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therascreen[®] BRAF V600E RGQ PCR Kit Instructions for Use (Handbook)



Version 1

For in vitro diagnostic use

Rx only (For prescription use only)

For use with the QIAamp[®] DSP DNA FFPE Tissue Kit

For use with Rotor-Gene[®] Q MDx (US) instruments

IVD

REF

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Intended Use

The *therascreen* BRAF V600E RGQ PCR Kit is a real-time PCR test for the qualitative detection of V600E mutations in the *BRAF* gene using genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) human colorectal cancer (CRC) tumor tissue.

The *therascreen* BRAF V600E RGQ PCR Kit is an in vitro diagnostic device intended to be used as an aid in selecting patients with metastatic colorectal cancer (mCRC) whose tumors carry the *BRAF* V600E mutation for treatment with BRAFTOVI (encorafenib) in combination with cetuximab.

The *therascreen* BRAF V600E RGQ PCR Kit is for use on the Rotor-Gene Q MDx (US) instrument.

The *therascreen* BRAF V600E RGQ PCR Kit is intended for in vitro diagnostic use.

Summary and Explanation of the Test

The *therascreen* BRAF V600E RGQ PCR Kit is a ready-to-use kit for the detection of the most commonly occurring somatic mutation in the *BRAF* gene using real-time polymerase chain reaction (PCR) on the Rotor-Gene Q MDx (US) instrument. Each kit contains material to analyze a maximum of 24 specimens.

Using amplification refractory mutation system (ARMS®) and Scorpions® technologies (1, 2), the *therascreen* BRAF V600E RGQ PCR Kit enables detection of the V600E (T1799A) mutation at codon 600 of the *BRAF* oncogene against a background of wild-type genomic DNA.

The methods used are highly selective and enable detection of a low percentage of mutant DNA in a background of wild-type genomic DNA depending on the total amount of DNA present.

Technologies

The *therascreen* BRAF V600E RGQ PCR Kit utilizes two technologies (ARMS and Scorpions) for detection of mutations in real-time PCR.

ARMS

Allele- or mutation-specific amplification is achieved using ARMS. *Taq* DNA polymerase (*Taq*) is effective at distinguishing between a match and a mismatch at the 3' end of a PCR primer. Specific mutated sequences are selectively amplified even in samples where the majority of the sequences do not carry the mutation. When the primer is fully matched, the amplification proceeds with full efficiency. When the 3' base is mismatched, only low level background amplification occurs.

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 measurable increase in fluorescence from the reaction tube.

Principle of the Procedure

Two reaction mixes are supplied in the *therascreen* BRAF V600E RGQ PCR Kit:

- Control Reaction Mix (CTRL)
- V600E Reaction Mix (V600E)

The V600E reaction mix detects both V600E and V600Ec mutations but does not distinguish between these mutations. All reaction mixes are duplex and contain reagents to detect targets that are labeled with FAM™ and an internal control that is labeled with HEX™. The internal control reaction controls for the presence of inhibitors that may lead to false-negative results.

Reaction mixes

The *therascreen* BRAF V600E RGQ PCR Kit comprises a two-step procedure. In the first step, the control test is performed to assess the total amplifiable *BRAF* DNA in a sample. In the second step, both the mutation and control tests are performed to determine the presence or absence of mutant DNA.

Control reaction

The control reaction, labeled with FAM, is used to assess the total amplifiable *BRAF* DNA in a sample. The control reaction amplifies a region of exon 3 of the *BRAF* gene. The primers and Scorpions probe have been designed to amplify independently of any known *BRAF* polymorphisms.

Mutation reaction

The mutation reaction contains a FAM-labeled Scorpions probe and an ARMS primer for discrimination between the wild-type DNA and V600E DNA.

Controls

All experimental runs must contain positive and negative controls.

Positive control

Each run must contain a positive control. The *therascreen* BRAF RGQ PCR Kit contains *BRAF* Positive Control (PC) to be used as the template in the Positive Control reaction. The positive control results will be assessed to ensure that the kit performs within the stated acceptance criteria.

Negative control

Each run must contain a negative control (“No Template Control”). The *therascreen* BRAF RGQ PCR Kit contains “Water for NTC” (NTC) to be used as the “template” for the No Template Control. The No Template Control is used to assess any potential contaminations during the run setup and to assess the performance of the internal control reaction.

Internal control reaction assessment

Each reaction mix contains an internal control in addition to the target reaction. A failure indicates that either there may be inhibitors present that could lead to an inaccurate result or an operator setup error has occurred for that tube.

Sample assessment

It is required to use the Control Reaction Mix (CTRL) supplied with the *therascreen* BRAF V600E RGQ PCR Kit to assess the total amplifiable *BRAF* DNA in a sample. The control reaction amplifies a region of exon 3 of the *BRAF* gene.

Set up samples with only the control reaction using the *BRAF* Positive Control (PC) as a positive control and Water for NTC (NTC) as the no template control.

Note: DNA assessment should be based on PCR and may differ from quantification based on absorbance readings. Additional Control Reaction Mix (CTRL) is supplied to enable assessment of quality and quantity of the DNA in samples before analysis with the *therascreen* BRAF V600E RGQ PCR Kit.

Materials Provided

Kit contents

<i>therascreen</i> BRAF V600E RGQ PCR Kit			(24)
Catalog no.			874821
Number of reactions			24
Contents	Cap color		Volume
Control Reaction Mix	Red	CTRL	2 x 720 μ l
V600E Reaction Mix	Purple	V600E	720 μ l
<i>BRAF</i> Positive Control	Beige	PC	250 μ l
<i>Taq</i> DNA Polymerase	Mint	<i>Taq</i>	80 μ l
Water for NTC	White	NTC	1.9 ml
Water for Dilution	White	Dil	1.9 ml
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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.

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

Reagents

- QIAamp DSP DNA FFPE Tissue Kit (cat. no. 60404; see "DNA extraction", page 21)
- Xylene
- Ethanol (96–100%)*
- Cleaning reagents following local procedures

Consumables

- 1.5 ml or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Eppendorf [Eppendorf Safe-Lock Tubes, 1.5 ml, cat. no. 0030 120.086] or Sarstedt [Safety Cap, cat. no. 72.690])†
- 0.1 ml Strip Tubes and Caps, for use with 72-well rotor (cat. no. 981103 or cat. no. 981106)
- Sterile pipette tips with filters (to avoid cross-contamination, we recommend pipette tips with aerosol barriers)
- Nuclease-free, low DNA-binding microcentrifuge tubes for preparing master mixes

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

† This is not a complete list of suppliers.

Equipment

- Thermomixer*, heated orbital incubator*, heating block*, or water bath* capable of incubation at 56°C and 90°C
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Vortex
- Dedicated pipettes (adjustable) for sample preparation
- Dedicated pipettes (adjustable) for PCR master mix preparation
- Dedicated pipettes (adjustable) for dispensing of template DNA
- Rotor-Gene Q MDx Platform (US)* (cat. no. 9002035) or Rotor-Gene Q MDx System (US)* (cat. no. 9002036)
- Loading Block 96 x 0.2 ml PCR tubes, aluminum block for manual reaction setup (cat. no. 9018905)
- Loading Block 72 x 0.1 ml Tubes, aluminum block for manual reaction setup (cat. no. 9018901)
- 72-Well Rotor (cat. no. 9018903)
- Rotor-Disc® 72 (cat. no. 9018899)
- Rotor-Disc 72 Locking Ring (cat. no. 9018900)
- Rotor Holder (cat. no. 9018908)
- Rotor-Gene AssayManager® v2.1
- Rotor-Gene AssayManager Gamma MDx Plug-in version 1.0.0
- BRAF V600E Sample Assessment Assay Profile, version 1.0.1:
therascreen_BRAF_V600E_FFPE_SA_MDx
- BRAF V600E Mutation Assessment Assay Profile, version 1.0.1:
therascreen_BRAF_V600E_FFPE_MA_MDx

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

Warnings and Precautions

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

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.

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

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

Discard sample and assay waste according to your local safety regulations.

General precautions

Always pay attention to the following:

- All chemicals and biological materials are potentially hazardous. FFPE specimen material and nucleic acids prepared from it are unlikely to pose an infection hazard, but local institutional Health and Safety procedures must always be adhered to.
- Discard sample and assay waste according to your local safety procedures.
- Reagents for the *therascreen* BRAF V600E 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 in the *therascreen* BRAF V600E RGQ PCR Kit are formulated specifically for optimal performance. All reagents supplied in the *therascreen* BRAF V600E RGQ PCR Kit are intended to be used solely with the other reagents in the same *therascreen* BRAF V600E RGQ PCR Kit. Substitutions to the reagents in the kit must not be made if optimal performance is to be maintained.
- Only use the *Taq* DNA polymerase (*Taq*) provided in the 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.
- Use extreme caution to prevent cross-contamination between samples. Cap the tubes promptly after addition of each sample.
- Thoroughly decontaminate the loading block before using it for preparation of assay master mixes. The loading block must be dry before use.
- Use individual, dedicated pipettes for setting up reaction mixes and adding positive control reagents.

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- Perform preparation and dispensing of reaction mixes in an area separate from the one used for the addition of the positive control.
 - Fluorescently labeled molecules included in the reaction mix reagents are light sensitive. Protect control and reaction mix reagents from light to avoid photo bleaching.
 - Do not open the Rotor-Gene Q MDx (US) instrument until the run has finished.
 - Do not open Rotor-Gene Q tubes after the run has finished.
 - Caution must be observed to ensure correct sample testing to avoid wrong sample entry, loading error and pipetting error.
 - Caution must be observed to ensure no empty wells are introduced between controls and samples or between the samples themselves.
 - Store and extract positive 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 PCRs with synthetic control material. We recommend using separate, dedicated pipettes for setting up reaction mixes and adding DNA 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.

Limitations of the assay

- The test is for use with formalin-fixed, paraffin-embedded primary and metastatic colorectal cancer (mCRC) specimens.
- The Kit is for use with the QIAGEN QIAamp DSP DNA FFPE Tissue Kit.
- Determination of DNA input for the assay is based on DNA qualification and is performed using the Control Reaction Mix to assess the amount of amplifiable DNA. Samples will be qualified for further Mutation Assessment if the amount of the amplifiable DNA (represented by the Ct value of the Control Reaction Mix) is determined to be within the pre-defined Control Working Range (CWR). Extracted DNA should be assessed for

amplifiable DNA using the Control Reaction Mix. The acceptable measurable range of the assay (Control C_T 20.95–33.00).

- The kit is validated for use only with the QIAGEN Rotor-Gene Q MDx Instrument (RGQ) MDx Instrument with Automated data analysis and results interpretation using Rotor-Gene AssayManager® (RGAM) software version 2.1 or higher, Gamma MDx plug-in and *therascreen* BRAF V600E FFPE Assay Profile.
- The BRAF Mutation Reaction mix is cross reactive for V600Ec and V600D. The assay will not distinguish between V600E and V600E Complex (V600Ec) mutations. V600Ec and V600D very rare somatic mutations in CRC. The assay is not analytically validated for V600Ec or V600D. V600Ec and V600D specimens were not identified in the trial used to support the clinical validity of the *therascreen* BRAF V600E RGQ PCR Kit.
- For efficient use of the *therascreen* BRAF V600E RGQ PCR Kit, samples must be grouped into batches of no less than six. Smaller batch sizes will mean that fewer samples can be tested with the *therascreen* BRAF V600E RGQ PCR Kit.

