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ALK RGQ RT-PCR Kit Handbook

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For Research Use Only. Not for use in diagnostic procedures.

For use with the Rotor-Gene® Q 5plex HRM instrument

REF 870501

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Sample to Insight

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Intended Use

The ALK RGQ RT-PCR Kit is intended for research use only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Principle of the Procedure

Technology

The ALK RGQ RT-PCR Kit uses Scorpions® technology, which enables the detection of RNA transcripts encoding the anaplastic lymphoma kinase (ALK) tyrosine kinase domain and the control region of the ABL1 RNA transcript. The kit is designed to detect the aberrant expression of mRNA encoding the ALK tyrosine kinase domain.

Scorpions

Detection of amplification is performed using Scorpions. Scorpions are bi functional molecules containing a PCR primer covalently linked to a fluorescently labeled probe. The fluorophore in this probe is associated with a quencher also incorporated into the probe, which reduces fluorescence. During PCR, when the probe binds to the amplicon, the fluorophore and quencher become separated. This leads to a measureable increase in fluorescence from the reaction tube.

Kit format

The ALK RGQ RT-PCR Kit contains two assays in a single multiplex reaction. The kit is designed to detect the aberrant expression of mRNA encoding the ALK tyrosine kinase

domain. The reaction mix contains reagents to convert RNA to cDNA (reverse transcription) and reagents to detect targets (ALK) that are labeled with FAM™ (amplified cDNA) and an internal control (ABL1) that is labeled with HEX™. The internal control ABL1 is an endogenous control that monitors the quantity of RNA and also the quality of RNA (to check for the presence of inhibitors that may lead to false negative results).

Each kit contains the following:

- One tube of reaction mix for the ABL1 and ALK multiplex assay
- One tube of OneStep Enzyme Mix
- One tube of positive control RNA
- One tube of RNase/DNase-free water for no template control (NTC)
- One tube of RNase/DNase-free water for dilution of sample

Assays

The ALK RGQ RT-PCR Kit comprises a one-step procedure during which RNA is reverse transcribed and tested using real-time PCR on the Rotor Gene Q 5plex HRM instrument. Expression assays are performed to monitor the presence of ALK and ABL1 RNA transcripts.

Control expression assay

The ABL1 internal control assay detection is via a HEX labeled Scorpion.

Target expression assay

The ALK assay detection is via a FAM labeled Scorpion.

Controls

All experimental runs must contain positive control, which controls for both ALK and ABL1 expression, and a negative control (NTC), which controls for contamination.

Positive control

The ALK RGQ RT-PCR Kit contains ALK and ABL1 Positive Control (PC) to be used as the template in the positive control reaction and each run must be performed with a positive control. The positive control contains in vitro transcripts that will be amplified by both the ALK and ABL1 assays in the reaction mixes. The positive control results will be assessed to ensure that the kit performs within the stated acceptance criteria.

Negative control

The ALK RGQ RT-PCR Kit contains water to be used as the template for the no template negative control. Each run must contain a no template control (NTC).

Materials Provided

Kit contents

ALK RGQ RT-PCR Kit			**(24)**
Catalog number			**870501**
Number of reactions			**24**
ALK/ABL1 Reaction Mix	Red	ALK	600 µl
ALK/ABL1 Positive Control	Beige	PC	100 µl
OneStep Enzyme Mix	Red	Enzyme Mix	45 µl
Water for NTC	White	NTC	1.9 ml
Water for Sample Dilution	White	Dil.	1.9 ml

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Reagents

- RNeasy® FFPE Extraction Kit (cat. no. 73504)
- Deparaffinization solution (cat. no. 19093)
- Ethanol (96–100%)*

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Consumables

- 0.1 ml PCR strip tubes and caps (RGQ tubes) for use with 72-well rotor (QIAGEN cat. nos. 981103 and 981106)
- Sterile microcentrifuge tubes for preparing master mixes. Recommendation is to use Lo-bind tubes.

Equipment

- Adjustable pipets, dedicated for sample preparation*
- Sterile pipet tips with aerosol barriers
- Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C and 80°C*
- Bench top centrifuge with rotor for 1.5 ml tubes*
- Bench top vortex*
- Adjustable pipets, dedicated for PCR master mix preparation*
- Adjustable pipets, dedicated for dispensing of positive control and template RNA*
- Rotor-Gene Q 5plex HRM System (cat. no. 9001650)
- Rotor-Gene Q Software, version 2.1.0.7 or higher.
- QIAGEN Loading Block 72 x 0.1 ml Tubes (cat. no. 9018901)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

General precautions

The user should always pay attention to the following:

- Use sterile pipet tips with aerosol barriers and make sure that pipets have been calibrated according to the manufacturer's instructions.
- Store and extract template nucleic acid materials (specimens and positive controls) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Use extreme caution to prevent contamination of PCR reactions with synthetic control material. We recommend using separate, dedicated pipets for setting up reaction mixes and adding RNA template. Preparation and dispensing of reaction mixes must be carried out in a separate area to the addition of template. Rotor-Gene Q tubes must not be opened after the PCR run has finished. This is to prevent laboratory contamination with post-PCR products.

Important notes

- All reagents in the ALK RGQ RT-PCR Kit are formulated specifically for use with the tests provided in the ALK RGQ RT-PCR Kit. All reagents supplied are intended to be used

solely with the other reagents in the same ALK RGQ RT-PCR Kit. Do not substitute the reagents in the ALK RGQ RT-PCR Kit, or between ALK RGQ RT-PCR Kits, as this may affect performance.

- Reagents for the ALK RGQ RT-PCR Kit have been optimally diluted. We do not recommend further dilution of reagents as this may result in a loss of performance. We do not recommend using reaction volumes of less than 25 µl as this will increase the risk of false negatives.
- The product is intended only for use on the Rotor-Gene Q 5plex HRM instrument.
- The product is to be used only by experienced laboratory personnel familiar with laboratory procedures and the Rotor-Gene Q 5plex HRM instrument.
- To ensure correct sample testing and analysis, the user must take particular care when pipetting, placing the PCR strip tubes into the appropriate positions of the 72-well rotor, and entering sample names.

The test is highly sensitive and specific, enabling detection of small proportions of mutant RNA against a background of wild-type RNA. The procedure should be repeated in the event that the control expression assay value indicates that the quantity of RNA is either insufficient or at a concentration too high for ALK and ABL1 expression analysis.

Reagent Storage and Handling

The ALK RGQ RT-PCR Kit is shipped on dry ice and must still be frozen on arrival. If the ALK RGQ RT-PCR Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note or the reagents, please contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

The ALK RGQ RT-PCR Kit should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer. Repeated thawing and freezing should be avoided.

Note: To ensure optimal activity and performance, Scorpions (as with all fluorescently labeled molecules) must be protected from light to avoid photo bleaching.

Specimen Handling and Storage

Note: All samples must be treated as potentially infectious material.

Sample material must be human lung tumor RNA from NSCLC, extracted from formalin-fixed paraffin-embedded (FFPE) tissue. Specimens must be transported according to standard pathology methodology to ensure specimen quality.

Procedure

RNA isolation

RNA isolation must be carried out with 3–5 µm FFPE sections using the RNeasy FFPE Kit (cat. no. 73504). For optimal de-crosslinking, incubate for 15 minutes at 80°C. Elute RNA with 30 µl water. RNA should be stored at –80°C until use.

Protocol: PCR and Data Analysis

Important points before starting

- Before beginning the procedure read “Important notes”, page 9.

Things to do before starting

- Before each use, thaw all reagents for a minimum of 30 minutes (but less than 90 minutes) at room temperature (15–25°C), mix by inverting 10 times, and centrifuge briefly to collect the contents at the bottom of the tube.

Procedure

ALK RGQ RT-PCR assay plate set-up

Note: Each PCR reaction final volume must be 25 µl (20 µl Master Mix + 5 µl Sample).

1. To prepare the master mix, pipet out the reaction mix and then add the OneStep Enzyme mix according to Table 1.

Table 1. Preparation of master mix

Master Mix		
Reaction Mix	Volume of Reaction Mix (Tube ALK)	Volume of OneStep Enzyme Mix (Tube Enzyme Mix)
ALK Kit	19.5 µl x (n+2)*	0.5 µl x (n+2)

*n = number of reactions (RNA samples plus 2 controls). Prepare enough for 2 extra samples (n + 2). n should not exceed 26 (24 samples plus 2 controls), as 24 is the maximum number of samples which can be tested using one ALK Kit.

2. Close micro centrifuge tube and mix by inverting 10 times. Where appropriate, collect master mix after mixing by centrifuging briefly (pulse) at max rpm.
3. Place PCR tubes into a loading block according Rotor-Gene Q ALK RGQ RT-PCR Analysis Template layout (Figure 1). Do not cap the tubes.

