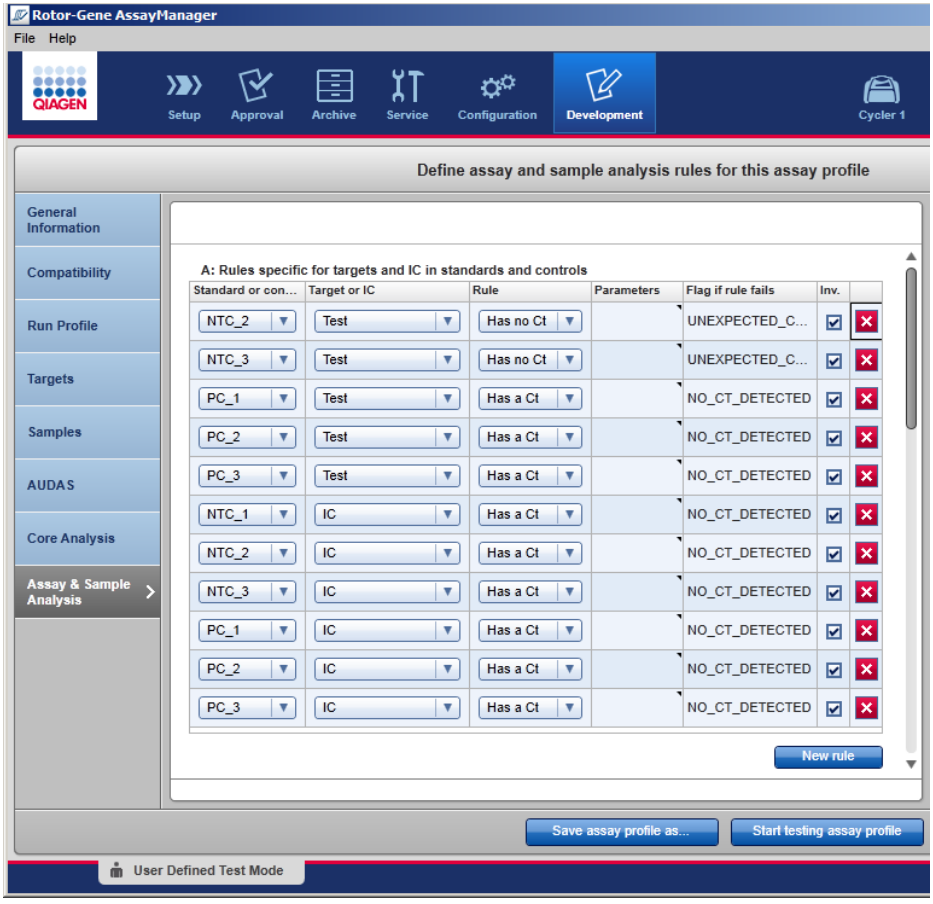
42. Change to the "Assay & Sample Analysis" tab.
    In the "Assay & Sample Analysis" tab different rules can be defined for evaluation of

sample, control, and assay results. The different rules are divided in six different sections:

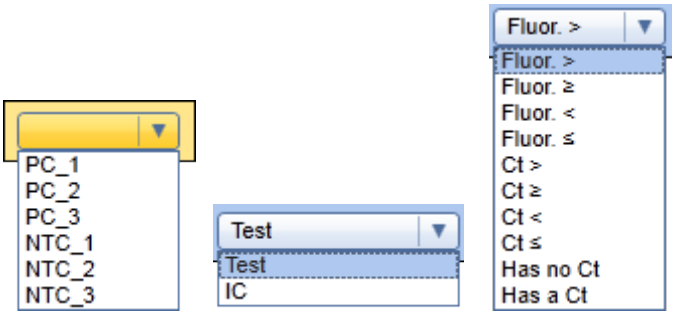
A: Rules specific for targets and IC in standards and controls
B: Rules for standard curve
C: Analysis rules for standards and controls
D: Analysis rules for the assay
E: Rules specific for targets and IC in test samples
F: Analysis rules for test samples

A: Rules specific for targets and IC in standards and controls

In this section rules specific for targets and IC in standards and controls can be defined.



Click "New rule" to create a new rule.



Several rules for a specific target may be defined in parallel. Rules can be defined by:

1. Selecting a specific external control from the "Standard or control" drop-down list.
2. Selecting a specific target from the "Target or IC" drop-down list.
3. Selecting a rule to be applied from the "Rule" drop-down list. The following rules are available:

Rule name	Rule function	Flag if rules fail
Fluor. >	Normalized fluorescence must be greater than the parameter value to be entered.	FLUORESCENCE_TOO_LOW
Fluor. ≥	Normalized fluorescence must be greater than or equal to the parameter value to be entered.	FLUORESCENCE_TOO_LOW
Fluor. <	Normalized fluorescence must be less than the parameter value to be entered.	FLUORESCENCE_TOO_STRONG
Fluor. ≤	Normalized fluorescence must be less than or equal to the parameter value to be entered.	FLUORESCENCE_TOO_STRONG
C_T >	C_T value must be greater than the parameter value to be entered.	CT_BELOW_ACCEPTED_RANGE
C_T ≥	C_T value must be greater than or equal to the parameter value to be entered.	CT_BELOW_ACCEPTED_RANGE
C_T <	C_T value must be less than the parameter value to be entered.	CT_ABOVE_ACCEPTED_RANGE
C_T ≤	C_T value must be less than or equal to the parameter value to be entered.	CT_ABOVE_ACCEPTED_RANGE
Conc.	Concentration must be greater than the	CONCENTRATION_

>*	parameter value to be entered.	BELOW_ ACCEPTED_RANG E
Conc. ≥*	Concentration must be greater than or equal to the parameter value to be entered.	CONCENTRATION_ BELOW_ ACCEPTED_RANG E
Conc. <*	Concentration must be less than the parameter value to be entered.	CONCENTRATION_ ABOVE_ ACCEPTED_RANG E
Conc. ≤*	Concentration must be less than or equal to the parameter value to be entered.	CONCENTRATION_ ABOVE_ ACCEPTED_RANG E
Has no C_T	The amplification curve may not have a C_T value.	UNEXPECTED_CT_ DETECTED
Has a C_T	The amplification curve must have a C_T value.	NO_CT_DETECTED

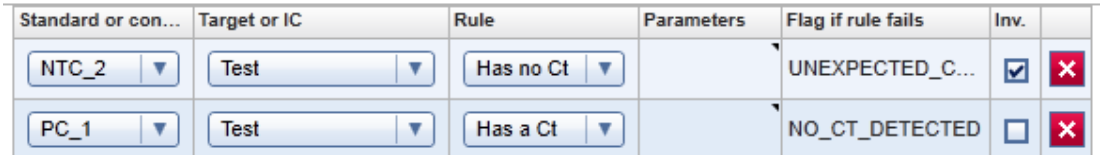
* These rules are only available for quantitative targets. They will only be applied if a valid standard curve has been calculated.

4. If applicable for the selected rule, enter a parameter value in the "Parameters" input box. The input format for the different parameters is as follows:

Parameter	Parameter value format
Fluorescence	Enter a value for the normalized fluorescence between 0 and 100.
C_T value	Enter a C_T value between 1 and 100. The value shall not be larger than the number of cycles of the run.
Concentration	Enter a concentration value. This value has to be in the default concentration unit and relates to the target concentration in the eluate. The default concentration unit is displayed in the "Targets" tab.

5. The "Flag if rule fails" column shows the flag that is assigned to the target and displayed if the rule fails.

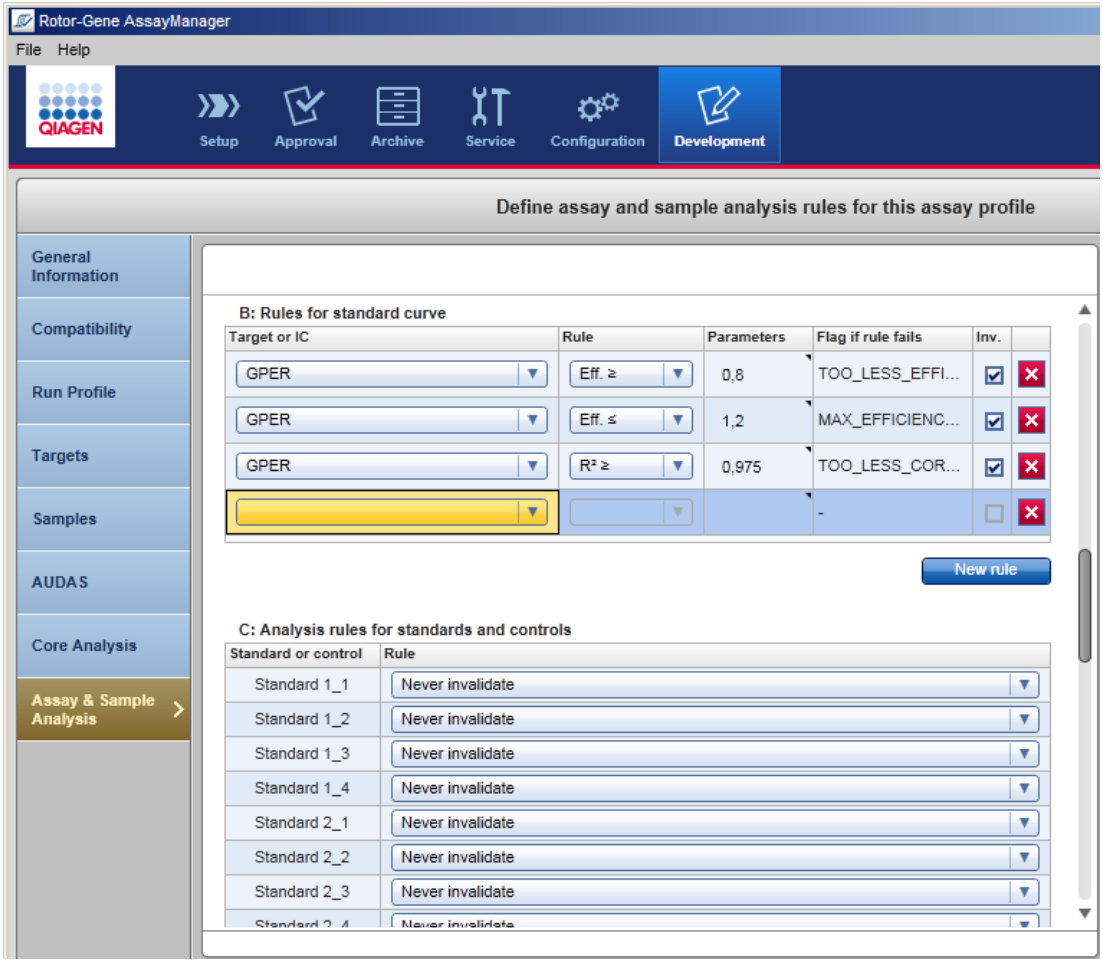
Example:



6. Check the check box in the "Inv." column if the result of the selected target should be set to invalid if the corresponding rule fails. If the check box is not checked, the flag will only be displayed as a "warning" and the target will be valid if no other rule or condition causes an invalid result for this target.