Reagent Handling and Storage

Shipping conditions

The *therascreen* BRAF V600E RGQ PCR Kit is shipped on dry ice and must still be frozen on arrival. If the *therascreen* BRAF V600E 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 or reagents, please contact the QIAGEN Technical services or local distributors (visit www.qiagen.com).

Storage conditions

The *therascreen* BRAF V600E RGQ PCR Kit must be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer and protected from light. Scorpions, as with all fluorescently labeled molecules, must be protected from light to avoid photo-bleaching and loss of performance.

Stability

Once opened, reagents can be stored in their original packaging at -30 to -15°C until the stated expiration date shown on the packaging. Do not exceed a maximum of 6 freeze thaw cycles.

therascreen BRAF V600E RGQ PCR Kit handling

The reagents must be thawed at room temperature (15–25°C) for a minimum of 1 hour prior to use. Once the reagents are ready to use, the PCR reactions can be set up. The Rotor-Gene Q tubes, containing the master mixes and the DNA eluate, should 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 hours if stored at ambient temperature, or 12 hours if stored at 2–8°C.

Note: This time includes both the PCR setup and storage.

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

Reagents in the *therascreen* BRAF V600E RGQ PCR Kit are diluted optimally and no further purification or treatment is required prior to their use.

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.

Specimen Handling and Storage

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

Sample material must be human genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue. Specimens must be transported according to standard pathology methodology to ensure specimen quality.

Tumor samples are non-homogeneous and data from a sample of tumor may not be concordant with other sections from the same tumor. Tumor samples may also contain non-tumor tissue. DNA from non-tumor tissue would not be expected to contain mutations detected by the *therascreen* BRAF V600E RGQ PCR Kit.

Specimen storage

FFPE blocks and slides should be stored at room temperature (15–25°C) prior to DNA extraction. Sectioned FFPE slides may be stored for a period of up to 8 weeks prior to extraction.

DNA may be stored following extraction with the QIAamp DSP DNA FFPE Tissue Kit, prior to testing. The DNA may be stored in a freezer (–30 to –15°C) for a maximum of 8 weeks prior to testing with the *therascreen* BRAF V600E RGQ PCR Kit.

Procedure

DNA extraction

DNA extraction must be carried out using the QIAamp DSP DNA FFPE Tissue Kit (QIAGEN, cat. no. 60404).

Note: The *therascreen* BRAF V600E RGQ PCR Kit has been developed for use with the QIAamp DSP DNA FFPE Tissue Kit with manual extraction technique, including the listed modifications. To ensure optimal performance, do not use any other DNA extraction product.

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

- Collect FFPE sections on glass slides.
- A trained individual (e.g., a pathologist) must assess a Hematoxylin & Eosin (H&E)-stained section to confirm that there is tumor tissue present and mark its boundaries. Sections will not qualify for extraction if there is no tumor tissue present.
- Store all FFPE blocks and slides at room temperature (15–25°C) in the dark.
- Use two 4–5 µm sections per extraction. Adjacent sections should be used for extraction, if necessary.
- Scrape excess paraffin away from around the tissue sections using a fresh, sterile scalpel.
- Scrape tissue sections into microcentrifuge tubes using a fresh scalpel for each sample to be extracted. Do not use the RNase step described in the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.
- The extraction process has **not** been validated with the use of deparaffinization solution. Only use the Xylene/Ethanol method for deparaffinization, as described in the *QIAamp DSP DNA FFPE Tissue Kit Handbook*.
- Proteinase K digestion (incubation at 56°C) must be performed at 56°C and for 1 hour.

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- De-crosslinking (incubation at 90°C) must not exceed 1 hour.
 - Purified genomic DNA must be eluted in 60 µl Buffer ATE (provided in the QIAamp DSP DNA FFPE Tissue Kit) and incubated at room temperature for 2.5 minutes. Centrifuge at full speed and then perform an additional 60 µl elution with Buffer ATE followed by an additional incubation at room temperature for 2.5 minutes.
 - Store purified genomic DNA at –30 to –15°C.

DNA assessment should be based on the Control Reaction Mix (CTRL) supplied with the *therascreen* BRAF V600E RGQ PCR Kit and may differ from quantification based on absorbance readings. Additional Control Reaction Mix (CTRL) is supplied to enable assessment of quality and quantity of the DNA in samples before analysis with the *therascreen* BRAF V600E RGQ PCR Kit. The acceptable measurable range of the assay (Control C_T 20.95–33.00).

Note: To ensure sufficient DNA for analysis, FFPE sections must be a minimum of 4 µm thick.

Both reaction mixes in the *therascreen* BRAF V600E RGQ PCR Kit generate short PCR products. However, the *therascreen* BRAF V600E RGQ PCR Kit will not work with heavily fragmented DNA (DNA samples will be qualified following a successful sample assessment PCR run).

Sample assessment

This protocol is used to assess the total amplifiable DNA in samples.

Important points before starting

- Before beginning the procedure, read “Warnings and Precautions”, page 14.
- Take time to familiarize yourself with the Rotor-Gene Q MDx (US) instrument before starting the protocol. See the instrument user manual.
- Do not vortex *Taq* DNA polymerase (*Taq*) or any mix containing *Taq* DNA polymerase, as this may inactivate the enzyme.
- Pipet *Taq* DNA polymerase (*Taq*) by placing the pipette tip just under the liquid surface to avoid the tip being coated in excess enzyme.
- Up to 24 samples can be assessed using the Control Reaction Mix (CTRL) available.

Things to do before starting

- Before each use, all reagents need to be thawed for a minimum of 1 hour at room temperature (15–25°C), mixed by inverting 10 times and centrifuged briefly to collect the contents at the bottom of the tube.
- Ensure that *Taq* DNA polymerase (*Taq*) is at room temperature before each use. Centrifuge the tube briefly to collect the enzyme at the bottom of the tube.
- The *therascreen* BRAF V600E RGQ PCR Kit must be run on the Rotor-Gene Q MDx (US) instrument. Rotor-Gene AssayManager v2.1 and Gamma MDx Plug-in must be installed before use.
- The Rotor-Gene AssayManager Core Application and Gamma MDx Plug-in(s) each have a separate user manual. For installation instructions, see the respective user manual.
- The *BRAF* V600E sample assessment and mutation analysis assay profile must be installed before use. The assay profile contains all the necessary parameters to execute a PCR run and perform automatic analysis and result interpretation.

Sample Assessment Assay Profile, version 1.0.1:

therascreen_BRAF_V600E_FFPE_SA_MDx

Mutation Assessment Assay Profile, version 1.0.1:

therascreen_BRAF_V600E_FFPE_MA_MDx

- A PDF viewer must be installed to view run reports produced by Rotor-Gene AssayManager.
- Before starting, the following settings must be configured from the configuration tab (Figure 1 and Figure 2).

Finish run

Run has to be released before starting approval

Release of run has to be signed

Figure 1. Ensure that “Run has to be released before starting approval” and “Release of run has to be signed” check boxes are not checked.

Work list

Format of generated work list names

WL_20110513_0430

User-definable section

WL

Date

Time

Operator

Enable processing of unclear samples

Enable checksum for LIMS import

Closed mode

Material number required

Valid expiry date required

Lot number required

UDT mode

Material number required

Valid expiry date required

Lot number required

Figure 2. Ensure that “Material number required” is not checked.

Preparing the sample assessment run

1. Thaw the Control Reaction Mix (CTRL), Water for No Template Control (NTC) and the Positive Control (PC) at room temperature (15–25°C) for a minimum of 1 hour. When the reagents have thawed, mix them by inverting each tube 10 times to avoid localized concentrations of salts and then centrifuge briefly to collect the contents at the bottom of the tube.
2. Prepare sufficient master mixes (Control Reaction Mix [CTRL] plus *Taq* DNA polymerase [*Taq*]) for the DNA samples, one Positive Control reaction and one No Template Control reaction, according to the volumes given in Table 1. The master mix contains all of the components needed for PCR except the sample.

Table 1. Preparation of the Control master mix*

Component	Volume
Control Reaction Mix (CTRL)	19.5 µl x (n+X)*
<i>Taq</i> DNA polymerase (<i>Taq</i>)	0.5 µl x (n+X)*
Total volume	20.0 µl/reaction

* **n** = number of reactions (samples plus controls). When preparing the master mix, prepare enough for n + X extra sample (where X = 1 sample when testing ≤ 4 samples plus controls and X = 2 when testing ≥ 5 samples plus controls) to allow for sufficient coverage for the PCR setup.

3. Thoroughly mix the master mix by pipetting up and down 10 times. Place the appropriate number of strip tubes in the loading block according to the layout in Table 2. Immediately add 20 µl master mix to each PCR strip tube (not provided).

Note: Caps will remain in the plastic container until required. For sample assessment, control master mix should be added to one positive control well, one negative control well and one well for each sample.

Table 2. Layout of sample assessment tests in the loading block (numbers denote positions in the loading block and indicate final rotor position)

Mix									
Control	1 (PC)	9	17	-	-	-	-	-	-
Control	2 (NTC)	10	18	-	-	-	-	-	-
Control	3	11	19	-	-	-	-	-	-
Control	4	12	20	-	-	-	-	-	-
Control	5	13	21	-	-	-	-	-	-
Control	6	14	22	-	-	-	-	-	-
Control	7	15	23	-	-	-	-	-	-
Control	8	16	24	-	-	-	-	-	-

4. Immediately perform the following steps:

- 4a. Add 5 µl of Water for No Template Control (NTC) to the No Template Control tube (PCR tube number 2) and cap the tube.
- 4b. Add 5 µl of each sample to the sample tubes (PCR tube numbers 3–24) and cap the tubes.
- 4c. Add 5 µl *BRAF* Positive Control (PC) to the positive control tube (PCR tube number 1) and cap the tube.

Note: Mark the lids of the tubes to show the direction to load the tubes into the Rotor-Gene Q MDx (US) instrument.

5. After all PCR tubes are capped, perform a visual check on sample tube fill levels to ensure sample has been added to all tubes.
6. Invert all PCR tubes (4 times) to mix samples and reaction mixes.
7. Place the PCR strip tubes into the appropriate positions in the 72-well rotor (Table 2). If the rotor is not fully occupied, all empty positions on the rotor must be filled with a capped, empty tube.
8. Immediately place the 72-well rotor into the Rotor-Gene Q MDx (US) instrument. Ensure that the locking ring (accessory of the Rotor-Gene Q MDx (US) instrument) is placed on top of the rotor to secure the tubes during the run.

Performing a *BRAF* V600E sample assessment run using Rotor-Gene AssayManager

Note: The *therascreen* BRAF V600E RGQ PCR Kit must be run on the Rotor-Gene Q MDx (US) instrument. Rotor-Gene AssayManager v2.1 and Gamma MDx Plug-in must be installed before use.

The following instructions detail the setup of a *BRAF* V600E run using Rotor-Gene AssayManager. For advanced instructions, refer to the respective user manuals.

