January 2022

Creating a Rotor-Gene AssayManager® Assay Profile

Introduction

This guide describes the creation of a Rotor-Gene AssayManager assay profile in detail (only available for the User Defined Test [UDT] mode). An example is used to illustrate a step-by-step guide through the complete workflow.

Assay settings/parameters need to be developed using the Rotor-Gene® Q software as Rotor-Gene AssayManager does not support this functionality. Afterwards, the **.ret** and .**qut** files of the Rotor-Gene Q software can be imported into Rotor-Gene AssayManager to create an assay profile together with additional settings and parameter values.

Assay example

Creation of an assay profile is shown for the following assay example:

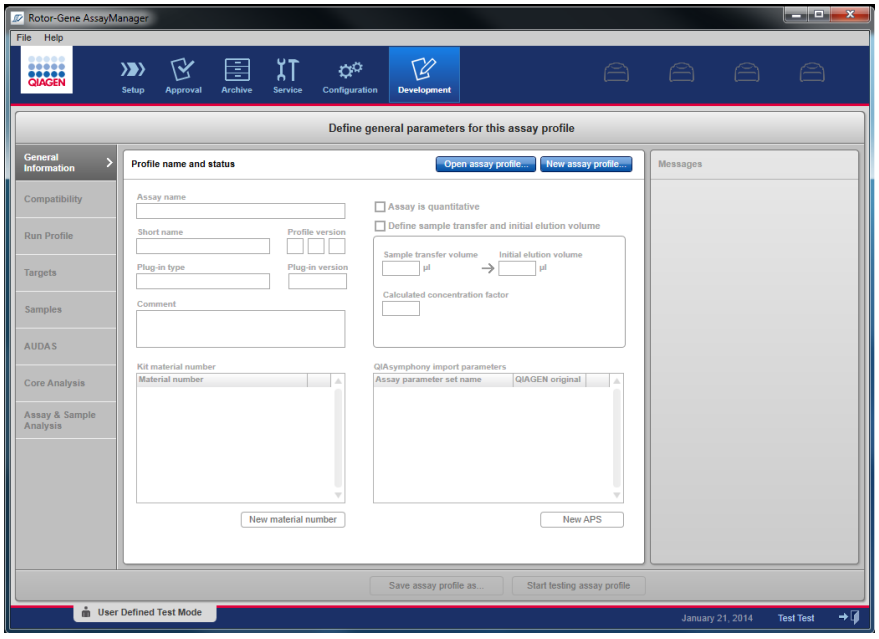
- RT-PCR for virus X
- Quantitative assay, target virus X, using the green channel
- Assay includes an internal control (IC), using the orange channel
- Assay includes 4 Quantitation Standards (10,000 IU/ml, 1000 IU/ml, 100 IU/ml, 10 IU/ml)
- Assay includes 1 no template control (NTC)
- Assay setup is done manually in the example, not using the QIAsymphony AS for automated assay setup
- 72-well rotor is used
- Reaction volume is 50 µl
- The assay must run exclusively, not combined with any other assay with a compatible cycling profile, on Rotor-Gene Q 5plex cyclers
- The result for target virus X is displayed in IU/ml by default, but is also convertible to IU/µl

- It is not possible with Rotor-Gene AssayManager to change the rotor layout from run to run, therefore the following rotor layout is defined:
  - First: all samples
  - Quantitation Standard 1 (QS 1)
  - QS 2
  - QS 3
  - QS 4
  - NTC
- The assay developer uses the following rules for analysis in this assay example:
  - QS 1 must have a C_T in the green channel; if not the target will be set to invalid
  - QS 2 must have a C_T in the green channel
  - NTC must have no C_T in the green channel
  - The C_T for QS 1 must be lower than 24; if not the target will be set to invalid
  - The standard curve for the virus X target has R > 0.99 (or "0,99", depending on language settings)

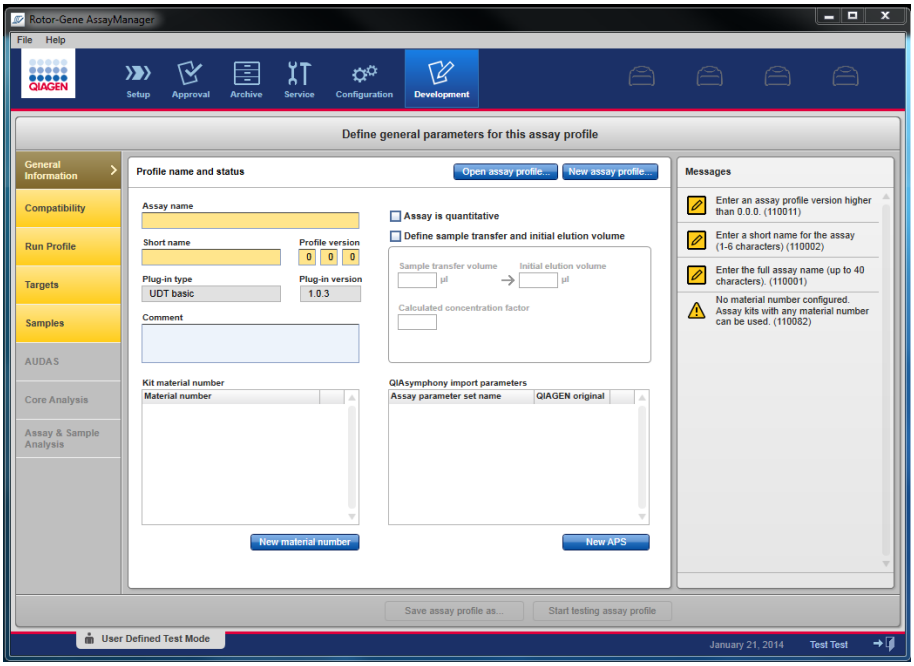
  Note: This set of rules is given as an example to show the general concept.

Define the general information

1. Log in to Rotor-Gene AssayManager in UDT mode as a user with the role “AssayDeveloper”.
2. Enter the “Development” environment.
3. Click “New assay profile…”.



4. The “Select plug-in” dialog appears.
5. Select the UDT basic plug-in from the drop-down list and click “OK”.
6. All mandatory fields appear yellow.



7. **Enter an assay name. (In this example, the assay name is "Assay Example".)**
8. **Enter a short name for the assay to be displayed in the "Setup" and "Approval" environments. The maximum allowed number of characters is 6. (In this example, the assay short name is "Assay".)**
9. **Enter a version for the assay profile in the "Profile version" field (e.g., 1.0.0).**
10. **Optional: A comment explaining assay details or history information of the assay profile can be entered. (The example uses the "Comment" field for a version history.)**
11. **Optional: Enter a kit material number.**

    The material number for QIAGEN kits can be found on the kit label. If a material number is entered in the assay profile and the configuration for the UDT mode is set to "Material number required", the operator must enter a material number when creating the work list. The software then validates whether the selected assay fits the entered material number. No material number is entered in the example.

12. **If the assay is a quantitative assay, check the box "Assay is quantitative". This example is for a quantitative assay.**

    For quantitative assays, the software can display the concentration in the eluate and in the original sample if a concentration factor is defined (see step 13).