B: Rules for standard curve

In this section rules specific for the standard curve of a quantitative assay can be defined. If the assay is not quantitative, no rules can be defined in that section.



Click "New rule" to create a new rule. Several rules may be defined in parallel. Rules

can be defined by:

1. Select the target the rule shall be defined for. Only quantitative targets can be found in the drop-down list.

2. Select a rule to be applied from the "Rule" drop-down list. The following rules are available:

Rule name	Rule function	Flag if rules fail
R >	The R value of the standard curve must be greater than the parameter value to be entered.	TOO_LESS_CORRELATION_IN_STANDARD_CURVE
R ≥	The R value of the standard curve must be greater than or equal to the parameter value to be entered.	TOO_LESS_CORRELATION_IN_STANDARD_CURVE
R <	The R value of the standard curve must be less than the parameter value to be entered.	MAX_CORRELATION_IN_STANDARD_CURVE_EXCEEDED
R ≤	The R value of the standard curve must be less than or equal to the parameter value to be entered.	MAX_CORRELATION_IN_STANDARD_CURVE_EXCEEDED
R^2 >	The R^2 value of the standard curve must be greater than the parameter value to be entered.	TOO_LESS_CORRELATION_IN_STANDARD_CURVE
R^2 ≥	The R^2 value of the standard curve must	TOO_LESS_CORR

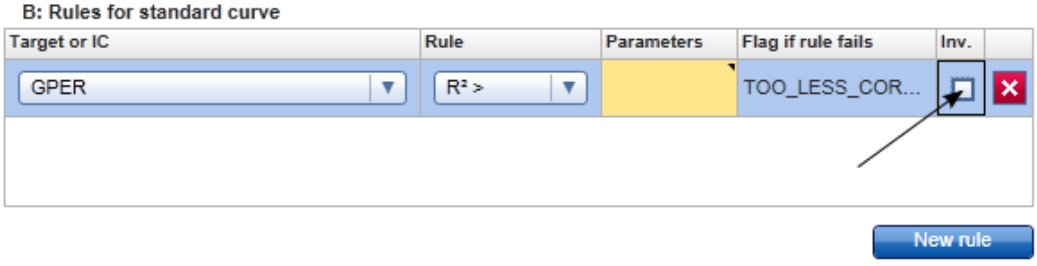
	be greater than or equal to the parameter value to be entered.	ELATION_ IN_STANDARD_CU RVE
R^2 <	The R^2 value of the standard curve must be less than the parameter value to be entered.	MAX_CORRELATIO N_IN_ STANDARD_CURVE _EXCEEDED
$R^2 \leq$	The R^2 value of the standard curve must be less than or equal to the parameter value to be entered.	MAX_CORRELATIO N_IN_ STANDARD_CURVE _EXCEEDED
Eff. >	The reaction efficiency must be greater than the parameter value to be entered.	TOO_LESS_EFFICI ENCY
Eff. ≥	The reaction efficiency must be greater than or equal to the parameter value to be entered.	TOO_LESS_EFFICI ENCY
Eff. <	The reaction efficiency must be less than the parameter value to be entered.	MAX_EFFICIENCY_E XCEEDED
Eff. ≤	The reaction efficiency must be less than or equal to the parameter value to be entered.	MAX_EFFICIENCY_E XCEEDED
# valid QS ≥	The number of valid quantification standards must be greater than or equal to the parameter value to be entered.	TOO_MANY_QUANT IFICATION_ STANDARDS_INVALI D

3. Enter a parameter value in the "Parameters" input box. The input format for the different parameters is as follows:

Parameter	Parameter value format
R value	Enter a value between 0 and 1.
R^2 value	Enter a value between 0 and 1.
Reaction efficiency	Enter a value between 0 and 2 (stands for 0–200%).
Number of valid quantification standards	Enter a value between 0 and 100. The number shall be equal or less than the number of

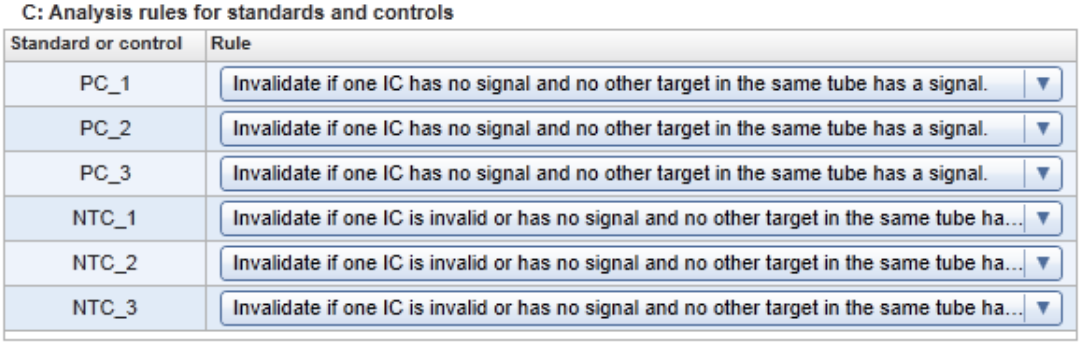
	quantitation standards available for the selected target. Please note that at least two valid quantitation standards with different given concentrations are required for a proper quantification.

4. The "Flag if rule fails" column shows the flag that is assigned to the target and displayed if the rule fails.
5. Check the check box in the "Inv." column if the quantitative target result of the standards should be set to invalid if the configured rule fails. If the check box is not checked, the flag will only be displayed as a "warning" and the target will be valid if no other rule or condition causes an invalid result for this target.



C: Analysis rules for standards and controls

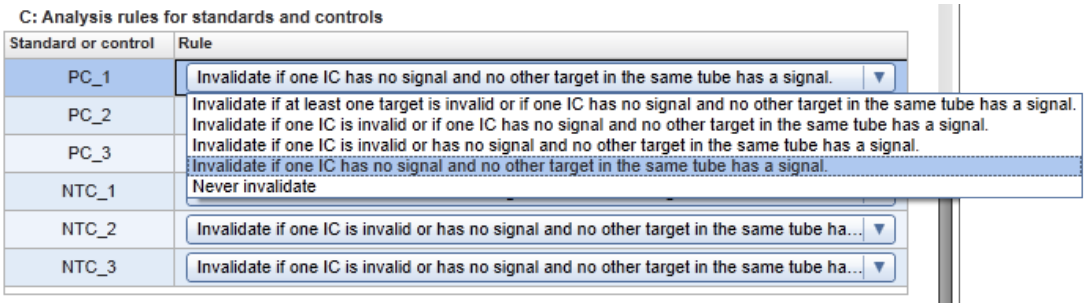
In this section analysis rules specific for standards and controls can be defined.



Section C defines the influence of individual targets with an invalid flag on the validity of the complete standard or control. Individual targets in this context mean all specific targets and internal controls (IC). Please note that all types of invalid flags are taken into account, no matter whether they have been set by the upstream process, the core analysis, or by the rules defined, for example, in Section A and B of the assay and sample analysis.
Furthermore, Section C describes the influence of an IC with no signal on the validity

of the complete standard or control. This takes into account the special role of the IC in real-time PCR to monitor the correct amplification of a sample. The IC signal alone is not conclusive in this context and must be compared to the signal of the corresponding targets in the same tube. For example, a missing signal for the IC only indicates missing amplification, if all other targets in the same tube also do not show amplification. If one of the rules defined in this section is true for a specific target or IC of a standard or control, the complete standard or control is set to invalid in the analysis. This means that all targets of that standard or control are given corresponding invalid flags.

In the "Standard or control" column, every standard or control as defined in the "Samples" sub tab is listed. Select for every standard or control a specific rule from the "Rule" drop-down list. The rules are sorted by stringency, i.e., the first rule of the drop-down list is the most stringent one resulting in more invalidations than rules lower in the table. The lowest rule "never invalidate" results consequently in no change of the validity status of other targets.



The rules are explained in more detail in the table below. The following rules can be applied:

Rule number	Rule name	Rule function	Comments
1	Invalidate if at least one target is invalid or if one IC has no signal and no other target in the same tube has a signal.	All targets of the selected standard or control will be set to invalid, if: ▪ At least one target is invalid. or ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This is the most stringent behavior that can be selected in this section. If any target of the standard or control has an invalid flag (set by the upstream process, the core analysis, or by rules defined in Section A or B), the complete standard or control is set to invalid. The same happens if the

			internal control has no signal (no C_T) and no other target in the same tube as the IC has a signal, which indicates that the PCR run has not correctly amplified the sample. Note: It is recommended to use this most stringent rule for any routine assays. The less stringent rules below can be applied if your assay profile is still under development and you want to see the target result even if there was a problem with another target or your PCR amplification.
2	Invalidate if one IC is invalid or if one IC has no signal and no other target in the same tube has a signal.	All targets of the selected standard or control will be set to invalid, if: ▪ Any internal control is invalid. or ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This rule detects an invalid IC in any case and invalidates the corresponding standard or control. Missing amplification by the IC is also detected and invalidates the standard or control. In comparison to rule 1, invalid specific targets have no effect on the validity of the standard or control. Note: Use with caution. For this rule the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid positive or negative control targets will not automatically invalidate other targets for this

			standard or control.
3	Invalidate if one IC is invalid or has no signal and no other target in the same tube has a signal.	All targets of the selected standard or control will be set to invalid, if: ▪ Any internal control is invalid, and no other target in the same tube has a signal. or ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This rule detects an invalid IC or missing amplification via the IC and invalidates in this case all other targets for this standard or control. However, if amplification is detected simultaneously for any non-IC target, no invalidation will occur. Note: Use with caution. For this rule the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid positive or negative control targets will not automatically invalidate other targets for this standard or control.
4	Invalidate if one IC has no signal and no other target in the same tube has a signal.	All targets of the selected standard or control will be set to invalid, if: ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This rule only detects missing amplification via a missing signal for the IC and invalidates in this case all other targets for this standard or control. Note: Use with caution. Invalidity of the IC for any other reason does not result in corresponding invalidity of other targets for this standard or control. Furthermore, for this rule the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid positive

			or negative control targets will not automatically invalidate other targets for this standard or control.
5	Never invalidate	The selected standard or control will never be set to invalid by that part of the analysis.	With this setting, there is no interdependency between targets. However, all individual targets with flags from previous steps keep their flags and any “invalid” status. Note: Use with caution: Any invalidity for any target will not result in the invalidity of any other target for this standard or control.