1. Double-click the Rotor-Gene AssayManager software version 2.1 icon on the desktop of the laptop connected to the Rotor-Gene Q MDx (US) instrument.
2. On the Rotor-Gene AssayManager login screen (Figure 3), enter the user ID and password. Select "Closed" from the Mode list and click "OK".
3. The main Rotor-Gene AssayManager window will open.

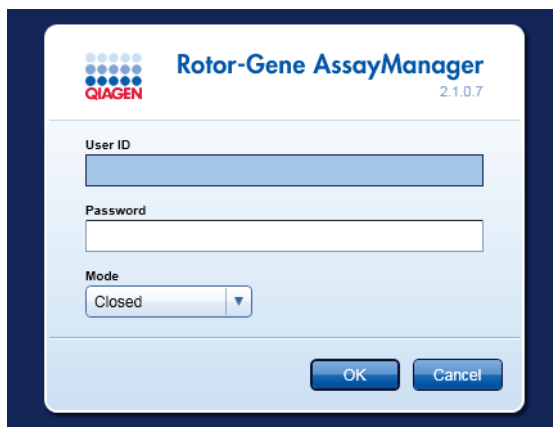


Figure 3. Rotor-Gene AssayManager login screen.

4. In the "Setup" tab, select "New manual work list" to create a new work list (Figure 4).

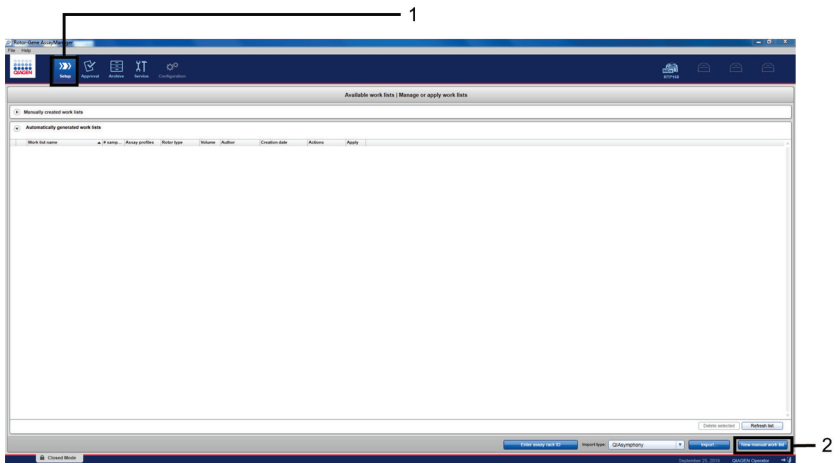


Figure 4. Rotor-Gene AssayManager work list setup. 1 = “Setup” tab. 2 = New manual work list.

5. To perform a *BRAF* V600E sample assessment run, select the “Assays” tab. Click “therascreen_BRAF_V600E_FFPE_SA_MDx” in the “Available assay profiles” section and click the blue arrow to select the assay profile. Mouse over the assay profile to see the full name.

The assay profile should now appear in the “Selected assay profiles” section (Figure 5). For mutation analysis, see “Performing a *BRAF* V600E mutation assessment analysis run using Rotor-Gene AssayManager”, page 38.

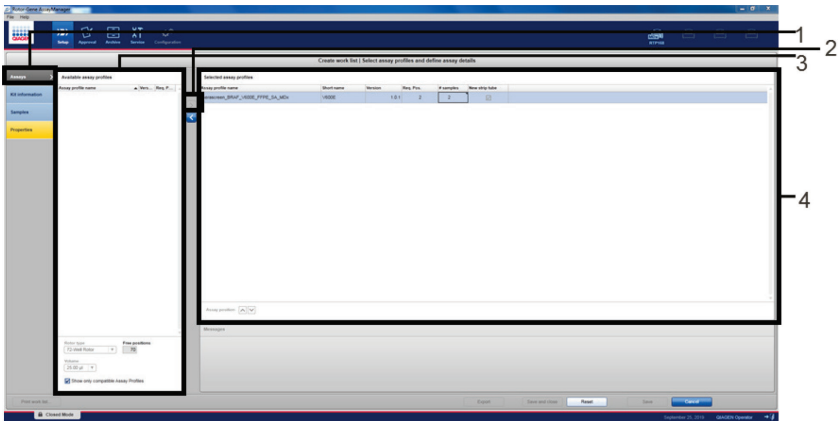


Figure 5. Assay profile Selection window. 1 = “Assays” tab. 2 = “Available assay profiles” section 3 = Arrow to select assay profile. 4 = “Selected assay profiles” section.

6. In the “Selected assay profiles” section, enter the following information:

- Number of samples to be tested (excluding controls)
- Kit expiry date
- Kit lot number (Figure 6).

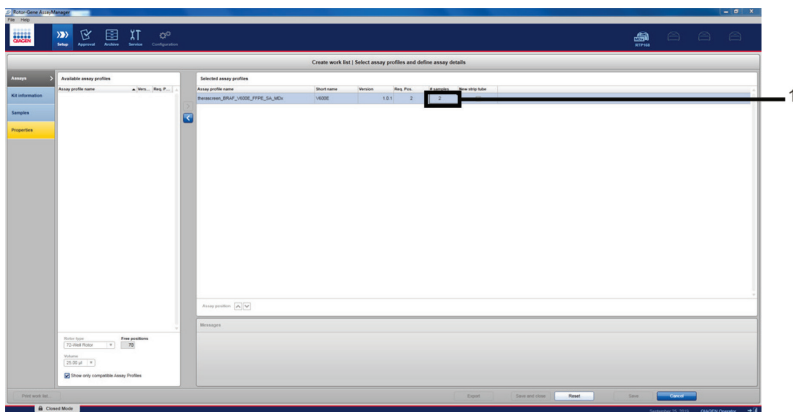


Figure 6. “Create work list” window on the “Assays” tab. 1 = Enter the sample number.

7. Click the “Kit information” tab and enter the following kit information (Figure 7):

- Kit barcode (optional)
- Material number (required)
- Lot number (optional)
- Kit expiry date (optional)

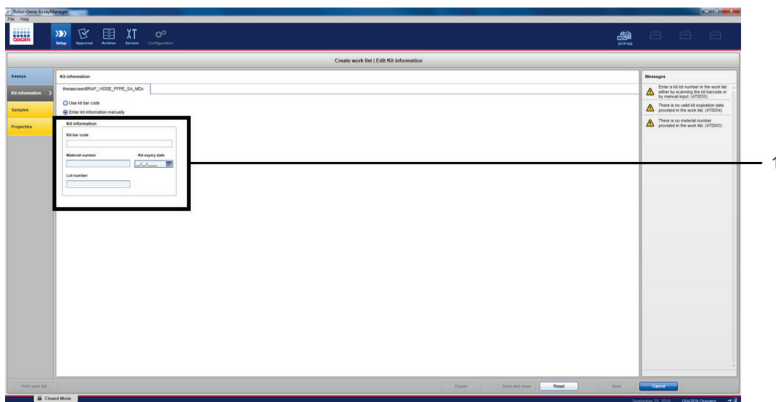


Figure 7. “Create work list” window on the “Kit information” tab. 1 = Enter kit information.

8. Click the “Samples” tab. Enter the sample names into the “Sample ID” column. Enter any comments into the “Comments” column (Figure 8).

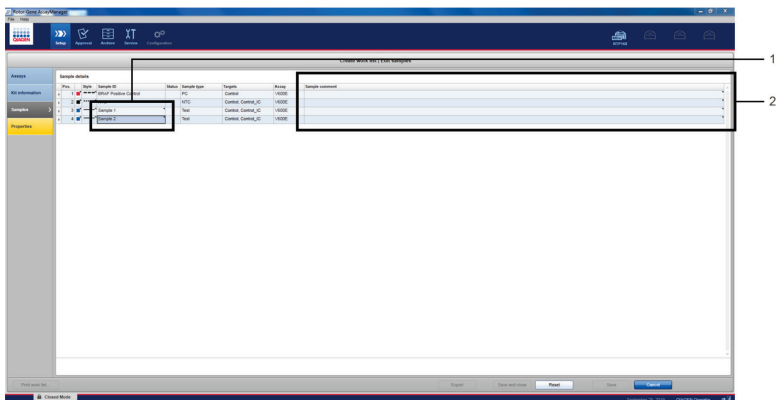


Figure 8. “Create work list” window on the “Samples” tab. 1 = Enter sample names. 2 = Enter sample comments.

9. Click the “Properties” tab. Enter a work list name in the “Work list name” field and check the “is editable” and “work list is complete” boxes. Click “Apply” to apply the work list (Figure 9).

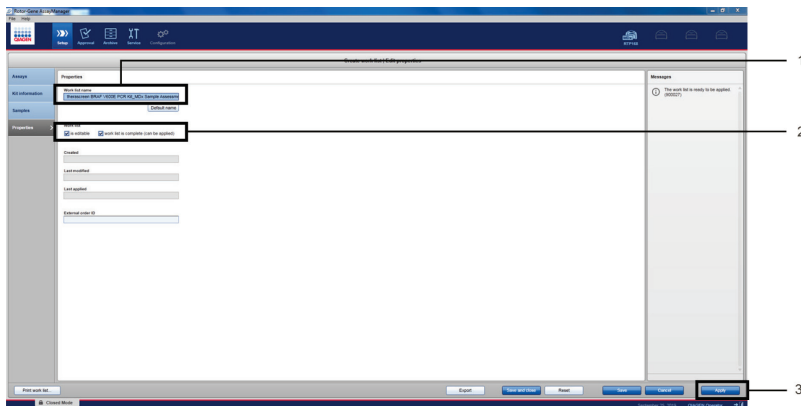


Figure 9. “Create work list” window on the Properties” tab. 1 = “Work list name” field. 2 = “is editable” and “work list is complete” check boxes. 3 = “Apply”.

10. Enter an experiment name in the “Experiment name” field. Ensure that the “Ring attached” check box is checked and click “Start run” (Figure 10).

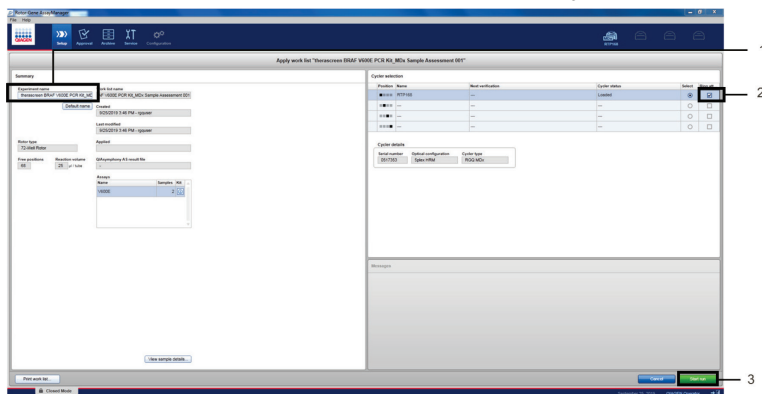


Figure 10. “Apply work list” window. 1 = “Experiment name” section. 2 = “Ring attached” check box. 3 = “Start run”.

11. On completion, click “Finish run” to release and approve the run (Figure 11).



Figure 11. “Cycler Environment” window. 1 = Finish run.

12. To view the run information and BRAF V600E assay results, click “Release and go to approval” (Figure 12).

This also releases the Rotor-Gene Q MDx (US) instrument ready for the next run.