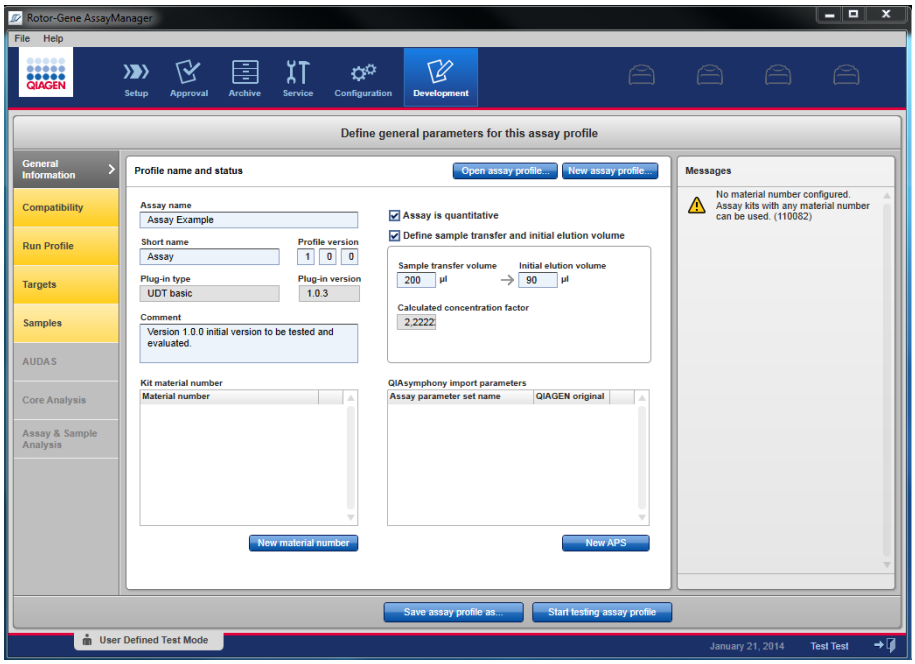
13. **To display the concentration in the sample, a concentration factor is required. To obtain the concentration factor, check the box "Define sample transfer and initial elution volume" and enter both values in the dialog boxes. For the example assay, the sample transfer volume is 200 µl and the initial elution volume is 90 µl.**

    Note: If the QIAsymphony SP/AS is used upfront for sample preparation and assay setup, values for these volumes can be found in the respective sample preparation or assay documentation.

    If the QIAsymphony SP/AS is used upfront, enter the related Assay Parameter Set (APS) name under "QIAsymphony import parameters" by clicking "New APS" and editing the name of the APS. Use the Assay Parameter Set name and not the APS file name.

    The QIAsymphony AS result file can be imported in the "Setup" environment and a corresponding work list is automatically generated. The check box "QIAGEN original" allows only QIAsymphony AS result files to be imported using the original APS from QIAGEN.

    Note: This example is set up manually and not by the QIAsymphony AS, so no entries are required in "QIAsymphony import parameters".


Rotor-Gene AssayManager
File
Help
QIAGEN
Setup
Approval
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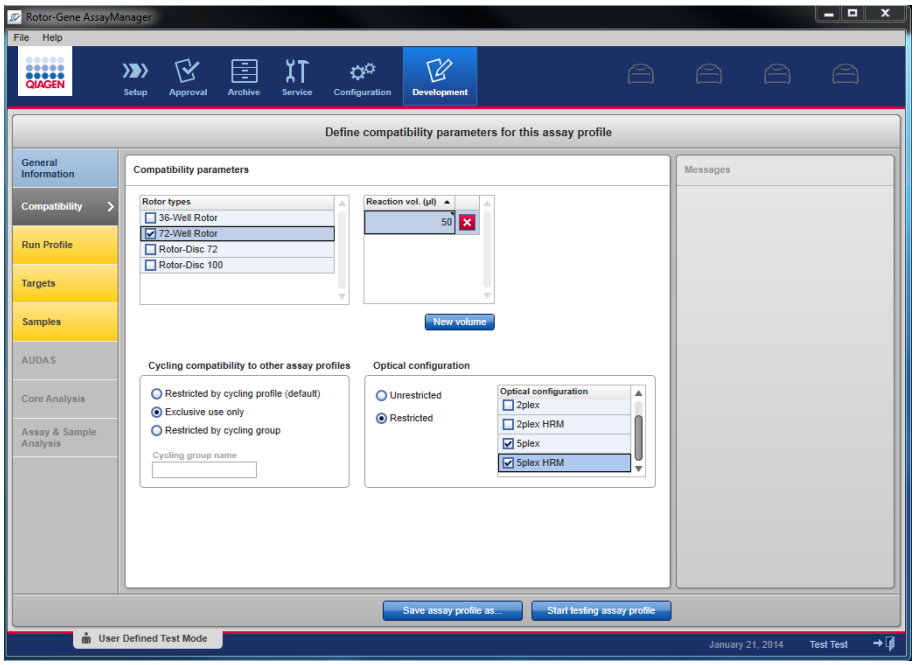
Configuration
Development
Define general parameters for this assay profile
General Information
Compatibility
Run Profile
Targets
Samples
AUDAS
Core Analysis
Assay & Sample Analysis
Profile name and status
Open assay profile...
New assay profile...
Assay name
Assay Example
Short name
Assay
Profile version
1
0
0
Plug-in type
UDT basic
Plug-in version
1.0.3
Comment
Version 1.0.0 initial version to be tested and evaluated.
Assay is quantitative
Define sample transfer and initial elution volume
Sample transfer volume
200
µl
Initial elution volume
90
µl
Calculated concentration factor
2.2222
Kit material number
Material number
New material number
QIAsymphony import parameters
Assay parameter set name
QIAGEN original
New APS
Messages
No material number configured.
Assay kits with any material number can be used. (110082)
Save assay profile as...
Start testing assay profile
User Defined Test Mode
January 21, 2014
Test Test

Define compatibility settings

1. **Select the "Compatibility" step.**
2. **Select the rotor type to be used for the assay. The example assay uses the 72-well rotor.**
3. **Enter the reaction volume in µl by clicking "New volume" and entering the volume in the yellow field. The example assay uses a reaction volume of 50 µl.**
4. **Select "Cycling compatibility to other assay profiles".**
   - If the assay is running exclusively without any other assay, select "Exclusive use only".
   - If the assay is running with all assays which have compatible cycling profiles, select "Restricted by cycling profile (default)".
   - If the assay is running with all other assays defined with the same cycling group name, select "Restricted by cycling group" and enter the name of the cycling group.

   The example assay is "Exclusive use only".
5. **Select the optical configuration of the cycler.**
   - If the assay can run on every cycler, select "Unrestricted".
   - If the assay can only run on certain cycler types, select "Restricted" and select the cyclers.

   The example assay can run on 5plex cyclers only.