Examples for Rule 1

Example 1a

Positive control sample of a duplex assay. The positive control consists of one target (PC_1) and an internal control (IC) in the same tube. There is only one rule defined in Section A for the target PC_1:

“C_T for PC_1 < 30“ (invalidate, if rules fail)

According to rule 1 the PC_1 is then valid only if

1)“C_T for PC_1 < 30“ and no other invalid flag for this target and the IC is valid and has signal.

2) “C_T for PC_1 < 30“and no other invalid flag for this target and the IC is valid but has no signal.

This second case may occur, for example, with a high concentration of PC_1 suppressing the IC signal.

Please note if the second case should be invalidated as well, an additional invalidity rule can be defined for the IC in Section A such as

“IC has a signal”.

Example 1b

NTC of the same duplex assay. There is only one rule defined in Section A for the target NTC:

“NTC has no signal” (invalidate, if rules fail)

According to rule 1, the NTC is then valid only if the ”NTC has no signal” and and no other invalid flag for this target and the IC is valid and has a signal Please note if the “IC has no signal” and the “NTC has no signal”, this rule correctly invalidates the NTC, as the IC has not detected a correct amplification.

Example 1c
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid specific target or an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 1, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 1d
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in any target but no invalid flag.
According to rule 1, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly amplified the sample.

Example 1e
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target or one IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 1, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 1f
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). In one tube both targets, the specific target and the corresponding IC, have no signal but also no invalid flags.
According to rule 1, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly amplified the sample in at least one tube.

Examples for rule 2
Example 2a
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid specific target (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 2, the control is maintained as valid. Only the invalid specific target remains invalid (the invalid flag is kept).

Example 2b
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 2, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 2c
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in any target but no invalid flag.
According to rule 2, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly amplified the sample.

Example 2d
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 2, the control is maintained as valid. Only the invalid specific target remains invalid (the invalid flag is kept).

Example 2e
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 2, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 2f
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). In one tube both targets, the specific target and the corresponding IC, have no signal but also no invalid flags.
According to rule 2, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly amplified the sample in at least one tube.

Examples for rule 3
Example 3a
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid specific target (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is maintained as valid. Only the invalid specific target remains invalid (the invalid flag is kept).

Example 3b
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with a specific target, which has a signal and an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is maintained as valid. Only the invalid IC target

remains invalid (the invalid flag is kept).

Example 3c
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in the specific targets and an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 3d
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in any target but no invalid flag.
According to rule 3, the control is set to invalid (all targets ([specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly amplified the sample.

Example 3e
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is maintained as valid. Only the invalid specific target remains invalid (the invalid flag is kept).

Example 3f
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target has a signal but the corresponding IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is maintained as valid. Only the invalid IC target remains invalid (the invalid flag is kept).

Example 3g
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target has no signal and the IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 3, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag).

Example 3h
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). In one tube both targets, the specific target and the corresponding IC, have no signal but also no invalid flags.
According to rule 3, the control is set to invalid (all targets [specific ones and the IC] are given an invalid flag) since the PCR process has obviously not correctly

amplified the sample in at least one tube.

Examples for rule 4

Example 4a

A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid specific target or an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).

According to rule 4, the control is maintained as valid. Only the invalid target remains invalid (the invalid flag is kept).

Example 4b

A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in any target but no invalid flag.

According to rule 4, the control is set to invalid (all targets [specific ones and the IC] get an invalid flag) since the PCR process has obviously not correctly amplified the sample.

Example 4c

An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target or one IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).

According to rule 4, the control is maintained as valid. Only the invalid target remains invalid (the invalid flag is kept).

Example 4d

An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). In one tube both targets, the specific target and the corresponding IC, have no signal but also no invalid flags.

According to rule 4, the control is set to invalid (all targets [specific ones and the IC] get an invalid flag) since the PCR process has obviously not correctly amplified the sample in at least one tube.

Examples for rule 5

Example 5a

A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with an invalid specific target or an invalid IC (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).

According to rule 5, the control is maintained as valid. The invalid target remains invalid (the invalid flag is kept).

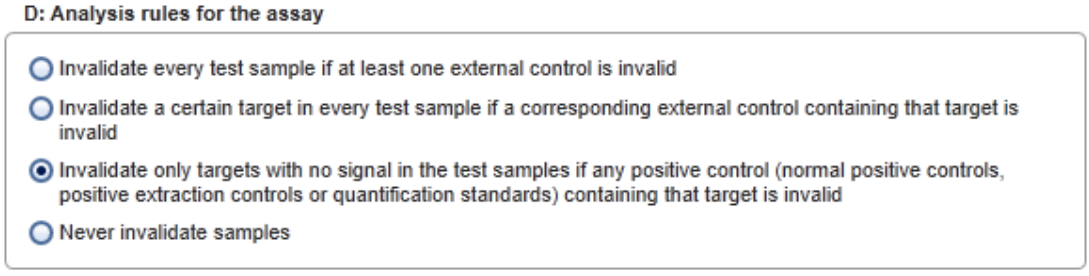
Example 5b
A 3-plex assay (two specific targets and one IC, all in the same tube) contains a control with no signal in any target but no invalid flag. According to rule 5, the control is maintained as valid.

Example 5c
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). One specific target or one IC has an invalid flag (no matter whether it is set to invalid by the upstream process, the core analysis, or rules defined in Section A or B).
According to rule 5, the control is maintained as valid. Only the invalid target remains invalid (the invalid flag is kept).

Example 5d
An assay with a sample divided over 4 tubes contains one specific target in every tube and a corresponding IC (8 targets overall). In one tube both targets, the specific target and the corresponding IC, have no signal but also no invalid flags.
According to rule 5, the control is maintained as valid.

D: Analysis rules for the assay

In this section, analysis rules specific for the complete assay can be defined. These rules define the consequences of any “invalid” results for standards and controls due to the rules described in Section C.



Select one of the four radio buttons to apply the corresponding analysis rule to the assay. The following rules are available:

Rule name	Rule function
Invalidate every test sample if at least one external control is invalid.	A flag is set to all targets of every test sample that the assay is invalid if at least one external control is invalid. If the rule is applied during assay analysis because of an invalid external control, the assay can be set manually

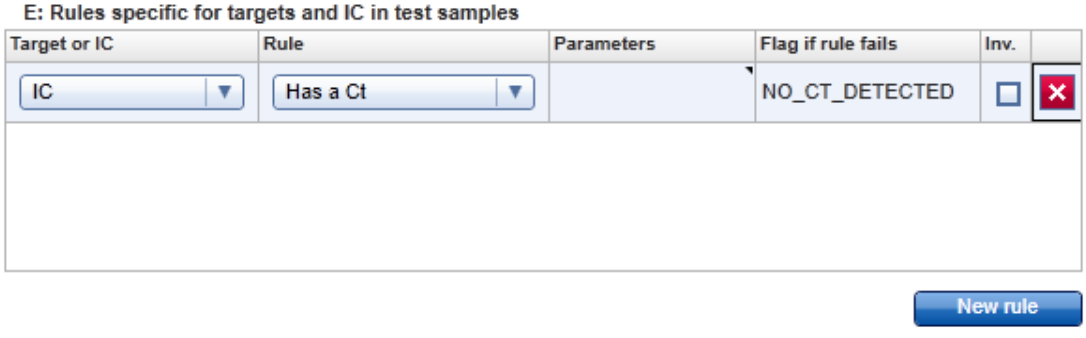
	to be valid by checking the check box "Set assay to be valid" in the "Approval" environment. This functionality must be enabled first in the “Configuration” environment. Further information can be found under ▶ Concept of approval buttons in UDT plug-in.
Invalidate a certain target in every test sample if a corresponding external control containing that target is invalid.	Certain targets of test samples are set to invalid if any standard or control containing the same target was set to invalid.
Invalidate only targets with no signal in the test samples if any positive control (normal positive controls, positive extraction controls, or quantification standards) containing that target is invalid.	Certain targets of test samples are set to invalid if the target result is "No signal" and any positive control containing the same target was set to invalid.
Never invalidate samples.	Samples will never be set to invalid by that part of the analysis.

Note

The rules in the drop-down menu are sorted for strictness in descending order.

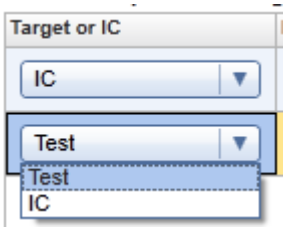
E: Rules specific for targets and IC in the test samples

In this section, analysis rules specific for targets and internal control in the test samples can be defined. Several rules for a specific target may be defined in parallel.

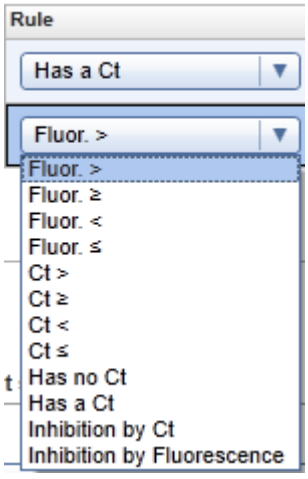


Click "New rule" to create a new rule.

