

therascreen® FGFR RGQ RT-PCR Kit Instructions for Use

IVD

For In Vitro Diagnostic Use

This Instructions for Use is applicable for:



REF

Version

therascreen FGFR RGQ RT-PCR Kit

24

874755

Version 2



QIAGEN, GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

Table of Contents

1. Intended Use	4
1.1. Intended User	4
2. Description and Principle	5
2.1. Summary and explanation	5
3. Materials Provided	11
3.1. Kit contents	11
3.2. Platform and software	11
4. Materials Required but Not Provided	13
4.1. Equipment, consumables, and reagents for RNA purification	13
4.2. Equipment and consumables for reverse transcription and real-time PCR	13
5. Warnings and Precautions	15
5.1. Safety information	15
5.2. Precautions	16
6. Disposal	18
7. Storage and Handling	19
7.1. Reagent storage and handling	19
7.2. Specimen Handling and Storage	20
8. Quality Control	22
9. Procedure	23
9.1. Protocol A: RNA extraction and preparation	23
9.2. Protocol B: Quantification and normalization of RNA	24
9.3. Protocol C: Reverse transcription	24
9.4. Real-time PCR on the instrument	27
9.5. Using Rotor-Gene AssayManager version 2.1 software	30
10. Interpretation of Results	42
11. Limitations	45
12. Performance Characteristics	47
12.1. Limit of blank	47
12.2. Assay cutoff and cross-reactivity	47
12.3. Assay cross-reactivity and analytical specificity	48
12.4. Limit of detection	49
12.5. Repeatability and reproducibility	49
12.6. Specimen handling	51
12.7. Lot-to-lot interchangeability	52
12.8. Cross-contamination/analytical carryover	52

12.9. Interfering substances	53
12.10. Correlation to reference method	53
12.11. Clinical outcome data	54
13. References	58
14. Troubleshooting Guide	59
15. Symbols	60
16. Contact Information	62
17. Ordering Information	63
18. Document Revision History	65

1. Intended Use

The *therascreen*® FGFR RGQ RT-PCR Kit is a real-time, reverse transcription PCR test for the qualitative detection of two point mutations in exon 7 [p.R248C (c.742C>T), p.S249C (c.746C>G)], two point mutations in exon 10 [p.G370C (c.1108G>T) and p.Y373C (c.1118A>G)] and two fusions (FGFR3-TACC3v1 and FGFR3-TACC3v3) in the fibroblast growth factor receptor 3 (FGFR3) gene in RNA samples derived from formalin-fixed paraffin-embedded (FFPE) urothelial tumor tissue. The test is indicated for use as an aid in identifying patients with cases of urothelial cancer (UC) that harbor these alterations and are therefore eligible for treatment with BALVERSA® (erdafitinib).

Specimens are processed using the RNeasy® DSP FFPE Kit for manual sample preparation followed by reverse transcription and then automated amplification and detection on the Rotor-Gene® Q MDx 5plex HRM instrument.

1.1. Intended User

This kit is intended for professional use.

The product is to be used by personnel specifically instructed and trained in molecular biology techniques and familiar with this technology.

2. Description and Principle

2.1. Summary and explanation

Fibroblast growth factor receptors (*FGFRs*) are tyrosine kinases (transmembrane receptors) present in many types of cells within the body. When activated, *FGFRs* become phosphorylated at specific tyrosine residues that mediate interaction with cytosolic adaptor proteins and the RAS-MAPK, PI3K-AKT, PLC γ and STAT intracellular signaling pathways. This signaling pathway is known to play an important role in the control of cell growth, survival, and migration (1) and is therefore an attractive target for cancer therapies.

Activating alterations within the *FGFR* genes have been found in a subset of UC patients (2,3), demonstrating the importance of this type of genetic defect as a driver of tumor development and progression.

The *therascreen* FGFR RGQ RT-PCR Kit is a real-time, reverse transcription PCR test for the qualitative detection of 2 point mutations in exon 7 [p.R248C (c.742C>T) and p.S249C (c.746C>G)], 2 point mutations in exon 10 [p.G370C (c.1108G>T) and p.Y373C (c.1118A>G)] and 2 fusions [FGFR3-TACC3v1 and FGFR3-TACC3v3] in the fibroblast growth factor receptor 3 (*FGFR3*) gene in RNA samples derived from formalin-fixed paraffin-embedded (FFPE) urothelial tumor tissue. The test is indicated for use as an aid in identifying patients with cases of UC that harbor these alterations and are therefore eligible for treatment with BALVERSA (erdafitinib).

Specimens are processed using the RNeasy DSP FFPE Kit for manual sample preparation followed by reverse transcription and then automated amplification and detection on the instrument.