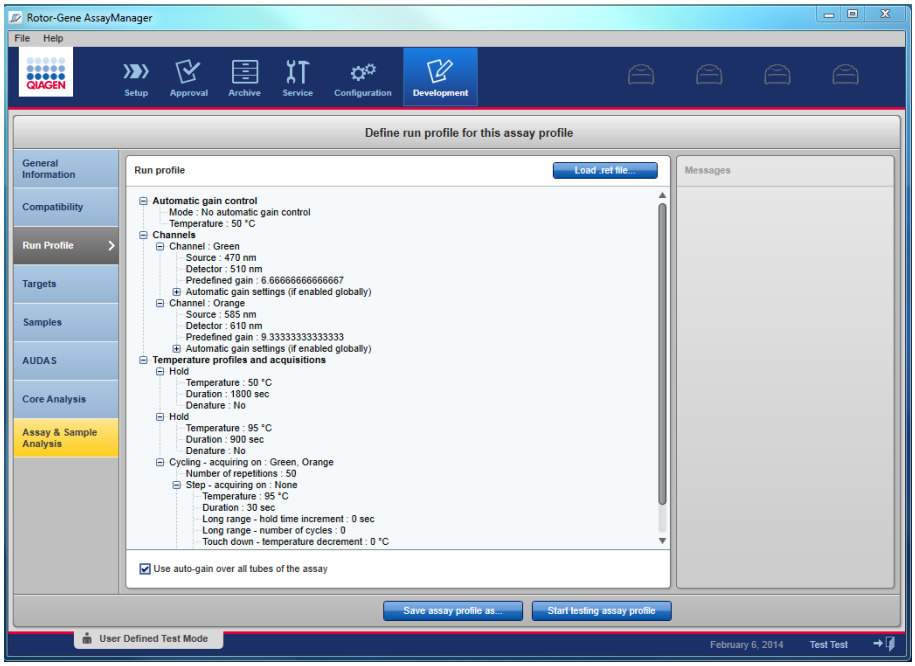
Define run profile

1. **Select the "Run Profile" step.**
2. **Click "Load .ret file".**
3. **Browse for the .ret file generated during assay development in the Rotor-Gene Q software.**

   The **.ret** file contains the cycling profile. For instructions on creating and saving **.ret** files, refer to Section 1.3.2.3.5, "Creating a .ret file", in the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

4. **Select the respective .ret file and click "Open".**

   The parameters of the **.ret** file are imported and can be reviewed.



5. **To use auto-gain over all tubes of the assay, check the box "Use auto-gain over all tubes of assay".**

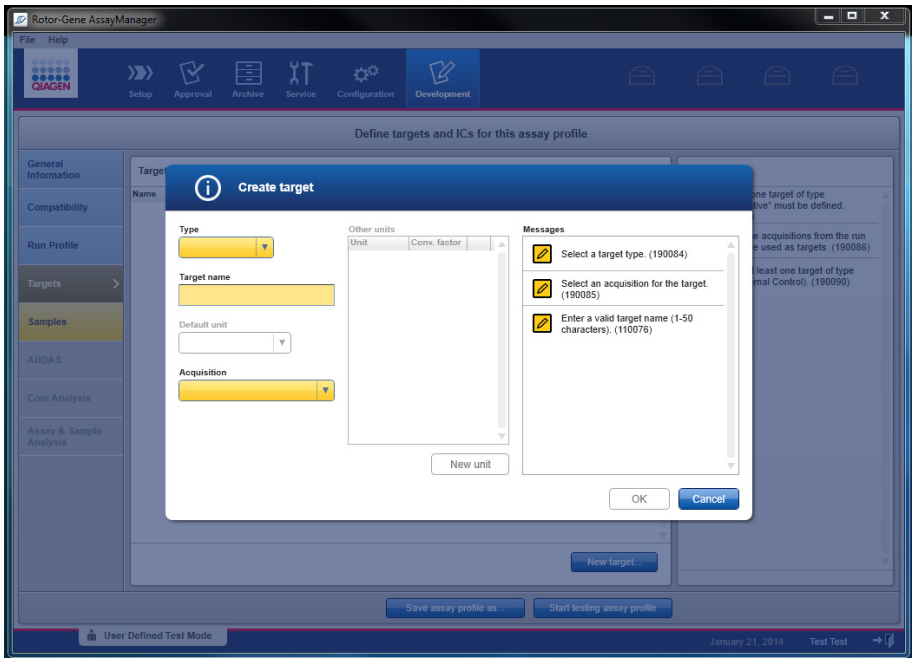
   If this box is checked, the median fluorescence measured in all tubes of the assay is used to optimize the gain settings.

Define targets and internal controls

1. **Select the "Targets" step.**

   The assay example includes a target, "Virus X", and an internal control (IC). "Virus X" is measured with the green channel and the IC is measured with the orange channel.

2. **Click "New target" to define the first target.**



3. **Select the type of target from the drop-down list.**

   - Select "IC" if the target is an internal control.
   - Select "Quantitative" if the target is a quantitative target.
   - Select "Qualitative" if the target is a qualitative target.

4. **Enter a target name.**

   This name is used to identify the result for this target in the approval. The target name is "Virus X" in the example.

5. **Select a default unit to display the results.**

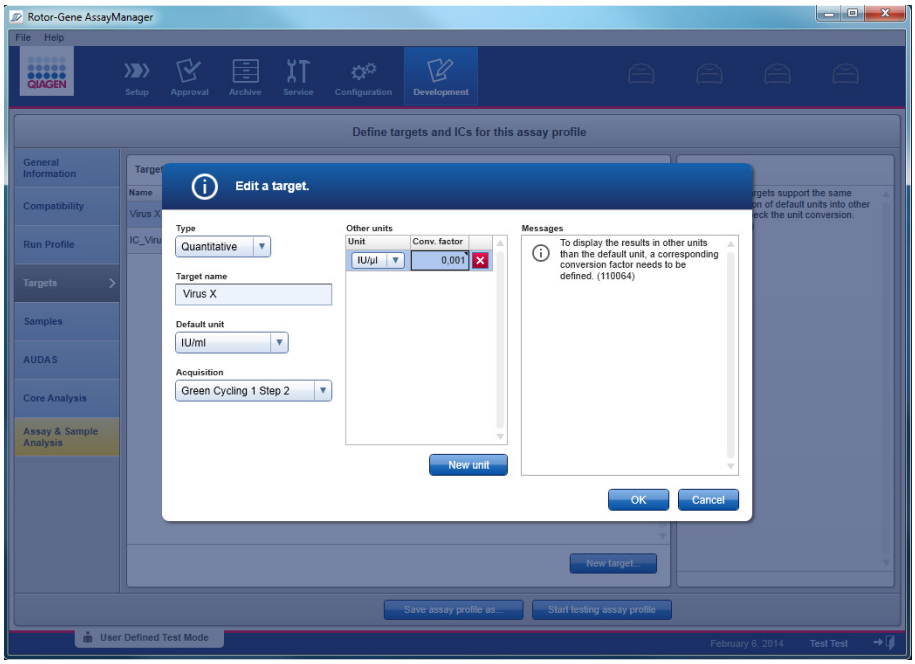
   The example assay uses the default unit IU/ml.