1. Select a specific target from the "Target or IC" drop-down list.



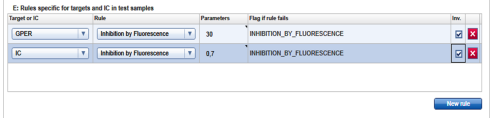
2. Select a rule to be applied from the "Rule" drop-down list. The following rules are available:



Rule name	Rule function	Flag if rules fail
Fluor. >	Normalized fluorescence must be greater than the parameter value to be entered.	FLUORESCENCE_TOO_LOW
Fluor. ≥	Normalized fluorescence must be greater than or equal to the parameter value to be entered.	FLUORESCENCE_TOO_LOW
Fluor. <	Normalized fluorescence must be less than the parameter value to be entered.	FLUORESCENCE_TOO_STRONG
Fluor. ≤	Normalized fluorescence must be less than or equal to the parameter	FLUORESCENCE_TOO_STRONG

	value to be entered.	
C_T >	C_T value must be greater than the parameter value to be entered.	CT_BELOW_ACCEPTED_RANGE
$C_T \geq$	C_T value must be greater than or equal to the parameter value to be entered.	CT_BELOW_ACCEPTED_RANGE
C_T <	C_T value must be less than the parameter value to be entered.	CT_ABOVE_ACCEPTED_RANGE
$C_T \leq$	C_T value must be less than or equal to the parameter value to be entered.	CT_ABOVE_ACCEPTED_RANGE
Conc. >*	Concentration must be greater than the parameter value to be entered.	CONCENTRATION_BELOW_ ACCEPTED_RANGE
Conc. ≥*	Concentration must be greater than or equal to the parameter value to be entered.	CONCENTRATION_BELOW_ ACCEPTED_RANGE
Conc. <*	Concentration must be less than the parameter value to be entered.	CONCENTRATION_ABOVE_ ACCEPTED_RANGE
Conc. ≤*	Concentration must be less than or equal to the parameter value to be entered.	CONCENTRATION_ABOVE_ ACCEPTED_RANGE
Has no C_T	The amplification curve may not have a C_T value.	UNEXPECTED_CT_ DETECTED
Has a C_T	The amplification curve must have a C_T value.	NO_CT_DETECTED
Inhibition by C_T	For inhibition testing by C_T this rule has to be applied to every single target of a test sample. Note that the rule has a different meaning depending on whether it is applied to an internal control or to another target. Inhibition testing is only useful for multiplex PCRs with all targets of a sample analyzed in the same tube.	INHIBITION_BY_CT

	If this rule is applied to a target that is not the IC: Enter the minimum C_T value for which the inhibition rule should be applied. If the C_T value of this target is greater than the entered value or there is no signal at all, the inhibition check will be applied. If the entered C_T value is not exceeded or if another test target has a signal, the inhibition check will not be applied. If applied to the IC: The difference between the C_T value of the internal control of the test sample and the mean C_T value of the internal control of the NTCs has to be less than the value to be entered. x = (C_T of test sample IC) – (mean C_T of all NTC ICs) x must be less than the value to be entered.	
Inhibition by fluorescence	For inhibition testing by fluorescence this rule must be applied to every single target of a test sample. Note that the rule has a different meaning depending on whether it is applied to an internal control or to another target. Inhibition testing is only useful for multiplex PCRs with all targets of a sample analyzed in the same tube. If this rule is applied to a target that is not the IC: Enter the minimum C_T value for which the inhibition rule should be applied. If the C_T value of this target is greater than the entered value or there is no signal at all, the inhibition check will be applied. If the entered	INHIBITION_BY_FLUORESCENCE

	C_T value is not exceeded or if another test target has a signal, the inhibition check will not be applied. If applied to the IC: The difference between the mean normalized fluorescence value of the internal control of the NTCs and the normalized fluorescence value of the internal control of the test sample must be within a certain range depending on the parameter value to be entered. The normalized fluorescence values are taken from the last cycle of the PCR. $x = (FI_{IC\ NTC} - FI_{IC\ Test}) / (FI_{IC\ NTC})$ $FI_{IC\ NTC}$: Mean normalized fluorescence of all NTC ICs $FI_{IC\ Test}$: Normalized fluorescence of test sample IC x must be less than the parameter value to be entered. In the following example an inhibition by fluorescence check is applied for all test samples with a C_T of greater than 30 in the test target "GPER". If the calculated factor "x" is greater than 0.7 the test sample will get an "INHIBITION_BY_FLUORESCENCE" flag.  	
> Upper LOQ*	This rule is only applied if a signal was detected for the selected target. LOQ stands for Limit Of Quantification. The concentration of the target must be less than the	ABOVE_UPPER_LOQ

	parameter value to be entered. If the target concentration is greater than the parameter value to be entered the displayed target result depends on the status of the invalidation check box: 1) If the invalidation check box is activated, the result will be "INVALID". 2) If the invalidation check box is deactivated, only a qualitative result will be presented ("Signal detected").	
< Lower LOQ*	This rule is only applied if a signal was detected for the selected target. LOQ stands for Limit Of Quantification. The concentration of the target must be greater than the parameter value to be entered. If the target concentration is less than the parameter value to be entered the displayed target result depends on the status of the invalidation check box: 1) If the invalidation check box is activated, the result will be "INVALID". 2) If the invalidation check box is deactivated, only a qualitative result will be presented ("Signal detected").	BELOW_LOWER_LOQ

* These rules are only available for quantitative targets. They will only be applied is a valid standard curve has been calculated.

3. If applicable for the selected rule, enter a parameter value in the "Parameters" input box. The input format for the different parameters is as follows:

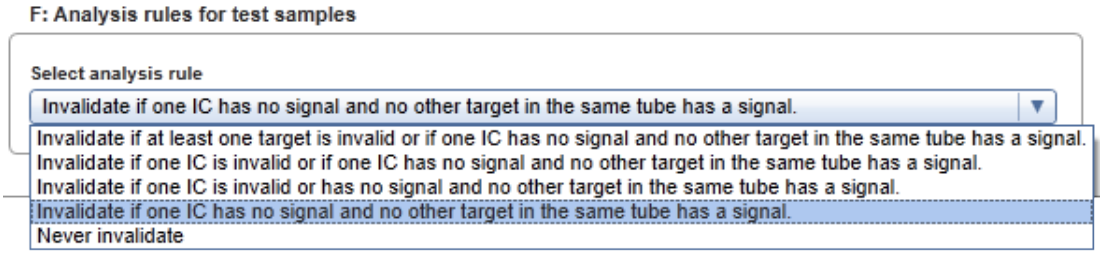
Parameter	Parameter value format
Fluorescence	Enter a value for the normalized fluorescence between 0 and 100.
C_T value	Enter a C_T value between 1 and 100. The value shall not be larger than the number of cycles of the run.

Concentration	Enter a concentration value. This value must be in the default concentration unit and is relating to the target concentration in the eluate.
Inhibition by C_T	For a target that is not the IC: Enter a C_T value between 1 and the number of cycles defined in the assay profile. For IC: Enter a value for the maximum Delta C_T between IC_{Test} and IC_{NTC} which may not be exceeded.
Inhibition by fluorescence	For a target that is not the IC: Enter a C_T value between 1 and the number of cycles defined in the assay profile. For IC: Enter a value for x that has to be between 0 and 1. $x = (Fl_{IC\ NTC} - Fl_{IC\ Test}) / (Fl_{IC\ NTC})$ $Fl_{IC\ NTC}$: Mean normalized fluorescence of all NTC ICs $Fl_{IC\ Test}$: Normalized fluorescence of test sample IC
> Upper LOQ	Enter the maximum concentration within the linear range of the target. This value must be in the default concentration unit and is related to the target concentration in the eluate.
< Lower LOQ	Enter the minimum concentration within the linear range of the target. This value must be in the default concentration unit and is related to the target concentration in the eluate.

4. In the "Flag if rule fails" box the flag that will be applied if the rule fails is automatically displayed.
5. Check the check box in the "Inv." column if the target result should be set to invalid if the configured rule fails. If the check box is not checked, the flag will only be added as warning to a valid result.

F: Analysis rules for test samples

In this section, analysis rules specific for test samples can be defined.



The function of Section F corresponds to Section C above, but describes the impact of the analysis result for individual targets on the validity of the whole test sample. Individual targets in this context means all specific targets and internal controls (IC). Please note that all types of invalid flags are taken into account, no matter whether they have been set by the upstream process, the core analysis, or by the rules defined, for example, in Sections A and B of the assay and sample analysis. Furthermore, Section C describes the influence of an IC with no signal on the validity of the test sample. This takes into account the special role of the IC in real-time PCR to monitor the correct amplification of a sample. The IC signal alone is not conclusive in this context and must be compared to the signal of the corresponding targets in the same tube. For example, a missing signal for the IC only indicates a missing amplification, if also all other targets in the same tube do not show amplification. If one of the rules defined in this section is true for a specific target or IC of a test sample, the complete test sample is set to invalid in the analysis. This means that all targets of that test sample are given corresponding invalid flags.

