

June 2023

therascreen® PDGFRA RGQ PCR Kit Instructions for Use



Version 1

IVD

For In Vitro Diagnostic Use

R

For prescription use only

For use with Rotor-Gene® Q MDx (US) instrument

For use with QIAamp® DSP DNA FFPE Tissue Kit

REF

874921



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— Sample to Insight —





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Intended Use

The QIAGEN *therascreen*[®] PDGFRA RGQ PCR Kit is a real-time qualitative *in vitro* diagnostic assay for the detection of the D842V somatic mutation in the *PDGFRA* gene using genomic DNA extracted from Gastrointestinal Stromal Tumor (GIST) patient's formalin-fixed paraffin-embedded (FFPE) tumor tissue.

The *therascreen* PDGFRA RGQ PCR Kit is intended for use as a companion diagnostic test, to aid clinicians in identification of patients with GIST who may be eligible for treatment with AYVAKIT[™] (avapritinib) based on a *PDGFRA* mutation detected result. FFPE tumor specimens are processed using the QIAamp[®] DSP DNA FFPE Tissue Kit for manual sample preparation and the Rotor-Gene[®] Q (RGQ) MDx instrument for automated amplification and detection.

Description and Principle

The *therascreen* PDGFRA RGQ PCR Kit comprises one multiplex PCR amplification reaction to detect the point mutation of interest and a control region downstream of this in the same *PDGFRA* gene. The principal components of the kit are explained below.

PDGFRA reaction mix

The PDGFRA Reaction Mix contains primers and probes for both the PDGFRA D842V mutation and a control reaction:

Mutation reaction

Patient DNA is selectively amplified by qPCR using a forward and reverse primer and mutation specific labeled probe which is detected in the Texas-Red (Orange) channel of the Rotor-Gene Q MDx (US) instrument.

Control reaction

The Control reaction uses a forward and reverse primer and labeled probe which is detected in the Green (FAM) channel to amplify a short conserved sequence of the *PDGFRA* gene. The Control reaction is used to determine if an appropriate level of amplifiable DNA is present in the sample and is a factor in the analytical calculations that determine mutation status.

The control target is used to both define a sample as mutant or wild type and as a DNA loading control. All samples will give a signal in the Green (FAM) channel in a predefined C_T range if sufficient DNA has been added to the reaction tube.

Positive control

The positive control (tube PC) comprises a single plasmid representing both the mutant and control regions of the *PDGFRA* gene. Detection of both targets within acceptable ranges confirms the proper functioning of the reaction mix in the kit and overall assay set up.

Negative control

The no template control (tube NTC) contains nuclease-free water to be used for the "no template control" (NTC) reaction. The NTC serves as a negative control and assesses potential contamination during assay setup.

Sample diluent

The sample diluent (tube Dil.) contains nuclease-free water.

Taq DNA polymerase

Taq DNA polymerase is the enzyme for the polymerase chain reaction used by the *therascreen* PDGFRA RGQ PCR Kit.

Summary and explanation

The *PDGFRA* gene codes for a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family. These growth factors are mitogens for cells of mesenchymal origin. The identity of the growth factor bound to a receptor monomer, determines whether the functional receptor is a homodimer or a heterodimer, composed of both platelet-derived growth factor receptor alpha and beta polypeptides¹⁻². Studies suggest that this gene plays a role in organ development³, wound healing⁴, and tumor progression^{2,5}. Mutations in this gene have been associated with idiopathic hypereosinophilic syndrome⁶⁻⁷, somatic and familial gastrointestinal stromal tumors⁸⁻⁹, and a variety of other cancers^{2,5}.

The assay will contain the reagents necessary for the amplification and detection of the target DNA sequence using real-time qualitative PCR. It is designed to be used with the RGQ MDx instrument in conjunction with Rotor-Gene AssayManager® (RGAM) software.

The assay will include a number of controls to be used as part of each assay run. A no template control (NTC) will be used to detect any contamination. A positive control plasmid containing the sequences of interest will be used to ensure the PCR run has performed within expected parameters.

The Rotor-Gene AssayManager (RGAM) MDx software generates a C_T value for the D842V and control sequence present in a sample. The C_T value difference (ΔC_T) between the D842V C_T value and the control C_T value is used by the software to generate a mutation call ('Mutation Detected' or 'No Mutation Detected' if the D842V mutation is present or absent).

Materials Provided

Kit contents

<i>therascreen PDGFRA RGQ PCR Kit</i>			(24)
Catalog no.			874921
Number of reactions			24
Color	Identity	Tube Identification	Volume
Violet	Reaction mix	PDGFRA Reaction Mix	720 µl
Mint	<i>Taq</i> DNA Polymerase	<i>Taq</i>	1 x 85 µl
Red	Positive Control	PC	40 µl
Transparent	Nuclease-free water for No Template Control	NTC	1 x 1.9 ml
Transparent	Nuclease-free water for Dilution	Dil.	1 x 1.9 ml
<i>therascreen PDGFRA RGQ PCR Kit Instructions for Use</i>			1

Components of the kit

Reaction mixes are multiplex, containing fluorescently labeled reagents to detect targets and an internal control.

The principal components of the kit are explained below.

Table 1. Reagents supplied in the *therascreen* PDGFRA RGQ PCR Kit

Reagent	Active Ingredients	Volume
Reaction mix	Control Unlabeled Primers, Control probe labeled with Green (FAM) fluorophore, Mutant Unlabeled Primers, Mutant Probe labeled with Orange (Texas-Red) fluorophore, Blocker Unlabeled Primer, Deoxynucleotide Triphosphates, Magnesium Chloride, Tris EDTA Buffer, PCR Buffer and Nuclease-free Water	1 x 720 μ l
Positive Control	Single plasmid, Poly A RNA, Tris EDTA Buffer	1 x 40 μ l
Taq DNA Polymerase	Taq Polymerase; 50% Glycerol/Nuclease-free water	1 x 85 μ l
No template control	Nuclease-Free Water	1 x 1.9 mL
For sample dilution	Nuclease-Free Water	1 x 1.9 mL

Platform and software

The *therascreen* PDGFRA RGQ PCR Kit is specifically designed for use with the Rotor-Gene Q MDx (US) instrument installed with Rotor-Gene Assay Manager v2.1 or later. Refer to the instrument user manual for information concerning the instrument.

The Rotor-Gene AssayManager software needs to be loaded with the *therascreen*_PDGFRA_FFPE_MDx_V1_0_0 Assay Profile for cycle parameters specific to the *therascreen* PDGFRA RGQ PCR Kit.

The Rotor-Gene Q MDx (US) instrument must be maintained according to the requirements in the instrument user manual.

Materials Required but Not Provided

Additional reagents

- QIAamp® DSP DNA FFPE Tissue Kit (QIAGEN, cat. no. 60404; see Protocol: DNA Extraction)

Consumables

- 0.1 mL Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or 981106)
- Nuclease-free, low DNA-binding microcentrifuge tubes for preparing master mixes
- Nuclease-free pipet tips with aerosol barriers

Equipment*

- Permanent marker
- Rotor-Gene Q MDx (US) instrument with 72-well rotor (QIAGEN, cat. no. 9002035)
- Rotor-Gene AssayManager v2.1 or later
- *therascreen_PDGFR_A_FFPE_MDx_V1_0_0* Assay Profile
- Loading Block 72 x 0.1 mL Tubes, aluminum block for manual reaction set up (QIAGEN, cat. no. 9018901)
- Dedicated pipets (adjustable) for sample preparation
- Dedicated pipets (adjustable) for PCR master mix preparation
- Dedicated pipets (adjustable) for dispensing of template DNA

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

-
- Benchtop centrifuge with rotor for 1.5 mL tubes
 - Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C, 70°C, and 90°C*

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

For in vitro diagnostic use.

For prescription use only.

For use only with Rotor-Gene Q MDx (US) instrument.

For use only with QIAamp DSP DNA FFPE Tissue Kit.

Note: For safety information regarding the Rotor-Gene Q MDx (US) instrument, see the user manual supplied with the instrument.

Note: For safety information regarding the QIAamp DSP DNA FFPE Tissue Kit (cat. no. 60404), see the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.

Note: The *therascreen* PDGFRA RGQ PCR Kit is to be used by trained personnel in a professional laboratory environment.

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.

- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

Precautions

- The test is for use with formalin-fixed, paraffin-embedded GIST specimens.
- All chemicals and biological materials are potentially hazardous. Specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard sample and assay waste according to your local safety procedures.
- Reagents for the *therascreen* PDGFRA RGQ PCR Kit are diluted optimally. Do not dilute reagents further as this may result in a loss of performance. Do not use reaction volumes (reaction mix plus sample) of less than 25 μ l.
- All reagents supplied in the *therascreen* PDGFRA RGQ PCR Kit are intended to be used solely with other reagents supplied in the same *therascreen* PDGFRA RGQ PCR Kit. Do not substitute the reagents in the *therascreen* PDGFRA RGQ PCR Kit or between *therascreen* RGQ PCR Kits, as this may affect performance.
- Only use the *Taq* DNA polymerase (tube *Taq*) that is provided in the *therascreen* PDGFRA RGQ PCR Kit. Do not substitute with *Taq* DNA polymerase from other kits of the same or any other type or with *Taq* DNA polymerase from another supplier.
- Refer to the Rotor-Gene Q MDx (US) instrument user manual for additional warnings, precautions, and procedures.
- Do not use expired or incorrectly stored components.

Note: Use extreme caution to prevent contamination of the control and reaction mix reagents with the synthetic materials that are contained in the positive control reagent.

Note: Use extreme caution to prevent cross-contamination between samples. Cap the tubes promptly after addition of each sample.

Note: Thoroughly decontaminate the loading block before using it for preparation of assay master mixes. The use of DNAZap solutions followed by Distel and IPA wash is recommended. Loading block must be dry before use.

Note: Use individual, dedicated pipets for setting up reaction mixes and adding positive control reagents.

Note: Perform preparation and dispensing of reaction mixes in an area separate from the one used for the addition of the positive control.

Note: Do not open the Rotor-Gene Q MDx (US) instrument until the run has finished.

Note: Do not open Rotor-Gene Q tubes after the run has finished. This is to prevent laboratory contamination with post-PCR products.

Note: Caution must be observed to ensure correct sample testing with emphasis on eliminating wrong sample entry, loading error, pipetting error, and ensuring the PCR strip tubes are placed into the appropriate positions of the 72-well rotor.

Note: Ensure kit reagents are thawed and mixed. Absence of sufficient mixing may affect performance.

Reagent Storage and Handling

Shipping conditions

The *therascreen* PDGFRA RGQ PCR Kit is shipped on dry ice. If any component of the *therascreen* PDGFRA RGQ PCR Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note, Instructions for Use, or the reagents, please contact QIAGEN Technical Services or local distributors (visit www.qiagen.com).

Storage conditions

The *therascreen* PDGFRA RGQ PCR Kit should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer and protected from light until the stated expiration date.