   Note: Leave the caps in the plastic container until required
4. Dispense 20 µl of Master Mix to the relevant PCR strip tubes, using the run layout shown in Figure 1.

01. PC	09. sample	17. sample	25. sample	33.	41.	49.	57.	65.
02. NTC	10. sample	18. sample	26. sample	34.	42.	50.	58.	66.
03. sample	11. sample	19. sample	27.	35.	43.	51.	59.	67.
04. sample	12. sample	20. sample	28.	36.	44.	52.	60.	68.
05. sample	13. sample	21. sample	29.	37.	45.	53.	61.	69.
06. sample	14. sample	22. sample	30.	38.	46.	54.	62.	70.
07. sample	15. sample	23. sample	31.	39.	47.	55.	63.	71.
08. sample	16. sample	24. sample	32.	40.	48.	56.	64.	72.

Figure 1. Rotor-Gene Q ALK RGQ RT-PCR Analysis Template layout. Suggested run and loading block layout. Numbers denote position in the loading block and indicate final rotor position.

5. Add 5 µl Water for NTC to the NTC tube (tube 02) and cap the tube.

   Note: Water for NTC must be added and tubes must be capped before adding samples or positive control to prevent cross-contamination.

6. Add 5 µl RNA template to the sample tubes (tube 3–26) and cap the tubes.
7. Add 5 µl Positive Control (PC) to the PC tube (tube 01) and cap the tube.
8. Flick the capped strip tubes 3 times to mix the contents.
9. Transfer the PCR strip tubes to the 72-well rotor in the RGQ instrument.

Note: Tube 01 must always contain Positive Control (PC) and tube 02 must always contain NTC.

Run the ALK RT-PCR assay using the PCR cycling conditions shown in the table below.

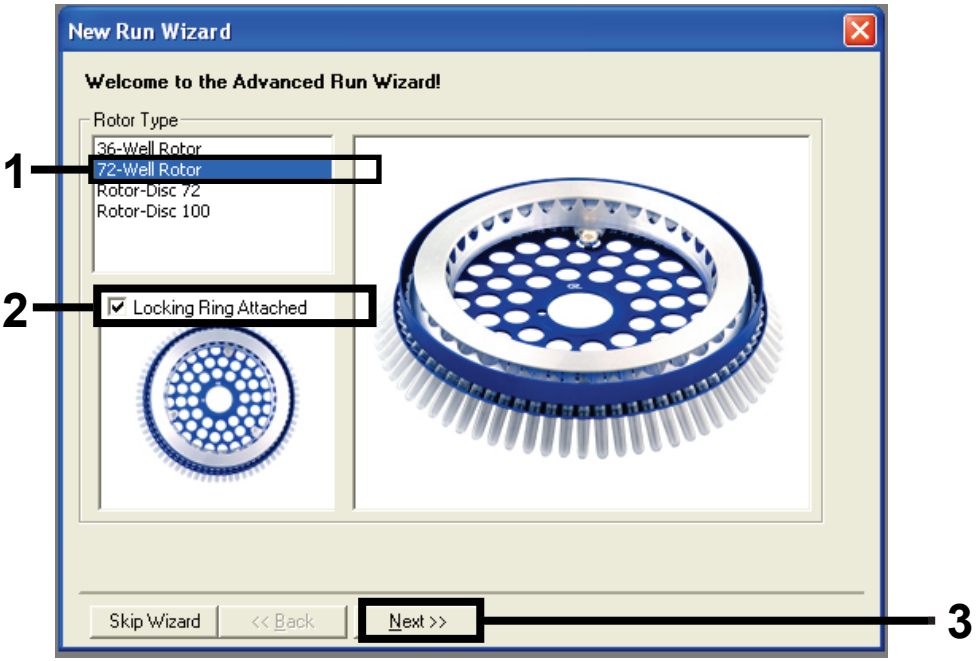
Overview of PCR cycling conditions

Cycle	Step	Temperature Setting	Duration	Acquisition Channels	Number of Cycles
Hold	Reverse transcription	50°C	30 min	N/A	1
Hold	Denaturation and *Taq* activation	95°C	15 min	N/A	1
Cycling	Denaturation	95°C	30 s	N/A	40
	Annealing	60°C	1 min	Green and Yellow	

N/A: Not applicable

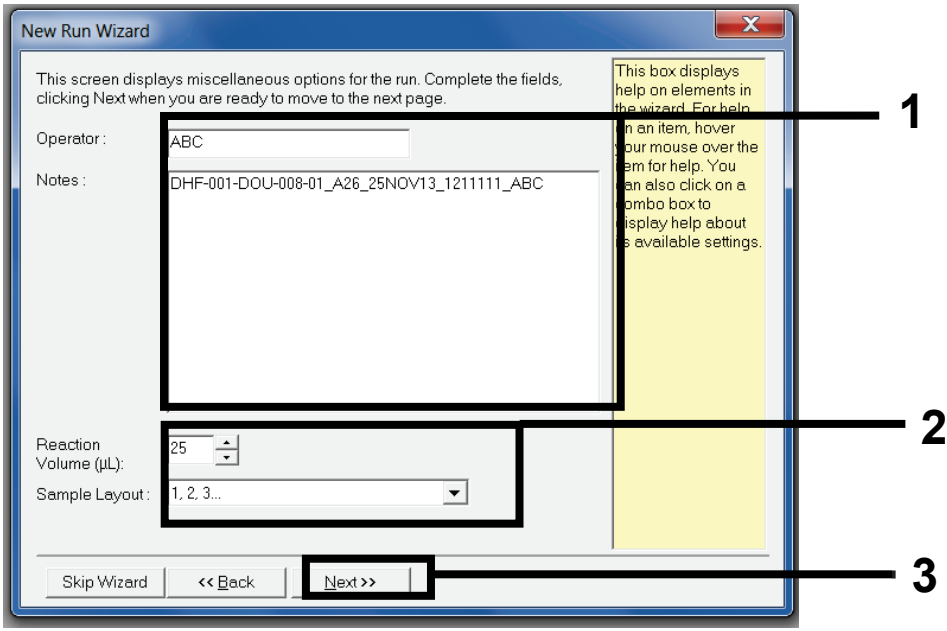
Rotor-Gene Q run setup

1. Open the "New Run Wizard" dialog box and select "72-Well Rotor".
   Click "Locking Ring Attached" and then click "Next".



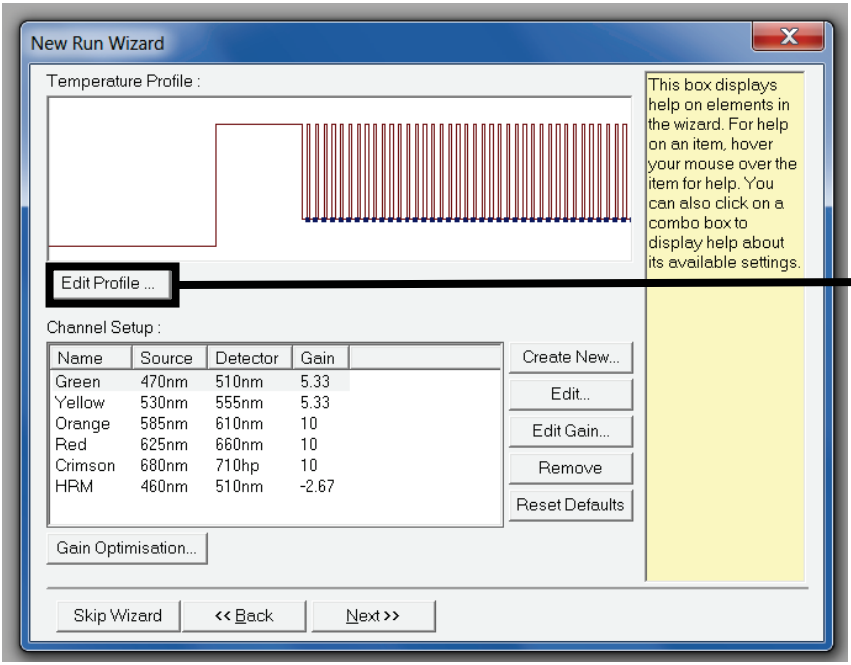
The "New Run Wizard" dialog box.

2. Enter the name of the operator. Add any notes and check that the reaction volume is set to "25" and the "Sample Layout" box reads "1, 2, 3…". Click "Next".