Select an analysis rule from the drop-down list. The following rules can be applied:

Rule name	Rule function	Comments
Invalidate if at least one target is invalid or if one IC has no signal and no other target in the same tube has a signal.	All targets of the test samples will be set to invalid, if: ▪ At least one target is invalid. or ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This is the most stringent behavior that can be selected in this section. If any target of a test sample has an invalid flag (set by the upstream process, the core analysis, or by rules defined in Section A or B), the complete test sample is set to invalid. The same happens if the internal control has no signal (no C_T) and no other target in the

		same tube as the IC has a signal which indicates that the PCR run has not correctly amplified the sample. Note: It is recommended to use this most stringent rule for any routine assays. The less stringent rules below can be applied if your assay profile is still under development and you want to see target result even if there was a problem with another target or your PCR amplification.
Invalidate if one IC is invalid or if one IC has no signal and no other target in the same tube has a signal.	All targets of the test samples will be set to invalid, if: ▪ Any internal control is invalid. or ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This rule detects an invalid IC in any case and invalidates the corresponding test sample. A missing amplification by the IC is also detected and invalidates the test sample. In comparison to rule 1, invalid specific targets have no effect on the validity of the test sample. Note: Use with caution. For this rule the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid individual targets will not automatically invalidate other targets for this test sample.
Invalidate if one IC is invalid or has no signal and no other target in the same tube has a signal.	All targets of the test sample will be set to invalid, if: ▪ Any internal control is invalid, and no other target in the same tube has a signal. or	This rule detects an invalid IC or a missing amplification via the IC and invalidates in this case all other targets for this test sample. However, if amplification is detected simultaneously for any non-

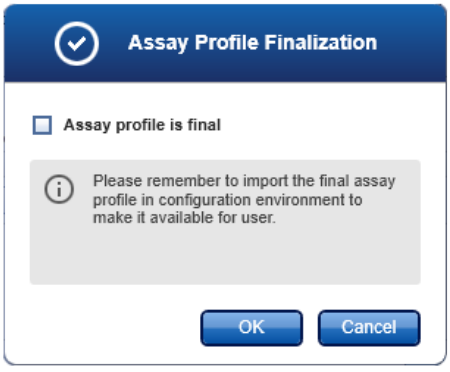
	▪ Any internal control has no signal, and no other target in the same tube has a signal.	IC target, no invalidation will occur. Note: Use with caution. For this rule the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid individual targets will not automatically invalidate other targets for this test sample.
Invalidate if one IC has no signal and no other target in the same tube has a signal.	All targets of the selected test sample will be set to invalid, if: ▪ Any internal control has no signal, and no other target in the same tube has a signal.	This rule only detects missing amplification via a missing signal for the IC and invalidates in this case all other targets for this test sample. Note: Use with caution. An invalidity for the IC for any other reason does not result in a corresponding invalidity of other targets for this test sample. Also, for this rule, the validity status of any non-IC target is not relevant for other targets. For higher multiplex assays, this may have the result that invalid individual targets will not automatically invalidate other targets for this test sample.
Never invalidate	The selected standard or control will never be set to invalid.	With this setting, there is no interdependency between targets. However, all individual targets with flags from previous steps keep their flags and any “invalid” status. Note: Use with caution: Any invalidity for any target will not result in the invalidity of any other target for this test

sample.

Note
The rules in the drop-down list are sorted for stringency in descending order.

For examples of how the different rules can be applied, please refer to Section C above.

43. After all assay and sample analysis rules are set, click "Save assay profile as...".
44. The following dialog is displayed:



45. Confirm that the assay profile is final by activating the "Assay profile is final" check box (if this check box is not checked, the assay profile cannot be imported for work list setup in Rotor-Gene AssayManager).
46. Click "OK".
47. The "Save assay profile as..." dialog is displayed.
48. Browse the target directory and click "OK".

Note
Before the new assay profile can be used for setting up a work list, it must be imported to the Rotor-Gene AssayManager database. Go to the "Assay Profiles" tab in the "Configuration" environment, click "Import...", and select the file to be imported. Click "Open" to import the new assay profile to the Rotor-Gene AssayManager database.

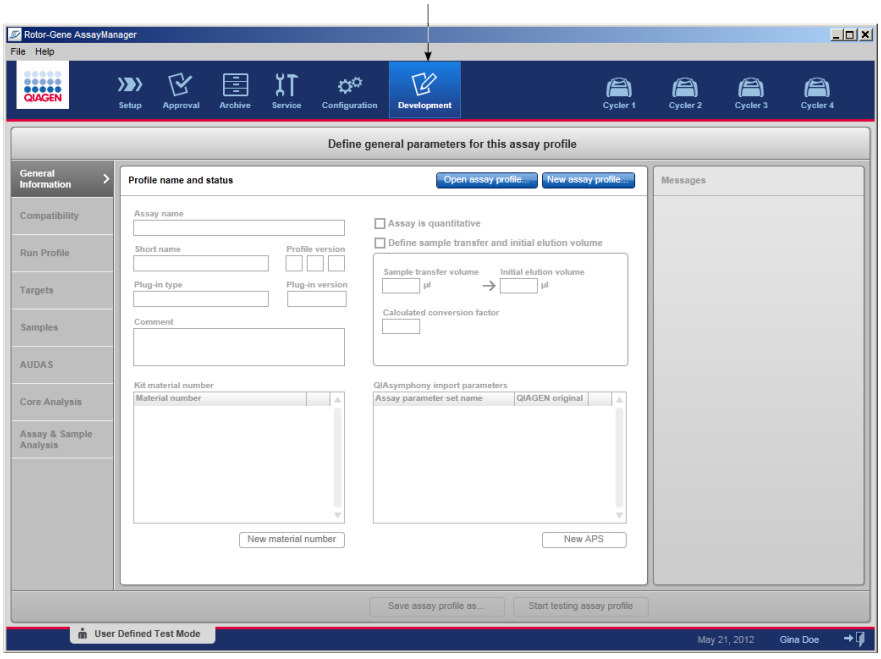
Related topics
- Testing an assay profile

Modifying an Assay Profile

The alternative to creating an assay profile from scratch is to import an existing one and modify it accordingly. The work flow for modifying an existing assay profile is the same as described in ▸ Creating an Assay Profile. The only difference is that instead of clicking "New assay profile...", "Open assay profile..." is used.

Step-by-step procedure to modify an assay profile

1. Click the "Development" icon to change to the "Development" environment.



2. The "Development" environment opens. In this initial state only the two start buttons, "Open assay profile..." and "New assay profile...", are enabled. All other elements are disabled.
3. Click "Open assay profile...".

   The "Select assay profile to load" dialog opens.
4. Browse the directory containing the assay profile to be used, select it, and click "OK".
5. Continue with step 7 in the procedure described in ▸ Creating an assay profile.

Related topics

▸ Testing an assay profile

Testing an Assay Profile

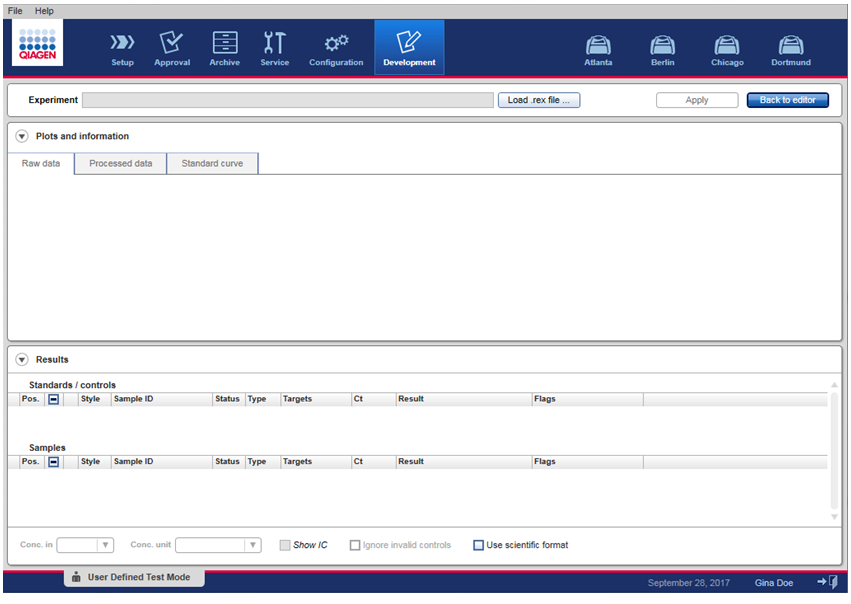
An assay profile currently in the development process can be tested by performing a virtual analysis of a previously finished PCR experiment. The current assay profile can be tested using real experiment data. The outcome of this process is the answer to the question "What would the results have been if a previously finished experiment would be run with the currently developed assay profile?".

A *.rex file (containing raw experiment data and sample data) from an experiment performed with the Rotor-Gene software or Rotor-Gene AssayManager can be loaded. The data of the *.rex file are analyzed with the currently developed assay profile — specifically the rules and parameters defined in the "Core Analysis" and "Assay & Sample Analysis" sub tabs. Raw data, processed data, and — for quantitative assays — also the standard curve can be checked and compared to the results generated by the assay profile.

Test screen

The screen to test assay profiles has three parts:

- An interactive button bar at the top
- "Plots and information" area
- "Results" area



A *.rex file is loaded using the "Load .rex file..." button at the top of the screen. Clicking "Apply" starts the analysis process using the loaded *.rex file and the currently developed assay profile. Clicking "Back to editor" changes to the "Development" environment.

Note

The assay profile test environment is designed to be very similar to the "Approval" environment. For further information about the functionalities, refer to the description of the "Approval" environment in the *Rotor-Gene AssayManager v1.0 Core Application User Manual* .

Step-by-step procedure to test an assay profile

1. Click "Start testing assay profile" in the button bar of the "Development" environment.

   The screen to test assay profiles is opened.

2. Click "Load *.rex file" in the button bar.

   The "Select *.rex file to load" dialog opens.

3. Change to the directory containing the *.rex file, select it, and click "OK".

Note

The run profile of the *.rex file must match the run profile of the assay profile exactly. Even the positions of external controls and test samples on the rotor must be identical.
If run settings or sample type definitions differ between the two files, a corresponding error message will be displayed.

Note

Empty rotor positions must have the sample type "None" in the rex file to be loaded. Only test sample positions may be of the sample type "Unknown".

Note

The testing environment only supports rex files with samples defined on one page. Rex files with samples defined on several pages cannot be loaded.

4. Click "Apply" in the button bar to start the analysis process using the currently developed assay profile.

   Raw experiment data from the *.rex file are analyzed using the assay profile.

The results are presented in the "Plots and information" area and the "Results" table.

Note

If changes were made to the assay profile, the results in the test environment will not automatically be updated when returning. The "Apply" button must be clicked to update the results.

Note

The loaded *.rex file must contain only raw experiment data and sample data. If the “crop cycles” function has already been used on the file, the *.rex file cannot be used in the Assay Profile test environment and will be indicated by a corresponding message. Therefore, re-open the *.rex file with the Rotor-Gene Q software and delete the crop cycled raw channel. Click on “Options of the corresponding raw channel and select “Delete this raw channel”. After the export of the *.rex file, it can be used in the Rotor-Gene AssayManager v1.0 assay profile test environment.