In-use stability

Once opened, reagents can be stored in their original packaging at -30 to -15°C for 24 months (or until the stated expiry date shown on the packaging if this is less than 24 months once the reagents have been opened for the first time). Repeated thawing and freezing should be avoided. Do not exceed a maximum of five freeze-thaw cycles.

The reagents must be thawed at room temperature (15 – 25°C) for 1 hour (and up to a maximum of 4.5 hours) before use. Once the reagents are ready to use, the PCR reactions can be set up. The Rotor-Gene Q tubes, containing the master mix and the DNA sample, can be loaded onto the Rotor-Gene Q MDx (US) immediately. The total time from start of PCR setup to the start of the run should not exceed 4.5 hours if stored at room temperature.

Note: Fluorescently labeled molecules included in the reaction mix reagents are light sensitive. Protect reaction mix reagents from light to avoid photo bleaching.

Reagents in the *therascreen PDGFRA RGQ PCR Kit* are diluted optimally and no further purification or treatment is required prior to their use in analysis as directed by the *therascreen PDGFRA RGQ PCR Kit Instructions for Use* (this document).

Attention should be paid to the expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

Note: Reagents required for extraction should be stored as described in the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.

Specimen Storage and Handling

The *therascreen* PDGFRA RGQ PCR Kit is for use with DNA samples extracted from GIST patient's formalin-fixed paraffin-embedded (FFPE) tumor tissue. Tumors are heterogeneous in terms of both genotype and phenotype. Mutation-positive tumors can contain wild type DNA and similarly histology can show regions of non-tumor tissue. All tissue specimens should be treated as potentially hazardous. All samples should be treated as potentially infectious. Discard sample and assay waste according to your local safety procedures.

Store FFPE blocks and slides at room temperature.

Genomic DNA may be stored at 2–8°C for 1 week post-extraction, then at –30 to –15°C for up to 8 weeks before use.

The workflow is described in Figure 1.

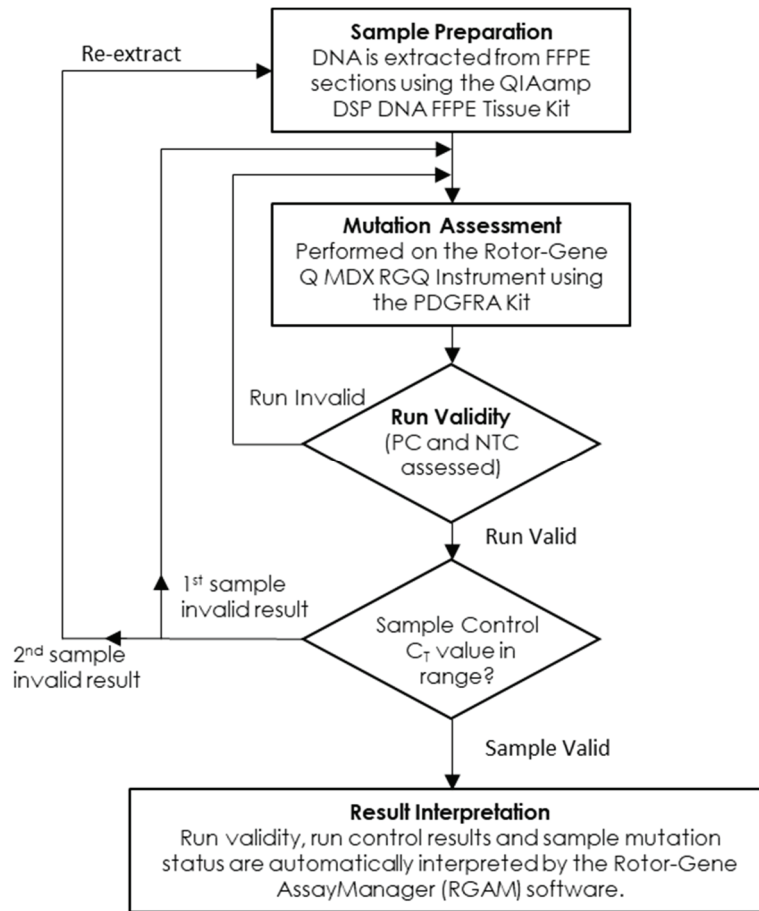


Figure 1. Workflow for the qualitative detection of PDGFRA D842V Mutation.

Repeat PCR testing of an invalid sample should only be done once. If the required conditions are not met, samples should be re-extracted from fresh FFPE slides. In case of low slide availability, slides from a new specimen can be used.

Protocol: DNA Extraction

Important points before starting

- Use the QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, cat. no. 60404) with the stipulations described below for purifying genomic DNA from samples prepared from FFPE GIST specimens.
- The *therascreen* PDGFRA RGQ PCR Kit has been developed using DNA extracted using the QIAamp DSP DNA FFPE Tissue Kit. Do not use any other DNA extraction product.
- Use dry scalpels. Do not perform this step in a laminar flow or fume hood.

Handling reagents

- Label, handle, and store tumor specimens, blocks, slides, samples, and microcentrifuge tubes ready for extraction in a controlled fashion according to local procedures.
- Store FFPE blocks and slides at room temperature.

Things to do before starting

- Using standard materials and methods, fix the tissue specimen in 10% neutral buffered formalin (NBF), and embed the tissue specimen in paraffin. Using a microtome, cut 5 μ m serial sections from the paraffin block and mount them on glass slides.

Procedure

Carry out the DNA extraction according to instructions in the *QIAamp DSP DNA FFPE Tissue Kit Handbook* noting the following:

- Use a trained individual (e.g., a pathologist) to assess a Hematoxylin & Eosin (H&E)-stained section to confirm that there is tumor present.

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- The stained sections must not be used for DNA extraction.
 - The QIAamp DSP DNA FFPE Tissue Kit must be used manually only. Do not use the automated protocol for the QIAcube® described in the QIAamp DSP DNA FFPE Tissue Kit Handbook.
 - Use number of slides and elution volumes as recommended in the FFPE tissue resections section of this handbook.
 - Do not use the QIAGEN Deparaffinization Solution. Use only the xylene/ethanol method for deparaffinization described in the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.
 - Allow additional centrifugation (repeat Step 4 in *QIAamp DSP DNA FFPE Tissue Kit Handbook*) if tissue not pelleted after first centrifugation.
 - Ensure that molecular biology grade ethanol is used for all required steps.*
 - Incubate open tube at 37°C for 10 minutes or until all residual ethanol has evaporated (Step 7 in *QIAamp DSP DNA FFPE Tissue Kit Handbook*.)
 - Do not use the RNase step described in the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.

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

FFPE tissue resections

Scrape the entire tissue area from 2 sections (5µm) into labelled microcentrifuge tubes using a fresh scalpel for each specimen.

Proteinase K digestion must be performed for 1 hour.

For resected tissue specimens, purified genomic DNA must be eluted as a multi-step process.

- 1a. Pipette 60 µl of Buffer ATE (provided in the QIAamp DSP DNA FFPE Tissue Kit) and incubate at room temperature (15–25°C) for 2.5 minutes.
- 1b. Centrifuge at full speed for 1 minute.
- 1c. Retaining the collection tube and flow-through, carefully open the lid of the QIAamp MinElute column again and apply a further 60 µl Buffer ATE to the center of the membrane.
- 1d. Incubate at room temperature (15–25°C) for 2.5 minutes.
- 1e. Centrifuge at full speed for 1 minute.
- 1f. Discard the QIAamp MinElute column and store extracts at –30 to –15°C as needed.

Note: Genomic DNA may be stored at 2–8°C for 1 week post-extraction, then at –30 to –15°C for up to 8 weeks before use.

Protocol: Detection of PDGFRA Mutation

Important points before starting

- Up to 24 samples can be assessed over four runs using the Reaction Mix available in each kit. The optimal use is four runs with each run containing a maximum of six samples. Smaller sample batch sizes will mean that fewer samples can be tested with the Kit.
- Do not vortex the *Taq* DNA polymerase (tube *Taq*) as this may inactivate the enzyme. However, once the *Taq* has been added to reaction mix to make the master mix, the *Taq* can be vortexed as part of the master mix.
- Pipette the *Taq* DNA polymerase by carefully placing the pipette tip just under the liquid surface to avoid the tip being coated in excess enzyme.

Things to do before starting

- Ensure that the Rotor-Gene AssayManager v2.1 or later software and the `therascreen_PDGFRA_FFPE_MDx_V1_0_0` Assay Profile is installed before first use of the Rotor-Gene Q MDx (US) instrument.
- Before each use, all reagents (except the *Taq* DNA polymerase (tube *Taq*)) and DNA samples need to be thawed completely for 1 hour at room temperature (15–25°C), mixed by vortexing for 3–5 seconds, and centrifuged briefly to collect the contents at the bottom of the tube.
- Ensure that the *Taq* DNA polymerase (tube *Taq*) is at room temperature (15–25°C) for 1 hour and mixed by inverting 10 times before each use. Centrifuge the tube briefly to collect the enzyme at the bottom of the tube.

Procedure

Completely thaw the reaction mix, nuclease-free water for no template control (tube NTC), positive control (tube PC), and DNA samples at room temperature (15–25°C) for 1 hour. Bring the *Taq* DNA polymerase to room temperature at the same time as other reagents. After 1 hour mix all reagents (except the *Taq* DNA polymerase) by vortexing each tube for 3–5 seconds to avoid localized concentration of salts. Mix the *Taq* DNA polymerase by inverting the tube 10 times. Centrifuge all reagents briefly to collect the contents at the bottom of the tube.

Important: Insufficient mixing of the reagents could produce invalid assay results.

Label Lo-Bind tubes with appropriate assay contents.

Prepare sufficient master mix (reaction mix plus *Taq* DNA polymerase) for the DNA samples, one positive control (tube PC) reaction, and one nuclease-free water for no template control (tube NTC) reaction according to the volumes in Table 2.

The master mix contains all of the components needed for the PCR except the sample.

Table 2. Preparation of assay master mix*

Volume of reaction mix	Volume of <i>Taq</i> DNA polymerase (tube <i>Taq</i>)
19.8 μ l x (n+2)	0.2 μ l x (n+2)

* n = number of samples. Two extra reactions are counted to ensure sufficient overage for the PCR setup and the controls.

Note: When preparing the master mix, the required volume of reaction mix is added to the relevant tube first and the *Taq* DNA polymerase is added last.

Mix the tube containing the master mix by vortexing for 3–5 seconds followed by brief centrifugation.

Immediately after master mix is ready, place the appropriate number of PCR 4-strip tubes (each strip has 4 tubes) in the loading block according to the example layout in Table 3 (adding more tubes if required, depending on the number of samples to be tested). Do not cap the tubes. Immediately add 20 µl master mix to each PCR strip tube.

Note: Leave the caps in the plastic bag container until required.

Note: Ensure that loading block is appropriately decontaminated (see Precautions, page 16) and dry before use.

Note: Refer to Table 3 for the example run layout while setting up the reaction mixes.

Note: Change pipette tips between each template addition to avoid contamination.