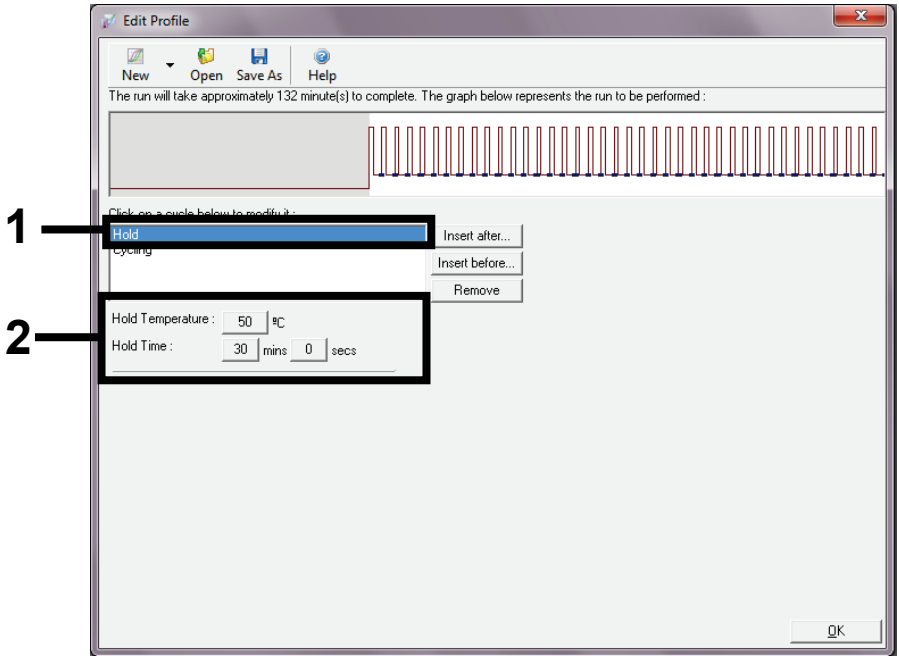
The "New Run Wizard" dialog box.

3. The next window enables editing of the temperature profile. Select "Edit Profile" to modify the temperatures.



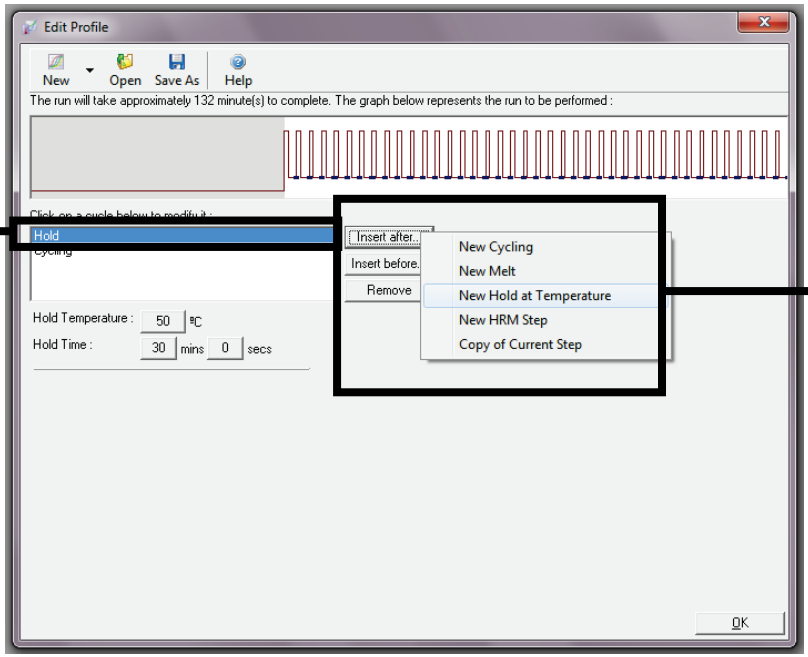
Editing the temperature profile.

4. Edit the "Hold" temperature to 50°C and 30 minutes.



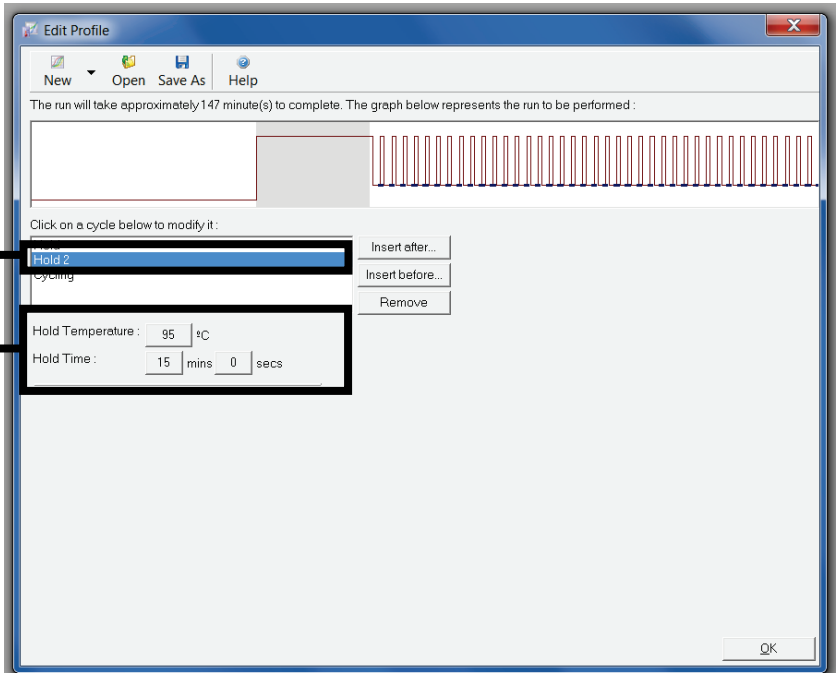
Editing the "Hold" temperature.

5. Then, add a second hold step. Select "Hold", click "Insert after…" and select "New Hold at Temperature".



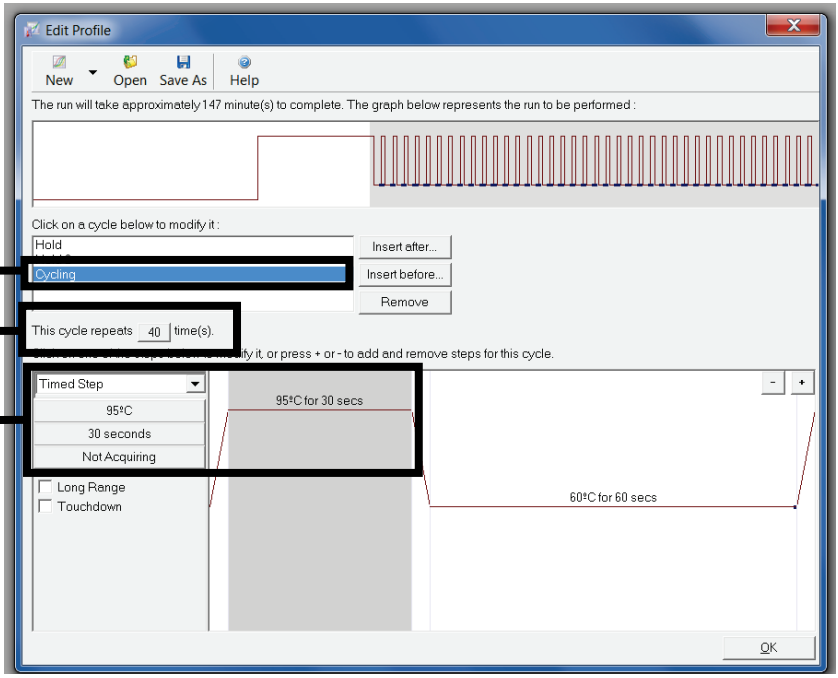
Adding a second "Hold" step.

6. Edit the temperature profile for the second "Hold" step. Select "Hold 2" and set the values to 95°C and 15 minutes.



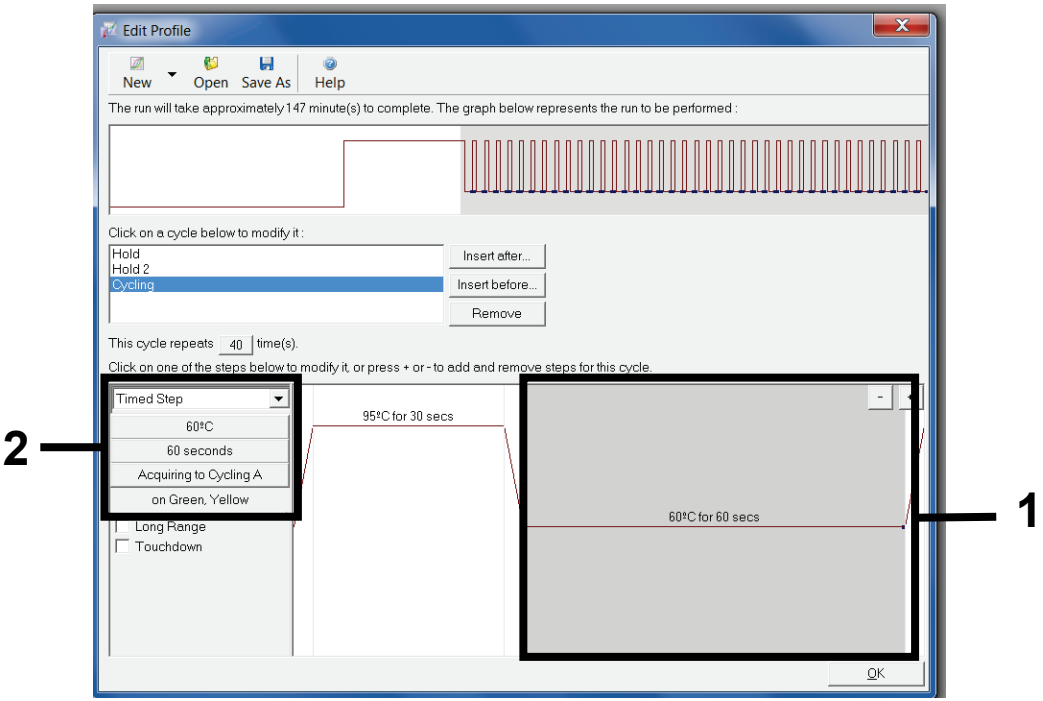
Editing the "Hold 2" temperature.