Creating a .qut file

The core analysis defines algorithms for the normalization of the amplification curves and quantification of the targets. In the "Core Analysis" tab most of the parameter values must be imported from a Rotor-Gene quantification template file. This *.qut file can be generated after analysis of an assay in the standard Rotor-Gene software.

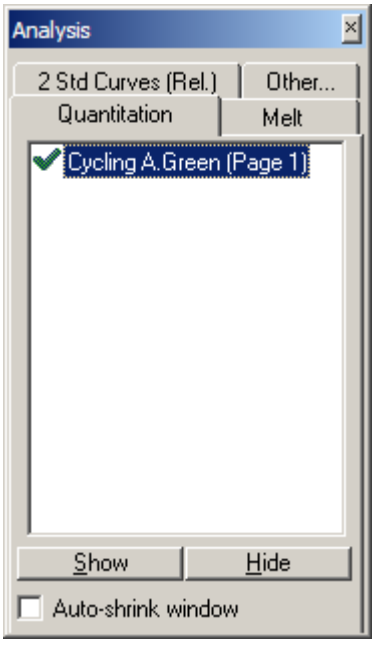
Generating *.qut files in Rotor-Gene software

Analysis

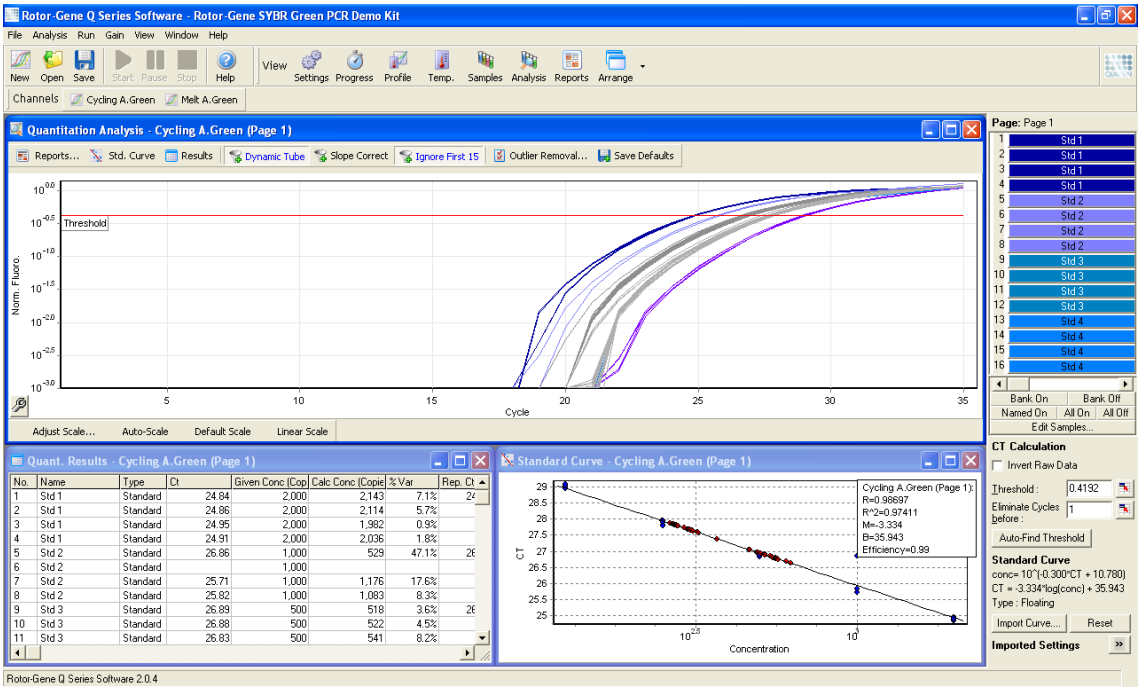
After opening the raw data of a PCR run and clicking "Analysis", the “Analysis” window appears.

Saving a *.qut file

Select the “Quantitation” tab in the “Analysis” window. Double-click on the channel name or select the channel and click "Show" to open the channel of interest.



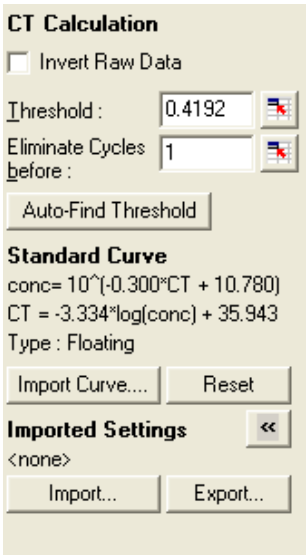
Three windows appear: the main screen, the standard curve, and the results. Adapt the analysis options as required (e.g., set threshold, activate dynamic tube normalization, apply slope correction, etc.).



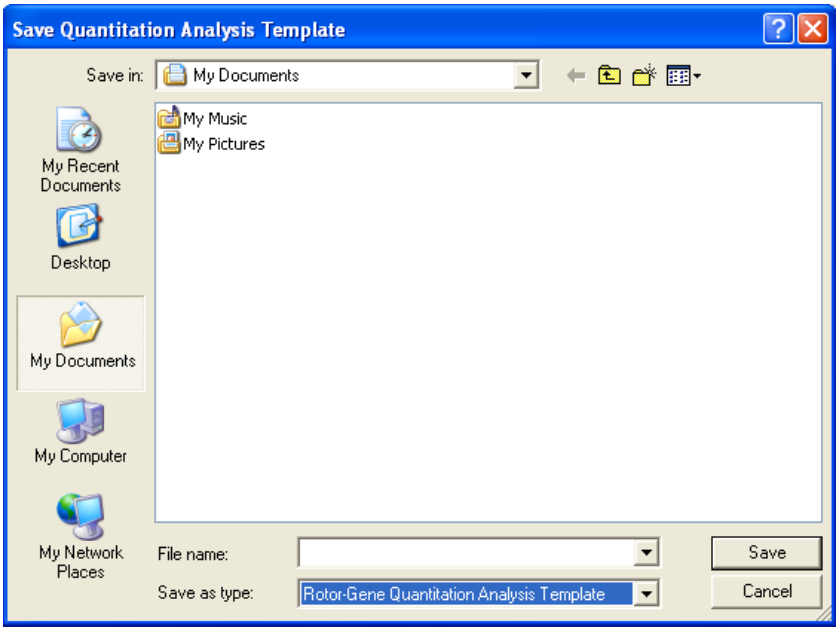
Note

For details regarding the different analysis options in the Rotor-Gene software refer to the *Rotor-Gene Q User Manual* .

At the bottom right of the screen expand the “Imported Settings” by clicking  .



Click "Export..." to export the selected analysis options to a Rotor-Gene Quantitation Analysis Template.



Enter a file name, browse the target directory, and confirm by clicking "Save". The Rotor-Gene Quantitation Template file extension is *.qut.

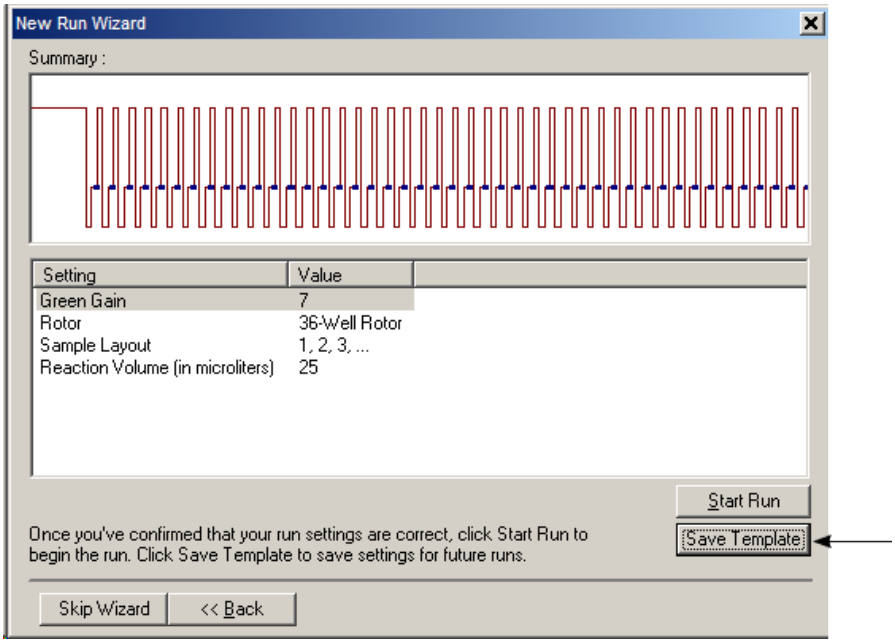
> Note
> For every single acquisition channel an individual *.qut file has to be generated.

Creating a .ret file

The "Run Profile" tab allows loading of a Rotor-Gene experiment template file (*.ret file) to define the cycling conditions and the acquisition channels for the assay profile. These parameters cannot directly be configured or modified in Rotor-Gene AssayManager. The configuration can only be done in the standard Rotor-Gene software. See the *Rotor-Gene Q User Manual* for details.