6. **Use the drop-down list to select the acquisition (channel) to measure the target.**

   The example assay uses the green acquisition channel for the target "Virus X". Acquisitions are retrieved from the loaded **.ret** file.

7. **For the results of the currently defined target to be converted and displayed in alternative units, click "New unit", select the chosen unit, and enter a conversion factor.**

   The assay example uses IU/µl as another unit. The conversion factor from IU/ml to IU/µl is 0.001 (if the operating system language settings are in English, be aware of other language settings when entering parameters).

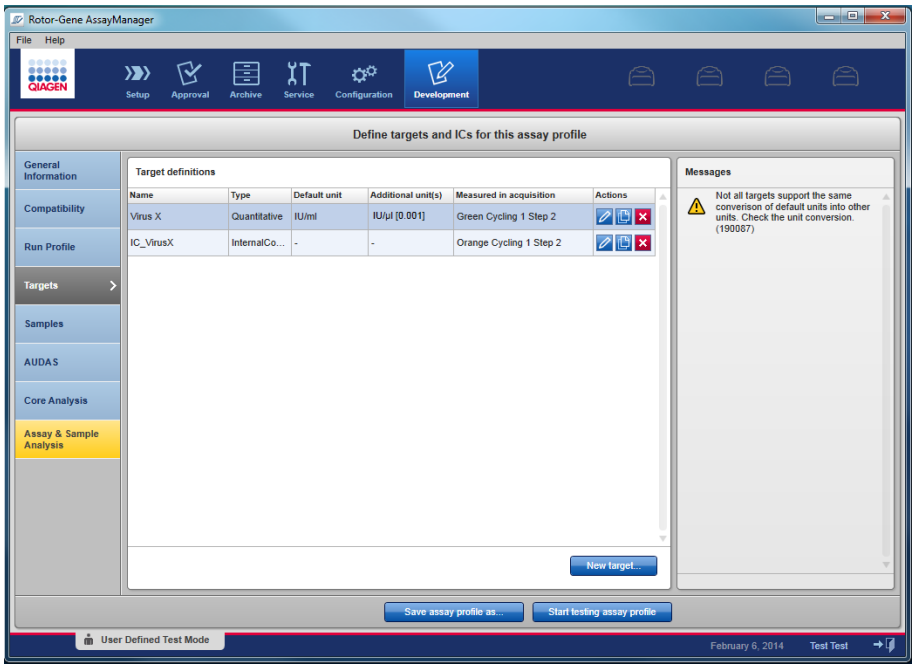


8. **Click "OK".**

   The target "Virus X" is defined.

9. **To define the internal control, click "New target".**
10. **Select "IC" as the type.**
11. **Enter the name of the internal control as target name. The name IC_VirusX is used in this example assay.**
12. **Select the acquisition (channel). The example assay IC uses the orange channel.**
13. **Click "OK".**

The targets for the example assay are now defined.


Rotor-Gene AssayManager
File Help
QIAGEN
Setup
Approval
Archive
Service
Configuration
Development
Define targets and ICs for this assay profile
General Information
Compatibility
Run Profile
Targets
Samples
AUDAS
Core Analysis
Assay & Sample Analysis
Target definitions
Name
Type
Default unit
Additional unit(s)
Measured in acquisition
Actions
Virus X
Quantitative
IU/ml
IU/µl [0.001]
Green Cycling 1 Step 2
IC_VirusX
InternalCo...
-
-
Orange Cycling 1 Step 2
New target...
Messages
Not all targets support the same conversion of default units into other units. Check the unit conversion. (190087)
Save assay profile as...
Start testing assay profile
User Defined Test Mode
February 6, 2014
Test Test

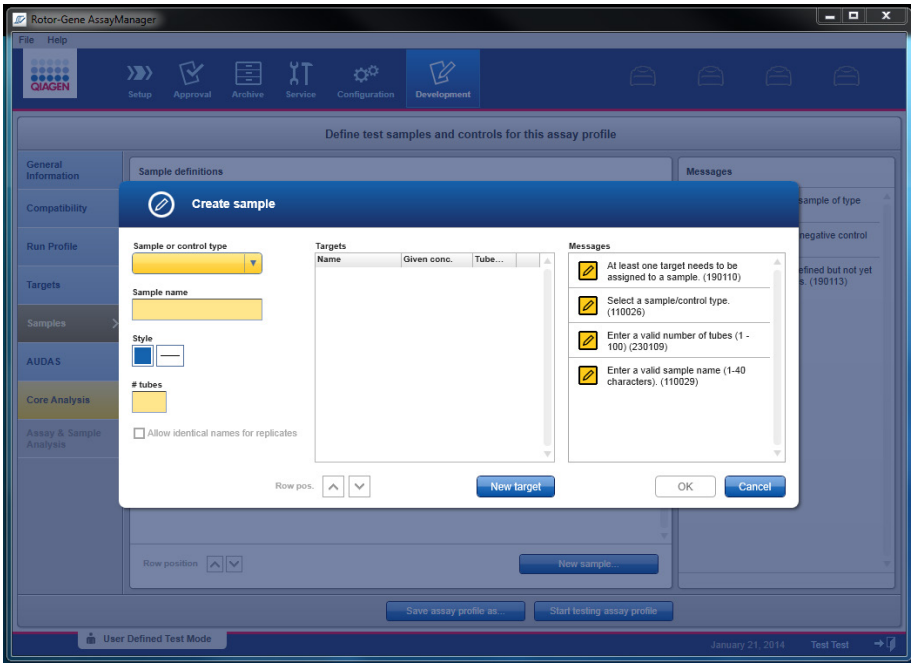
Define samples, standards and controls

1. **Select the "Samples" step.**

   The example assay uses a standard curve of 4 quantitation standards. In addition, an NTC is required. The rotor layout needs to be defined in Rotor-Gene AssayManager so that the rules defined in the "Assay & Sample Analysis" step can be applied. The layout order for the example assay is as follows:

   - All test samples first
   - QS 1
   - QS 2
   - QS 3
   - QS 4
   - NTC.

2. **Click "New sample" to define all test samples first.**



3. **Select "Test" from the "Sample or control type" drop-down list.**

4. **Select "Style" (color and line) by clicking on the respective fields.**

   The test samples for the assay example are displayed in blue.

5. **Enter 1 for "# tubes" if the sample is not divided into several tubes and all targets are measured in one tube.**

   The assay example has one target, "Virus X", and the target IC. Both are measured in the same tube. The samples are not split.