7. Edit the "Cycling" step. Select "Cycling". Set the "This cycle repeats X times" box to 40. Ensure that the first part of the cycle is selected (shown in gray on the figure). Select "Timed Step" and set the values to 95°C, 30 seconds and Not Acquiring.



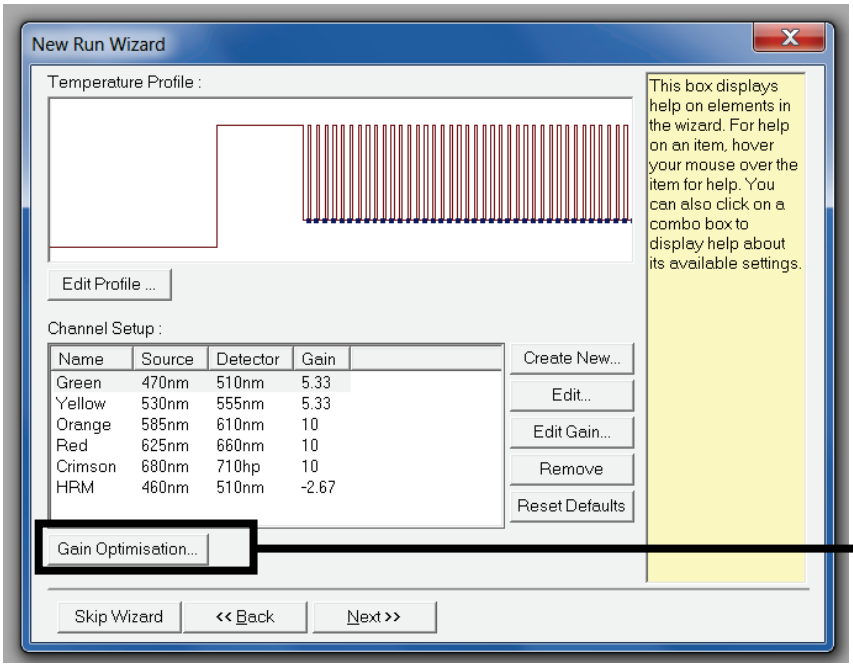
Editing the "Cycling" step.

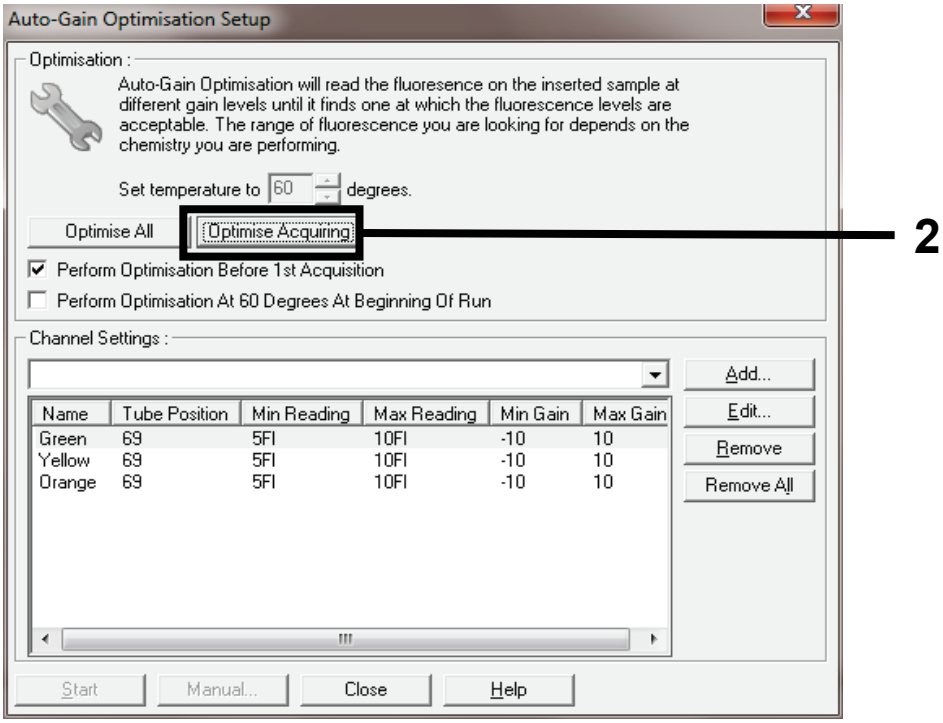
8. Select the second temperature in the cycle diagram (shown in gray on the figure). Select "Timed Step" and set the values to 60°C, 60 seconds, and Acquiring to cycling A on Green, Yellow. Click "OK".



Editing the "Cycling" step.

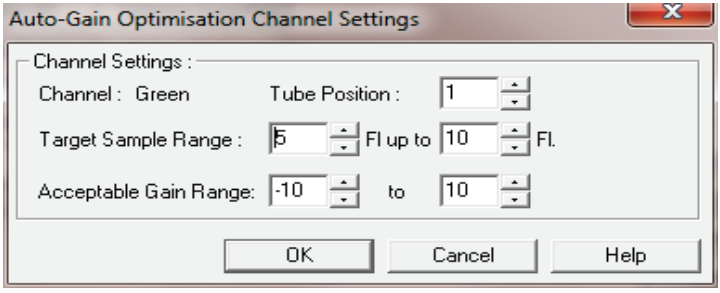
9. Select "Gain Optimisation" from the "Temperature Profile" menu. Select "Optimise Acquiring" and when prompted by the text, "This will remove your existing settings for Auto-Gain. Continue?", select "Yes".


New Run Wizard
Temperature Profile :
This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also click on a combo box to display help about its available settings.
Edit Profile ...
Channel Setup :
Name Source Detector Gain
Green 470nm 510nm 5.33
Yellow 530nm 555nm 5.33
Orange 585nm 610nm 10
Red 625nm 660nm 10
Crimson 680nm 710hp 10
HRM 460nm 510nm -2.67
Create New...
Edit...
Edit Gain...
Remove
Reset Defaults
Gain Optimisation...
Skip Wizard
<< Back
Next >>
1



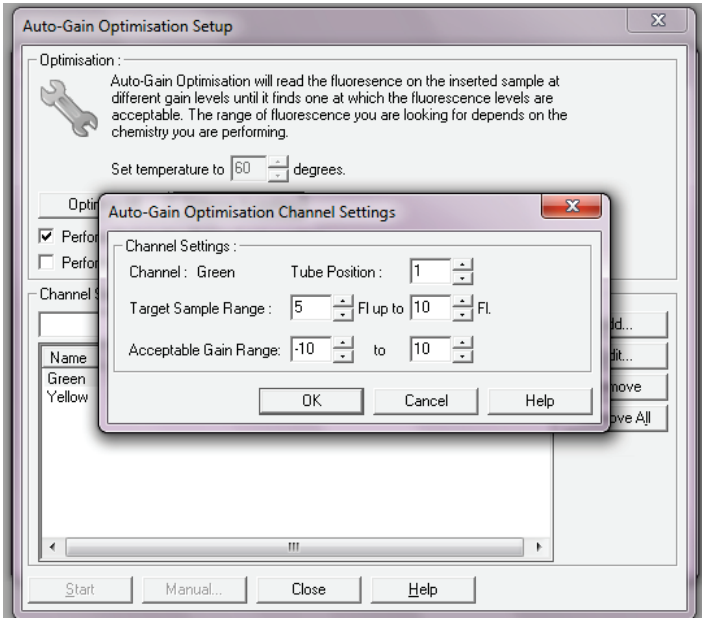
"Gain Optimisation" setup.