Saving templates in the Rotor-Gene software

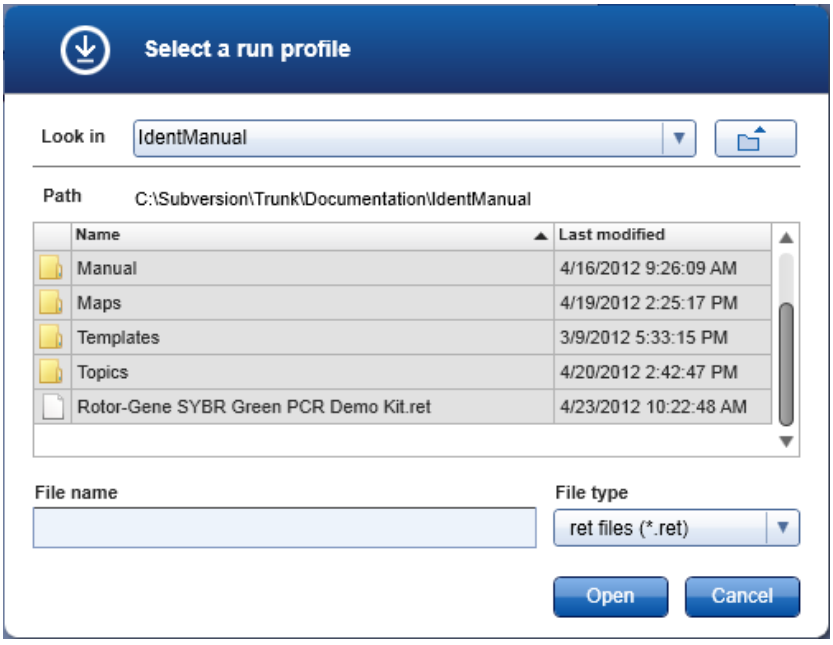
Set up a run in the Rotor-Gene software using the Advanced wizard according to the assay requirements. In the "New Run Wizard window 4" the run settings are summarized and can be saved as a template using "Save Template". Alternatively open a finished run and select the "Save As Template..." function from the file menu. For details regarding saving templates refer to the *Rotor-Gene Q User Manual* .



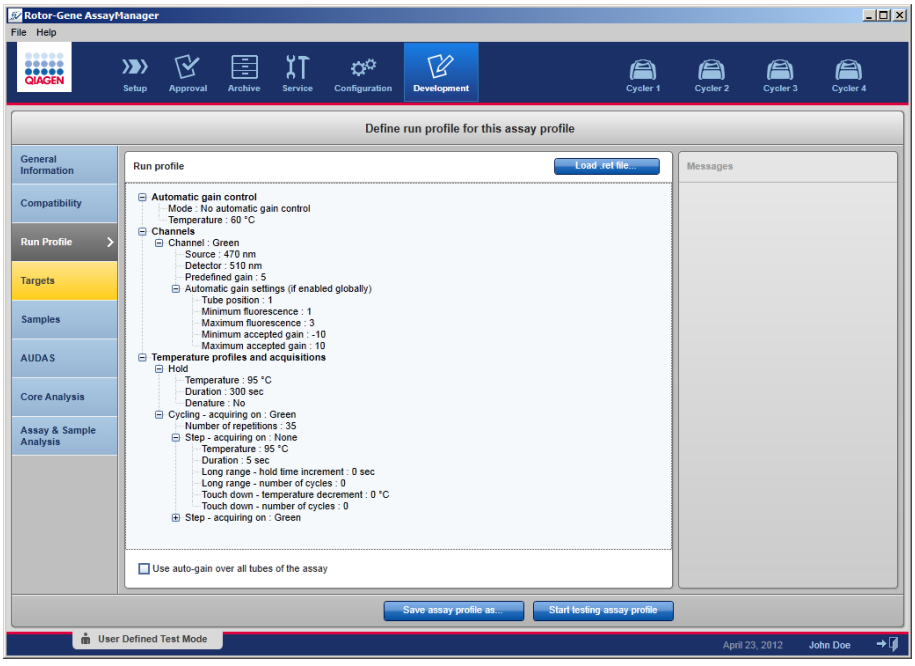
Loading templates in Rotor-Gene AssayManager

To load a Rotor-Gene experiment template file (*.ret file) in Rotor-Gene AssayManager click "Load *.ret file...." 

.
A dialog box opens where the source directory can be selected. Select the desired *.ret file and click "Open".



After successful loading of the template file the detailed run settings can be checked. The different run settings can be enlarged or collapsed using the "+" or "–" buttons in the list.



Note

The run settings cannot be altered using Rotor-Gene AssayManager v1.0.

At the bottom of the screen there is a check box labeled "Use auto-gain over all tubes of the assay". Activate this check box to apply the auto-gain optimization to all reserved rotor positions and not only on the one rotor position defined during run setup in the Rotor-Gene software.

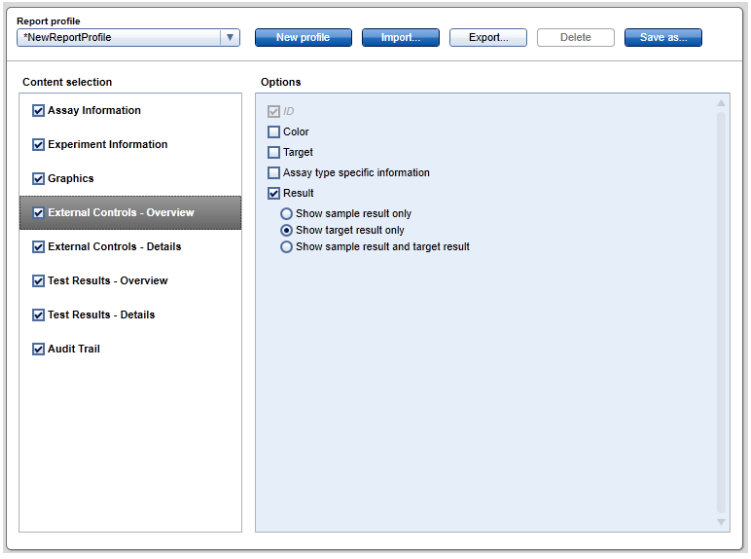
If "Use auto-gain over all tubes of the assay" is checked, the median gain determined on all reserved rotor positions of that assay will be applied during data acquisition. This option applies to all different acquisition channels and steps defined in that assay profile.

1.3.2.4 Report Profiles for UDT Assays

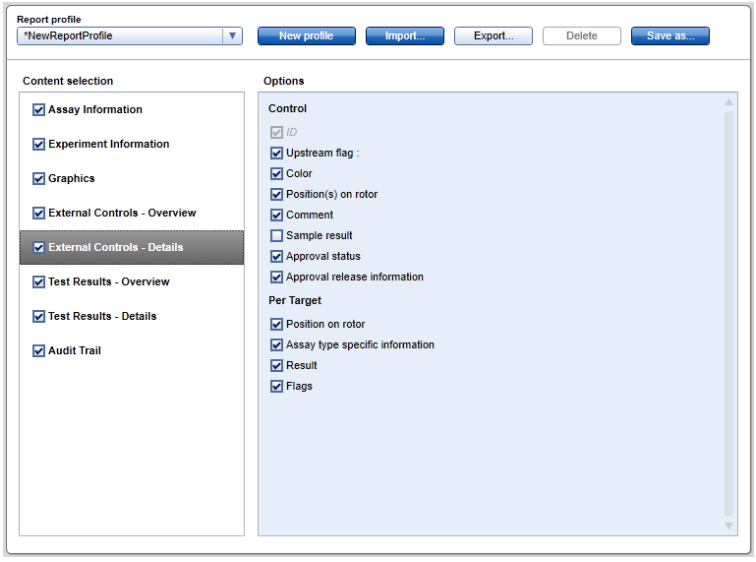
In a report profile used to report data for a UDT Basic Plug-in assay several options must be set in a certain way in order to get an appropriate PDF report. Report profiles can be created and managed in the "Report Profiles" tab of the "Configuration" environment.

The following configuration is useful for report profiles used for standard UDT Basic Plug-in assays with one rotor position per sample ID:

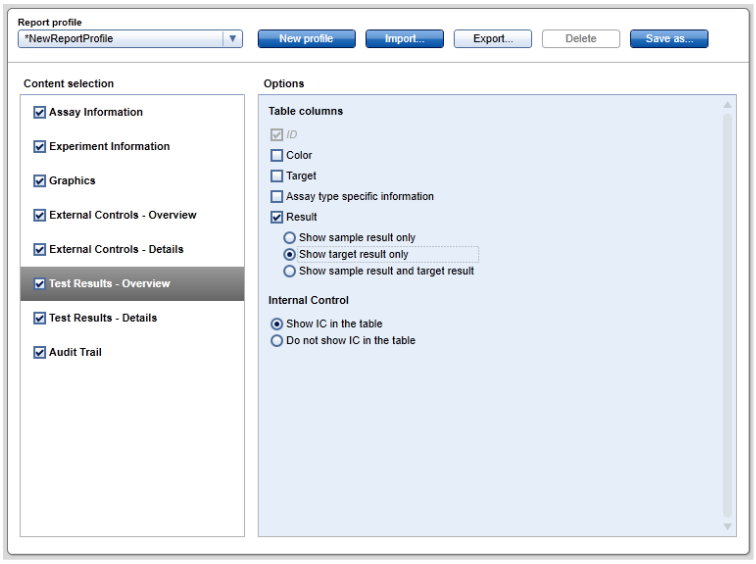
1. Go to "External Controls - Overview" in the "Content selection" area and select the "Show target result only" radio button.



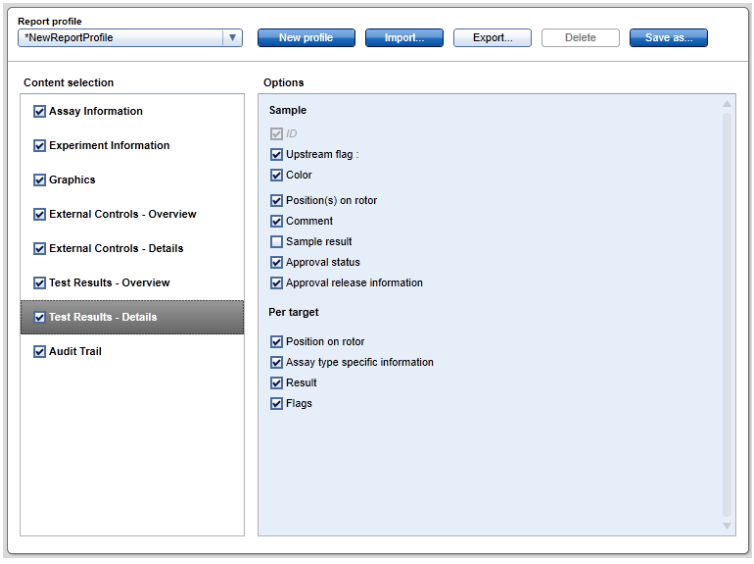
2. Go to "External Controls - Details" in the "Content selection" area and deselect the "Sample result" check box.