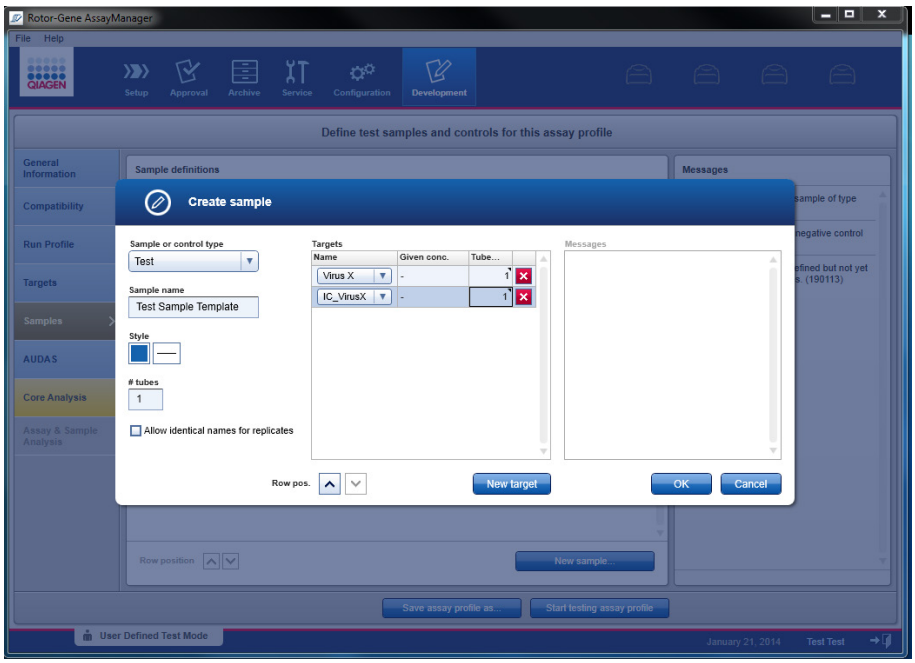
6. **To allow identical sample IDs for the work list, check the box "Allow identical names for replicates".**

   Note: While it is possible to have samples with identical names, there is no support for handling of replicates in Rotor-Gene AssayManager.

7. **Click "New target". Select the target "Virus X" from the "Name" drop-down list and enter 1 for "Tube position".**

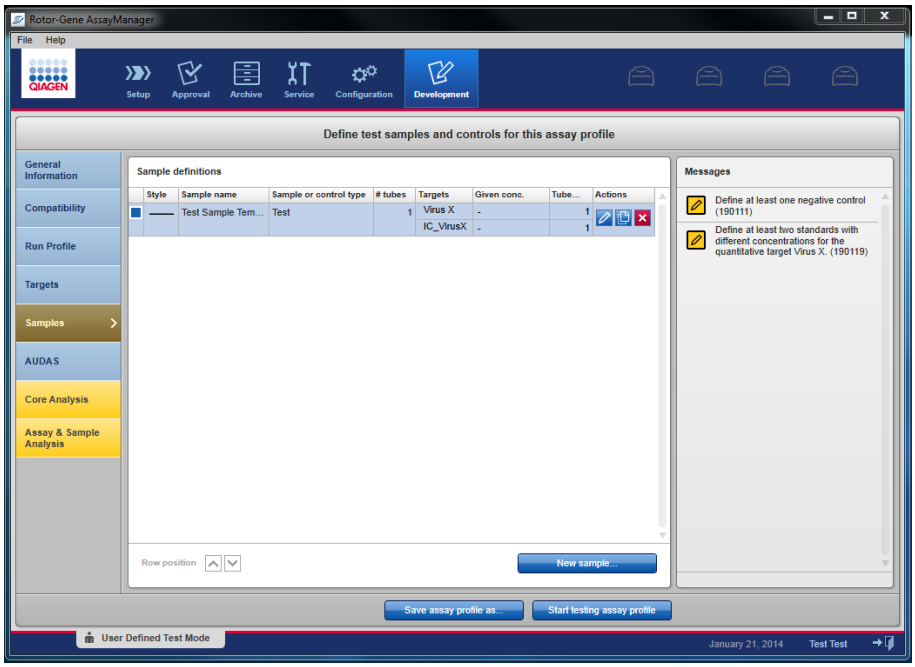
   Note: Always enter 1 if "# tubes" is equal to 1.

8. **Click "New target" again, select "IC_VirusX" from the "Name" drop-down list, and enter 1 for "Tube position".**



9. **Click "OK".**

   All information for the test samples (Virus X and IC_VirusX) is entered.



The next position (control or standard) of the rotor layout can now be defined.

This is QS 1 for the example assay.

10. **Click "New sample".**
11. **Select "Quantification Standard" from the "Sample or control type" drop-down list.**
12. **Enter the standard name "QS 1" in the "Sample name" field.**
13. **Select color and line style.**

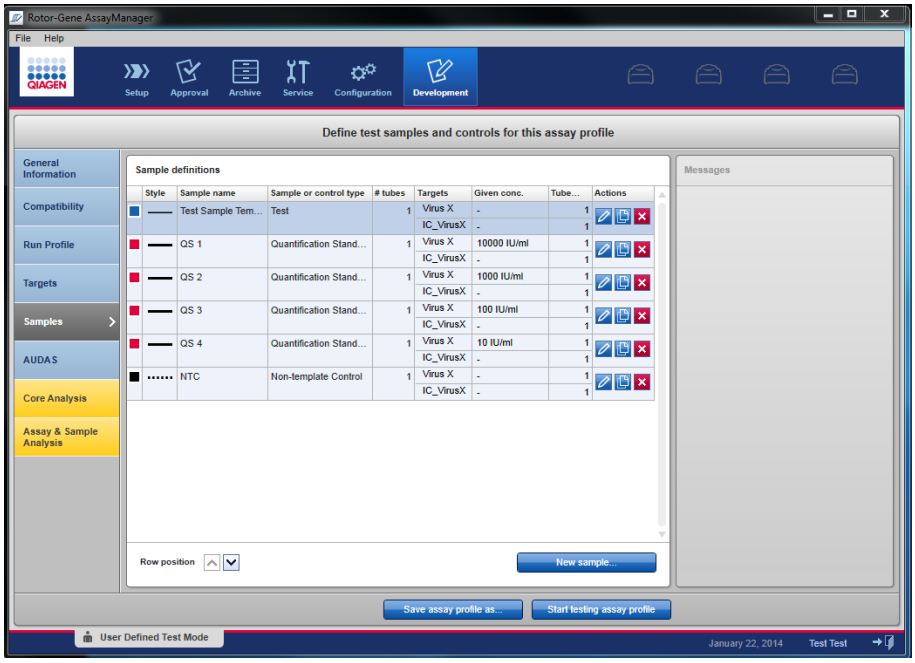
    The example assay uses the color red for the quantitation standards.
14. **Enter 1 for the number of tubes in the"# tubes" field.**
15. **Click "New target". Select target "Virus X", and enter 1 for "Tube position".**
16. **Enter the given concentration for the standard in the previously defined default unit. (Click near the check box to enter the value.)**

    The example assay uses QS 1 with 10,000 IU/ml.
17. **Click "New target". Select target "IC_VirusX" and enter 1 for "Tube position".**
18. **Click "OK".**
19. **Click the "Duplicate sample" button in the QS 1 row.**
20. **Change the name of the standard to QS 2 and change the given concentration value to 1000 IU/ml. Click "OK".**
21. **Repeat steps 19 and 20 for QS 3 and QS 4, entering 100 IU/ml and 10 IU/ml respectively.**

    All information for the quantitation standards has now been entered. The NTC can now be defined.
22. **Click "New sample".**

23. **Select "Non-template Control" for "Sample or control type" from the drop-down list.**
24. **Enter the name "NTC" in the "Sample name" field.**
25. **Select color and line style.**

    The example assay uses black and a broken line for the NTC.
26. **Enter 1 for number of tubes in the"# tubes" field.**
27. **Click "New target". Select target "Virus X", and enter 1 for "Tube position".**
28. **Click "New target". Select target "IC_VirusX" and enter 1 for "Tube position".**
29. **Click "OK".**



All information for the samples, quantitation standards, and NTC has now been entered.