The *therascreen* FGFR RGQ RT-PCR Kit is also designed to identify *FGFR3* fusion *FGFR3*-*BAIAP2L1* and *FGFR2* fusions *FGFR2*-*BICC1* and *FGFR2*-*CASP7*, because patients harboring these *FGFR* fusions were eligible for the 42756493-BLC2001 clinical trial of BALVERSA (erdafitinib). However, the test is not clinically validated to detect these 3 fusions. Drug safety and efficacy has not been established for cases of UC harboring these fusions and no claims are made for the use of the *therascreen* FGFR RGQ RT-PCR Kit as an aid in the selection of such patients for treatment with BALVERSA (erdafitinib). Therefore, results for these alterations are not reported for samples. However, these targets are relevant for the purpose of PC and NTC validity.

The *FGFR* alteration targets of the *therascreen* *FGFR* RGQ RT-PCR Kit assays are detailed in Table 1 and Table 2.

Table 1. *therascreen* *FGFR* RGQ RT-PCR Kit assays targets: Point mutations

Gene	Amino acid variant	CDS mutation	Cosmic ID	Exons
FGFR3	p.R248C	c.742C>T	COSM714	7
FGFR3	p.G370C	c.1108G>T	COSM716	10
FGFR3	p.S249C	c.746C>G	COSM715	7
FGFR3	p.Y373C	c.1118A>G	COSM718	10

Table 2. *therascreen* *FGFR* RGQ RT-PCR Kit assay targets: Fusions

Fusion ID	Genes involved	Genomic breakpoints	Exons
FGFR3-TACC3v1	FGFR3	chr4:1808661 C	17
	TACC3	G chr4:1741428	11
FGFR3-TACC3v3	FGFR3	chr4:1808661 C	17
	TACC3	G chr4:1739324	10
FGFR3-BAIAP2L1	FGFR3	chr4:1808661 C	17
	BAIAP2L1 *	A chr7:97991744	2
FGFR2-BICC1	FGFR2	chr10:123243211 G	17
	BICC1 *	A chr10:60461834	3
FGFR2-CASP7	FGFR2	chr10:123243211 G	17
	CASP7*	A chr10:115457252	2

* The test was designed to identify FGFR2 fusions FGFR2-BICC1 and FGFR2-CASP7 and FGFR3 fusion FGFR3-BAIAP2L1 because these *FGFR* fusion patients were eligible for the trial. However, the QIAGEN test is not clinically validated to detect these 3 fusions and results are not reported for samples.

The *therascreen* *FGFR* RGQ RT-PCR Kit is based on the detection by selective amplification of 6 *FGFR3* alterations in RNA extracted from FFPE UC specimens, using the Rotor-Gene Q MDx 5plex HRM system. Allele-specific technology allows accurate and highly reproducible detection of target *FGFR* alterations, based on the use of specific forward and reverse primer and probe sets; only a perfect match between the primers and probes with the target cDNA allows extension and amplification in the PCR reaction. Result reporting is fully automated. If both the run controls and the sample results are valid and assay target amplification occurs

below the pre-determined cycle number cutoff threshold, the report will display the *FGFR* alteration(s) detected in each sample.

2.1.1. RNA purification

Formalin fixed, paraffin embedded (FFPE) samples are the most widely available form of solid tumor material. While they are stable and easy to transport and store, the fixation and embedding process damages nucleic acids and mixes them with waxy hydrocarbon materials. Specialist purification techniques must therefore be employed to obtain samples suitable for in vitro diagnostic analysis. The RNeasy DSP FFPE Kit has been developed specifically to deal with the challenges posed by RNA purification from FFPE material and must be used to prepare RNA that is to be tested using this kit.

2.1.2. Reverse transcription

To carry out the test procedure, complementary DNA (cDNA) is first synthesized from sample RNA using Reverse Transcriptase. It is this cDNA that then acts as the initial template in an analytical PCR reaction.

Reverse transcription (RT) is performed using a master mix prepared from Reverse Transcriptase enzyme, RT Buffers, and RT Primer Mix, which are all provided with the *therascreen* FGFR RGQ RT-PCR Kit. The RT reaction takes place at 42°C and is then stopped by incubation at 95°C.

Reverse Transcriptase is a multifunctional enzyme with 3 distinct enzymatic activities: an RNA-dependent DNA polymerase, a hybrid-dependent exoribonuclease (RNase H), and a DNA-dependent DNA polymerase. For reverse transcription in vitro, the first 2 activities are utilized to produce single-stranded cDNA. First, the RNA-dependent DNA-polymerase activity (reverse transcription) transcribes cDNA from an RNA template, forming a DNA:RNA hybrid. Subsequently, the RNase H exonuclease activity specifically degrades only the RNA strand of these hybrids (Figure 1). Therefore, this activity affects RNA hybridized to cDNA, but has no effect on pure RNA. A separate RNA degradation step using RNase H enzyme is not necessary prior to real-time PCR.