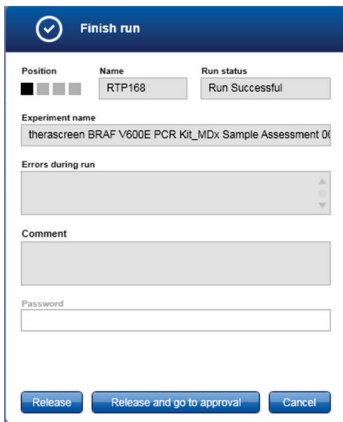


Figure 12. “Finish Run” dialog. 1 = Release and go to approval.

Note: Raw data, processed data, experiment information, assay and audit trail information can be found in the “Plots and information” window. Assay results can be found in the “Results” window. If the Positive Control and No Template Control results are within an acceptable range, the “Overall sample result” column will report “Valid”, otherwise an “Invalid” result will appear (Figure 13).

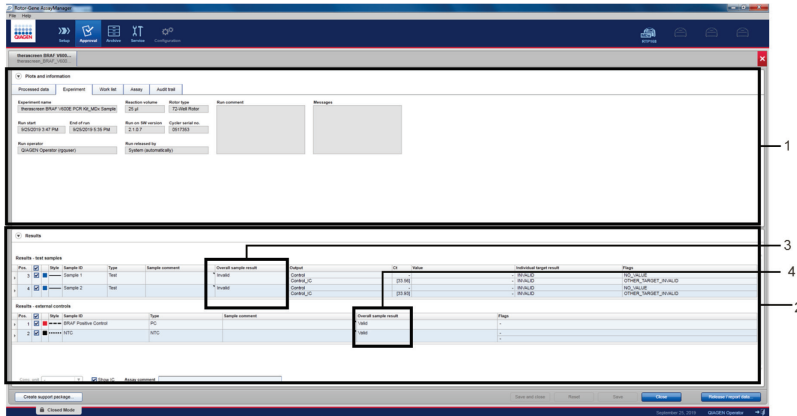


Figure 13. “Approval” window. 1 = “Plots and Information” window. 2 = Approval window. 3 = “Overall sample result” column for test samples. 4 = “Overall sample result” column for run controls.

Note: The BRAF V600E assay profile 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. If the Sample Control results are within an acceptable range, the “Overall sample result” column will report “Valid”. This indicates that the sample DNA concentration is suitable for mutation analysis.

- Click "OK" to save the experiment to the archive and create a LIMS output and run report (Figure 14). 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.

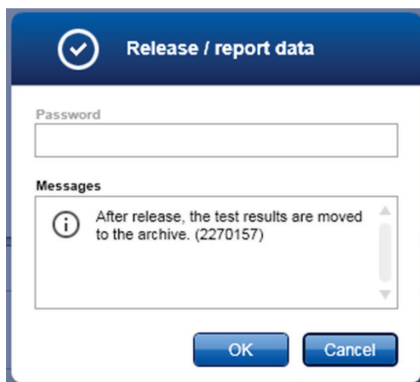


Figure 14. "Release/report data" dialog.

- Click "OK" when the Status of release dialog box appears. (Figure 15).

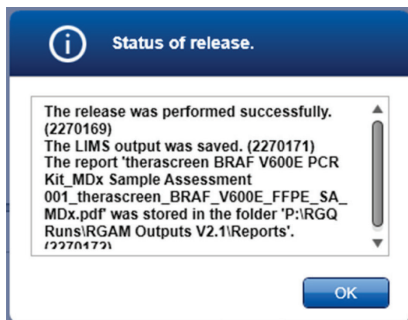


Figure 15. "Status of release" dialog.

Note: Released experiments can now be retrieved from the "Archive" environment. To find an experiment, click the "Archive" tab and enter the necessary search criteria.

V600E mutation detection

This protocol is for the detection of *BRAF* V600E mutation. Once a sample has passed the sample assessment, it can be tested using the *BRAF* V600E reaction mix.

Important points before starting

- Before beginning the procedure, read “Warnings and Precautions”, page 14.
- Take time to familiarize yourself with the Rotor-Gene Q MDx (US) before starting the protocol. See the instrument user manual.
- Do not vortex *Taq* DNA polymerase (*Taq*), or any mix containing *Taq* DNA polymerase, as this may inactivate the enzyme.
- For efficient use of the *therascreen* *BRAF* V600E RGQ PCR Kit, samples must be grouped into batches of no less than six. Smaller batch sizes will mean that fewer samples can be tested with the *therascreen* *BRAF* V600E RGQ PCR Kit.
- Pipet *Taq* DNA polymerase (*Taq*) by placing the pipette tip just under the liquid surface to avoid the tip being coated in excess enzyme.

Things to do before starting

Before each use, all reagents need to be thawed for a minimum of 1 hour at room temperature (15–25°C), mixed by inverting 10 times and centrifuged briefly to collect the contents at the bottom of the tube.

Ensure that *Taq* DNA polymerase (*Taq*) is at room temperature (15–25°C) before each use. Centrifuge the tube briefly to collect the enzyme at the bottom of the tube.

Procedure

1. Thaw the reaction mixes, Water for No Template Control (NTC) and *BRAF* Positive Control (PC) at room temperature (15–25°C) for a minimum of 1 hour. When the reagents have thawed, mix them by inverting each tube 10 times to avoid localized concentrations of salts and then centrifuge briefly to collect the contents at the bottom of the tube.
2. Prepare sufficient master mixes (reaction mix plus *Taq* DNA polymerase [*Taq*]) for the DNA samples, one Positive Control reaction and one No Template Control reaction according to the volumes given in Table 3. Include reagents for $n + x$ extra sample to allow sufficient overage for the PCR setup.

Note: The master mixes contain all of the components needed for PCR except the sample.

Table 3. Preparation of the master mixes*

Component	Volume of reaction mix	Volume of <i>Taq</i> DNA polymerase (<i>Taq</i>)
Control	19.5 μ l \times (n+x)	0.5 μ l \times (n+x)
V600E	19.5 μ l \times (n+x)	0.5 μ l \times (n+x)

* n = number of reactions (samples plus controls). When preparing the master mix, prepare enough for $n + X$ extra sample (where $X = 1$ sample when testing ≤ 4 samples plus controls and $X = 2$ when testing ≥ 5 samples plus controls) to allow for sufficient overage for the PCR setup.

3. Thoroughly mix the master mix by pipetting up and down 10 times. Place the appropriate number of strip tubes in the loading block according to the layout in Table 4. Immediately add 20 μ l master mix to each PCR strip tube (not provided).

Caps will remain in the plastic container until required.

Table 4. Layout of control and mutation reactions in the loading block (numbers denote position in the loading block and indicate final rotor position. Numbers in bold indicate sample number)

Mix	PC	3	7	11	15	19	23	-	-
Control	1	9	17	25	33	41	49	-	-
BRAF	2	10	18	26	34	42	50	-	-
	NTC	4	8	12	16	20	24	-	-
Control	3	11	19	27	35	43	51	-	-
BRAF	4	12	20	28	36	44	52	-	-
	1	5	9	13	17	21	-	-	-
Control	5	13	21	29	37	45	-	-	-
BRAF	6	14	22	30	38	46	-	-	-
	2	6	10	14	18	22	-	-	-
Control	7	15	23	31	39	47	-	-	-
BRAF	8	16	24	32	40	48	-	-	-

4. Immediately perform the following steps:

- 4a. Add 5 µl Water for No Template Control (NTC) to the No Template Control PCR strip tubes (PCR tube numbers 3–4) and cap the tubes.
- 4b. Add 5 µl of each sample to the sample tubes (PCR tube numbers 5–6, 7–8, 9–10, etc., up to n samples) and cap the tubes.
- 4c. Add 5 µl BRAF Positive Control (PC) to the Positive Control tubes (PCR tube numbers 1–2) and cap the tubes.

Note: Each DNA sample must be tested with both the control and the mutation mixes.

Note: Mark the lids of the tubes to show the direction to load the tubes into the Rotor-Gene Q MDx (US) instrument.

5. After all PCR tubes are capped, perform a visual check on sample tube fill levels to ensure sample has been added to all tubes.
6. Invert all PCR tubes (4 times) to mix samples and reaction mixes.

- Place the PCR strip tubes into the appropriate positions in the 72-well rotor (Table 4).
Note: A maximum of 24 samples can be included in each PCR run. If the rotor is not fully occupied, all empty positions on the rotor must be filled with a capped, empty tube.
- Immediately place the 72-well rotor into the Rotor-Gene Q MDx (US) instrument. Make sure that the locking ring (accessory of the Rotor-Gene Q MDx (US) instrument) is placed on top of the rotor to secure the tubes during the run.

Performing a *BRAF* V600E mutation assessment analysis run using Rotor-Gene AssayManager

- To perform a *BRAF* V600E Mutation Analysis run, ensure the “Assays” tab is selected on the left-hand side of the main window. Click “therascreen_BRAF_V600E_FFPE_MA_MDx” in the “Available assay profiles” window and click the blue arrow to select the assay profile. Mouse over the assay profile to see the full name (Figure 16).

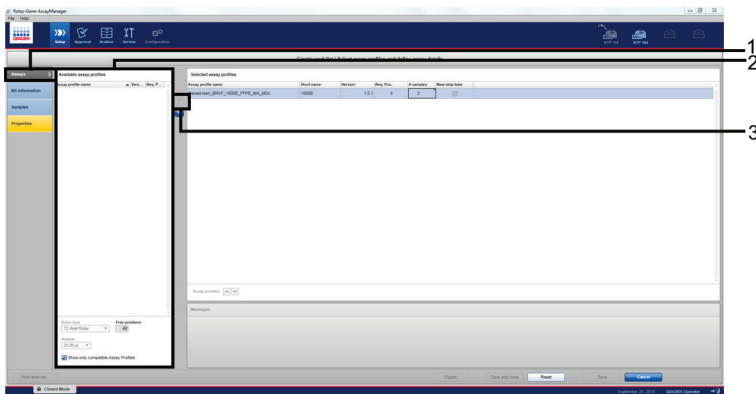


Figure 16. Create work list window. 1 = “Assays” tab. 2 = “Available assay profile” section. 3 = Arrow to select the assay profile.

2. In the “Selected assay profiles” window, enter the number of samples to be tested (excluding controls) (Figure 17).

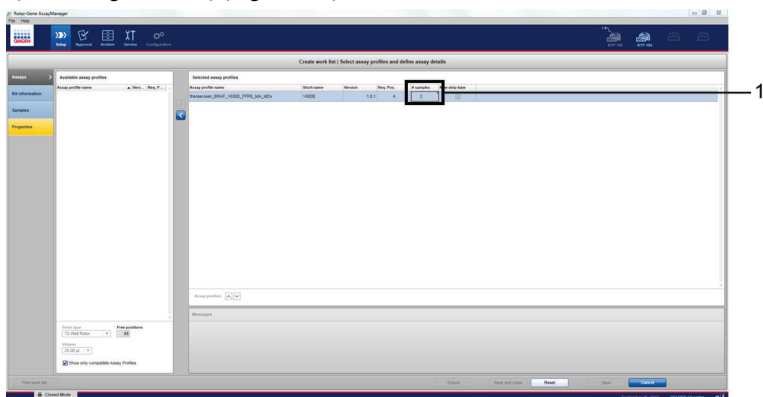


Figure 17. “Create work list” main window on the “Assays” tab. 1 = Enter the sample number.