AUDAS analysis

AUDAS (Automated Data Scan) is not available in UDT mode.

Define core analysis

A **.qut** file is loaded to assign the analysis settings for each acquisition. The .**qut** file contains the analysis data for a specific target.

1. **Select the "Core Analysis" step.**
2. **Select the target "Virus X" to import the .qut file with core analysis parameters for this target.**

   The **.qut** files can be exported from Rotor-Gene Q software. (For more information, refer to Section 1.3.2.3.4, "Creating a .qut file", in the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.) "Virus X" is selected for the example assay.

3. **Click "Load .qut file".**

   The "Browse" dialog opens.

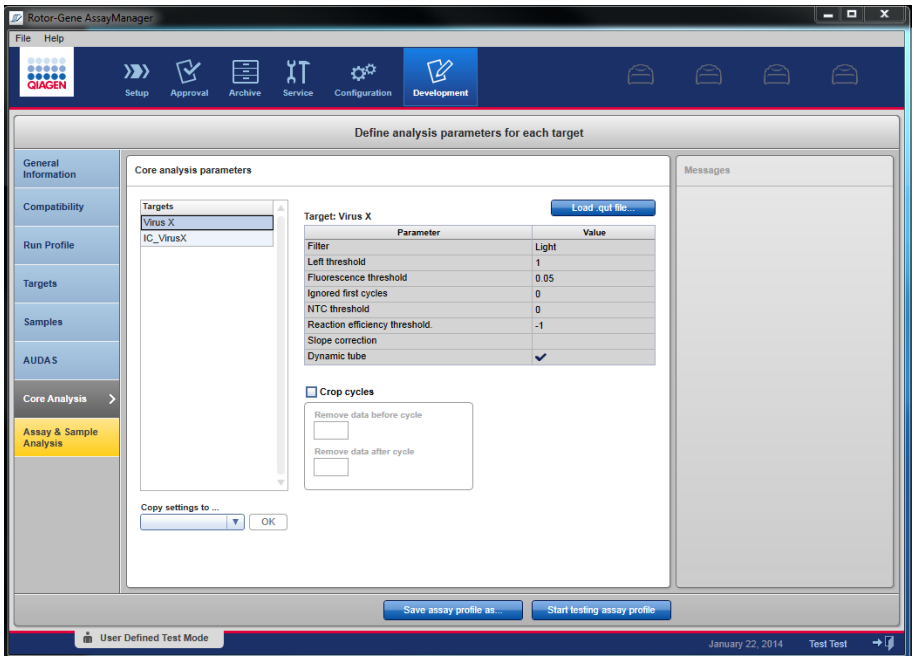
   Note: The **.qut** files can be exported from Rotor-Gene Q software. (For more information, refer to Section 1.3.2.3.4, "Creating a .qut file", in the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.)

4. **Browse for the .qut file to be loaded and select the file.**

   The parameters for target virus X are displayed.

5. **Select the target "IC_VirusX" and load the respective .qut file.**
6. **The crop cycles set value is not defined by loading the .qut file. Check the "Crop cycles" box if cycles are to be cropped, and enter the required values.**

   "Crop cycles" is not required for the example assay.



Define assay and sample analysis rules

Note: In the assay and sample analysis step, rules can be defined for each control and test sample. A rule defines the circumstances whereby a control or sample is considered to be valid. The checkbox in the "Inv." column determines whether breaking the rule makes the target invalid. If a rule fails, Rotor-Gene AssayManager sets the respective flag in the "Approval" environment. If "Inv." is activated and a rule is defined in this way, the target result will be "INVALID".

Note: The rules given in this example are only for demonstrating to users how to edit these rules. It is the responsibility of the assay developer to define the rules specific for the developed assay. Information about the rules used in the example assay and sample analysis, together with examples of their application, can be found in Section 1.3.2.3.1 of the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

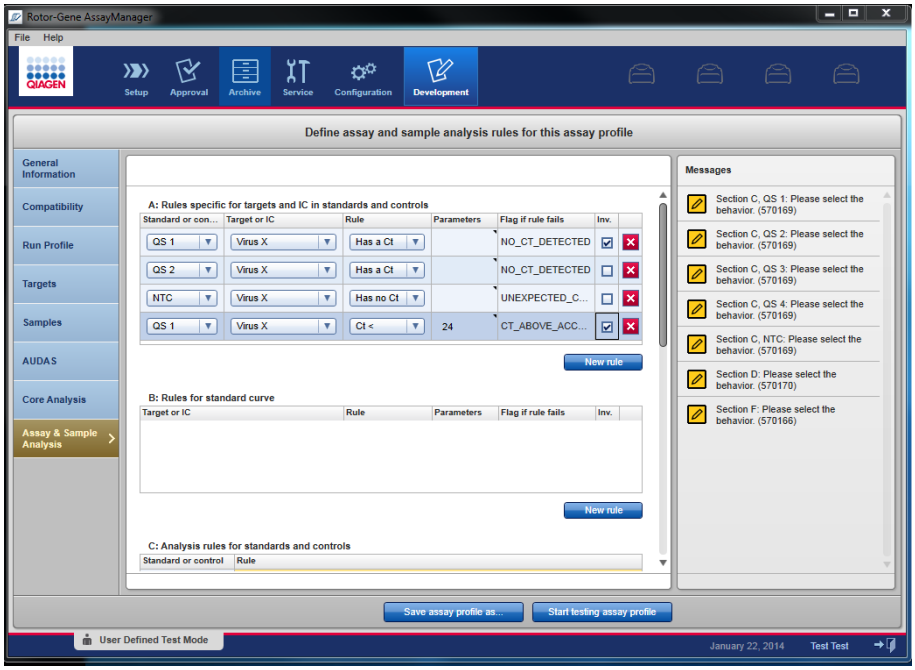
1. Click the "Assay & Sample Analysis" step.

The rules are entered in 6 panels labeled A, B, C, D, E, and F.