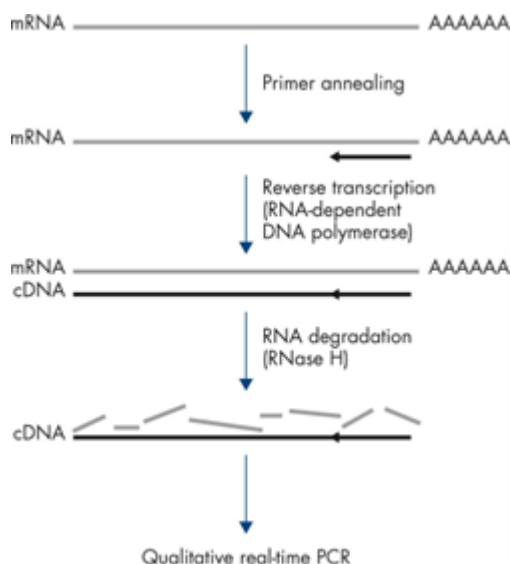


Figure 1. cDNA synthesis. Reverse transcription and first-strand cDNA synthesis.

2.1.3. Real-time PCR

The use of real-time PCR enables the detection of reaction products during the exponential phase of the PCR amplification process, rather than at its end, as is the case with endpoint PCR. This enhances test specificity and reduces the amount of time required to perform the test process.

The assay utilizes the real-time PCR oligonucleotide hydrolysis reaction principle with TaqMan® probes (4). Three types of oligonucleotides are used to detect each target base sequence; a pair of conventional PCR primers which are complementary to sequences up and downstream of the target sequence and form a PCR amplicon, and a probe molecule which is complementary to the precise target sequence and incorporates both a fluorescent reporter dye and a fluorescence quencher in close proximity. The 3' end of the probe is a di-deoxy base, to prevent its extension and hence prevent it from acting as another PCR primer in the reaction.

During PCR, if the target sequence of interest is present, the oligonucleotide probe will anneal to it, while the PCR primers anneal to their complementary sequences up- and downstream of the probe binding region. In the primer extension phase of the reaction, the 5' to 3' exonuclease activity of *Thermus aquaticus* (Taq) DNA polymerase cleaves the probe

oligonucleotide, freeing both the fluorescent reporter and quencher molecules. As these diffuse away from each other into solution, the effect of the quencher upon the reporter diminishes, leading to an increase in detectable fluorescence. This process occurs in every PCR cycle and does not interfere with the exponential accumulation of product (Figure 2).

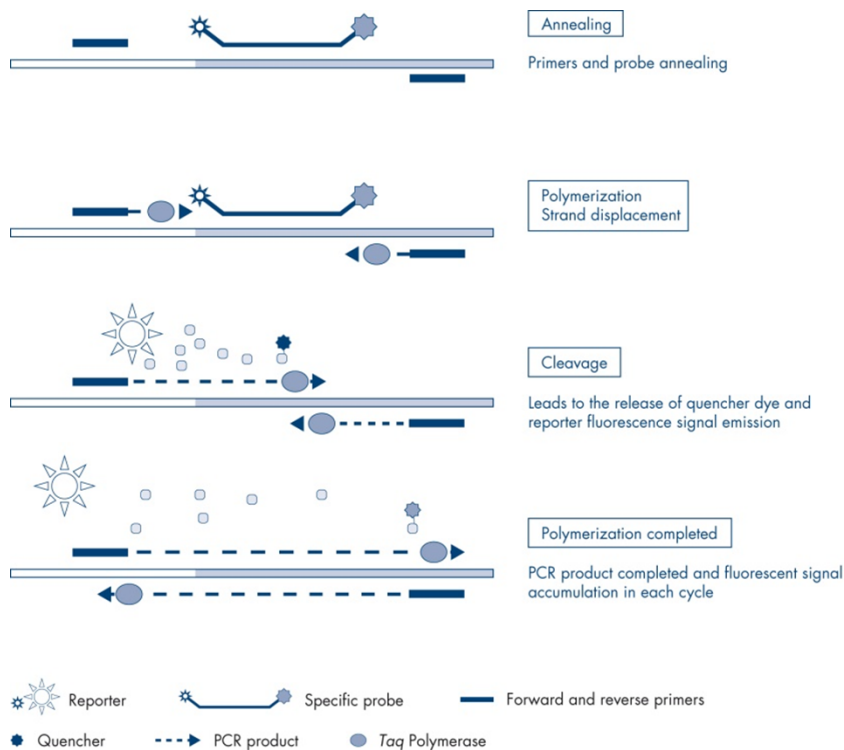


Figure 2. The TaqMan real-time reaction principle.

An increase in fluorescence signal is detected only if the target sequence, which is complementary to both the primers and probe, is present in the reaction. If the target sequence is absent, no probe cleavage will take place, no dissociation of fluorescent reporter from quencher will occur and hence no increase in fluorescence will be observed. The number of PCR cycles necessary to detect a fluorescent signal above a pre-determined threshold is called the cycle threshold (Ct) and is directly proportional to the amount of target present at the beginning of the reaction, allowing a sensitivity limit to be set for the test.