10. Select tube position 1 (PC tubes) and ensure that the "Target Sample Range" is set to "5 to 10". Then, set the "Acceptable Gain Range" from –10 to 10 and click "OK".



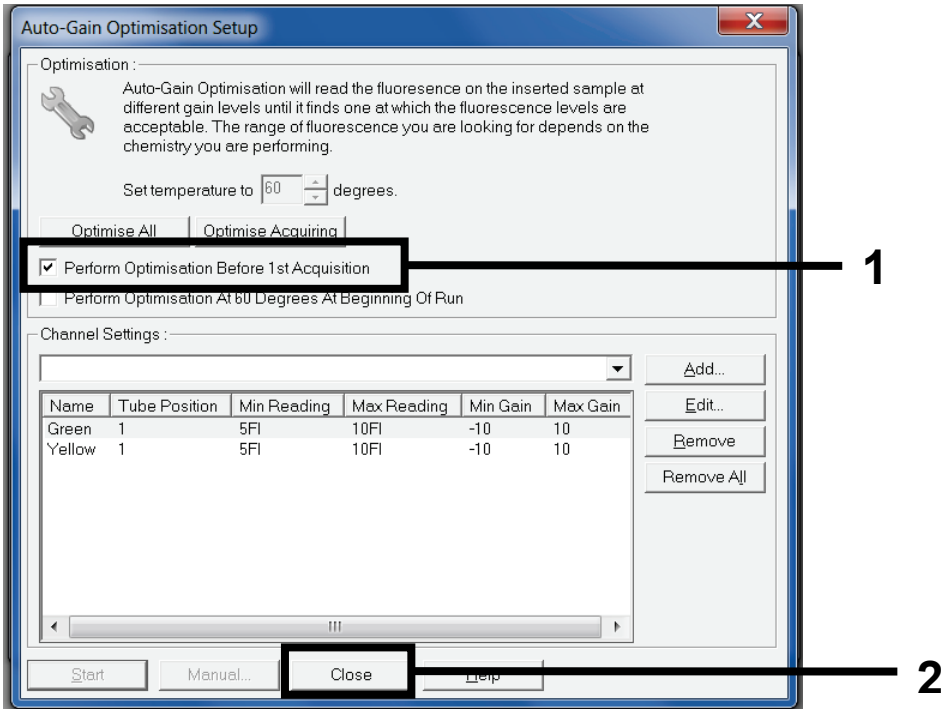
"Acceptable Gain Range".

11. Ensure this is completed for both the Green and Yellow channels.



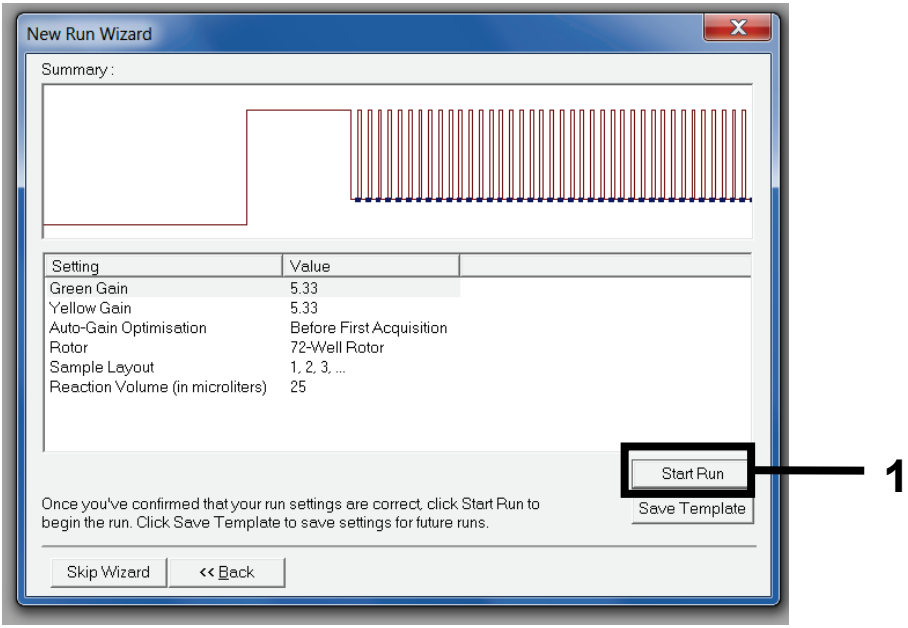
"Gain Optimisation" setup.

12. Check the "Perform Optimisation before 1st Acquisition" box and click "Close". Select "Next" in the "New Run Wizard" to move to the "Summary" screen.



"Gain Optimisation" setup.

13. In the "New Run Wizard" dialog box, Click "Start run" to begin the Rotor-Gene Q run.



The "New Run Wizard" dialog box.

Results

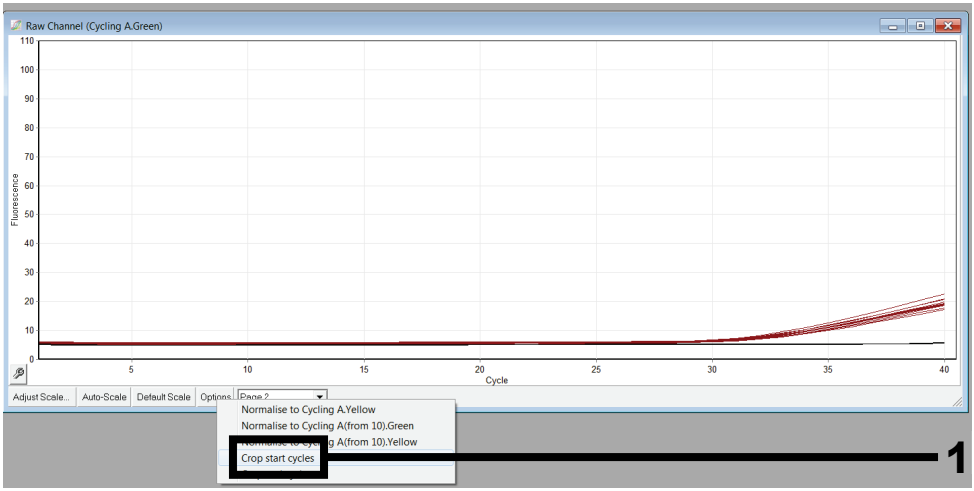
Sample assessment data analysis

After the run is complete, analyze the data according to the following procedure.

Software analysis settings

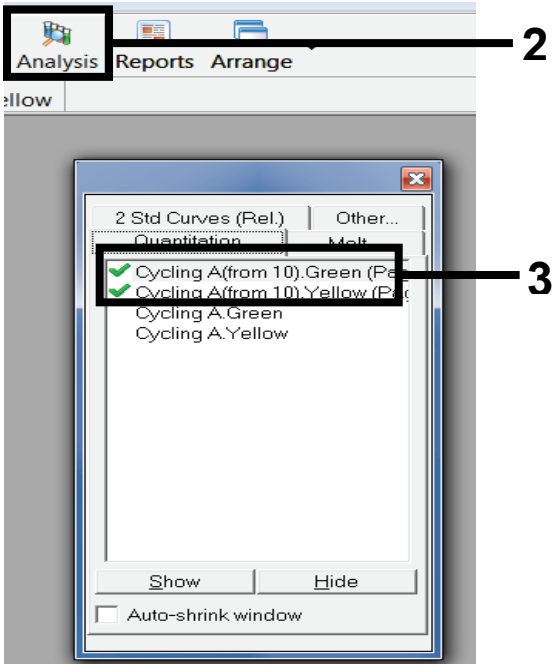
1. Open the appropriate run file using the Rotor-Gene Q series software (2.1.0.7 or higher).
2. If the samples were not named before starting the run, click "Edit Samples".
3. Insert the sample names in the "Name" column.

   Note: Leave the names of any empty wells blank.
4. Go to the "Channels" tab and select "Raw Channel" (Cycling A Green or Cycling A Yellow). Click "Options", followed by "Crop start cycles" and enter a value of 10. Repeat for each channel.