Table 3. Run layout for detection of mutation

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9
PC	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
NTC	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 1	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 2	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 3	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 4	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 5	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
Sample 6	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY

Immediately add 5 µl of nuclease-free water for no template control (tube NTC) to the NTC tube and cap the tube.

Add 5 µl of each DNA sample to the sample tubes and cap the tubes immediately after each sample to avoid sample to sample cross-contamination.

Add 5 µl of positive control (tube PC) to the PC tube and cap the tube.

Important: Run should be performed immediately after the run setup.

Using a permanent marker, mark the lids of the first tubes in the lowest numerical position in each PCR 4-strip tube to show the orientation to load the tubes into the 72-well rotor of the Rotor-Gene Q MDx (US) instrument.

Place all PCR 4-strip tubes into the appropriate positions of the 72-well rotor according to the run layout (Table 3). Take extra care to ensure that tubes will be transferred to the correct positions in the 72-well rotor (tube position in 72-well rotor should be the same as tube position in the Loading Block).

Note: All unused positions on the rotor must be filled with capped, empty tubes. This ensures that the thermal efficiency of the Rotor-Gene Q MDx (US) instrument is maintained.

Place the 72-well rotor into the Rotor-Gene Q MDx (US) instrument. Ensure that the locking ring (supplied with the Rotor-Gene Q MDx (US) instrument) is placed on top of the rotor to secure the tubes during the run.

The cycling parameters are shown in Table 4.

Table 4. Cycling parameters

Cycles	Temperature	Time	Data Acquisition
1	95°C	15 minutes	None
40	95°C	30 seconds	None
	61°C	60 seconds	Green (FAM) and Orange (Texas-Red)

Double-click the Rotor-Gene AssayManager v2.1 (or later) icon on the desktop of the laptop connected to the RGQ instrument.



The Setup environment appears by default.

Click New Manual worklist to create a new worklist.

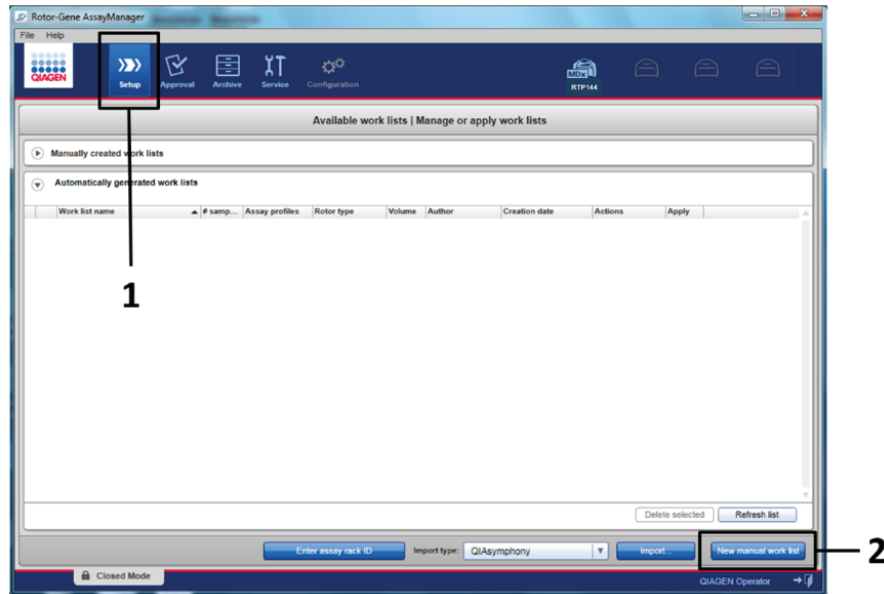


Figure 2. Setting up new manual worklist. 1 – Select Setup tab, 2 – Click New manual worklist.

Click the Assays tab (1) on the left-hand side of the main window. Click the screen_PDGFRA_FFPE_MDx (2) from the Available assay profiles list. Then, click the blue arrow (3) to move the selected assay profile to the Selected assay profiles

section. If the assay profile name is truncated, move the mouse pointer over the assay profile to see the full name (Figure 3).

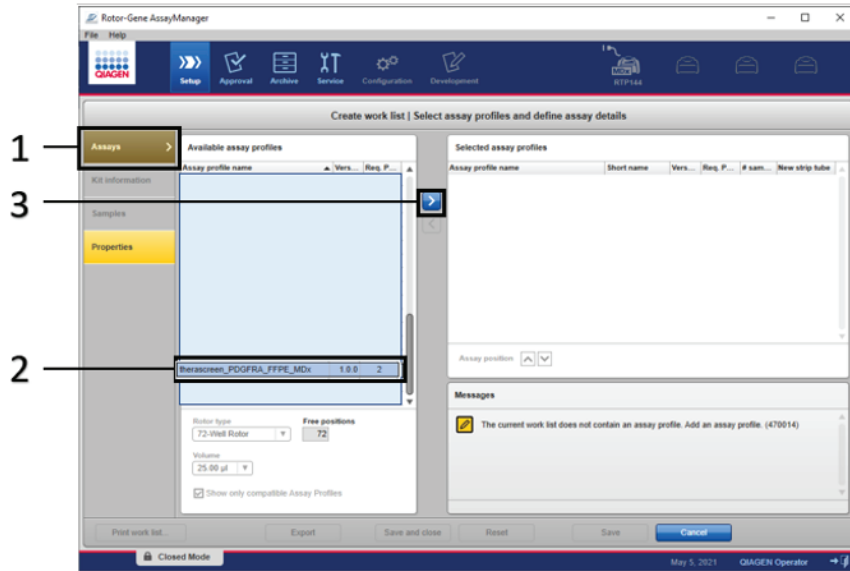


Figure 3. Setting up new manual worklist — select assay profile name. 1 – Click Assays tab, 2 – Select therascreen_PDGFR_A_FFPE_MDx from the available assay profiles, 3 - Click the arrow.

In the Selected assay profiles section, enter the number of test samples to be tested excluding the number of run controls (1) (Figure 4)

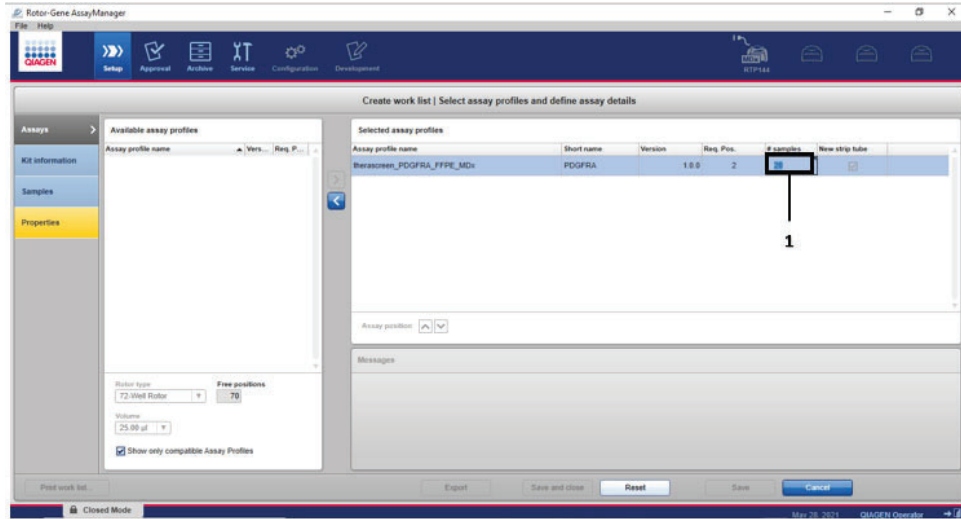


Figure 4. Create work list main window. 1 - Enter the number of samples in the Selected assay profiles section.

Click the Kit Information tab. Select Enter kit information manually (1) and enter the following information:

- Material number (2)
- Kit expiry date (3)
- Lot number (4), if available (Figure 5).

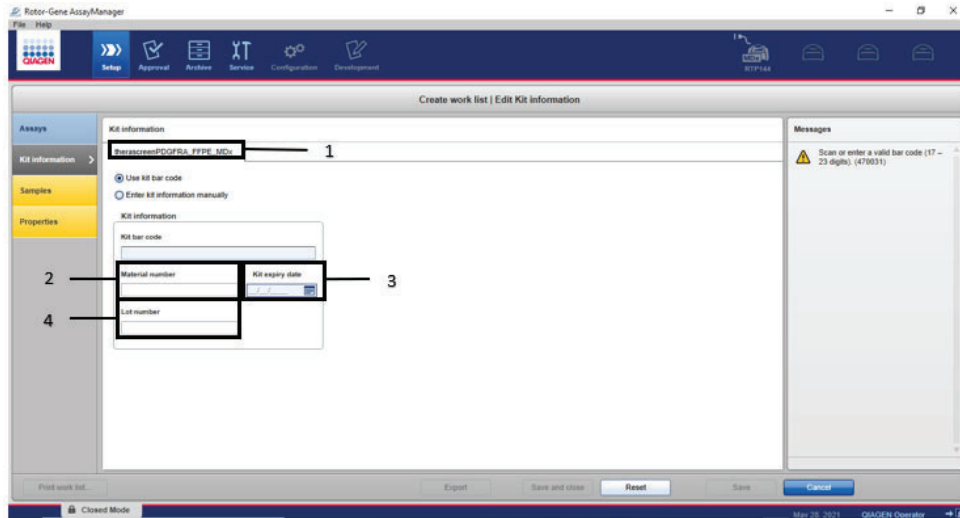


Figure 5. Create work list main window. In the Kit information tab, select Enter kit information manually. 1. Enter the Material number 2, Kit expiry date 3 and Lot number 4.

Click the Samples tab (1) to enter sample information. Enter the sample names (2) manually.

Important: If using a previously generated work list, ensure that the correct sample names are entered. Sample names must be ≤ 40 characters in length. All sample names must be unique. Multiple entries of the same sample name will be highlighted and the work list cannot be applied until resolved.

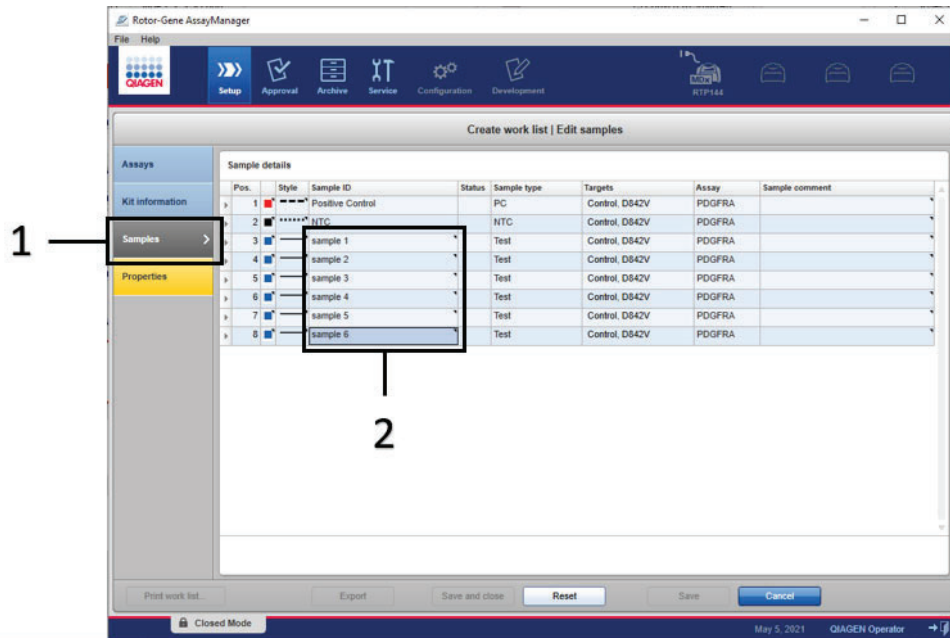


Figure 6. Create work list main window – 1 - "Samples" tab, 2 - Entering sample names. Sample names can also be entered by pasting from a notepad document.