The *therascreen* FGFR RGQ RT-PCR Kit PCR reaction mixes contain HotStarTaq® DNA Polymerase, a modified form of QIAGEN *Taq* DNA Polymerase. It is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer-dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity. The enzyme is activated at the start of a reaction by a 15-minute, 95°C incubation step. The hot start enables reactions to be set up rapidly and conveniently at room temperature.

The *therascreen* FGFR RGQ RT-PCR Kit reaction mixes also contain a PCR Buffer which has been specifically developed for multiplex, real-time PCR using sequence-specific probes. This buffer contains a specially optimized combination of KCl and (NH₄)₂SO₄, which promotes a high ratio of specific to nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity. When using this buffer, primer annealing is only marginally influenced by the MgCl₂ concentration, so optimization by titration of Mg²⁺ is not required. The buffer also contains synthetic Factor MP, which facilitates multiplex PCR. Factor MP increases the local concentration of primers and probes at the cDNA template and stabilizes specifically bound primers and probes, allowing efficient annealing and extension. The combination of these various components in the PCR Buffer prevents the multiple amplification reactions from interfering with each other.

3. Materials Provided

3.1. Kit contents

<i>therascreen</i> FGFR RGQ RT-PCR Kit		(24)
Catalog no.	874755	
Number of reactions	24	
Contents	Cap color	Volume
RT Buffer 1	White	200 µL
RT Buffer 2*	Brown	100 µL
RT Primer Mix	Violet	50 µL
Reverse Transcriptase	Blue	50 µL
Positive Control	Red	100 µL
PC Diluent	Red	200 µL
Mutations-1 Reaction Mix†	Orange	720 µL
Mutations-2 Reaction Mix†	Violet	720 µL
Fusions-1 Reaction Mix†	Yellow	720 µL
Fusions-2 Reaction Mix†	Green	720 µL
Water for Sample Dil.	Clear	1.9 mL
Water for NTC	Clear	1.9 mL

* Contains deoxyribonuclease. For more information, see "Warnings and Precautions" on page 15.

† PCR reaction mix containing all the required components, except the sample to be analyzed.

3.2. Platform and software

The *therascreen* FGFR RGQ RT-PCR Kit is specifically designed for use with the Rotor-Gene Q MDx 5plex HRM instrument installed with the following:

- Rotor-Gene AssayManager® version 2.1 or later
- Gamma MDx Plug-in version 1.0.0 or later

- FGFR FFPE JP Assay Profile version 1.0.4 Assay Profile

Refer to the *Rotor-Gene Q MDx 5plex HRM User Manual* for information concerning the Rotor-Gene Q MDx 5plex HRM instrument. The Rotor-Gene Q MDx 5plex HRM instrument must be maintained according to the requirements in the user manual.

4. Materials Required but Not Provided

4.1. Equipment, consumables, and reagents for RNA purification

- RNeasy DSP FFPE Kit (cat. no. 73604)
- Calibrated, dedicated pipettes* (adjustable) for sample processing (20 µL, 200 µL, and 1 mL)
- RNase and nuclease-free, aerosol-resistant, sterile PCR pipette tips with hydrophobic filters
- Benchtop centrifuge* with rotor for 2 mL tubes
- Vortex mixer*
- Molecular biology grade 100% ethanol†
- Fresh scalpel, razor blade or similar sharp bladed instrument
- Calibrated heating block capable of incubation between 56°C and 80°C and shaking at 1100 rpm
- Calibrated spectrophotometer

4.2. Equipment and consumables for reverse transcription and real-time PCR

- Calibrated dedicated pipettes* (adjustable) for sample preparation, master mix preparation, and dispensing of RNA and cDNA (20 µL, 200 µL, and 1 mL)
- RNase and nuclease-free, aerosol-resistant, sterile PCR pipette tips with hydrophobic filters
- 1.5 or 2.0 mL nuclease-free, low-binding PCR tubes
- 0.2 mL PCR Tubes (cat. no. 981005)
- 0.1 mL Strip Tubes and Caps (cat. no. 981103 or 981106)
- Heating block*, water bath* or thermal cycler* capable of incubating 0.2 mL PCR tubes at 42–95°C
- Cooling system* capable of holding 1.5 and 2.0 mL tubes at 0–8°C

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

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

- Benchtop centrifuge* with rotor for 0.2, 1.5, and 2.0 mL tubes
- Vortex mixer
- Loading Block 96 × 0.2 mL PCR tubes, aluminum block for manual reaction setup (cat. no. 9018905)
- Loading Block 72 × 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 Q MDx 5plex HRM instrument with 72-well rotor (QIAGEN, cat. no. 9002030)
- Rotor-Gene AssayManager version 2.1 or later
- Rotor-Gene AssayManager Gamma MDx Plug-in version 1.0.0 or later
- *therascreen* FGFR FFPE JP Assay Profile version 1.0.4 Assay Profile

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

5. 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/or the regulatory authority in which the user and/or the patient is established.