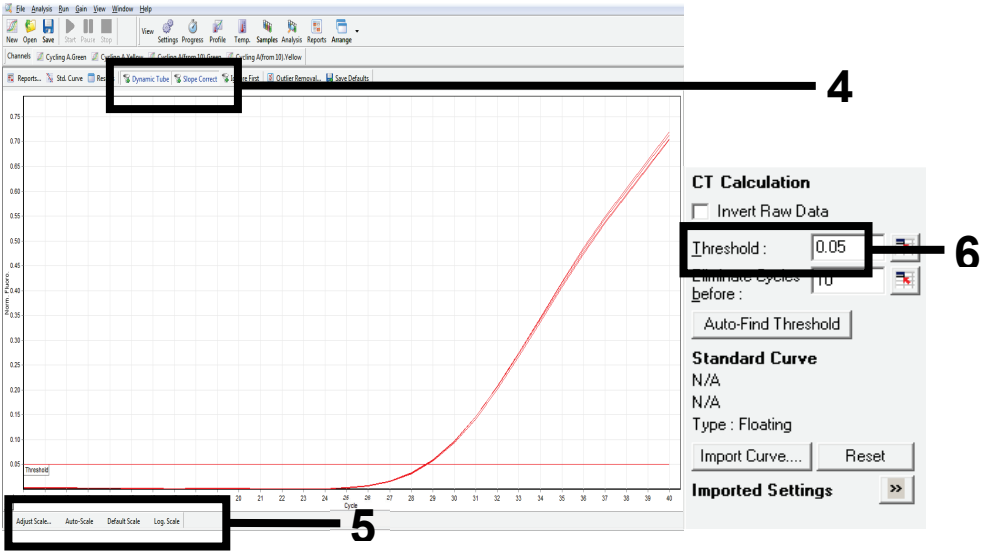
The Raw Channel "Crop start cycles".

5. Go to the “Analysis” tab. Click “Cycling A. Yellow (from 10)” to view the Yellow channel.



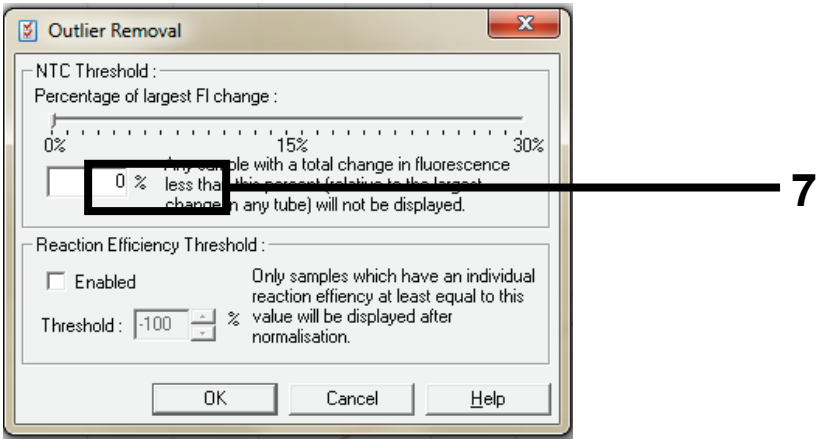
The “Analysis” tab for Cycling A.

6. Check that “Dynamic Tube” and “Slope Correct” are selected.
7. Click “Linear scale” and set the threshold to 0.05.



Setting the linear scale.

8. Click "Outlier Removal" and set "NTC Threshold" to 0% and select "OK".

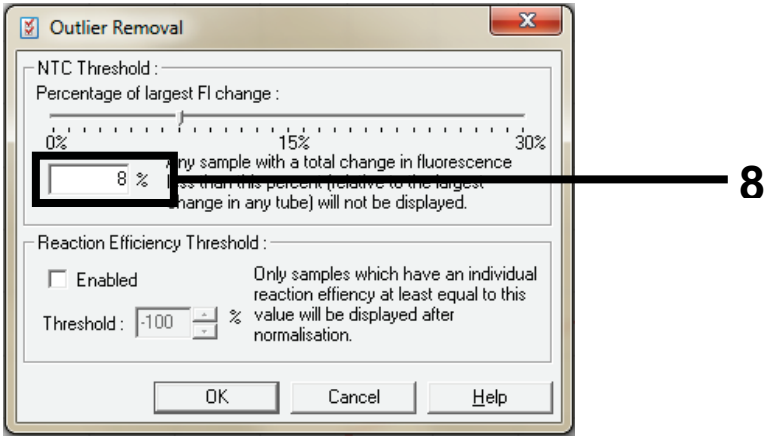


Setting Outlier Removal

9. Check that there are no curves crossing the threshold as a result of linear amplification.

   Note: See "Linear amplification", page 36 for identification of linear amplification and to differentiate linear and true amplification.

10. Check the Yellow channel C_T values.
11. Go to the "Analysis" tab. Click "Cycling A. Green (from 10)" to view the Green channel.
12. Check that "Dynamic Tube" and "Slope Correct" are selected.
13. Click "Linear scale" and set the threshold to 0.05.
14. Click "Outlier Removal" and set to 8% and select "OK".



Setting Outlier Removal

15. Check that there are no curves crossing the threshold as a result of linear amplification.

    Note: See "Linear amplification", page 36 for identification of linear amplification and to differentiate linear and true amplification.

16. Check the Green channel C_T values.

The analysis and expression calls are performed manually after analysis by the Rotor-Gene Q software when a run is completed. The following information explains how to analyze the data obtained.

The PCR cycle at which the fluorescence from a particular reaction crosses a threshold, is defined as the C_T value. C_T values in the Yellow (HEX) channel correlate with the quantity of specific input RNA. Low C_T values indicate higher input RNA levels and high C_T values indicate lower input RNA levels.

Samples with a C_T value in the Green (FAM) channel equal to or less than the cutoff are classified as positive expression.

The Rotor-Gene Q software interpolates fluorescence signals between any two recorded values. C_T values can therefore be any real number (not limited to integers) within the range 0 to 40.

For the ALK RGQ RT-PCR Kit, the threshold value is set at 0.05 relative fluorescence units for the Green (FAM) and Yellow (HEX) channels. The threshold value was defined during development of the ALK RGQ RT-PCR Kit.

Analysis of run controls

The run controls (positive control and NTC) are assessed to ensure that acceptable C_T values are achieved and that the reactions are performing correctly. Only if the run controls meet the acceptance criteria, will the sample wells be analyzed (Table 2).

Table 2. Acceptance criteria for controls

Run control	Channel	Lower limit ≥	Upper limit ≤	Range
Positive Control ALK FAM	Green	25.44	32.16	6.72
Positive Control ABL1 HEX	Yellow	24.55	33.79	9.24
NTC ALK FAM	Green	No C_T value below 40		
NTC ABL1 HEX	Yellow	No C_T value below 40		

After the RGQ run is completed, Sample C_T values are determined for the Green (ALK; target expression assay) and the Yellow (ABL1; control expression assay) channels.

Note: The target expression assay C_T and control expression assay C_T values come from the same sample well.

Sample analysis: sample expression control assay (Yellow channel)

Table 3. Established value for ABL1 expression control assay

	ABL1 expression control assay (C_T) (HEX; Yellow channel)
Working range	24.23–31.09

There are 2 possible outcomes for each sample:

- If the sample control HEX CT falls within the specified range (24.23–31.09, Table 3), it is amplification positive and valid.
- If the sample control HEX CT is above the specified range (>31.09, Table 3), the tube is amplification negative and invalid.

- The sample can be re-tested one more time and the sample validity determined. If the sample control HEX C_T is above the specified range after the re-test, a second extraction will be performed the sample validity re-assessed like the first time. If the sample still fails the acceptance criteria for Control HEX, the sample is invalid.

Sample analysis: sample target expression assay (Green channel)

If a sample is determined valid, sample ΔC_T values are calculated using the equation:

$$\Delta C_T = [\text{Target Expression Assay Ct value (FAM } C_T)] - [\text{Expression Control Assay } C_T \text{ value (HEX } C_T)]$$

Note: The Target Expression Assay C_T and Expression Control Assay C_T values are from the same sample well.

- ΔC_T value should be checked against the ΔC_T values indicated in Table 4. If the sample control HEX C_T falls within the specified range (24.23–31.09) and the ΔC_T value is equal to or below 8.0; the sample is called as "Expression Detected".
- If the sample control HEX C_T falls within the specified range (24.23–31.09) and the ΔC_T value is above 8.0; the sample is called as "No Expression Detected".
- If the sample control HEX C_T is above the specified range (>31.09), and, if there is amplification in the FAM (Green) channel for that sample, the sample is called as "Invalid".

Table 4. Established value for ALK target expression assay

	ALK target expression assay (ΔC_T) (FAM; Green channel)
Cutoff (C_T)	$\leq$8.0

The cutoff is a point above which the positive signal may be due to background signal of the "Scorpion" primer or background RNA. If a sample has a ΔC_T value higher than the cutoff, it is classified as negative and beyond the limit of detection of the assay.

For every sample, the ALK RT-PCR assay will be assigned a status of "Expression Detected", "No Expression Detected", "Sample Invalid", or in the case where a run control fails, "Run Control Fail".