3. Click the “Kit information” tab and enter the following kit information (Figure 18):

- Kit barcode (optional)
- Material number (required)
- Lot number (optional)
- Kit expiry date (optional)

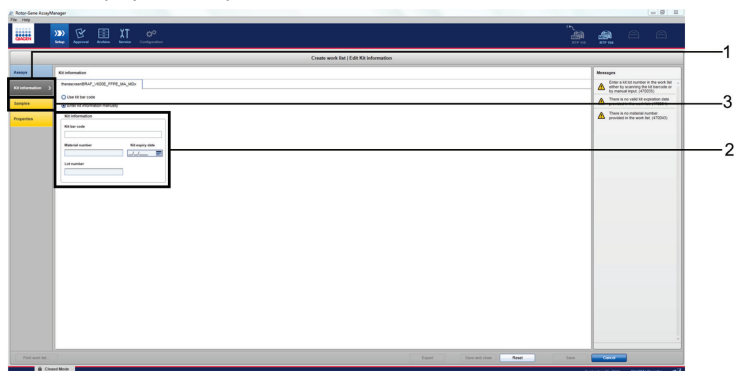


Figure 18. “Create work list” window on the “Kit information” tab. 1 = “Enter kit information. 2 = Kit information. 3 = “Samples” tab.

- Click the “Samples” tab to enter sample information (Figure 19). Enter the sample names into the “Sample ID” column. Enter any comments into the “Comments” column. (Figure 19).

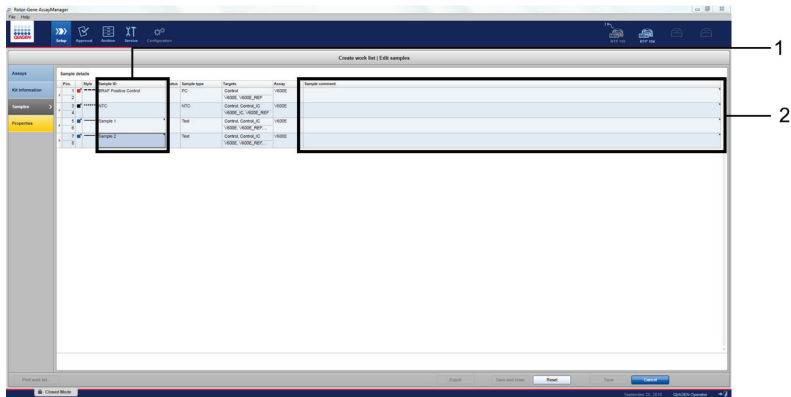


Figure 19. “Create work list” main window on the “Samples” tab. 1 = Enter sample ID. 2 = Enter sample comments.

- Click the “Properties” tab. Enter a work list name in the “Work list name” field and check the “is applicable” box. Click “Apply” to apply the work list (Figure 20).

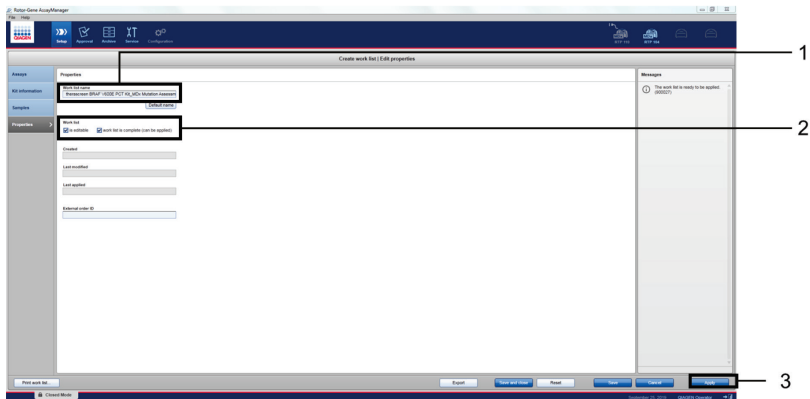


Figure 20. “Create work list” main window on the “Properties” tab. 1 = “Work list name” section. 2 = “is editable” and “work list is complete” check boxes. 3 = “Apply”.

- Enter an experiment name in the "Experiment name" field. Ensure that the "Ring attached" box is checked and click Start run (Figure 21).

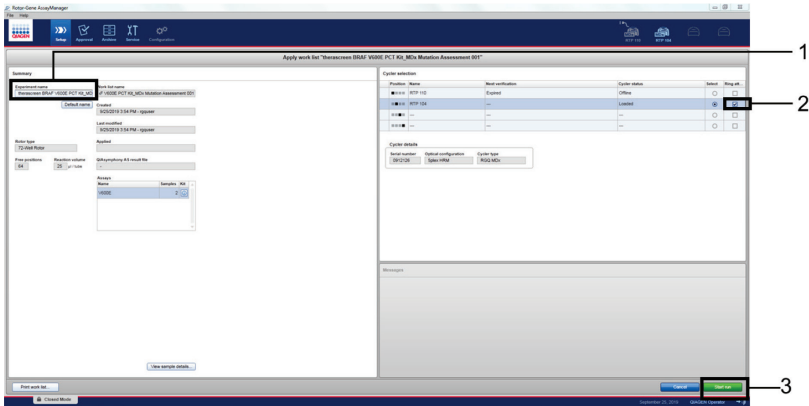


Figure 21. "Apply work list" window. 1 = "Experiment name" section. 2 = "Ring attached" checkbox. 3 = "Start run".

- On completion, click "Finish run" to release and approve the run (Figure 22).



Figure 22. "Cycler Environment" window. 1 = "Finish run".

8. To view the run information and BRAF V600E assay results, click “Release and go to approval”. This also releases the RGQ instrument ready for the next run (Figure 23).

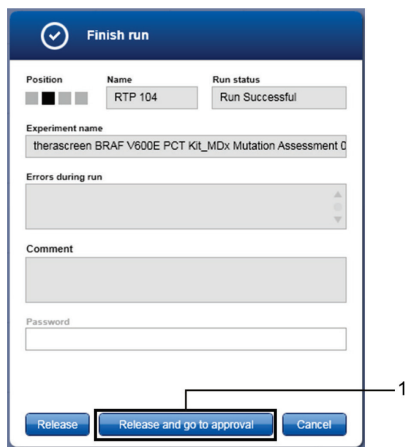


Figure 23. “Finish Run” window. 1 = Release and go to approval.

9. **Note:** Raw data, processed data, experiment information, assay and audit trail information can be found in the “Plots and information” window. Assay results can be found in the “Assay results” window. If the Positive Control and No Template Control results are within an acceptable range, the “Sample status” column will report “Valid”, otherwise an “Invalid” result will appear.

10. Click “Release/report data” to open the “Release/report data” window (Figure 24).

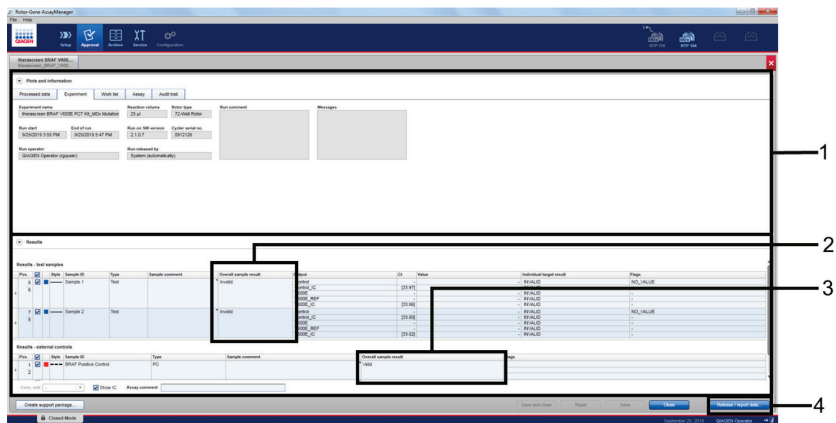


Figure 24. “Assay results” window. 1 = “Plots and information” section. 2 = “Results” section. 3 = “Overall sample results” section. 4 = “Release/Report data”.

Note: The *BRAF* V600E assay profile 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. If the Sample Control results are within an acceptable range, the “Sample status” column will report “Valid”. This indicates that the sample DNA concentration is suitable for mutation analysis.

See information related to the re-testing procedure from page 49, for an explanation of the software flags.

11. Click "OK" to save the experiment to the archive and create a LIMS output and run report (Figure 25).

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.

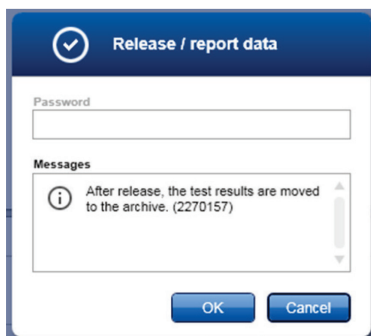


Figure 25. "Release/report data" dialog.

12. Click "OK" when the "Status of release" dialog appears. (Figure 26).

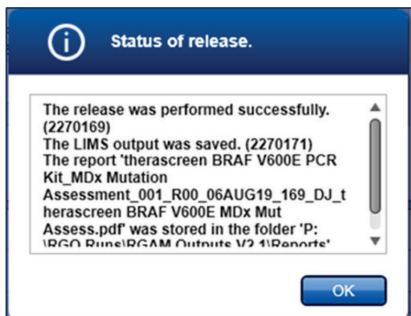


Figure 26. "Status of release" dialog.

13. To view an experiment stored in the experiment archive, click the “Archive” tab and search for the experiment using the search criteria in the “Filter Options” section. Click “Apply filter” to search. Select an experiment(s) by checking the box next to the experiment(s) you want to view and click “Show assays” (Figure 27).

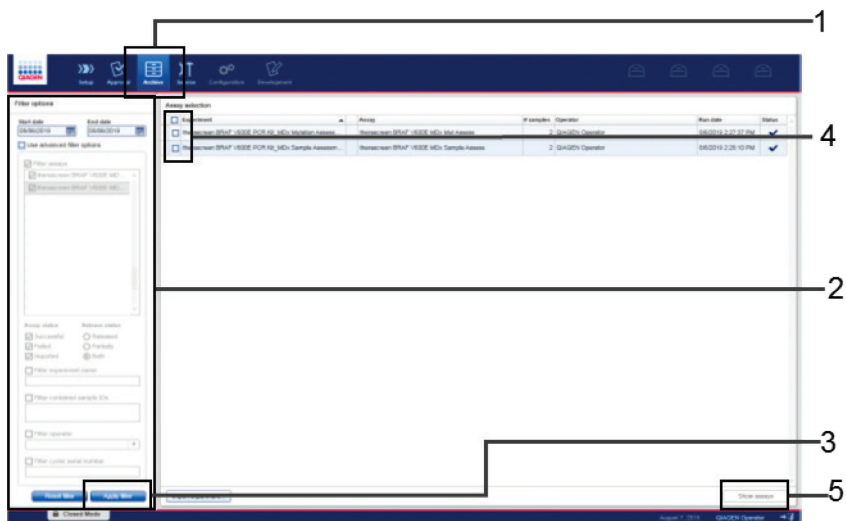


Figure 27. “Experiment Archive” window. 1 = “Archive” tab. 2 = “Filter options” section. 3 = “Apply filter”. 4 = Check box to select experiment. 5 = “Show assays” button.