The overall run control failure rate (PC and NTC) observed when using the *therascreen* FGFR RGQ RT-PCR Kit is approximately 5.2% (1% for the PC and 4.2% for NTC). In the event that an invalid run result is obtained, sample testing should be repeated as described in the "Troubleshooting Guide" on page 59, using available sample RNA. If insufficient RNA is available for retest from the original FFPE extraction, re-extraction from FFPE material is required.

5.1. 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 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 and must be treated as biohazardous materials.
- Discard sample and assay waste according to your local safety procedures.
- For safety information regarding the Rotor-Gene Q MDx 5plex HRM instrument, see the user manual supplied with the instrument.
- For safety information for the RNeasy DSP FFPE Kit (cat. no. 73604), see the *RNeasy DSP FFPE Kit Handbook*.
- The *therascreen* FGFR RGQ RT-PCR Kit is to be used by trained personnel in a professional laboratory environment.
- For use with the Rotor-Gene Q MDx 5plex HRM instrument
- For use with the RNeasy DSP FFPE Kit.

CAUTION



DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The following hazard and precautionary statements apply to components of the *therascreen* FGFR RGQ RT-PCR Kit:

RT BUFFER 2



Contains: DNase. Danger! May cause an allergic skin reaction. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing mist or vapors. Wear protective gloves/ protective clothing/ eye protection/ face protection. Wear respiratory protection. IF exposed or concerned: Call a POISON CENTER or doctor/ physician. Remove person to fresh air and keep comfortable for breathing. Wash contaminated clothing before reuse.

5.1.1. Emergency information

CHEMTREC

Outside USA & Canada +1 703-527-3887

5.2. Precautions

Always pay attention to the following:

Performance of RT-PCR tests requires good laboratory practices, including equipment maintenance, that are appropriate for molecular biology and compliant with applicable regulations and relevant standards.

- This test is for use with clinical FFPE UC specimens only.
- All chemicals and biological materials are potentially hazardous and should be treated accordingly. 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 supplied with *therascreen* FGFR RGQ RT-PCR Kit are optimally diluted. 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.

- The reagents supplied in the *therascreen* FGFR RGQ RT-PCR Kit are intended to be used only with other reagents from the same kit lot. Do not substitute any supplied reagents with the same reagents from a different *therascreen* FGFR RGQ RT-PCR Kit production lot, as this may affect performance.
- Refer to the Rotor-Gene Q MDx 5plex HRM instrument user manuals for additional warnings, precautions, and procedures.
- Alteration of incubation times and temperatures may result in erroneous or discordant data.
- Do not use expired or incorrectly stored components.
- Reaction mixes may be degraded if exposed to light.
- Use extreme caution to prevent RNA, cDNA, or PCR product carryover contamination which may result in a false-positive signal.
- Use extreme caution to prevent contamination by RNase, which may cause degradation of the template RNA and test failure.
- Use individual, dedicated pipettes for setting up reaction mixes and adding templates.
- Do not open the Rotor-Gene Q MDx 5plex HRM instrument until the run is finished.
- Do not open Rotor-Gene Q tubes after the real-time PCR run is finished.
- Caution must be observed to ensure correct sample testing with emphasis placed upon avoiding incorrect sample entry, loading errors, and pipetting errors.
- Make sure the samples are handled in a systematic way and clearly labeled to ensure correct identification at all times, thereby maintaining traceability.

We recommend the following precautions are taken:

- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials) and wear gloves when performing the assay.
- Use fresh aerosol-resistant pipette tips for all pipetting steps to avoid cross-contamination of the samples and reagents.
- Handle the RT and PCR reagents with dedicated labware (pipettes, tips, etc.) in a dedicated area where no RNA or DNA matrices (RNA, DNA, cDNA, plasmid, or PCR products) are introduced. Add samples to be analyzed in a separate zone (preferably in a separate room or PCR sample preparation cabinet) with dedicated equipment (pipettes, tips, etc.).

6. Disposal

Dispose of as hazardous waste in compliance with local and national regulations. This also applies to unused products.

Follow recommendations in the Safety Data Sheet (SDS).

7. Storage and Handling

7.1. Reagent storage and handling

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

7.1.1. Storage conditions

Upon receipt, the *therascreen* FGFR RGQ RT-PCR Kit must be stored immediately at -30 to -15°C in a constant-temperature freezer and protected from light, with the exception of the PC Diluent, which must be removed from the kit box and immediately stored at -90 to -65°C until the stated expiration date.

For storage information for the RNeasy DSP FFPE Kit (cat. no. 73604), see the *RNeasy DSP FFPE Kit Handbook*.

7.1.2. Stability

Reagents can be stored in their original packaging at -30 to -15°C (with the exception of the PC Diluent, which should always be stored at -90 to -65°C) until the stated expiration date. Do not exceed a maximum of 5 freeze-thaw cycles.