- Expression Detected: if the C_T value in the Yellow channel is valid and the ΔC_T value is less than or equal to the cutoff C_T value (Table 4).
- No Expression Detected: if the C_T value in the Yellow channel is valid (Table 3), and the ΔC_T value is greater than the cutoff C_T value (Table 4) or there is no C_T observed in the Green Channel.
- Sample Invalid: When the C_T value in the Yellow channel is invalid (Table 3).
- Run control Fail: When the C_T values in the Green and the Yellow channel are not with in specifications in the Positive control well (Table 2) and there is C_T value <40 in the NTC well.

Notes for data analysis

Linear amplification

Rotor-Gene Q plots from all reactions should be checked. Occasionally, an increase in fluorescence signal is seen in the NTC (negative samples and empty tubes). If this occurs and a C_T value is obtained in the NTC well, the user must distinguish between a true amplification event (which would indicate contamination in the NTC; see Figure 2) and a linear increase in fluorescence, which may have arisen due to a fluorescent artifact. Linear amplification in empty tubes is due to a fluorescence artifact. C_T data for the empty tubes should therefore be discarded. An increase in the fluorescence signal in an empty tube can be disregarded.

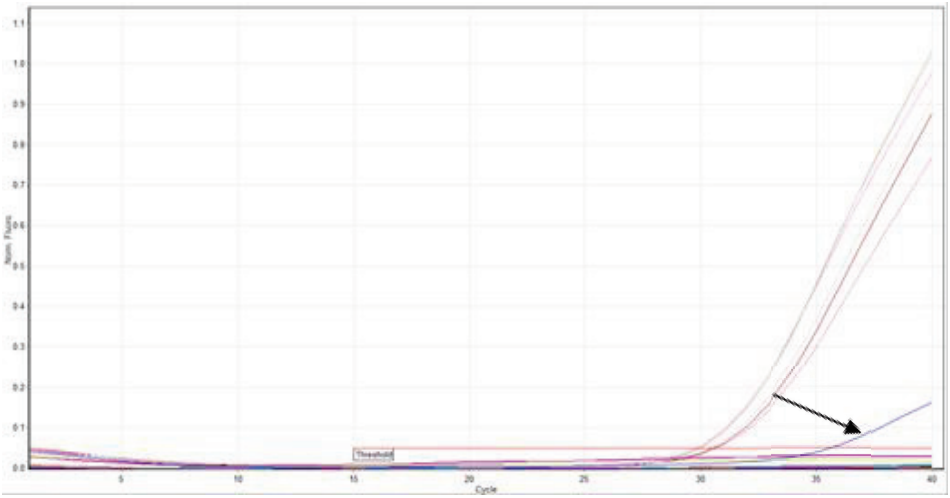


Figure 2. Contamination in a NTC of an assay in an analyzed run. The arrow indicates amplification showing contamination in an NTC tube.

Analysis of the NTC

Figure 2 and Figure 3 show two examples of abnormal behavior of NTC samples. In Figure 2, nonlinear (true) amplification due to sample contamination is seen. This run should be discarded and samples retested. In Figure 3, linear amplification in an NTC is seen. In this case, the raw fluorescence should be examined. The corresponding raw fluorescence plot is presented in Figure 4, showing a linear increase in fluorescence rather than a true amplification event. The data from this run can be used, but only if the positive control has passed. For comparison with Figure 4, Figure 5 shows raw fluorescence data where true amplification has taken place. In this case, the data should be discarded and the samples retested as this indicates that contamination is present.

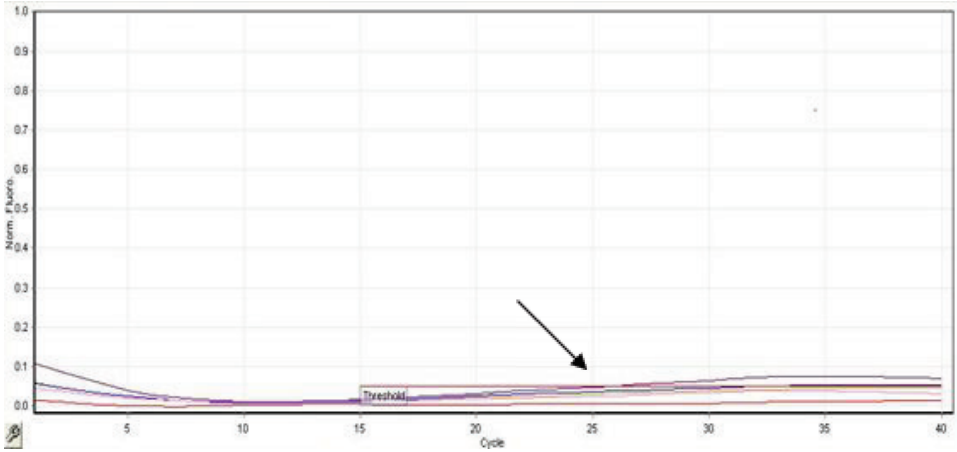


Figure 3. Example of a linear increase in fluorescence in an NTC tube. The arrow indicates linear increase in fluorescence in a NTC tube.

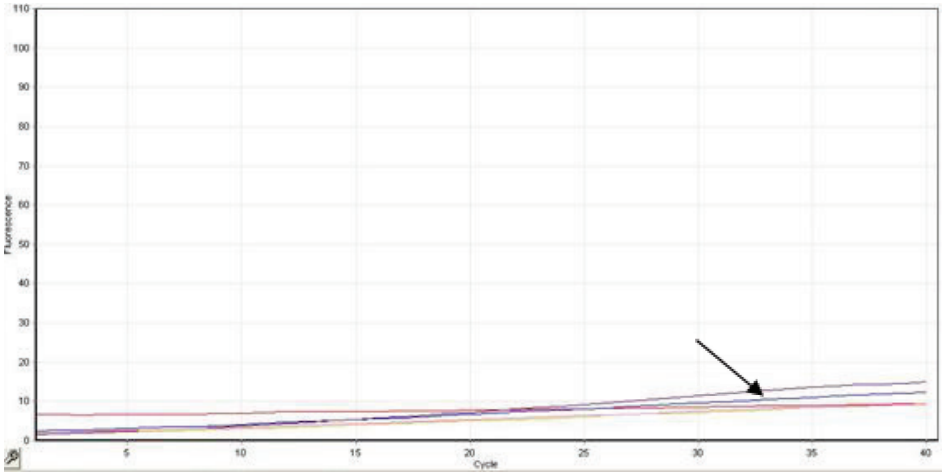


Figure 4. Raw fluorescence data in NTC well. The arrow indicates linear amplification in an NTC well.

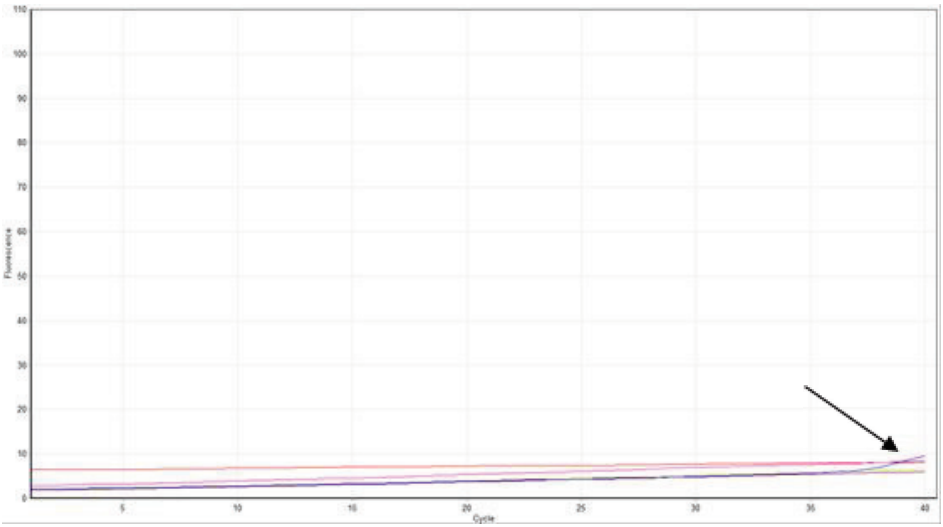


Figure 5. Raw fluorescence data showing an NTC tube with a true amplification event. The arrow indicates contamination in an NTC well. All other wells show no contaminant present.

Analysis of samples

Figure 6 and Figure 7 are two examples of amplification in sample reactions. Figure 6 is an example of true amplification in a sample tube. If a run shows this type of sigmoidal amplification curve, this is true amplification and the data from this run can be used, but only if the positive control and the internal control have passed.