Click the Properties tab (1) and enter the name of the worklist (2). After the worklist name is entered, ensure that the "is editable" and "work list is complete" boxes are checked (3). Click Apply.

A new window will appear.

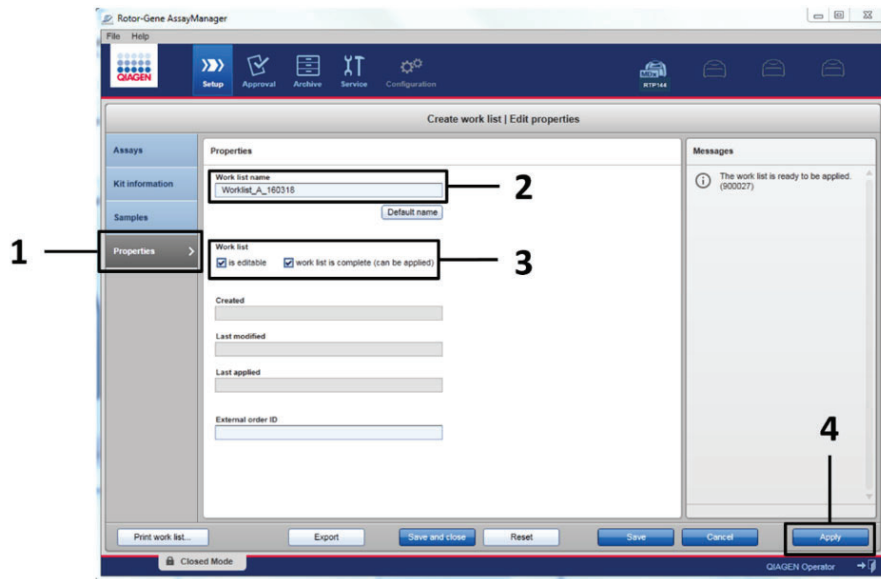


Figure 7. Create work list main window – 1 - Properties tab, 2 - Entering work list name, 3 - Select "is editable" and "work list" is complete, 4 - Apply button.

Enter the experiment name in the Experiment name (1) field. Select a cyclor from the Cyclor selection list (only compatible cyclors will be available) (2) and ensure that the Ring attached box (3) is checked. After all steps have been performed, click Start run (4). Note: A progress indicator will appear below the Rotor-Gene Q MDx icon at the top of right of the screen to indicate that the run has started (Figure 8).

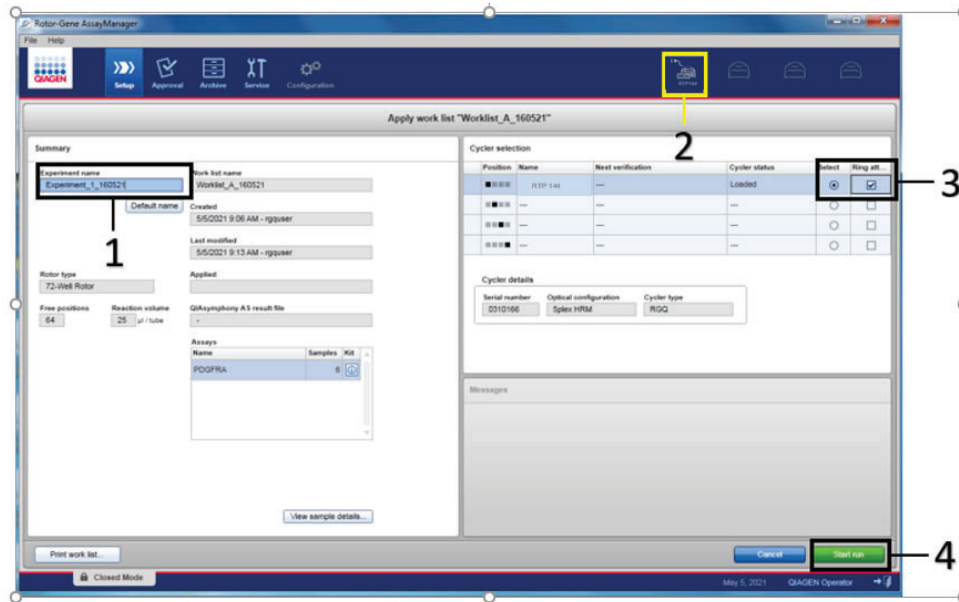


Figure 8. Applying work list and run start. 1 - Enter experiment name, 2 - Select the instrument, 3 - Ensure that Ring attached is selected, 4 - Start the run.

Note: The Cyclor icon changes its appearance depending on the progress and result of the run. Full descriptions of these cyclor icons can be found in the *Rotor-Gene AssayManager v2.1 Core Application User Manual*.

Example cycler icons are shown in Figure 9.

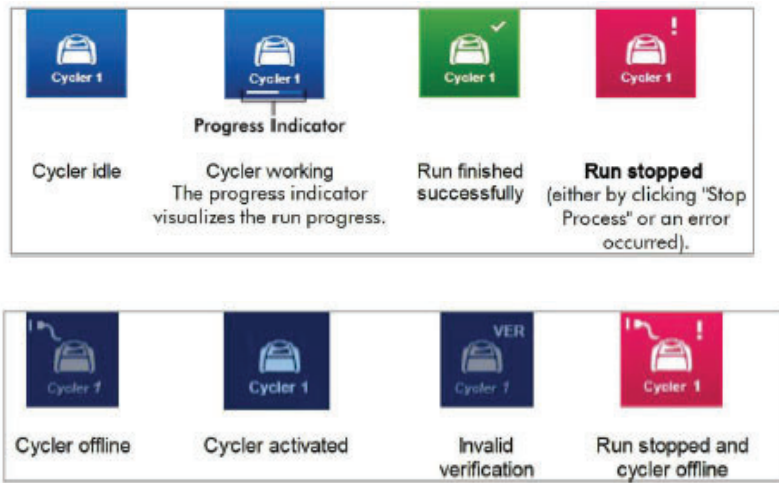


Figure 9. Cycler icons that may be displayed.

After the run is complete, click Finish run (Figure 10).

Note: During the run process, the amplification curves will be displayed and updated in real time. A progress indicator at the bottom left will show the remaining time.

Important: Do not close the window when the run is in progress as data loss may occur!

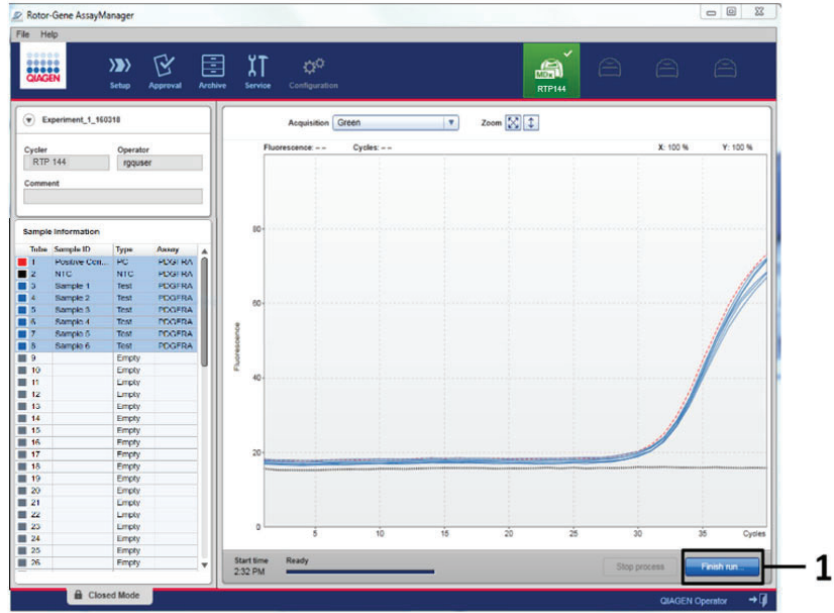


Figure 10. Finishing a run. 1 - Finish run button.

Click Release and go to approval (1) to enter the Approval tab and release the RGQ instrument. The RGQ icon at the top-right of the screen will change from green to blue indicating that the RGQ instrument is ready to perform another run (Figure 11). Regardless if a run is successful or not, the run must be released and approved.

For a list of potential failures and error codes presented in RGAM, see the *Rotor-Gene AssayManager v2.1 Core Application User Manual* and the *Rotor-Gene AssayManager v2.1 Gamma MDx Plug-in User Manual* (available from the QIAGEN website, visit www.qiagen.com)

Position: [Black square icon] Name: RTP 144 Run status: Run Successful

Experiment name: Experiment_1_160318

Errors during run: [Empty text area]

Comment: [Empty text area]

Password: [Empty text area]

Buttons: Release, Release and go to approval, Cancel

1

Figure 11. Finish Run pop up window. 1 - Release and go to approval button.

Select the experiment in the Assay selection section of the Approval environment (1). Click Start approval (2) (Figure 12). If the software has been closed after the run has finished, it may be necessary to search for the experiment.

Note: To view a stored experiment, click the Approval tab (A) and search for the experiment using the search criteria in the Filter Options section (B). Click Apply filter (C) to search. Check the box corresponding to the experiment you want to approve (1) and click Start approval (2).