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

The example assay requires the following set of rules for standards and controls:

- QS 1 has a C_T (for target "Virus X"); if not, the target "Virus X" is marked as invalid
- QS 2 has a C_T (for target "Virus X")
- NTC does not have a C_T (for target "Virus X")
- QS 1: C_T must be lower than 24 (for target "Virus X"); if not, the results are marked as invalid



To enter this set of rules in panel A: Rules specific for targets and IC in standards and controls:

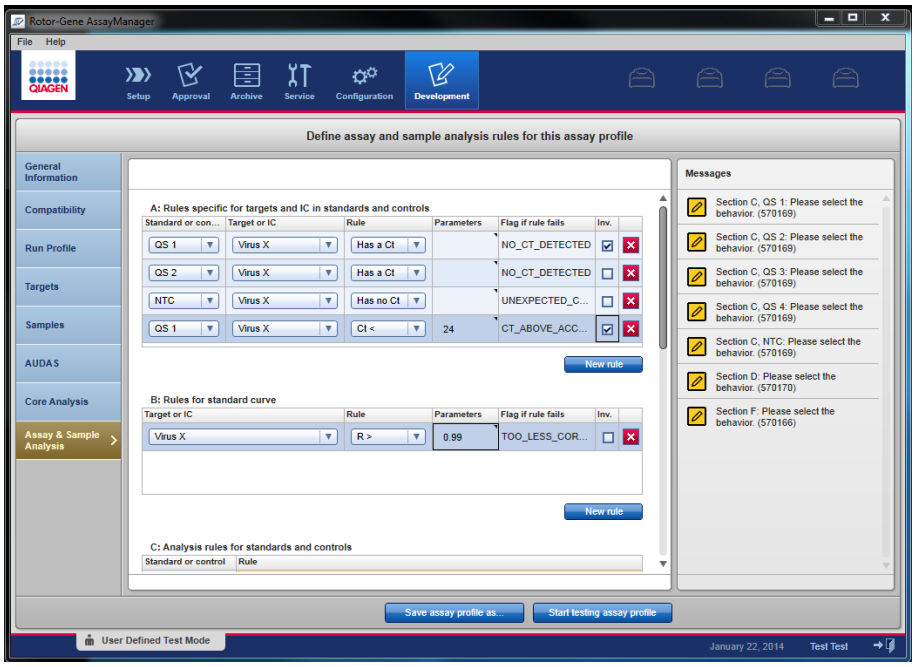
2. **Enter the rule for QS 1.**
   - Click "New rule"
   - Select the Quantitation Standard QS 1
   - Select the target ("Virus X")
   - Select the rule "Has a Ct"
   - Check the box "Inv." (Invalidate)
3. **Enter the rule for QS 2.**
   - Click "New rule"
   - Select the Quantitation Standard QS 2
   - Select the target ("Virus X")
   - Select the rule "Has a Ct"
4. **Enter the rule for NTC.**
   - Click "New rule"
   - Select the control NTC
   - Select the target ("Virus X")
   - Select the rule "Has no Ct"
5. **Enter the parameter rule for QS 1.**
   - Click "New rule"
   - Select the Quantitation Standard QS 1
   - Select the target ("Virus X")
   - Select the rule "Ct <"
   - Enter the parameter "24"
   - Check the box "Inv." (Invalidate)

Section B: Rules for standard curve

The example assay requires the following rule for the standard curve:

For target "Virus X", R must be greater than 0.99.

1. **Click "New rule" for panel B: Rules for standard curve.**
   - Select target "Virus X"
   - Select rule "R >"
   - Enter the value 0.99 (take care when entering parameters as the delimiter will vary with the language settings of the operating system)



Section C: Analysis rules for standards and controls

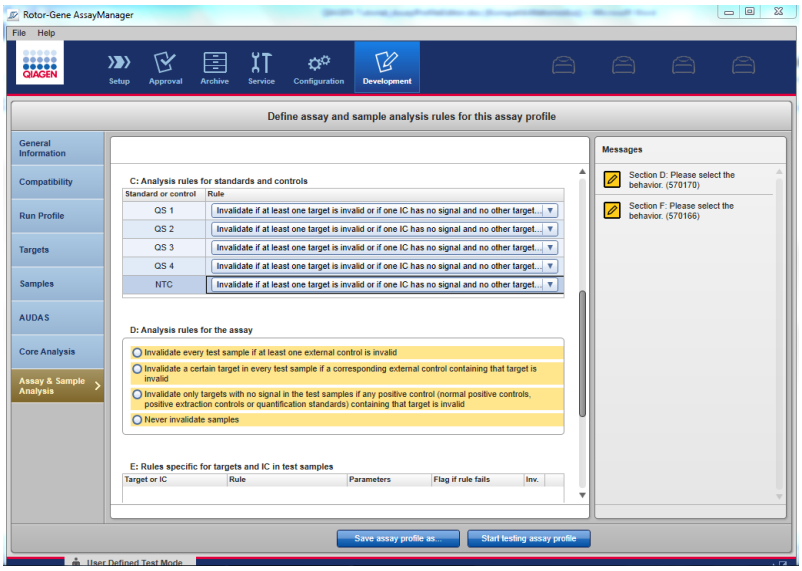
Section C defines the influence of individual targets with an invalid flag on the validity of the complete standard or control. For a detailed description of this rule, refer to Section 1.3.2.3.1 in the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

The example assay uses the strictest rule for all quantitation standards and controls.

1. **Enter the rule for QS 1.**

   Select "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" for Quantitation Standard QS 1.

2. **Repeat this step for all quantitation standards and NTC.**



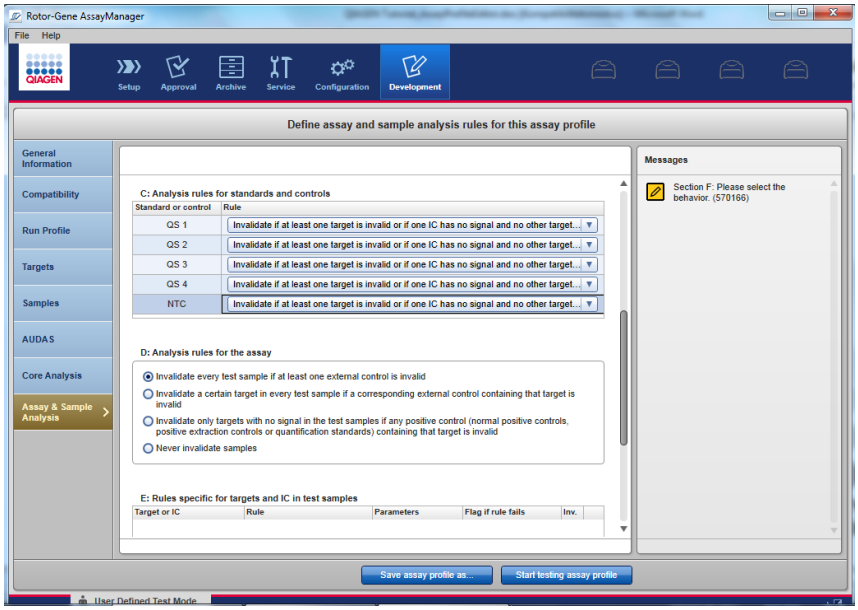
Section D: Analysis rules for the assay

These rules define the consequences of any "invalid" results for standards and controls due to the rules described in Section C. Further details about the rules can be found in Section 1.3.2.3.1 of the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

The example assay uses the rule:

Activate: "Invalidate every test sample if at least one external control is invalid".