3. Go to "Test Results - Overview" in the "Content selection" area and select the "Show target result only" radio button.



4. Go to "Test Results - Details" in the "Content selection" area and deselect the "Sample result" check box.



In addition to these configurations, the report profiles can be adapted to the individual needs for the report.

Only for UDT Basic Plug-in assays, where a sample is split into several rotor positions, the "Sample result" option in the report profile mentioned above is essential.

1.4 Hint for Online Documentation

Rotor-Gene AssayManager uses plug-ins to increase its functionality. In order to have a clear distinction between the core application user manual and the plug-in user manuals and to keep the documentation short and focused, general topics are explained in the core application user manual.

Providing you with the best information depends on the environment you are currently in, especially for the following items:

- Help for "Plots and information" table
- Help for "Results" table
- Help for testing an assay profile

1.4.1 Help for Plots and Information Table

The help information for the "Plots and Information" table is available either in the *UDT Basic Plug-in User Manual* or in the *Rotor-Gene AssayManager Core Application User Manual* .

The table below shows — depending on the current environment — where to find more information.

Environment	Help file and topic
Approval	*UDT Basic Plug-in User Manual* (i.e., this manual) Topic: ▸ General information about approving samples
Archive	*Rotor-Gene AssayManager Core Application User Manual* Topics: ▪ Basic Concepts ➔ Environments ➔ "Archive" Environment ▪ Using Rotor-Gene AssayManager ➔ Administrative Tasks ➔ Managing Archives
Development	*UDT Basic Plug-in User Manual* (i.e., this manual) Topic: ▸ Testing an assay profile

In case the information cross-references the *Rotor-Gene AssayManager Core Application User Manual* , open the help file using the Windows Start menu:

Start ➜ All Programs ➜ QIAGEN ➜ Rotor-Gene AssayManager

1.4.2 Help for Result Table

The help information for the "Results Table" is available either in the *UDT Basic Plug-in User Manual* or in the *Rotor-Gene AssayManager Core Application User Manual* .

The table below shows — depending on the current environment — where to find more information.

Environment	Help file and topic
Approval	*Rotor-Gene AssayManager Core Application User Manual* Topic: ▪ Using Rotor-Gene AssayManager ➜ Standard Tasks ➜ Approving a Run
Archive	*Rotor-Gene AssayManager Core Application User Manual* Topic: ▪ Using Rotor-Gene AssayManager ➜ Administrative Tasks ➜ Managing Archives
Development	*UDT Basic Plug-in User Manual* (i.e., this manual) Topic: Testing an Assay Profile

in case the information cross-references the *Rotor-Gene AssayManager Core Application User Manual* , open the help file using the Windows Start menu:

Start ➜ All Programs ➜ QIAGEN ➜ Rotor-Gene AssayManager

1.4.3 Core Analysis

The help information for the "Core Analysis" is available in the "Creating an Assay Profile" section. Click the link below to jump to the corresponding section:

Core Analysis

1.4.4 Assay and Sample Analysis

The help information for "Assay and Sample Analysis" is available in the "Creating an Assay Profile" section. Click the link below to jump to the corresponding section:

Assay and Sample Analysis

1.5 Error messages

The following list provides all error messages that might occur during the operation of this plug-in provide the following information to the service specialist:

- Actions performed before the error message occurred
- Error ID

> Note
> The error ID is unique and helps QIAGEN Technical Services to clearly identify the error message.

Error ID	Error Text
560010	The assay '{0}' could not be found.
560011	The external control '{0}' could not be found.
560012	The target '{0}' could not be found.
560014	An error occurred while retrieving test samples for assay profile {0}.
560015	Rule parameter for rule '{0}' could not be found.
560017	Could not create rule because of unexpected rule parameter {0}.
560018	Could not create rule of type {0}.
560019	Could not create rule description of type {0}.
560020	No rule with rule name {0} was found.
560021	No rule type {0} was found.
560022	Could not create rule because of unexpected rule parameter count: expected was {0}, but was {1}.
560023	No rule description type {0} was found.
560024	samples collection should at least contain one sample
570003	The provided curve is invalid.
570012	Slope correction cannot be performed without activation of 'DynamicTube' option. Check Rotor-Gene .qut-file and retry.
570014	The provided cycle threshold value is zero. Check Rotor-Gene .qut-file and retry.
570015	The slope of the provided regression line is zero.
570016	Schema validation failed: {0}

Error ID	Error Text
570017	Quantitation template could not be loaded. File reading failed. Check Rotor-Gene .qut-file and retry.
570018	Quantitation template could not be loaded. The file does not contain all mandatory fields. Create a file where all fields including the threshold are set.
570026	The entered number for N1 is invalid. Enter a valid number (1 - {1}).
570027	N2 for target {0} must not be greater than {1}. Enter a valid number in the N2 field.
570031	Enter a valid number for N2 (1 to maximum number of cycles).
570033	The run template does not contain any cycling parameters.
570034	The run profile must only contain "Cycling" and "Hold" steps. Check the run profile and the assay profile for consistency.
570035	Enter a valid number for N1 (1 to maximum number of cycles).
570036	The loaded rex-file contains a melt step. The assay profile does not allow melt steps. Check the rex-file and the assay profile for consistency.
570037	Enter a valid value for {0} of target {1} ({2}-{3}).
570057	No target profile with the name {0} was found.
570066	Shorten the sample comment to max. 256 characters.
570067	Shorten the assay comment to max. 256 characters.
570070	Failed to generate report. Reason: {0}
570073	Failed to launch the application {0}. Reason:
570074	File {0} not found.
570106	The concentration value must be less than the parameter value to be entered.
570107	The R value must be greater than the parameter value to be entered.
570112	The concentration value must be less than the parameter value to be entered.
570113	The concentration value must be less than or equal to the parameter value to be entered.
570114	The Ct value must be less than the parameter value to be entered.
570115	The Ct value must be less than or equal to the parameter value to be entered.
570116	The concentration value must be greater than the parameter value to be entered.
570117	The concentration value must be greater than or equal to the parameter value to be entered.
570118	The Ct value must be greater than the parameter value to be entered.
570119	The Ct value must be greater than or equal to the parameter value to be entered.
570120	The fluorescence must be greater than the parameter value to be

Error ID	Error Text
	entered. (Rule is only evaluated, if a Ct value is present.)
570121	The fluorescence must be greater than or equal to the parameter value to be entered. (Rule is only evaluated, if a Ct value is present.)
570135	The R value must be greater than or equal to the parameter value to be entered.
570136	The efficiency must be greater than the parameter value to be entered.
570137	The efficiency value must be greater than or equal to the parameter value to be entered.
570138	The number of valid quantification standards must be greater than or equal to the parameter value to be entered.
570156	Invalidate if one IC has no signal and no other target in the same tube has a signal.
570157	Invalidate if one IC is invalid or has no signal and no other target in the same tube has a signal.
570158	Invalidate if one IC is invalid or if one IC has no signal and no other target in the same tube has a signal.
570159	Invalidate if at least one target is invalid or if one IC has no signal and no other target in the same tube has a signal.
570172	{0}Please enter valid parameters. For more information, place the cursor over the rule name.
570175	Defines the lower limit of quantification. For concentrations below the parameter value to be entered, only a qualitative result is presented.
570176	Defines the upper limit of quantification. For concentrations above the parameter value to be entered, only a qualitative result is presented.
570186	The fluorescence must be less than the parameter value to be entered.
570187	The fluorescence must be less than or equal to the parameter value to be entered.
570192	This assay type is not supported by AUDAS.
570195	Sample result not supported
570202	Enter a valid password.
570203	This user is deactivated. Contact your local administrator.
570205	Password expired
570206	Enter a valid number for target {0} in the "Remove data after cycle field".
570207	Enter a valid number for target {0} in the „Remove data before cycle“ field (1 – 40).
570208	The value for „Remove data after cycle“ must be higher than the value of „Remove data before cycle“. The difference between these values must be at least 7.
570209	The value in the Remove data after cycle field for target {0} must not be greater than {1}.
570210	Enter a valid number lower than {1} in the "Remove data before cycle"

Error ID	Error Text
	field for target {0}.
570211	The value in the Remove data after cycle field for target {0} must not be smaller than {1}.
570212	The value for "Remove data before cycle" for target {0} must be higher than {1}.
570220	Copying of the selected cells failed. Only adjacent cells can be copied. Copy and paste the selected cells individually.
570222	Paste operation is cancelled. Selected cell(s) must be contiguous.
570223	Paste operation is cancelled. Selected cell(s) must be contiguous.
570224	Paste operation is cancelled. Selected cell(s) must be editable for pasting.
570225	Pasting failed. The selected target area is smaller than the clipboard entry. Select a different target area or reduce data to be copied.
570226	Paste operation is cancelled. Select some cell(s).
570229	There is not enough space for the information to be pasted.
570231	This user was deactivated because the password was entered wrong too many times. Contact your local administrator. The current session will be closed.
570237	The release was not performed but data was saved.
570238	The customized report generation is not supported by this plug-in.
570249	The R value must be less than the parameter value to be entered.
570250	The R value must be less than or equal to the parameter value to be entered.
570251	The efficiency must be less than the parameter value to be entered.
570252	The efficiency value must be less than or equal to the parameter value to be entered.
570253	The R^2 value must be less than the parameter value to be entered.
570254	The R^2 value must be less than or equal to the parameter value to be entered.
570255	The R^2 value must be greater than the parameter value to be entered.
570256	The R^2 value must be greater than or equal to the parameter value to be entered.
570274	The initial elution volume is invalid. Enter a valid volume (1 – 999 999 999).
570276	The sample transfer volume is invalid. Enter a valid volume (1 – 999 999 999).
570279	Sample results will be reported as valid despite one or more invalid external controls. You are abou to ignore analysis rules from the assay profile.
570280	The generated report could not be opened. Verify that you have installed a pdf viewer on your system

1.6 Appendix

The appendix contains the Liability Clause and the License Terms for the UDT basic plug-in.

> Note
> Further information, such as a glossary, can be found in the *Rotor-Gene AssayManager Core Application User Manual* .

Liability Clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.
All materials replaced under this warranty will be warranted only for the duration of the original warranty period and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices, and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

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