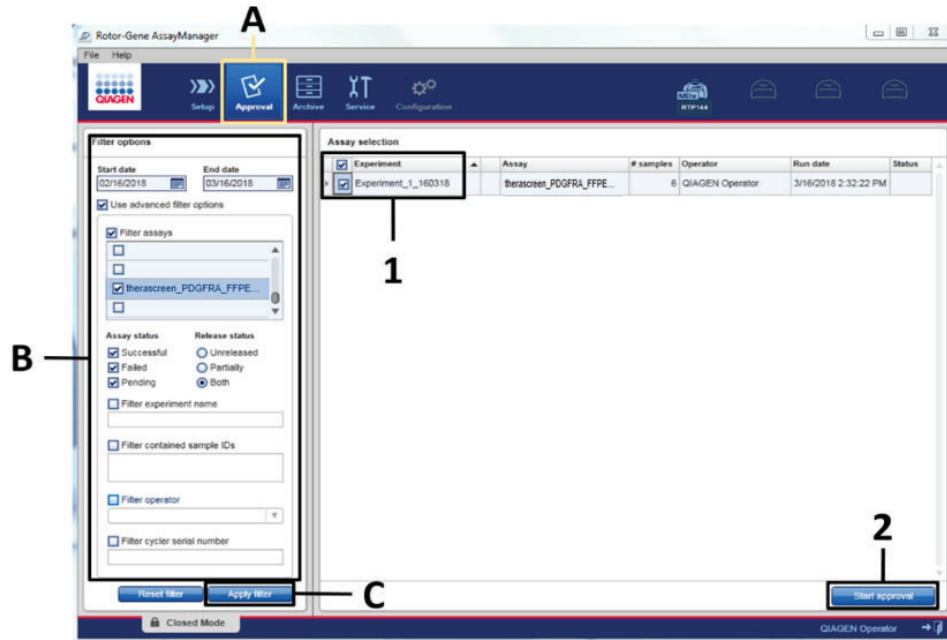


Figure 12. Starting the release process in the Approval Environment. 1 - Assay selected to approve, 2 - Start approval button, A – Approval tab, B – Filter options, C – Apply filter.

Raw Data, Processed data, Experiment information, Assay and Audit trail information can be found in the Plots and information section (1). Assay results can be found in the Results section (Figure 13).

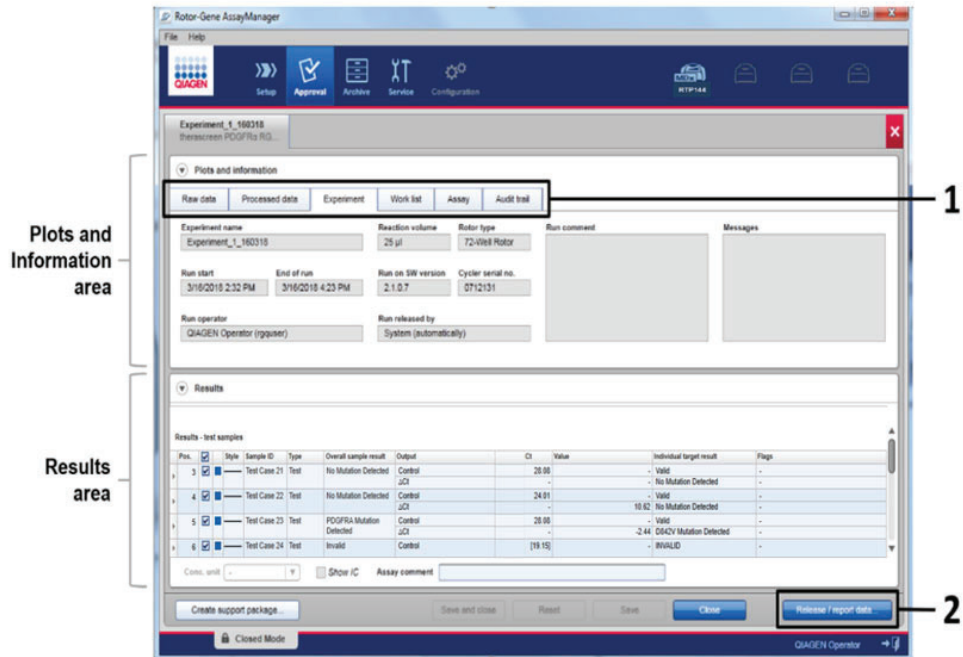


Figure 13. Example of assay results main windows. 1 - Experiment tab in the Plots and information. 2 - Release / report data button.

- If the Positive Control and No Template Control are within an acceptable range, the Sample Status column will report Valid; otherwise, an Invalid sample status will be reported.
- If either of the run controls fail, the run will be invalidated. All samples will be flagged as ASSAY_INVALID.
- See Results, page 47, for instructions on how to proceed.

Note: The Assay Profile for the *therascreen* PDGFRA RGQ PCR Kit contains all of the rules for automatic assay and sample analysis and result interpretation. The software will therefore assess the validity or invalidity of samples and controls automatically.

Click Release / report data. The Release / report data window will open (Figure 14).

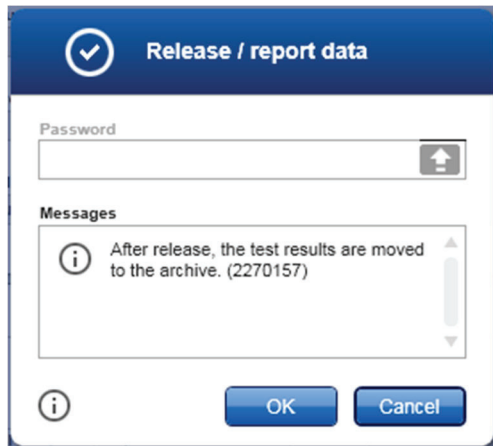


Figure 14. Example of "Release / report data" window.

To save the experiment to the archive, create a LIMS output, and run report, click OK. The Status of release window will open (Figure 15). Run reports and LIMS exports will be saved in the default report directory. The default directory can be found in the Default data export directories in the Configuration tab.



Figure 15. Example of Status of release window.

Click OK. The Assay is fully released dialog box will open (Figure 16).

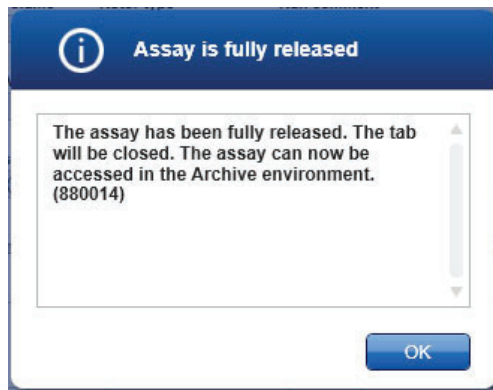


Figure 16. Example of Assay is fully released window.

Click OK.

To view an experiment stored in the experiment archive, click the Archive tab (1) and search for the experiment using the search criteria in the Filter Options section (2). Click Apply filter to search (3). Check the box next to the experiment you want to view (4) and click Show assays (5).

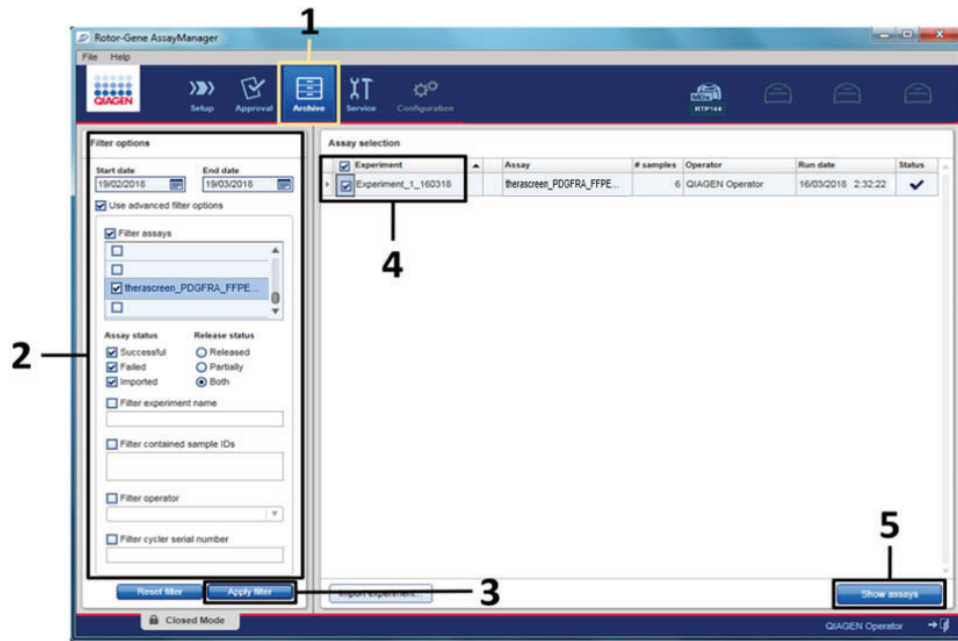


Figure 17. Example of “Experiment Archive” main window. 1 - Archive tab, 2 - Search options, 3 – Apply filter, 4 - Selecting experiment name, 5 – Show assays tab.

Results

Based on predetermined analytical C_T values, the RGAM software qualitatively determines the mutation status of the DNA samples and reports which samples contain a mutation.

The run controls (PC and NTC) are assessed to ensure that acceptable C_T values are met, and the reactions have been performed successfully. If the sample Control C_T value is below the acceptable range, this means that the DNA input is too high, and the sample needs to be diluted as described in the Control C_T value section of this handbook.

All of these assessments are performed automatically and require no manual interpretation. The system automatically checks the run validity and sample validity criteria and will not report mutation status in the event of an invalid sample or invalid run.

Rotor-Gene AssayManager v2.1(or later) determines the result of a target by combining all relevant analysis results according to core analysis algorithms such as normalization, and sample and assay rules defined in the corresponding assay profile.

The following results could be assigned to an individual sample:

- D842V Mutation Detected
- No Mutation Detected
- INVALID – if one or more flags are assigned to the sample during analysis by Rotor-Gene AssayManager v2.1 (or later) that are defined to set the target result to INVALID.

All possible flags corresponding to Rotor-Gene AssayManager v2.1 Gamma MDx Plug-in are listed in the *Rotor-Gene AssayManager v2.1 Gamma MDx Plug-in User Manual*. Additional flags which are specific to the *therascreen_PDGFR_A_FFPE_MDx_V1_0_0* Assay Profile are shown in Table 5.

Note: If an error has occurred during the run, the samples in the RGQ must be disposed of and not be retested.

Table 5. Software flags used in the PDGFRA Assay Profile

Software flags	Description	Action to be taken
PC_CONTROL_HIGH_CT	PC control C _T value is above the specification range	Invalid Run - the run must be repeated
PC_CONTROL_LOW_CT	PC control C _T value is below the specification range	Invalid Run - the run must be repeated
PC_CONTROL_NO_CT_VALUE	No C _T value detected for the PC in the control	Invalid Run - the run must be repeated
PC_D842V_HIGH_CT	PC mutant C _T value is above the specification range	Invalid Run - the run must be repeated
PC_D842V_LOW_CT	PC mutant C _T value is below the specification range	Invalid Run - the run must be repeated
PC_D842V_NO_CT_VALUE	No C _T value detected for the PC in the mutant channel	Invalid Run - the run must be repeated
NTC_CONTROL_UNEXPECTED_CT	A C _T value has been detected in the NTC control channel	Invalid Run - the run must be repeated
NTC_D842V_UNEXPECTED_CT	A C _T value has been detected in the NTC mutant channel	Invalid Run - the run must be repeated
CONTROL_HIGH_CT	Sample control C _T value is above the specification range	Sample is invalid. Retest the sample once. If the same result is obtained after the retest then re-extract the sample and test the re-extracted sample. If the re-extracted sample is invalid for the same reason, then retest one more time. If the sample is still above specification the sample should be reported as indeterminate.