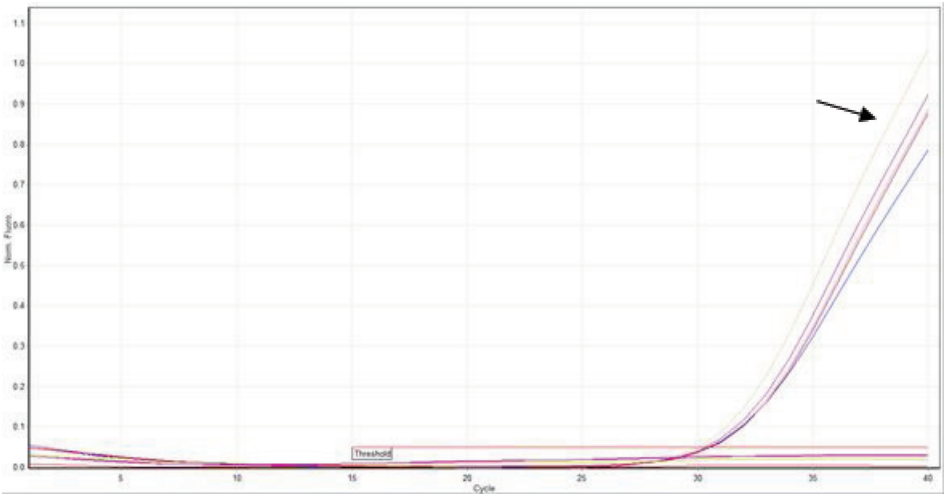


Figure 6. True amplification in a sample tube in an analyzed run. The arrow indicates the plots showing true amplification.

Figure 7 shows an example of linear amplification in a sample reaction. Here, the raw fluorescence data should be examined. The corresponding raw fluorescence plot (Figure 8) indicates that the linear increase observed corresponds to a linear increase in the raw fluorescence and is not a true amplification. If the positive and internal control checks have passed, sample results can be used from these runs with caution, such that linear amplification is called as "no C_T".

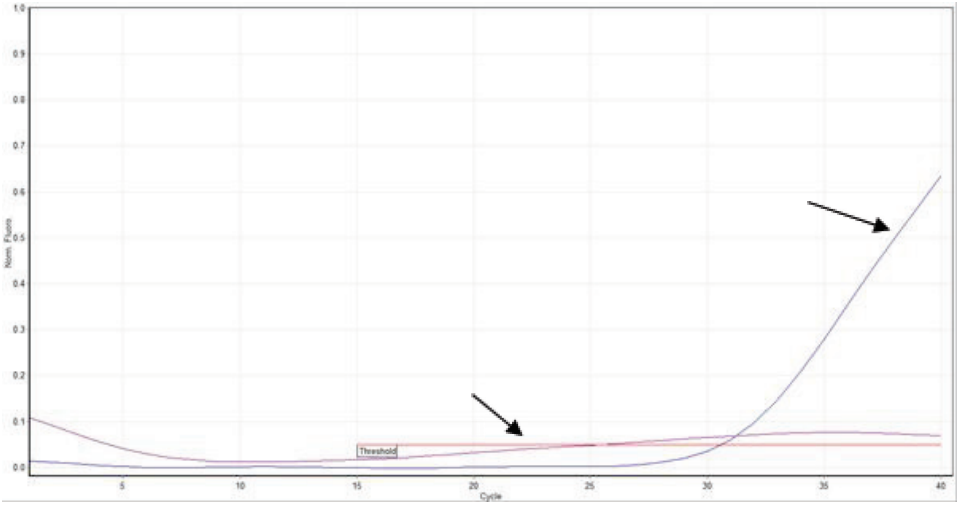


Figure 7. Example of a linear increase in fluorescence in a sample tube. The upper arrow indicates a plot showing true amplification. The lower arrow indicates linear increase in fluorescence in a sample well.

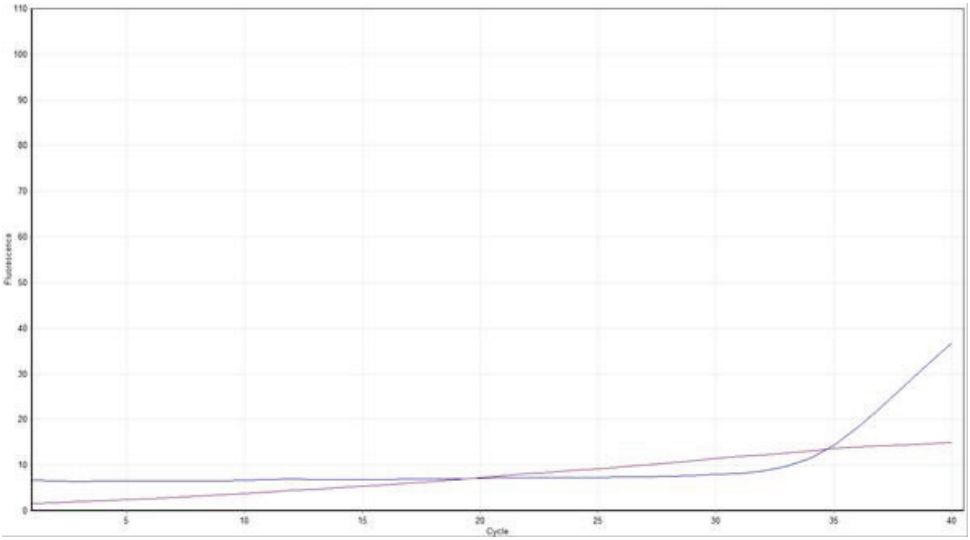


Figure 8. Raw fluorescence of Figure 7.

Analytical performance

The ALK RGQ RT-PCR Kit is for research use only and not for use in diagnostic procedures.

The analytical performance specifications are given as indicative data and should be re-evaluated by the user.

Limit of blank and initial cutoff

FFPE samples were tested using a method adhering to the guidance of the Clinical and Laboratory Standards Institute (CLSI). Data from 16 negative samples tested in a total of 128 replicates (16 samples, 8 replicates) were used to assess the limit of blank. Cut-off was determined using an eight point serial dilution of in vitro transcribed RNA and tested in eight replicates per run over three RGQ runs (24 reps per dilution).

The ABL1 control expression assay C_T range was established as 24.23–31.09 C_T.

The cutoff for the ALK target expression assay was established at a ΔC_T value of $\leq$8.0.

Troubleshooting Guide

Refer to this section for error handling and troubleshooting. If the recommended steps do not resolve the problem, contact QIAGEN Technical Services for assistance.

Possible problem or cause	Corrective action
Invalid results	
a) The storage conditions for one or more components did not comply with the instructions given in "Reagent Storage and Handling"	Check the storage conditions (see the label) of the reagents and use a new kit, if necessary.
b) FAM C_T out of range for positive control for the ALK reaction	Repeat the entire PCR run.
c) HEX C_T out of range for the ABL1 control reaction	Repeat the entire PCR run.
d) Fluorescence data in positive control (Green channel) cannot be interpreted	Repeat the entire PCR run.

Possible problem or cause	Corrective action
NTC samples show positive results in the FAM and/or HEX channel	
a) Contamination occurred during preparation of the PCR	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure that work space and platforms are decontaminated at regular intervals. Be sure to use sterile pipet tips with aerosol barriers.
Invalid sample	
a) Fluorescence data in sample control (Yellow channel) cannot be interpreted	Set up a new PCR run to repeat the relevant sample(s).
b) ABL1 HEX C_T too low in sample control (Yellow channel) reaction	Dilute sample to increase control C_T value. The dilution should be calculated on the assumption that diluting 1:1 with the water supplied in the kit will increase the C_T by 1.0. Once the sample is diluted, set up a new PCR run to repeat the sample.
c) ABL1 HEX C_T too high in sample control (Yellow channel) reaction	Set up a new PCR run to repeat the sample. If the sample is invalid again, repeat the PCR run, extracting the sample from a fresh FFPE section(s). If still invalid, repeat this second extraction. If the sample does not give a valid result after this run, the sample is given a quantity not sufficient status and no further testing should be carried out.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of ALK RGQ RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

Development studies were performed using human RNA extracted from FFPE sample as appropriate for the individual studies.

The product has been developed using the RNeasy FFPE Kit from QIAGEN.

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Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
 **< 24>**	Contains reagents sufficient for <24> reactions
	Positive Control
	Use by
	Catalog number
	Lot number
	Contains
	Number

Symbol	Symbol definition
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Keep away from sunlight

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
ALK RGQ RT-PCR Kit (24)	For 24 reactions: one tube of OneStep Enzyme Mix, one tube of ALK/ABL1 Reaction Mix, Positive Control, RNase/DNase-free Water for NTC and RNase/DNase-free Water for Sample Dilution	870501
Rotor-Gene Q and other accessories		
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (Green, Yellow, Orange, Red, Crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training included	9001650
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (Green, Yellow, Orange, Red, Crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training not included	9001580
Loading Block 72 x 0.1ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901

Product	Contents	Cat. no.
Strip Tubes and Caps, 0.1ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
RNeasy FFPE Kit	50 RNeasy MinElute® Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	73504

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