Retesting: Run validity

The run validity flags discussed within this section apply to both sample assessment and mutation assessment.

All possible flags corresponding to the Rotor-Gene AssayManager v2.1 Gamma MDx Plug-in are listed in the *Rotor-Gene AssayManager v2.1 Gamma MDx (US) Plug-in User Manual*.

Table 5 lists the possible flags that may be generated by the *therascreen* BRAF V600E Assay Profile, their meaning and actions to be taken.

Table 5. Run validity flags

Flag	Description
BELOW_ACCEPTED_RANGE	The PC or IC C _T value is below the specification range for the target. Action: The run should be repeated.
ABOVE_ACCEPTED_RANGE	The PC or IC C _T value is above the specification range for the target. Action: The run should be repeated.
NO_VALUE	There is no C _T value observed where a value is expected. Action: The run should be repeated.
UNEXPECTED_VALUE	There is a C _T value present where none was expected, this is indicative of contamination. Action: Clean your workspace as per local procedures and repeat the run.

Retesting: Sample assessment

If a sample fails the sample assessment run, it may be re-tested one more time.

DNA input too low

If a sample fails the sample assessment run because the DNA input is too low, the software will determine the sample as invalid and display flags as described in Table 6.

Table 6. Sample assessment flag for samples with low DNA input

Flag	Description
NO_VALUE	This target has no value but it is expected to have one.
ABOVE_ACCEPTED_RANGE	The target value is higher than a defined range; this can be a C _t Value above control working (measurable) range of the assay or above IC or PC specifications.

The sample will be re-tested in a sample assessment run.

If the sample fails the sample assessment run due to DNA input too low for a second time, a fresh extraction of two sections from the same sample may be undertaken. This may then go through two rounds of sample assessment as noted above.

If the sample does not return a valid result after all retests described above, it is deemed not suitable for use. This sample will be recorded as indeterminate.

Note: For indeterminate samples, a second specimen from the same patient may be requested and tested.

DNA input too high

If a sample fails the sample assessment run because the DNA input is too high, the software will determine the sample as invalid and display the flag in Table 7.

Table 7. Sample assessment flag for highly concentrated samples

Flag	Description
SAMPLE_CTRL_HIGH_CONC	Sample invalid. Sample DNA concentration too high. Dilute sample to increase control C_T value. This dilution should be calculated on the assumption that diluting 1 in 2 with the water supplied in the kit will increase the C_T by 1.0; once sample is diluted, set up new PCR run to repeat sample.

The sample may be diluted using water for dilution provided in the kit to bring the DNA input within the acceptable measurable range of the assay (Control C_T 20.95–33.00). A 1-in-2 dilution of the sample is expected to increase the C_T by approximately 1.

The diluted sample should then undergo a sample assessment run. If the flag is shown again after dilution, repeat dilution steps as required.

Once the appropriate dilution of the sample has been identified by the sample assessment procedures detailed above, the mutation assessment should be performed on that diluted sample.

Retesting: Mutation assessment

DNA input too low

If a sample has passed sample assessment and proceeded to mutation assessment, but produces an invalid result due to low DNA input, one of the two flags as listed in Table 6 will be shown.

The sample should be re-tested one more time. If the same flags are shown again upon retest of the mutation assessment, then a re-extraction from two sections from the same specimen should be executed. The newly extracted DNA sample then enters the workflow at the sample assessment stage.

If the re-extracted sample is invalid after two rounds of mutation assessment, the sample status must be reported as “Indeterminate” and no further testing carried out.

Note: For indeterminate samples, a second specimen from the same patient may be requested and tested.

IC failure

If the sample is invalid due to IC failure (software displays flag `SAMPLE_INT_CTRL_FAIL`) after the mutation assessment, the sample should be diluted with the Water for Sample Dilution (included in the *therascreen* BRAF V600E RGQ PCR Kit) according to Table 8. The diluted sample should return to the start of the mutation assessment phase.

Table 8. Recommended dilution factor based on control C_T

Control reaction C _T	Recommended dilution factor
<30.00	8
≥30.00	4

If the DNA input is too low (flags as shown in Table 6) after 1 in 8 dilution, then a lower dilution factor (1 in 4) should be used and the mutation assessment performed again.

If the SAMPLE_INT_CTRL_FAIL flag is displayed after two rounds of mutation assessment failure following sample dilution, then a re-extraction from two sections of the same specimen should be executed. The newly extracted DNA sample then enters the workflow at the sample assessment stage.

If the re-extracted sample is invalid due to the IC after two rounds of assessment following the sample assessment retesting (page 46) and mutation assessment retesting (page 49), then the result should be reported as "Indeterminate" and no further testing carried out.

Note: For indeterminate samples, a second specimen from the same patient may be requested and tested.

Interpretation of Results

The *BRAF* V600E assay and sample analysis is performed automatically by *BRAF* V600E Assay Profiles once a run is completed. The following information explains how the analysis is performed by the software.

The PCR cycle at which the fluorescence from a particular reaction crosses a threshold value is defined as the C_T value. C_T values indicate the quantity of specific input DNA. Low C_T values indicate higher input DNA levels and high C_T values indicate lower input DNA levels. Rotor-Gene AssayManager interpolates fluorescence signals between any two recorded values. The C_T values can therefore be any real number (not limited to integers) within the range of 0 to 40.

The run controls (positive control, NTC and internal controls) are assessed to ensure that all C_T values are within the acceptable ranges. Sample ΔC_T values are calculated as the difference between the mutation reaction C_T and control reaction C_T from the same sample. The sample status will be displayed as "Valid" or "Invalid".

The sample result is either "*BRAF* Mutation detected", "No Mutation Detected" or "Invalid". Any condition that indicates an issue with the tube or sample will generate a flag or warning that describes the cause of the issue. The flags and warnings will be displayed on the result page and on the report.

Software flags

For a list of software flags, see the *Rotor-Gene AssayManager v2.1 Gamma MDx (US) Plug-in User Manual*. Additional flags not covered by the *Rotor-Gene AssayManager v2.1 Gamma MDx (US) Plug-in User Manual* and that are specific to the BRAF V600E assay are listed in Table 9.

Table 9. BRAF V600E assay software flags

Flag	Description
SAMPLE_CTRL_HIGH_CONC	Sample invalid. Sample DNA concentration too high. Dilute sample to increase control C_T value. This dilution should be calculated on the assumption that diluting 1 in 2 with the water supplied in the kit will increase the C_T by 1.0; once sample is diluted. Set up new PCR run to repeat sample.
SAMPLE_CTRL_INT_CTRL_FAIL	C_T too high (or no C_T) for internal control (HEX) and C_T too high (or no C_T) for the control reaction (FAM).
SAMPLE_INT_CTRL_FAIL	C_T too high (or no C_T) for internal control (HEX) and no C_T for the mutation reaction (FAM).
UNEXPECTED_VALUE	The target has a value where it is not expected to have one. This can be a C_T , endpoint – fluorescence, concentration, or calculated value, e.g., mean C_T , or delta C_T
NO_VALUE	This target has no value where it is expected to have one.
BELOW_ACCEPTED_RANGE	The target value is below a defined range. This can be a C_T value below measurable range of the assay or below IC or PC specifications.
ABOVE_ACCEPTED_RANGE	The target value is higher than a defined range. This can be a C_T Value above measurable range of the assay or above IC or PC specifications.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Comments and suggestions

“Low Fluorescence” flag in Positive Control (PC) tube

Low fluorescence in the mutation reaction	Repeat the PCR ensuring accurate pipetting and mixing of reagents.
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“No value” flag in Positive Control (PC)

- | | |
|--|---|
| 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 Handling and Storage”, page 18 | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary. |

“Unexpected value” flag in NTC

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

“Above acceptable range” or “Below acceptable range” flag in PC or NTC

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

Comments and suggestions

“Below acceptable range” or “Sample Ctrl High Conc” flag in the sample tube

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.]).
----------------------------	---

“Above acceptable range” flag in the sample tube

Insufficient starting DNA template present in sample	Retest one more time. If system shows the same flag second time, re-extract DNA using two slides from the same sample of resected tissue and repeat PCR. 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 should be recorded as “Indeterminate” and no further testing carried out.
--	--

“No value” flag in Control (sample) tube

No amplifiable DNA template present in sample	Retest one more time. If system shows the same flag second time, re-extract DNA using two slides from the same sample of resected tissue and repeat PCR. 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 should be recorded as “Indeterminate” and no further testing carried out.
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“Sample Internal Control (Int Ctrl) Fail” flag in sample tube

C_T too high (or no C_T) for internal control and no C_T for the mutation reaction (FAM)	Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]) as described in “IC failure”, page 49.
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Quality Control

In accordance with QIAGEN’s ISO-certified Quality Management System, each lot of *therascreen* BRAF V600E RGQ PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Performance Characteristics

Analytical performance

Assay cutoff

A study was conducted to establish the assay cutoff for BRAF V600E mutation detection using 82 FFPE CRC samples (RES) that met the DNA quality assessment, i.e., the control reaction C_T values fell within the assay measurable range (Control Working Range [CWR] C_T 20.95 to 33.00). The cutoff value is based on the C_T of the Control reaction subtracted from the C_T of the V600E Mutant reaction (ΔC_T) are shown in Table 10. Samples with $\Delta C_T \leq 7.0$ are BRAF V600E "BRAF Mutation Detected". Samples with $\Delta C_T > 7.0$ are BRAF V600E "No Mutation Detected".

Table 10. Established cutoff value for the *therascreen* BRAF V600E RGQ PCR Kit

ΔC_T (Control reaction C_T – V600E Mutation reaction C_T)	
Cutoff ($\leq \Delta C_T$)	7.0

Limit of blank

To assess performance of the *therascreen* BRAF V600E RGQ PCR Kit in the absence of mutant-positive template and to ensure that a blank sample does not generate an analytical signal that may indicate a low concentration of mutation, samples with no template were evaluated. The results demonstrated no detectable control or mutant C_T value in any of the control or mutation reaction tubes.

Limit of detection (LoD)

The measurable range (CWR) for the *therascreen* BRAF V600E RGQ PCR Kit is based on the amount of amplifiable DNA in the specimen as determined by the Control reaction C_T value. The stated measurable range for the assay is defined by the control C_T pre-specified range of 20.95 to 33.00. The LoD is the minimum percentage of mutant DNA that can be detected in a background of wild-type DNA, when the total amplifiable DNA is within the stated input range and still below the threshold cutoff ΔC_T value.

Studies were conducted to determine and verify the LoD of the V600E mutation for the *therascreen* BRAF V600E RGQ PCR Kit. The limit of detecting mutant DNA in a background of wild-type DNA is defined as the lowest Mutation Allelic Fraction (MAF) at which 95% of the test replicates for V600E mutation-positive sample were determined to be positive. The LoD values determined by Probit analysis (Table 11), were successfully verified using additional clinical FFPE CRC BRAF V600E positive specimens (diluted to the determined LoD levels). The overall LoD for the BRAF V600E system is reported as 7.8% (MAF), which represents the highest LoD value observed across all DNA inputs.