Table continued on next page

Table continued from previous page

Table 5. Software flags used in the PDGFRA Assay Profile (cont'd)

Software flags	Description	Action to be taken
CONTROL_LOW_CT	Sample control C _T value is below the specification range	Sample is invalid. Sample is too concentrated and must be diluted. Follow instructions in the <i>Control C_T Value</i> section of this handbook.
CONTROL_NO_CT_VALUE	No C _T value detected for the sample in the control channel	Sample is invalid. Retest the sample once. If the same result is obtained after the retest then re-extract the sample and test the re-extracted sample. If the re-extracted sample is invalid for the same reason, then retest one more time. If the sample still has no C _T the sample should be reported as indeterminate.
		If this flag is seen for a Run Control either with or without another PC and/or NTC flag; Invalid Run - the run must be repeated.
CURVE_SHAPE_ANOMALY	A PCR curve anomaly has been detected in the Control channel	If this flag is seen for a sample; Sample is invalid. Retest the sample once. If the same result is obtained after the retest then re-extract the sample and test the re-extracted sample. If the re-extracted sample is invalid for the same reason, then retest one more time. If the sample still has no C _T the sample should be reported as indeterminate.
OTHER_TARGET_INVALID	A PCR curve anomaly has been detected in the control channel or the mutant channel	If this flag is seen for a Run Control either with or without another PC and/or NTC flag;

Invalid Run - the run must be repeated.

If this flag is seen for a sample;
Sample is invalid.

Retest the sample once. If the same result is obtained after the retest then re-extract the sample and test the re-extracted sample. If the re-extracted sample is invalid for the same reason, then retest one more time. If the sample still has no C_T the sample should be reported as indeterminate.

Control C_T value

Based on the C_T values obtained in the Control reaction, the system automatically checks the sample validity criteria and will not report 'mutation detected' or 'mutation not detected' in the event of an invalid sample.

There are three possible flags for an invalid sample due to Control C_T value:

Control _LOW_CT

Sample is too concentrated and will overload the mutation assay. In order to obtain a valid sample result, the sample must be diluted. Samples should be diluted on the basis that diluting by half will increase the C_T value by 1. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).

To calculate the required Control C_T shift (X_R) and estimate the dilution factor required (as detailed in Table 6):

$$X_R = 25 - X$$

where 25 is given as an example target Control C_T for the diluted sample and X is an actual Control C_T of sample to be diluted.

If X is not a whole number, round up to the next whole number, e.g., 2.1 is rounded up to 3.0. This value is X_R . Obtain the Dilution Factor required from Table 6.

Table 6. Calculation of dilution factor

X_R	Dilution factor	Volume of sample	Volume of H ₂ O
1	2-fold	1	1
2	4-fold	1	3
3	8-fold	1	7
4	16-fold	1	15
5	32-fold	1	31
6	64-fold	1	63

CONTROL_HIGH_CT

Quantity of DNA is not sufficient for mutation analysis. Retest the sample where sufficient DNA eluate is available (>5 μ l). If the quantity of DNA is still insufficient upon retest, re-extract from fresh FFPE sections. If not possible, the sample will be reported as indeterminate.

CONTROL_NO_CT_VALUE

Quantity of DNA is either not sufficient for detection or not present in the sample. Retest the sample where sufficient DNA eluate is available (>5 μ l). If the quantity of DNA is still insufficient upon retest, re-extract from fresh FFPE sections. If not possible, the sample will be reported as indeterminate.

Contraindication

There are no known contraindications.

Limitations

Samples with results reported as No Mutation Detected may harbor *PDGFRA* mutations, which were not detected by the *therascreen* PDGFRA RGQ PCR Kit.

Detection of mutations is dependent on sample integrity and the amount of amplifiable DNA present in the specimen. The procedure should be repeated if DNA in the sample indicates that the quantity is either not sufficient or too high for mutation analysis.

Determination of DNA input for the assay is based on DNA qualification and is performed using the Control Reaction C_T to assess the amount of amplifiable DNA. The amount of amplifiable DNA in a sample (represented by the C_T value of the Control Reaction) needs to be within the pre-defined Control Working Range (CWR) in order for the sample to proceed to mutation assessment. The acceptable measurable range of the Control assay is Control C_T 20.85-30.99. The PDGFRA assay profile will automatically determine if the sample has a Control C_T within the acceptable range.

Macrodissection of FFPE specimens is not performed prior to extraction of DNA. The performance of the *therascreen* PDGFRA RGQ PCR Kit was validated with DNA extracted from FFPE GIST specimens with 2.0–99.0% tumor content.

The *therascreen* PDGFRA RGQ PCR Kit is used in a polymerase chain reaction (PCR) procedure. As with all PCR procedures, samples may become contaminated by external sources of DNA in the test environment and the DNA in the positive control. Use caution to avoid contamination of samples and kit reagents.

The *therascreen* PDGFRA RGQ PCR Kit is only for use with DNA samples extracted from formalin-fixed, paraffin-embedded GIST specimens.

The *therascreen* PDGFRA RGQ PCR Kit is only for use with the QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, cat. no. 60404) and users should follow the extraction protocol stipulations as stated in this handbook.

Only the Rotor-Gene Q MDx (US) instrument has been validated for use with the *therascreen* PDGFRA RGQ PCR Kit. No other thermal cycler with real-time optical detection can be used with this product.

There is no information if *therascreen* PDGFRA RGQ PCR Kit shows cross-reactivity (results of Mutation Detected) to additional *PDGFRA* mutations different to that included in the kit.

The *therascreen* PDGFRA RGQ PCR Kit is a qualitative test. The test is not for quantitative measurements of percent mutation.

Performance of the *therascreen* PDGFRA RGQ PCR Kit is unknown if microbial contamination is introduced during assay procedures.

Performance of the *therascreen* PDGFRA RGQ PCR Kit could be affected in the presence of hemoglobin and triglycerides in the test samples.

Performance Characteristics

Analytical performance

The specific performance characteristics of the *therascreen* PDGFRA RGQ PCR Kit were determined using formalin-fixed paraffin-embedded (FFPE) specimens collected from Gastrointestinal Stromal Tumor (GIST) patients.

Limit of Blank (LoB)

The Limit of Blank (LoB) is defined in CLSI guideline EP17-A2 as “the highest measurement result that is likely to be observed (with a stated probability) for a blank sample”. For the *therascreen* PDGFRA RGQ PCR Kit, this is the data point that corresponds to the upper 95% percentile in the blank (wild type, *i.e.* D842V mutation negative) samples.

The LoB has been determined by measuring the levels of breakthrough for the *PDGFRA* D842V mutation reaction, where breakthrough is defined as the non-specific, low level amplification of a DNA template. For the *therascreen* PDGFRA RGQ PCR Kit, this refers to non-specific binding of the mutant probe to the wild type DNA.

Based on this analysis, the LOB for the assay has been calculated as a ΔC_T of 9.854.

ΔC_T cutoff value

The assay cutoff is a specific ΔC_T value used to determine whether a sample is classed as positive or negative for the *PDGFRA* D842V mutation. Samples which generate ΔC_T values at or below the cutoff are classified as D842V mutation positive (*i.e.*, *PDGFRA* D842V mutation detected) and ΔC_T values generated above the cutoff are classified as D842V mutation negative (*i.e.*, no mutation detected). The

false-negative and false-positive rates for the D842V assay were used to determine a cutoff value such that a result equal or less than the cutoff will result in a D842V mutation detected classification. The cutoff for the *therascreen* PDGFRA RGQ PCR Kit is 9.0 ΔC_T .

Effect of DNA input on ΔC_T values (linearity)

The DNA input level is defined as the total quantity of amplifiable DNA in a sample as determined by the C_T value from the Control reaction. To demonstrate that the performance of the *therascreen* PDGFRA RGQ PCR Kit is consistent across the Control reaction working range (CWR) (20.85 – 30.99 C_T), a 9-level serial dilution of a D842V mutation positive sample with varying DNA input levels (with the lower levels being outside of the CWR) and 13.5% MAF (representing 1.5x LoD) was tested.

The evaluation was performed using one *therascreen* PDGFRA RGQ PCR Kit lot with six replicates tested per DNA input level. The data was analyzed using regression analysis to determine the linear range. For the assay to be determined as linear across the DNA input range, there should be no change across the range in ΔC_T , i.e. no statistically significant linear, quadratic, or cubic effect.

Parameter estimates for the tested dilutions were reported along with the corresponding standard errors, 95% confidence limits, and p-values, and the data showed no significant linear, quadratic, or cubic trends. A linear ΔC_T range was observed across the DNA input range tested for the assay.

Limit of Detection (LoD)

The LoD is defined as the lowest amount of the *PDGFRA* D842V mutation (in a background of wild type DNA) that is possible to detect 95% of the time. For the *PDGFRA* D842V assay, the LoD is reported as % mutant allele frequency (MAF).

Genomic DNA was extracted from D842V mutation positive clinical GIST FFPE specimens and was diluted into normalized DNA extracted from wild type clinical GIST FFPE specimens and serially diluted to % MAF levels above, at, and below the estimated LoD.

At least sixty replicates of each % MAF dilution in the series per DNA input level (medium and low DNA input) were tested using two *therascreen* PDGFRA RGQ PCR Kit lots. The LoD was determined using a Probit model and reported as the maximum observed LoD value across the two *therascreen* PDGFRA RGQ PCR Kit lots tested.

The LoD of the *therascreen* PDGFRA RGQ PCR Kit was determined at 9% MAF. The LoD was verified in a low level of background DNA and where the level of background DNA was not limiting.

Cross-reactivity

The *therascreen* PDGFRA RGQ PCR Kit is comprised of one multiplex PCR amplification reaction to detect the D842V mutation at codon 842 in Exon 18 of the *PDGFRA* gene, and a control region downstream of this in the *PDGFRA* gene. There is no reaction that specifically measures the wild type sequence at codon 842 in Exon 18. The *therascreen* PDGFRA RGQ PCR Kit "No Mutation Detected" result is inferred by the absence of any positive mutation result.

The level of potential cross-reactivity between primers, probes, and the blocker used within the *therascreen* PDGFRA RGQ PCR Kit and nonspecific targets within human and non-human genomes, and the level of potential heterodimer formation was investigated. An *in silico* analysis was performed to determine whether the primers, probes, and blocker used with the *therascreen* PDGFRA RGQ PCR Kit assays bind nonspecifically within any genome, including the human genome. An additional *in silico* analysis was performed to determine whether the oligonucleotides used within the multiplex assay bind nonspecifically to each other.

The *in silico* analysis of oligo heterodimers indicated that there is a low prevalence of heterodimer formation. The primers and probes do not cross react with any targets not detected by the *therascreen* PDGFRA RGQ PCR Kit and therefore will not generate a false signal.

Repeatability and reproducibility

The precision and reproducibility of the *therascreen* PDGFRA RGQ PCR Kit was investigated by testing DNA extracted from D842V mutation positive clinical GIST FFPE specimens and wild type clinical GIST FFPE specimens.