The *therascreen* FGFR RGQ RT-PCR Kit reagents must be thawed for a minimum of 30 minutes and a maximum of 3 hours. Once the reagents are ready to use, the RT or PCR reactions can be set up. The total setup time prior to RT or PCR run should not exceed 4 hours.

7.1.3. Shipping conditions

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

7.2. Specimen Handling and Storage

7.2.1. Specimen storage and handling

7.2.1.1. FFPE specimens

The *therascreen* FGFR RGQ RT-PCR Kit is for use with RNA samples extracted from FFPE urothelial cancer (UC) specimens using the RNeasy DSP FFPE Kit. All samples should be treated as potentially hazardous.

7.2.1.2. RNA samples

Once extracted, RNA samples should either be tested immediately with the *therascreen* FGFR RGQ RT-PCR Kit or stored at -90 to -65°C . Do not exceed a maximum of 5 freeze-thaw cycles.

To prepare tissue samples for RNA extraction:

- Using a microtome, cut 4–5 μm serial sections from the FFPE sample block and mount them 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. A slide adjacent to the sections to be extracted is required to identify the tumor surface area and guide macrodissection, if necessary.
- The H&E stained sections must not be used for RNA extraction.
- Store all FFPE blocks and slides at room temperature (15 – 25°C).
- The FFPE input required is equivalent to a 4–5 μm slide thickness with a total tumor surface area between 100 mm^2 and 500 mm^2 (inclusive). This can be created by combining material from multiple slides (Figure 3).

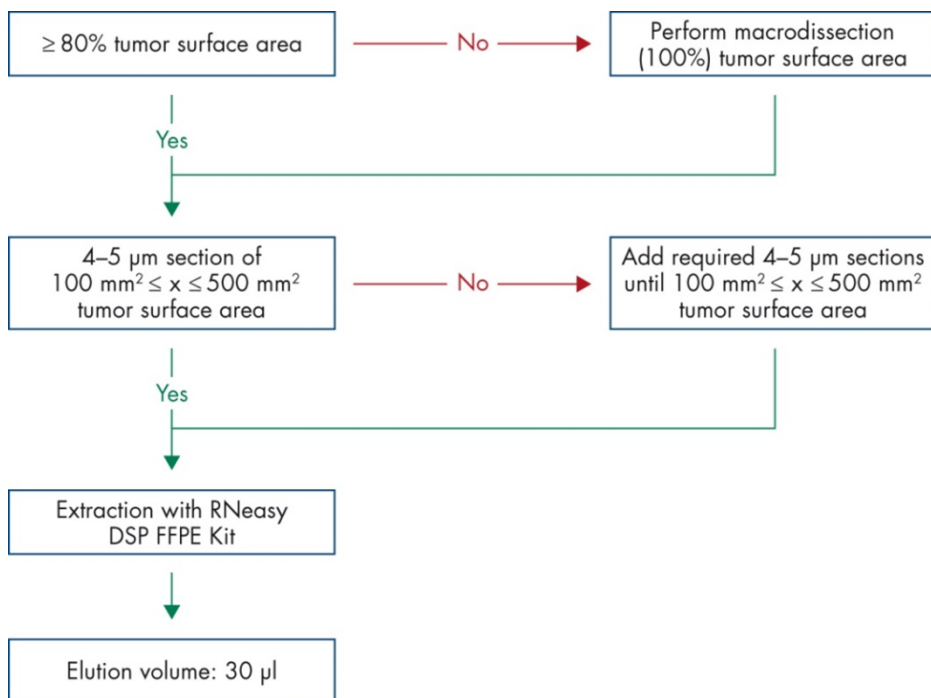


Figure 3. Clinical FFPE UC sample purification workflow to be used with the *therascreen* FGFR RGQ RT-PCR System.

- Macrodissection should be performed to attain a minimum of 80% tumor surface area. If tumor surface area is below 100 mm² and/or the tumor surface area is lower than 80%, additional sections must be used to achieve the minimum sample requirements.

Note: Exercise due care and attention when handling scalpels and ensure the scalpel blade is pointed away from the body at all times.

8. Quality Control

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

9. Procedure

9.1. Protocol A: RNA extraction and preparation

RNA should be purified using the RNeasy DSP FFPE Kit (cat. no. 73604).

Ensure reagents to be used have not expired and have been transported and stored under the correct conditions.

Note: Performance of the *therascreen* FGFR RGQ RT-PCR Kit has only been validated in combination with the RNeasy DSP FFPE Kit (cat. no. 73604). Do not use any other RNA purification product.