Table 11. LoD values

DNA input level	Control C_T range	LoD verified (MAF%)	Results of LoD verification (positive/total replicates, %)
High	≥ 20.95 to ≤ 25.00	2%	108/108, 100%
Medium	> 25.00 to ≤ 29.38	3.5%	107/108, 99.07%
Low	> 29.38 to ≤ 33.00	7.8%	107/107, 100%
Low (CNB)	> 29.38 to ≤ 33.00	7.8%	81/81, 100%

Effect of DNA Input on Results

When samples at different total DNA levels contain the same proportion of mutant DNA, it is expected that the measured ΔC_T values will remain consistent. DNA extracted from four FFPE CRC samples (two wild-type and two mutant) were used to prepare pools of DNA with the lowest achievable control reaction C_T and diluted across a minimum of six dilution levels that span the entirety of the CWR (measurable range of the assay).

For each sample tested, the linear, quadratic and cubic regression models were fitted to the data with ΔC_T (for positive samples) or Control C_T values (for negative samples) as the response variable and dilution number as the continuous explanatory variable. The regression model results were reported along with the corresponding standard errors, 95% confidence limits and p-values. Linearity was demonstrated for all samples throughout the measurable range tested, there were no statistically significant differences in the regression models. Some quadratic and cubic effects were observed but the maximum difference predicted was within the precision limit (i.e., $<2x$ standard deviation). The results demonstrated that DNA input, when within the CWR, will not yield different conclusions.

Interfering substances

Exogenous substances

Potential interfering substances present in the DNA extraction process were tested in mutant and wild-type samples at the highest concentration of the substance that could remain in the final eluate after the washing and purification steps. The substances assessed were: paraffin wax, xylene, ethanol, Buffer ATL, proteinase K, Buffer AL, Buffer AW1 and Buffer AW2. The results demonstrated that these substances did not interfere with the call results of the *therascreen* BRAF V600E RGQ PCR Kit.

Hemoglobin

Clinical CRC specimens with spiked hemoglobin content up to 2 mg/ml (or 2 µg/µl, according to the CLSI EP7-A2) did not interfere with call results of the *therascreen* BRAF V600E RGQ PCR Kit for both BRAF V600E mutant and wild-type specimens.

Necrotic tissue

The presence of necrotic tissue in the range of 30–100% within CRC FFPE samples (one RES sample and six CNB samples) was shown to not impact the amplification of the control or the internal control reactions, nor does it impact on the ability of the *therascreen* BRAF V600E RGQ PCR Kit to produce valid and correct results.

Cross-contamination

Tests were carried out to verify that the system does not introduce cross-contamination (carryover) between samples and runs, which could potentially lead to false-positive results. Potential sources of cross-contamination include:

- Sample extraction (e.g., scraping of slides)
- Pipetting of samples
- Closure (“capping”) of sample tubes
- Contamination of kit reagents during use
- Loading of assay tubes onto the Rotor-Gene Q MDx (US) instrument

The assessment of cross-contamination was carried out by extracting high-positive mutant samples (mutant samples with low Control C_T and low ΔC_T values) and wild-type (WT) samples (wild-type to *BRAF* V600E) within the same DNA extraction runs (four extraction runs performed) and followed by testing these samples in four “checker board” (containing both WT and MT samples) PCR runs and two “WT only” PCR runs. Any WT samples that produced false-positive results would be used as evidence of cross-contamination.

The results of this study indicated no detectable cross-contamination and 100% correct mutation calls were observed for all WT replicates (131 in total).

Cross-reactivity (assay specificity)

The absence of cross-reactivity of the *therascreen* BRAF V600E RGQ PCR Kit with other BRAF V600 mutations such as V600Ec, V600D, V600K, V600M, V600R and V600G was assessed using concentrated cell-line gDNA at high MAF.

The assay showed no cross reactivity to V600K, V600R, V600M and V600G at all concentrations (up to 100% MAF) tested. Cross-reactivity was observed for V600Ec and V600D using cell-line gDNA. The design of *therascreen* BRAF V600E RGQ PCR Kit does not distinguish between V600E and V600Ec mutations. However, V600Ec was not able to be validated as a target because no clinical specimens were identified in the BEACON study.

The cross reactivity of BRAF V600D was confirmed using cell-line DNA. No BRAF V600D positive clinical specimens were identified in the clinical study due to the absence of BRAF V600D in the CRC population. The clinical impact of the cross-reactivity observed for the *therascreen* BRAF V600E RGQ PCR Kit is small.

Precision: Repeatability

The precision of the *therascreen* BRAF V600E RGQ PCR Kit within-laboratory (repeatability) was assessed. Both correctness of mutation call results and the precision of ΔC_T values (the difference in C_T values between a Mutation Reaction and the Control Reaction) for positive test samples and Control C_T values for negative test samples are reported.

Clinical CRC (primary and metastatic) FFPE samples representing different DNA input levels and different mutation percentage levels were used for this evaluation. The proportion of correct call is presented in Table 12 for each test panel and the quantitative precision values are presented in Table 13.

Table 12. Proportion of correct calls overall for Repeatability study

Sample	Grouping variables		Proportion Fraction, %	Two-sided 95% confidence limit	
	Acquisition	Mutation level		Lower	Upper
Test Panel 1	RES	WT	81/81, 100.00	95.55%	100.00%
Test Panel 2	RES	WT	81/81, 100.00	95.55%	100.00%
Test Panel 3	CNB	Negative to Low Positive (below LoD)	78/81, 96.30%	89.56%	99.23%
Test Panel 4	RES	LOD	81/81, 100.00	95.55%	100.00%
Test Panel 5	RES	LOD	81/81, 100.00	95.55%	100.00%
Test Panel 6	CNB	LOD	80/81, 98.77%	93.31%	99.97%
Test Panel 7	RES	LOD	81/81, 100.00	95.55%	100.00%
Test Panel 8	RES	2 x LoD	81/81, 100.00	95.55%	100.00%
Test Panel 9	RES	2 x LoD	79/79, 100.00	95.44%	100.00%

CNB: Core needle biopsy; RES: Surgical resection.

Table 13. Repeatability and intermediate precision v values

Mutation level	No. amplified	Mean	Between run key order (SD, %CV)	Between kit lot (SD, %CV)	Between operator (SD, %CV)	Between instrument (SD, %CV)	Between day (SD, %CV)	Between template (SD, %CV)	Residual(SD, %CV)	Total (SD, %CV)
2 x LoD	160	3.57	(0.0844, 2.36%)	(0.0647, 1.81%)	(0.0559, 1.56%)	(0.0000, 0.00%)	(0.0445, 1.25%)	(1.5281, 42.75%)	(0.4967, 13.90%)	(1.1981, 33.52%)
LoD	323	3.90	(0.0653, 1.68%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(1.3368, 34.32%)	(0.4267, 10.95%)	(1.2365, 31.74%)
2 x LoD	160	28.75	(0.1349, 0.47%)	(0.0000, 0.00%)	(0.0926, 0.32%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(2.9368, 10.22%)	(0.2591, 0.90%)	(2.1049, 7.32%)
LoD	324	27.73	(0.0913, 0.33%)	(0.0476, 0.17%)	(0.0361, 0.13%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(3.8626, 13.93%)	(0.1695, 0.61%)	(3.3562, 12.10%)
WT	243	29.91	(0.0517, 0.17%)	(0.0314, 0.11%)	(0.0000, 0.00%)	(0.0063, 0.02%)	(0.0000, 0.00%)	(2.1832, 7.30%)	(0.2490, 0.83%)	(1.8045, 6.03%)
2 x LoD	160	32.32	(0.0000, 0.00%)	(0.0934, 0.29%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(1.4071, 4.35%)	(0.4463, 1.38%)	(1.0955, 3.39%)
LoD	324	31.63	(0.1527, 0.48%)	(0.0419, 0.13%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(2.9075, 9.19%)	(0.4400, 1.39%)	(2.5646, 8.11%)

Reproducibility

The precision of the *therascreen* BRAF V600E RGQ PCR Kit between-laboratories (reproducibility) was assessed. Three different laboratories (test sites) were used. The same test panel was used for this study as for the repeatability study. The proportion of correct mutation calls and concordance of calls between kit lots for each sample was calculated and reported in Table 14. The quantitative precision values (including site to site) are presented in Table 15.

Table 14. Proportion of correct calls for all sample panel members across all sites

Grouping variables		Fraction		Two-sided 95% confidence limit	
Sample	Mutation level	Proportion	Percentage	Lower	Upper
Test Panel 1	WT	81 / 81	100.00%	95.55%	100.00%
Test Panel 2	WT	80 / 80	100.00%	95.49%	100.00%
Test Panel 3	WT	81 / 81	100.00%	95.55%	100.00%
Test Panel 4	LoD	81 / 81	100.00%	95.55%	100.00%
Test Panel 5	LoD	81 / 81	100.00%	95.55%	100.00%
Test Panel 6	LoD	80 / 81	98.77%	93.31%	99.97%
Test Panel 7	LoD	81 / 81	100.00%	95.55%	100.00%
Test Panel 8	2 x LoD	81 / 81	100.00%	95.55%	100.00%
Test Panel 9	2 x LoD	81 / 81	100.00%	95.55%	100.00%

Table 15. Reproducibility precision values

Analysis variable	Mutation level	No. amplified	Mean	Between Site (SD, %CV)	Between run key Order (SD, %CV)	Between template (SD, % CV)	Between operator within site (SD, % CV)	Between instrument (SD, % CV)	Between day within Site (SD, %CV)	Residual (SD, %CV)	#Total (SD, % CV)
ΔC_T	2 x LoD	162	3.64	(0.0800, 2.20%)	(0.0587, 1.61%)	(1.4756, 40.50%)	(0.1004, 2.75%)	(0.0439, 1.21%)	(0.1006, 2.76%)	(0.4654, 12.77%)	(1.1570, 31.75%)
	LoD	323	3.89	(0.0460, 1.19%)	(0.0747, 1.92%)	(1.3340, 34.33%)	(0.0507, 1.31%)	(0.0000, 0.00%)	(0.0704, 1.81%)	(0.4486, 11.54%)	(1.2459, 32.07%)
Green C_T Control assay	2 x LoD	162	28.61	(0.1208, 0.42%)	(0.0803, 0.28%)	(2.9372, 10.27%)	(0.0830, 0.29%)	(0.0511, 0.18%)	(0.0000, 0.00%)	(0.1878, 0.66%)	(2.0976, 7.33%)
	LoD	324	27.62	(0.1260, 0.46%)	(0.0770, 0.28%)	(3.8494, 13.93%)	(0.0240, 0.09%)	(0.0624, 0.23%)	(0.0000, 0.00%)	(0.1518, 0.55%)	(3.3452, 12.11%)
Green C_T V600E assay	WT	242	29.82	(0.0821, 0.28%)	(0.0893, 0.30%)	(2.1804, 7.31%)	(0.0478, 0.16%)	(0.0279, 0.09%)	(0.0000, 0.00%)	(0.2150, 0.72%)	(1.7971, 6.03%)
	2 x LoD	162	32.25	(0.2626, 0.81%)	(0.1537, 0.48%)	(1.4599, 4.53%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.0000, 0.00%)	(0.4617, 1.43%)	(1.1639, 3.61%)
	LoD	323	31.50	(0.1957, 0.62%)	(0.0849, 0.27%)	(2.9052, 9.22%)	(0.0780, 0.25%)	(0.0000, 0.00%)	(0.0810, 0.26%)	(0.4253, 1.35%)	(2.5652, 8.14%)

Sample handling variability

Sample handling variability was assessed for the *therascreen* BRAF V600E RGQ PCR Kit, specifically DNA extraction at multiple sites.