To demonstrate repeatability, D842V mutation positive samples at two mutation levels (1x LoD and 1.5x LoD) and two DNA input levels (medium (C_T 26) and low (C_T 29) DNA input), and wild type samples also at medium and low DNA input levels were tested at one site (located in the United Kingdom). The samples were tested in triplicate with four runs per day across multiple days, Rotor-Gene Q MDx (US) instruments, and operators and using one lot of *therascreen* PDGFRA RGQ PCR Kit to generate a total of 108 replicates per sample (Table 7).

Table 7. Assay repeatability*

Template	No. of correct calls	Percentage of correct calls	Lower two-sided 95% confidence limit	Upper two-sided 95% confidence limit
D842V 1.5x LoD (C _T 26)	108/108	100.00%	96.64%	100.00%
D842V 1.5x LoD (C _T 29)	108/108	100.00%	96.64%	100.00%
D842V 1x LoD (C _T 26)	108/108	100.00%	96.64%	100.00%
D842V 1x LoD (C _T 29)	108/108	100.00%	96.64%	100.00%
WT (C _T 26)	108/108	100.00%	96.64%	100.00%
WT (C _T 29)	107/107	100.00%	96.61%	100.00%

* Number of correct calls and two-sided 95% confidence limits for D842V mutation positive samples at 1x LoD and 1.5x LoD and wild type samples tested across one site.

To demonstrate reproducibility, two runs per day were performed per operator (two operators per site) by two additional sites (both located in the USA) over six days to give an additional 108 data points for each additional site. The same test panel was used for this study as for the repeatability study and one lot of the *therascreen* PDGFRA RGQ PCR Kit (the same lot for all three sites) was used. The proportion of correct mutation calls for each sample across all three sites was calculated and is reported in Table 8.

Table 8. Assay reproducibility*

Template	No. of correct calls	Percentage of correct calls	Lower two-sided 95% confidence limit	Upper two-sided 95% confidence limit
D842V 1.5x LoD (C _T 26)	321/321	100.00%	98.86%	100.00%
D842V 1.5x LoD (C _T 29)	323/324	99.69%	98.29%	99.99%
D842V 1x LoD (C _T 26)	324/324	100.00%	98.87%	100.00%
D842V 1x LoD (C _T 29)	324/324	100.00%	98.87%	100.00%
WT (C _T 26)	324/324	100.00%	98.87%	100.00%
WT (C _T 29)	323/323	100.00%	98.86%	100.00%

* Number of correct calls and two-sided 95% confidence limits for D842V mutation positive samples at 1 x LoD and wild type samples tested across all sites.

Specimen handling (Extraction reproducibility)

This study assessed sample handling variability, specifically during the DNA extraction step using sections taken from four specimens; two wild type (i.e. D842V mutation negative) clinical GIST FFPE specimens and two D842V mutation positive clinical GIST FFPE specimens. Sections from the specimens were divided into three independent sets to be extracted with the QIAamp DSP DNA FFPE Tissue Kit at three independent laboratories. All specimens were blinded before extraction. Four extractions for each specimen were performed at each of the three sites, yielding a total of 12 data points per specimen. One QIAamp DSP DNA FFPE Tissue Kit lot was used for the extractions across all three sites. All DNA samples were then tested using the *therascreen* PDGFRA RGQ PCR Kit at one site.

The proportion of correct calls across all three sites was 100%, supporting the reproducibility and repeatability for the *therascreen* PDGFRA RGQ PCR System at the pre-analytical step of DNA isolation.

Lot interchangeability

The *therascreen* PDGFRA RGQ PCR System utilizes the QIAamp DSP DNA FFPE Tissue Kit, for the isolation of gDNA, and the *therascreen* PDGFRA RGQ PCR Kit for the amplification of gDNA and detection of *PDGFRA* D842V mutation status. Lot interchangeability was demonstrated using three lots of the QIAamp DSP DNA FFPE Tissue Kit and three lots of the *therascreen* PDGFRA RGQ PCR Kit to test five wild type and five D842V mutation positive clinical samples.

Each sample was extracted with three different lots of QIAamp DSP DNA FFPE Tissue Kit. Two replicate extractions were carried out per QIAamp DSP DNA FFPE Tissue Kit to give a total of six extractions per sample. All extracts were tested with three different lots of the *therascreen* PDGFRA RGQ PCR Kit.

The overall percentage of correct calls across lots for all mutation positive and wild type samples was 100% (180/180).

Cross-contamination / analytical carryover

The purpose of this study was to evaluate the *therascreen* PDGFRA RGQ PCR Kit for carryover when high *PDGFRA* D842V mutation positive samples are tested adjacent to *PDGFRA* D842V mutation negative samples. This study examined the entire *PDGFRA* Assay System from extraction to PCR amplification and investigated if carryover occurred between samples, extractions, and within or between runs.

Sections from one D842V mutation positive and three wild type clinical GIST FFPE specimens were divided into two independent sets, Set A and Set B. Two operators performed extractions for each set of samples, following a pre-defined extraction

matrix, to introduce the risk of cross-contamination in wild type samples. Nine extractions from the D842V mutation positive specimen and seven extractions from each of the three wild type specimens were extracted for each of the Set A and Set B sample sets.

The extracts were tested across five PCR runs following a run matrix designed to introduce the risk of sample cross-contamination. The five PCR runs were set up consecutively by the same operator using the same equipment and Rotor-Gene Q MDx (US) instrument, with no other runs set up using this instrument between these runs. Extracts were tested with one lot of the *therascreen* PDGFRA RGQ PCR Kit.

A total of 126 wild type replicates were tested, all of which generated the correct mutation status call ('no mutation detected', *i.e.* wild type sample). Therefore, the percentage of false-positive calls for the wild type samples was 0%, demonstrating no cross-contamination of the wild type samples by D842V mutation positive samples sharing the same DNA extraction and run set up procedure.

Interfering substances

The effects of potential interfering substances introduced from the QIAamp DSP DNA FFPE Tissue Kit (exogenous substances) or from the sample itself (endogenous substances) on assay performance were measured by comparison of ΔC_T between interferent-spiked and control-spiked extracts of D842V mutation positive DNA samples, and by comparison of the Control reaction C_T for wild type DNA samples and by comparison of mutation status calls.

The exogenous substances present in the DNA extraction process tested were:

- Paraffin wax
- Xylene
- Ethanol

-
- Buffer ATL
 - Proteinase K
 - Buffer AL
 - Buffer AW1
 - Buffer AW2

D842V mutation positive and wild type samples to be spiked with exogenous interferences were first normalized to Control reaction C_T 26.00 and Control reaction C_T 29.00. The mutation positive samples were then diluted with wild type DNA sample (also normalized to the corresponding Control reaction C_T) to give the % MAF representing 1.5x LoD.

Samples spiked with hemoglobin and triglycerides (endogenous interferences) during the extraction process were not normalized to Control reaction C_T 26.00, or C_T 29.00 nor diluted to 1.5 x LoD prior to mutation assessment, but used immediately following extraction. This was to avoid removing any variability which may have been introduced by the interference.

The study required the preparation of a test sample set and a blank sample set (Buffer ATE for exogenous substances, water for hemoglobin and Xylene for triglycerides). The test sample set included all D842V mutation positive samples and wild type samples spiked with an interference. The blank sample set included D842V mutation positive samples and wild type samples spiked with an appropriate control substance. Samples tested with hemoglobin and triglycerides were spiked during the extraction process to reflect what would be introduced via the FFPE sample.

The test concentrations of hemoglobin and triglycerides and the estimated tissue volume used in the extraction process were based on CLSI guidelines (CLSI EP07-A2, Appendix D, 2005, Interference Testing in Clinical Chemistry; Approved Guideline).

Both substances were tested at the highest (worst-case) and lowest feasible level of the interfering substance listed in the CLSI guidelines:

- Hemoglobin (2 mg/ml & 4 mg/ml)
- Triglycerides (3.7 μ mol & 7.4 μ mol)

Samples tested with potential exogenous interferents were spiked, following normalization to Control reaction C_T 26.00 and Control reaction C_T 29.00 and dilution of the D842V mutation positive samples to 1.5x LoD, at a concentration representing the highest (worst-case) feasible level of the interfering substance carryover into a sample.

In total, eight replicates of each sample/interferent combination were tested with one *therascreen* PDGFRA RGQ PCR Kit lot.

All mutation status calls in the D842V mutation positive samples and wild type samples were as expected.

All exogenous substances tested had no impact on the *therascreen* PDGFRA RGQ PCR Kit's performance. The study also showed that hemoglobin had no impact on the *therascreen* PDGFRA RGQ PCR Kit's performance when testing D842V mutation positive samples.

There was, however, a statistically significant impact on the *therascreen* PDGFRA RGQ PCR Kit's performance observed between the spiked and control samples for wild type (WT) samples spiked with low and high concentrations of hemoglobin, D842V mutation positive (MT) samples spiked with the low concentration of triglycerides, and MT and WT samples spiked with the high concentration of triglycerides (Table 9).

Table 9. Summary of the impact of the endogenous substances

Potential interferant (endogenous)	Concentration	Potential impact		
		Sample type	Green Channel/Delta Ct	Mutation status call
Hemoglobin	2 mg/ml (low)	WT	Higher Green channel Ct value	No change
Hemoglobin	4 mg/ml (high)	WT	Higher Green channel Ct value	No change
Triglycerides	3.7 µmol (low)	MT	Lower delta Ct value	No change
Triglycerides	7.4 µmol (high)	MT	Higher delta Ct value	No change
		WT	Higher Green channel Ct value	No change

Accuracy: Comparison to the analytical reference method

A study demonstrated the concordance in mutation detection of the *therascreen* PDGFRA RGQ PCR Kit relative to a validated next generation sequencing (NGS) method. In this study, a total of 166 FFPE samples from the BLU-285-1101 clinical trial were tested, 147 subjects received results from both methods, of which 40 were D842V positive by NGS. The Overall Percent Agreement (OPA), Positive Percent Agreement (PPA), and Negative Percent Agreement (NPA) between the *therascreen* PDGFRA RGQ PCR Kit and NGS (with NGS as the reference method) were 99.32%, 97.50%, and 100.00%, respectively.

Analytical accuracy was determined by comparing the result from the *therascreen* PDGFRA RGQ PCR Kit for both clinical trial samples and procured clinical samples

against the NGS result. Of the 166 clinical trial samples and 51 procured clinical samples, 194 samples gave a valid result with both NGS and the *therascreen* PDGFRA RGQ PCR Kit. Seven clinical trial samples had a valid result with NGS but could not be evaluated using the PDGFRA Kit. The OPA, PPA, and NPA between the *therascreen* PDGFRA RGQ PCR Kit and NGS (with NGS as the reference method, and excluding PDGFRA Kit invalid results) were 99.48%, 98.44%, and 100.00%, respectively.

The OPA, PPA, and NPA were also evaluated including the seven invalid samples (samples without PDGFRA Kit results), with corresponding two-sided exact 95% CI. The results demonstrate point estimates of PPA, NPA, and OPA of 96.92%, 95.59% and 96.02%, respectively.