9.1.1. Important points before starting

- If using the RNeasy DSP FFPE Kit for the first time, read "Important Notes" in the *RNeasy DSP FFPE Kit Handbook*.
- If working with RNA for the first time, read "Appendix: General Remarks on Handling RNA" in the *RNeasy DSP FFPE Kit Handbook*.
- Buffer RBC contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See the *RNeasy DSP FFPE Kit Handbook* for safety information.
- Unless otherwise indicated, perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly; do not stop in between steps.
- Perform all centrifugation steps using a centrifuge maintained at 15–25°C. If using a refrigerated microcentrifuge, set the instrument to room temperature, otherwise significant cooling below 15°C may occur.
- If using Buffer RPE and RNase-Free DNase I for the first time, reconstitute them as described in the *RNeasy DSP FFPE Kit Handbook*.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer RPE by shaking.
- Set a heating block with shaking function to 56°C for use in steps 5 and 9. To reduce waiting time, set a second heating block with shaking function to 80°C for use in step 9.

Note: Do not stop the purification procedure in between incubations as increased incubation times might lead to loss or degradation of RNA.

9.1.2. Procedure

1. Follow the RNA purification procedure as described in the *RNeasy DSP FFPE Kit Handbook*. In the protocol "Purification of Total RNA from FFPE Tissue Sections", ensure the volumes used for processing >3–4 sections (marked with •) are followed.
2. Elute RNA in 30 µL RNase-free water provided with the RNeasy DSP FFPE Kit.
3. Aliquot 3 µL of eluted RNA for quantification.
4. Store RNA eluates at –90 to –65°C.

9.2. Protocol B: Quantification and normalization of RNA

9.2.1. Procedure

1. Blank the spectrophotometer using the RNase-free water supplied with the RNeasy DSP FFPE Kit that was used to elute RNA.
2. To determine RNA quantity, measure optical density at 260 nm. RNA quantity is determined by measuring optical density at 260 nm.

Total amount of RNA purified = concentration x volume of sample in µL.

Note: If the RNA concentration is below 16.67 ng/µL, the sample must not be processed further. A fresh RNA extraction from a new FFPE specimen should be used for further analysis.

3. Dilute RNA to 16.67 ng/µL using the Water for Sample Dil. provided in the *therascreen* FGFR RGQ RT-PCR Kit.

Note: The reverse transcription reaction is optimized for 250 ng of purified RNA diluted in a final volume of 15 µL ($15\text{ }\mu\text{L} \times 16.67\text{ ng}/\mu\text{L} = 250\text{ ng RNA input}$).

9.3. Protocol C: Reverse transcription

The *therascreen* FGFR RGQ RT-PCR Kit provides high cDNA yields for sensitive detection of all target transcripts, even those present at low-abundance. Reverse transcription uses an RNA template and a blend of primers complementary to the 3' and 5' ends of the RNA to produce cDNA. Reverse transcriptase (RT) enzyme synthesizes the first-strand cDNA, which is then used as input into the *therascreen* FGFR RGQ RT-PCR Kit PCR reaction.

9.3.1. Important points before starting

- Set up all reactions at 0–8°C to minimize the risk of RNA degradation.
- RNase inhibitor and dNTPs are already included in the kit components. Do not add additional RNase inhibitor and dNTPs.
- RT Primer Mix (supplied) should be used. RT Primer Mix is optimized to provide high cDNA yields for all regions of RNA transcripts.
- Separate denaturation and annealing steps are not necessary before starting the reverse-transcription reaction.
- After reverse transcription, the reaction must be stopped by incubation at 95°C for 3 minutes to deactivate the reverse transcriptase.

9.3.2. Procedure

1. Place a loading block capable of holding 96 × 0.2 mL PCR tubes and a cooling block capable of holding 12 or more 2 mL tubes at 0–8°C for at least 60 minutes prior to reaction setup.
2. Thaw RT Buffer 1, RT Buffer 2, Reverse Transcriptase, RT Primer Mix, and Water for NTC provided with the *therascreen* FGFR RGQ RT-PCR Kit at room temperature for between 30 minutes and 3 hours prior to beginning the RT reaction setup.
3. Thaw the test samples, PC, and PC Diluent at 0–8°C for between 30 minutes and 3 hours prior to beginning the RT reaction setup.
4. Inspect all RT reagents closely to ensure they are completely thawed and dissolved in solution. Dissolve any precipitates in RT Buffer 2 by vortexing. If necessary, briefly incubate the buffer at 37°C until the precipitates dissolve.
5. Pulse vortex all RT reagents 3 times for 3 seconds each time, to ensure reagents are thoroughly mixed.
6. Centrifuge all RT reagents briefly to collect residual liquid from the lids and sides of the tubes.
7. Prepare enough reverse transcription master mix for the number of samples to be tested, plus 2 for the run controls; positive control (PC) and no template control (NTC). The reverse transcription master mix should be prepared at 0–8°C according to Table 3.

Note: Prepare sufficient master mix for 2 extra reactions ($n + 2$) if using ≤ 16 samples, and 4 extra reactions ($n + 4$) if using >16 samples, to allow sufficient additional volume for the RT setup.

Note: RT Buffer 1 and Reverse Transcriptase are viscous components. It is therefore recommended to pipet them slowly.