Sequential sections of 4–5 µm were cut from each of one CNB and eight FFPE RES samples, of which five were wild-type (including the CNB sample) and four were mutant (36 sections were cut per RES sample and 24 from the CNB CRC sample).

In total, 156 extractions were performed and the rate of correct calls observed across all samples from all sites was 100% for the RES and CNB mutant samples, a rate of 97.22% correct calls was observed for the RES wild-type samples (Table 16).

Table 16. Proportion of correct calls for all samples

Grouping variables		Proportion		Two-sided 95% confidence limit	
Sample level	Acquisition method	N correct/total	Proportion	Upper	Lower
MT	RES	72 / 72	100.00%	95.01%	100.00%
WT	CNB	12 / 12	100.00%	73.54%	100.00%
–	RES	70 / 72	97.22%	90.32%	99.66%

Accuracy: Comparison to the analytical reference method

A study demonstrated the concordance in mutation detection of the *therascreen* BRAF V600E RGQ PCR Kit relative to a validated bi-directional Sanger sequencing method. In this study, a total of 600 FFPE samples from the clinical trial were tested and received results from both methods.

Samples with both Sanger and *therascreen* BRAF V600E RGQ PCR Kit valid results were analyzed to assess the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA). These percentages, together with the corresponding two-sided 95% confidence intervals (CI), are summarized in Table 17. Of the 600 samples, approximately 13% of these samples were indeterminate or invalid by the *therascreen* BRAF

V600E RGQ PCR Kit. The data from these samples were used to determine the level of concordance between the two methods. The overall percent agreement, positive percent agreement and negative percent agreement were reported with the corresponding two-sided exact 95% confidence limits (Table 15) without adjusting for the enrichment of CTA positives. Agreement was demonstrated by assessing the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA) from both of sets of results. Table 17 demonstrates an overall agreement of 95%. Breakdown of the results including indeterminate or invalid are summarized in Table 18.

Table 17. Sanger and BRAF V600E RGQ PCR Kit agreements and confidence limits (Sanger as reference method)

Measure of agreement	Frequencies	Percent agreement	Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit	Clopper-Pearson (Exact) binomial upper two-sided 95% confidence limit
Overall percent agreement	397/417	95.20	92.69	97.05
Positive percent agreement	192/192	100.00	98.10	100.00
Negative percent agreement	205/225	91.11	86.61	94.49

Table 18. 3x3 table including Indeterminate or invalid results by both methods

Frequency		Bi-directional Sanger BRAF result			Total
		Indeterminate	Mutation Detected	No Mutation Detected	
<i>therascreen</i> BRAF V600E result	Indeterminate or invalid	32	24	23	79
	Mutation Detected	44	192	20	256
	No Mutation Detected	60	0	205	265
	Total	136	216	248	600

Clinical performance

The *therascreen* BRAF V600E PCR Kit is intended to be used as a companion diagnostic test to aid clinicians in identifying colorectal cancer (CRC) patients who may be eligible for treatment with encorafenib + cetuximab based on a BRAF V600E mutation detected in CRC tumor tissue samples.

Clinical study BEACON and outcome

The BEACON CRC Study (Binimetinib, Encorafenib And Cetuximab Combined to Treat BRAF-mutant Colorectal Cancer) was a multicenter, randomized, open-label, 3-arm phase 3 study of encorafenib + cetuximab plus (Triplet arm) or minus (Doublet arm) binimetinib vs. irinotecan/cetuximab or infusional 5-fluorouracil (5-FU)/ folinic acid (FA)/irinotecan (FOLFIRI)/cetuximab (Control arm) in patients with BRAF V600E-mutant metastatic CRC. There are 665 patients (224 Triplet arm; 220 Doublet arm; 221 Control arm) enrolled in the BEACON study. The primary endpoints of the study were Triplet arm vs. Control arm overall survival (OS) and overall response rate (ORR) by BICR (Blinded Independent Central Review per Response Evaluation Criteria in Solid Tumors (RECIST v1.1)). The secondary endpoints of the study for the Doublet arm vs. Control arm included overall survival (OS) and overall response rate (ORR) by BICR (Blinded Independent Central Review) per Response Evaluation Criteria in Solid Tumors (RECIST v1.1).

The study demonstrated a statistically significant clinical improvement in OS and confirmed ORR by BICR for the Doublet arm vs. the Control arm.

The analysis of the OS found 40% reduction in risk of death was observed for the Doublet arm compared to the Control arm (HR 0.60, 95% CI: 0.45, 0.79). The median OS in the Doublet arm was 2.99 months longer than that in the Control arm, with median OS estimates using Kaplan-Meier methodology of 8.41 months (95% CI: 7.46, 11.04) in the Doublet arm and 5.42 months (95% CI: 4.76, 6.57) in the Control arm (one sided $p=0.0002$, stratified log-rank test) (Figure 28).

The analysis of confirmed ORR by BICR found a significantly higher response rate in the Phase 3 Response Efficacy Set (the first 330 randomized patients and any additional patients randomized on the same day as the 330th randomized patient, into the Phase 3 portion of the study) in the Doublet arm compared to the Control arm (20.4% vs. 1.9%) (one sided $p < 0.0001$, Cochran-Mantel-Haenszel test). Confirmed CR by BICR was observed in 5.3% and 0.0% of patients in the Doublet and Control arms, respectively.

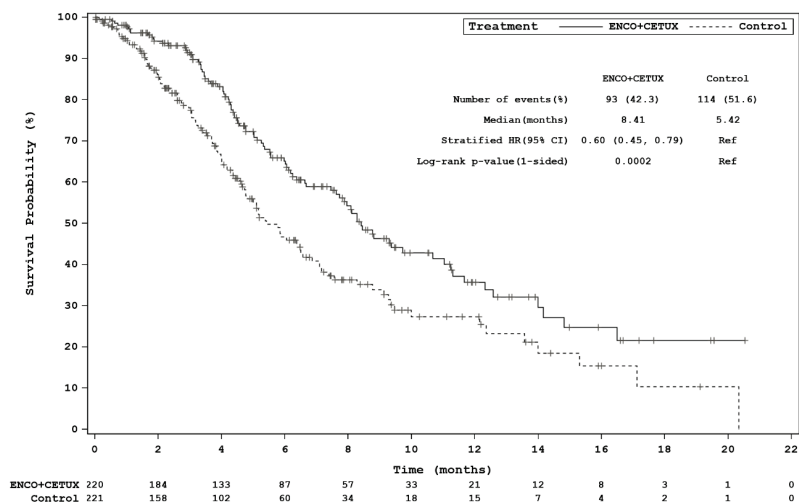


Figure 28. Kaplan-Meierplot of OS, Doublet arm (referred to as “ENCO +CETUX”) vs. Control arm (randomized Phase 3, full analysis set).

Concordance of CDx Assay vs CTA and LDT pre-screening

In the BEACON study, the *BRAF* V600E mutation status for screening and enrollment of patients was determined by a clinical trial assay (CTA) with/without pre-screening by a Laboratory Developed Test (LDT).

Concordance between the *therascreen* BRAF V600E RGQ PCR Kit (CDx) and the CTA was assessed. The measures of agreement including the respective Clopper-Pearson exact two-sided 95% confidence intervals for patients without prescreening by a LDT are provided in Table 19.

Table 19. Measures of agreement between CTA and CDx

Reference method	Measure of agreement	Frequencies	Percent agreement	Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit	Clopper-Pearson (exact) binomial upper two-sided 95% confidence limit
None	OPA	679/680	99.85	99.18	100.00
CDx	PPA	263/264	99.62	97.91	99.99
	NPA	416/416	100.00	99.12	100.00
CTA	PPA	263/263	100.00	98.61	100.00
	NPA	416/417	99.76	98.67	99.99

OPA: Overall percent agreement; PPA: Positive percent agreement; NPA: Negative percent agreement.

The *therascreen* BRAF V600E RGQ PCR Kit (CDx) demonstrated similar clinical efficacy to that determined by the CTA in the BEACON study. Efficacy analyses were conducted in terms of OS and ORR using data from randomized CDx-positive patients of the BEACON clinical trial population. For the OS efficacy analysis, the median survival time in the Doublet arm was 8.4 months, compared to a median survival time of 5.2 months in the Control arm and the stratified hazard ratio was 0.550 (95% CI: 0.406, 0.744). The CDx results are similar to those reported from the BEACON study (with the CTA), where a stratified hazard ratio of 0.60 (95% CI: 0.45, 0.79) was observed and median survival times in the Doublet and Control arms were 8.41 and 5.42 months, respectively.

The ORR in the Doublet arm was 17.6% (95% CI: 12.4%, 23.8%) compared to 1.1% (95% CI: 0.1%, 3.8%) in the Control arm. These results are in line with those reported from the BEACON trial where ORR values of 20.4% and 1.9% were observed in the Doublet and Control arms, respectively.















PPA and NPA between the *therascreen* BRAF V600E RGQ PCR Kit and LDT, with the LDT as the reference method, were 97.20% (95% CI: 95.42%, 98.42%) and 95.24% (95% CI: 89.92%, 98.23%), respectively. The high level of concordance between the LDT and the *therascreen* BRAF V600E RGQ PCR Kit shows that the impact of pre-screening samples with the LDT is minimal, as for 96.82% (95% CI: 95.18%, 98.02%; OPA) of patients the results would be unchanged from the LDT compared to the CDx.

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2. Whitcombe, D., Theaker, J., Guy, S.P., Brown, T., Little, S. (1999) Detection of PCR products using self-probing amplicons and fluorescence. *Nat. Biotechnol.* **17**, 804.

Symbols

The following symbols may appear 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
	Components
	Contains
	Number
	Global Trade Item Number
	Prescription use only
	Temperature limitation
	Manufacturer
	Consult instructions for use

Symbol

Symbol definition



Caution



Protect from light

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Ordering Information

Product	Contents	Cat. no.
<i>therascreen</i> BRAF V600E RGQ PCR Kit	For 24 reactions: Control Reaction Mix, V600E Reaction Mix, <i>BRAF</i> Positive Control, <i>Taq</i> DNA Polymerase, Water for NTC, Water for Dilution	874821
QIAamp DSP DNA FFPE Tissue Kit — for purification of genomic DNA from paraffin-embedded tissues		
QIAamp DSP DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	60404
Rotor-Gene Q MDx (US) and accessories		
Rotor-Gene Q MDx System (US)	Real-time PCR cycler with 6 channels, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002036
Rotor-Gene Q MDx Platform (US)	Real-time PCR cycler with 6 channels, laptop computer, software, accessories, 1-year warranty on parts and labor	9002035
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions of 20–50 µl	981005
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

Product	Contents	Cat. no.
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
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 (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions of 10–50 µl	981106

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Handbook Revision History

Date	Changes
R1, April 2020	Initial release

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