Clinical performance

The *therascreen* PDGFRA RGQ PCR Kit is intended to be used as a companion diagnostic test to aid clinicians in identifying GIST patients who may be eligible for treatment with AYVAKIT™ (avapritinib) based on a *PDGFRA* D842V mutation detected result in GIST tumor tissue samples¹⁰.

Clinical study BLU-285-1101 and outcome

The Clinical Study BLU-285-1101 was an open-label, multicenter, international, Phase 1, first-in-human (FIH) study to evaluate the safety and efficacy of AYVAKIT (avapritinib) in adult patients with unresectable or metastatic GIST. The study was expanded to include patients with advanced GIST based on initial efficacy observed in dose escalation. A total of 237 patients, with or without a mutation in the *PDGFRA* gene, were enrolled across all parts of the study and 204 of those patients were treated with at least 1 dose of either 300 mg or 400 mg once daily (*quaque die* [QD]) of AYVAKIT (avapritinib) (33 patients were treated with a dose of <300 mg QD).

The primary endpoints were to determine maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of avapritinib, to determine overall safety and tolerability of avapritinib, and to determine the overall response rate (ORR) by modified response evaluation criteria in solid tumors (mRECIST) version 1.1 at the MTD/RP2D of avapritinib in patients with GIST:

- A D842V mutation in *PDGFRA*;
- Progressed following treatment with imatinib and at least 1 other kinase inhibitor, and who are not known to have a D842V mutation in *PDGFRA*; or
- Progressed or experienced intolerance to imatinib, including in the adjuvant setting, and who had not received additional kinase inhibitor therapy and did not have a known D842V mutation in *PDGFRA*.

The analysis of effectiveness of avapritinib was based on the Primary Efficacy endpoints of Overall Response Rate (ORR) in 38 CTA D842V positive, evaluable patients at the database cut-off.

Of these 38 patients, 31 patients in the 300/400 mg starting dose group who had *PDGFRA* D842V mutations identified by *therascreen*[®] *PDGFRA* RGQ PCR Kit. 29 had confirmed complete response (CR) or partial response (PR) based on central radiology review for an ORR of 94% (95% CI: 78.6%, 99.2%). Best response for these 31 patients was CR in 2 patients, PR in 27 patients, and stable disease (SD) in 2 patients, for a clinical benefit rate (CBR) of 100%. Median duration of response (DOR) was not reached in the 300/400 mg dose group; 22 patients (76%) were censored at the time of the data cut-off based on FDA censoring rules with 64% and 51% estimated to be in response at 12 months and 18 months, respectively.

The BLU-285-1101 Study met its primary objective, demonstrating treatment with AYVAKIT (avapritinib) provides meaningful clinical benefit to *PDGFRA* D842V mutant GIST patients, as measured by ORR. ORR for patients with GIST harboring *PDGFRA* Exon 18 D842V mutations was 89% (with 95% CI 75%, 97%). Collectively, the efficacy and safety results from the BLU-285-1101 study demonstrate that AYVAKIT (avapritinib) has a favorable benefit-risk profile and is a useful treatment in patients with GIST who have *PDGFRA* D842V mutation. Please refer to AYVAKIT (avapritinib) label at Drugs@FDA.

Concordance between CDx assay and CTA (Bridging study)

In the BLU-285-1101 study, the *PDGFRA* D842V mutation status for screening and enrollment of patients was determined by a clinical trial assay (CTA).

Concordance between the *therascreen* *PDGFRA* RGQ PCR Kit (CDx) and the CTA was assessed on DNA extracted from GIST samples from 154 patients in study BLU-285-1101.

Of the 154 samples, 147 gave a valid *therascreen* PDGFRA RGQ PCR Kit result (CDx evaluable patients). The estimated PPA, NPA, and OPA between the *therascreen* PDGFRA RGQ PCR Kit and the CTA (with CTA as the reference method) for CDx evaluable patients are detailed in Table 10. The result of 98.64% OPA met the study acceptance criteria of $\geq 80\%$.

Table 10. Measures of agreement between CDx and CTA*

Measure of agreement	Frequencies	Percent agreement	Lower two-sided 95% confidence limit†	Upper two-sided 95% confidence limit†
Overall Percent Agreement	145/147	98.64%	95.17%	99.83%
Positive Percent Agreement	40/42	95.24%	83.84%	99.42%
Negative Percent Agreement	105/105	100.00%	96.55%	100.00%

* CTA as reference method and CDx evaluable patients

† Clopper-Pearson (Exact) Binomial¹¹

Seven (7) subjects with invalid *therascreen* PDGFRA RGQ PCR Kit results were then included as discordant results in the analysis across all CDx testable patients. The estimated PPA, NPA, and OPA between the *therascreen* PDGFRA RGQ PCR Kit and the CTA (with CTA as reference method) for all CDx testable patients are detailed in Table 11. The result of 94.16% OPA met the study acceptance criteria of ≥80%.

Table 11. Measures of agreement between CDx and CTA*

Measure of agreement	Frequencies	Percent agreement	Lower two-sided 95% confidence limit†	Upper two-sided 95% confidence limit†
Overall Percent Agreement	145/154	94.16%	89.20%	97.29%
Positive Percent Agreement	40/43	93.02%	80.94%	98.54%
Negative Percent Agreement	105/111	94.59%	88.61%	97.99%

* CTA as reference method and CDx testable patients

† Clopper-Pearson (Exact) Binomial¹¹

Effectiveness of treatment with AYVAKIT (avapritinib, when used with the *therascreen* PDGFRA RGQ PCR Kit, is shown in data collected in the BLU-285-1101 study of AYVAKIT (avapritinib), reanalyzed in the bridging study. The *therascreen* PDGFRA RGQ PCR Kit demonstrated overall agreement of 98.64% (95% CI: 95.17, 99.83) for CDx evaluable patients. Treatment with AYVAKIT (avapritinib) provides meaningful clinical benefit to *PDGFRA* D842V mutant GIST patients, as measured by ORR. the ORR for *PDGFRA* D842V mutation positive patients when using the *therascreen* PDGFRA RGQ PCR Kit was 0.94 (95% CI: 0.79, 0.99), corresponding to 94%.

Disposal

For disposal of chemicals, 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.

Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

References

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support (for contact information, visit www.qiagen.com).

Comments and suggestions

Positive Control (PC)

'PC_CONTROL_NO_CT_VALUE' or 'PC_D842V_NO_CT_VALUE' flags

- | | |
|---|---|
| a) Incorrect configuration of the PCR | Check your pipetting scheme and repeat the PCR. |
| b) The storage conditions for one or more kit components did not comply with the instructions given in Reagent Storage and Handling | Check the storage conditions (see kit label) of the reagents and use a new kit, if necessary. |

Positive Control (PC)

'PC_CONTROL_HIGH_CT', 'PC_D842V_HIGH_CT', 'PC_CONTROL_LOW_CT' or 'PC_D842V_LOW_CT' flags

- | | |
|--|---|
| a) Error during preparation of the PCR | Repeat the PCR ensuring accurate pipetting. |
|--|---|

No Template Control (NTC)

'NTC_CONTROL_UNEXPECTED_CT' or 'NTC_D842V_UNEXPECTED_CT' flags

- | | |
|---|--|
| a) Contamination occurred during preparation of the PCR | Repeat the PCR with new reagents. If possible, close the PCR tubes directly after addition of the sample to be tested. Ensure that the work space and instruments are decontaminated at regular intervals. |
|---|--|

Comments and suggestions

Sample

'CONTROL_LOW_CT' flag

- a) Sample is too concentrated

Dilute sample to increase C_T value. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).

Sample

'CONTROL_HIGH_CT' flag

- a) Insufficient starting DNA template present in sample

Retest one more time. If the system shows the same flag a second time, re-extract the DNA using slides from the same tissue specimen and repeat the assay. If after re-extraction the system shows the same flag for the sample, retest for a second time. If the flag occurs again, the sample is not suitable for use. It must be recorded as "indeterminate" and no further testing performed.

Sample

'CONTROL_NO_CT_VALUE' flag

- a) No amplifiable DNA template present in sample

Retest one more time. If the system shows the same flag a second time, re-extract the DNA using slides from the same tissue specimen and repeat the assay. If after re-extraction the system shows the same flag for the sample, retest for a second time. If the flag occurs again, the sample is not suitable for use. It must be recorded as "indeterminate" and no further testing performed.

Run Control or Sample

'CURVE_SHAPE_ANOMALY' or OTHER_TARGET_INVALID flags

Comments and suggestions

- a) Error during preparation of the PCR

Run Control

If either of these flags are seen for a Run Control with another flag for a Run Control then follow the comments and suggestions for the other flag.













If either of these flags are seen for a Run Control with no other flag then repeat the run ensuring accurate mixing and pipetting.





Sample

If either of these flags are seen for a sample then retest the sample ensuring accurate mixing and pipetting.

Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components
	Contains
	Number
	Global Trade Item Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Consult instructions for use
	Keep away from sunlight
	Prescription Use Only
	Warning/caution

Ordering Information

Product	Contents	Cat. no.
<i>therascreen</i> PDGFRA RGQ PCR Kit	For 24 reactions: Reaction mix, Positive Control, No-Template Control, Dilution Water, Taq DNA Polymerase	874921
Rotor-Gene Q <i>therascreen</i> PDGFRA Assay Profile	Software protocol package for use with the <i>therascreen</i> PDGFRA RGQ PCR Kit and Rotor-Gene Q MDx (US) instrument Compatible with Rotor-Gene Q software version 2.1 or later	9002820
Rotor-Gene Q MDx (US) and accessories		
Rotor-Gene Q MDx Platform (US)	Real-time PCR cycler, laptop computer, software, accessories, 1-year warranty on parts and labor	9002035
Rotor-Gene Q MDx (US) System	Real-time PCR cycler, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002036
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (250)	10 x 250 strips of 4 tubes and caps for 10000 reactions	981106

PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions of 20–50 µl.	981005
72-Well Rotor	For holding Strip Tubes and Caps, 0.1 ml, with reaction volumes of 10–50 µl; Requires Locking Ring 72-Well Rotor	9018903
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 ml in the 72-Well Rotor	9018904
Related products		
QIAamp DSP DNA FFPE Tissue Kit (50)	For 50 DNA preps; QIAamp MinElute® Columns, Proteinase K, Buffers, and Collection Tubes (2 ml)	60404

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Document Revision History

Revision	Description
R1, January 2021	Initial release
R2, February 2022	Update to the In-use stability and the number of freeze-thaw cycles on page18
R3, February 2023	Update to the In-use stability and the number of freeze-thaw cycles on page18 Update to the flags in Table 5; 'MUTANT' replaced with 'D842V' in the flags, and two additional flags have been added, 'CURVE_SHAPE_ANOMALY' and 'OTHER_TARGET_INVALID'
R4, June 2023	Clarified that kit reagents should be thawed and mixed to ensure performance of kit is not affected. Clarified patient numbers in Clinical Outcome Section

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