1. **Select the appropriate radio button in panel D: Analysis rules for the assay.**

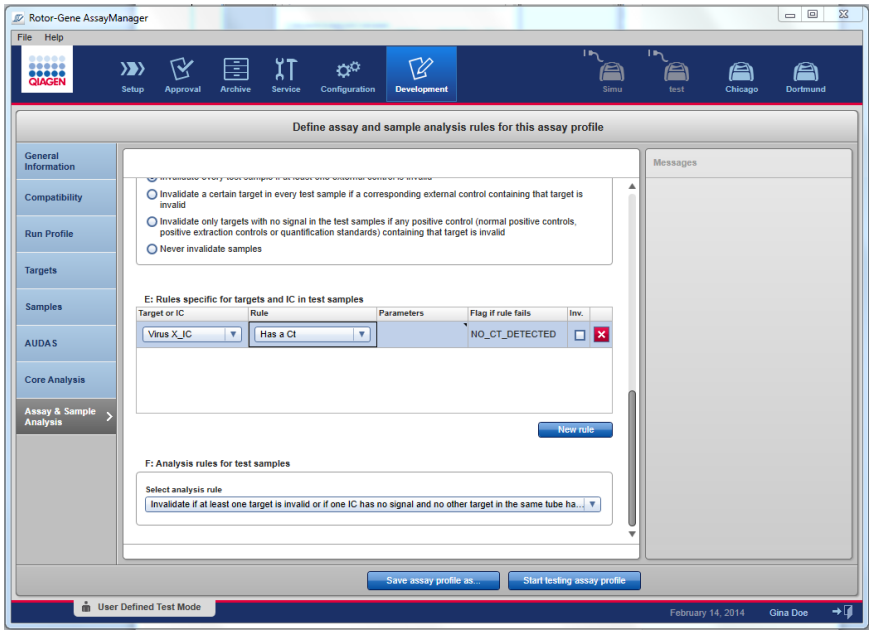


Section E: Rules specific for targets and IC in test samples

In this section, analysis rules specific for targets and internal control in the test samples can be defined. Further details about the rules can be found in Section 1.3.2.3.1 of the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

The example assay uses the rule:

If the internal control is negative, a flag is displayed for the internal control target in the "Approval" environment. This functions as a warning to users that samples are not automatically invalidated.



1. **Click "New rule" for panel E: Rules specific for targets and IC in test samples.**
2. **Select "Virus X_IC" as target.**
3. **Choose the rule "Has a Ct".**
4. **Do not activate the "Inv." checkbox.**

Section F: Analysis rules for test samples

Section F defines the influence of individual targets with an invalid flag on the validity of the complete sample. "Individual targets" in this context means all specific targets and internal controls (IC). Further details about the rules can be found in Section 1.3.2.3.1 of the *Rotor-Gene AssayManager UDT Basic Plug-in User Manual*.

The example assay uses the most stringent rule for test samples:

"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".

1. **Select "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" in panel F: Analysis rules for test samples.**

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.

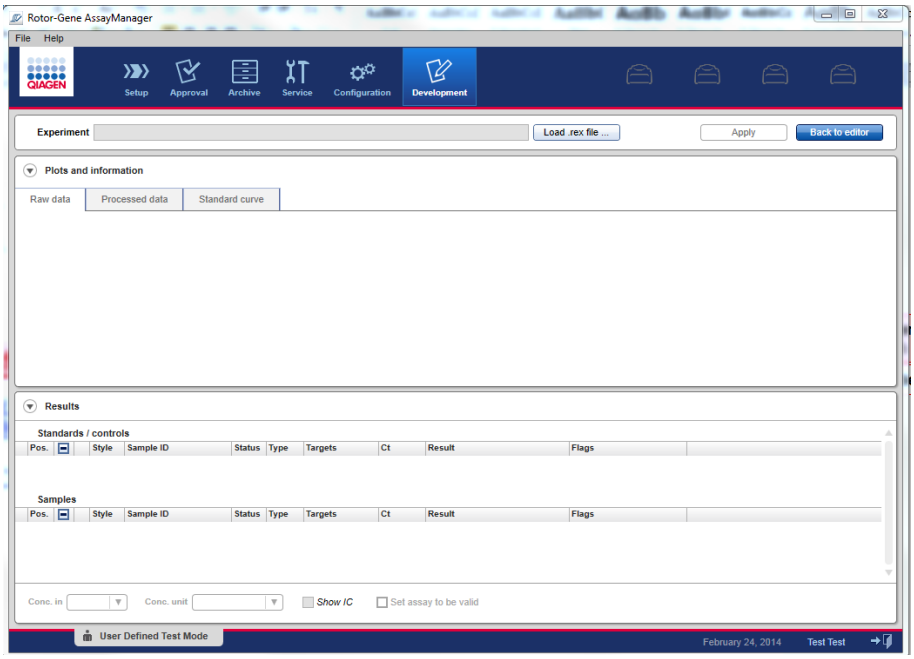
A **.rex** file (containing raw experiment data and sample data) from an experiment performed with the Rotor-Gene Q software or Rotor-Gene AssayManager can be loaded.

IMPORTANT: The **.rex** file must include the same sample layout as defined in the assay profile to be tested.

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 the standard curve can be checked and compared to the results generated by the 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".**

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

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

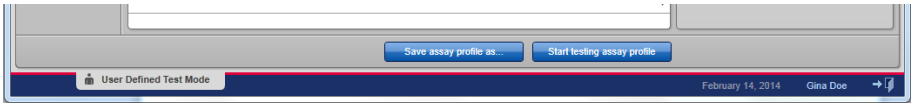
4. **Click "Apply" to start the analysis process using the currently developed assay profile.**

   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.

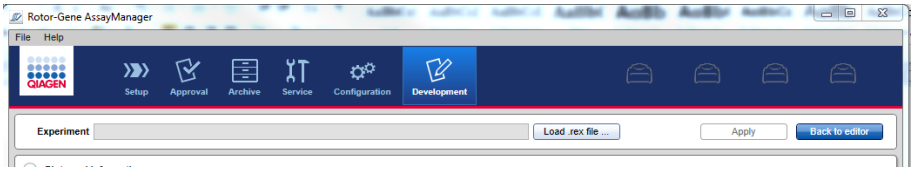
5. **Review the data.**

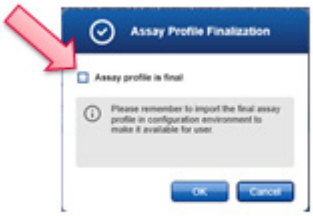
Saving the assay profile

1. **Click "Save assay profile as..."**



Note: The option to save the assay profile is located in the Assay Profile Editor. If the assay is currently being tested using the Assay Profile Tester, click "Back to editor" to access the Assay Profile Editor again.





2. **If the assay profile is not final and you want to save the current status, do not activate the checkbox. If the assay profile is final, activate the checkbox "Assay profile is final".**
3. **Click "OK".**
4. **Choose a location to save the file and enter a file name.**
5. **Click "OK".**
6. **If the assay profile is final and available for routine use, import the assay profile to the Rotor-Gene AssayManager database in the "Configuration" environment. A user with administrator privileges is required to import the assay profile.**

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Document Revision History	
R3 01/2022	This is revision 3 of the guide on *"Creating a Rotor-Gene AssayManager Assay Profile"*. Changes from the previous version are to remove a note stating different channel must be used for multiple acquisition steps throughout the run template.

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