Table 3. Reverse transcription reaction mix

Component	Volume per RT reaction (uL)
RT Buffer 1 *	5
RT Buffer 2	2.5
RT Primer Mix	1.25
Reverse Transcriptase†	1.25
Total volume of master mix	10

* Includes Mg²⁺ and dNTPs.

† Also contains RNase inhibitor.

- Pulse vortex the RT reaction mix 3 times for 3 seconds each time, to ensure each reagent is thoroughly mixed.
- Briefly centrifuge the RT reaction mix to collect residual liquid from the lid and sides of the tube.
- Store the RT reaction mix in the cooling block.
- Fill the loading block with the required number of 0.2 mL PCR tubes.
- Aliquot 10 µL of RT reaction mix into each 0.2 mL tube.
- Pulse vortex the Water for NTC, PC Diluent, and PC provided with the *therascreen* FGFR RGQ RT-PCR Kit and normalized RNA samples 3 times for 3 seconds each, to ensure each is thoroughly mixed.
- Briefly centrifuge the Water for NTC, PC Diluent, PC, and normalized RNA samples to collect residual liquid from the lids and sides of the tubes.
- Perform the following steps:
 - Add the samples to each tube containing reverse transcription master mix according to Table 4.
 - Pipet each sample directly into the RT reaction mix of the tube
Note: For the PC sample, pipet the PC first followed by the PC Diluent.
 - After adding each sample, set the pipette to 15 uL, pipet up and down 5–10 times to mix, and immediately cap tubes.

Note: The RT reaction mix and sample (PC, NTC, or test sample) must be mixed thoroughly for the RT reaction to proceed efficiently.

Table 4. Sample added to each reverse-transcription reaction mix

Sample name	Sample type	Volume (µL)
No template control	Water for NTC	15
Test sample	Sample	15
Positive control	Positive control (PC)	5
	PC Diluent	10

- 16. Pulse vortex all tubes for 3 seconds to ensure the RT reagents and template are mixed.
- 17. Ensure that all bubbles are removed and that the RT reagents and template are at the base of the tube.
- 18. Leave the tubes for 15 minutes in the loading block at room temperature.
- 19. Incubate the samples in a heating block, water bath, or thermal cycler for 30 minutes at 42°C to reverse transcribe the RNA.
- 20. Incubate the samples in a heating block, water bath, or thermal cycler for 3 minutes at 95°C to inactivate the Reverse Transcriptase enzyme.
- 21. Store the cDNA samples until they are used as input for the PCR step. For short-term storage, samples can be stored at 2–8°C for a maximum of 5 days, or at –30 to –15°C for a maximum of 10 days.

9.4. Real-time PCR on the instrument

The *therascreen* FGFR RGQ RT-PCR Kit provides accurate real-time PCR detection of the specified *FGFR* alterations in a multiplex format. The *therascreen* FGFR RGQ RT-PCR Kit contains 4 PCR reaction mixes which are provided in a ready-to-use format, including HotStarTaq DNA Polymerase and PCR Buffer.

9.4.1. Important point before starting

- The PCR must begin with an initial incubation step of 15 minutes at 95°C to activate the HotStarTaq DNA Polymerase.

9.4.2. Procedure

- 1. Keep a loading block capable of holding 72 × 0.1 mL tubes at 0–8°C for at least 60 minutes prior to reaction setup.
- 2. Thaw the *therascreen* FGFR RGQ RT-PCR Kit PCR reaction mixes at room temperature for between 30 minutes and 3 hours prior to PCR reaction setup.
- 3. Pulse vortex all PCR reaction mixes 3 times for 3 seconds each time to ensure each is thoroughly mixed.
- 4. Centrifuge all PCR reaction mixes briefly to collect residual liquid from the lids and sides of the tubes.
- 5. Fill the loading block with the required number of 0.1 mL PCR strip tubes.
- 6. Dispense 20 µL PCR reaction mix into the 0.1 mL PCR strip tubes, according to the pipetting scheme in Figure 4.

72 Well Plate									
	1	2	3	4	5	6	7	8	9
A	Tube 1 Mut-1	Tube 9 Mut-1	Tube 17 Mut-1	Tube 25 Mut-1	Tube 33 Mut-1	Tube 41 Mut-1	Tube 49 Mut-1	Tube 57 Mut-1	Tube 65 Mut-1
B	Tube 2 Mut-2	Tube 10 Mut-2	Tube 18 Mut-2	Tube 26 Mut-2	Tube 34 Mut-2	Tube 42 Mut-2	Tube 50 Mut-2	Tube 58 Mut-2	Tube 66 Mut-2
C	Tube 3 Fus-1	Tube 11 Fus-1	Tube 19 Fus-1	Tube 27 Fus-1	Tube 35 Fus-1	Tube 43 Fus-1	Tube 51 Fus-1	Tube 59 Fus-1	Tube 67 Fus-1
D	Tube 4 Fus-2	Tube 12 Fus-2	Tube 20 Fus-2	Tube 28 Fus-2	Tube 36 Fus-2	Tube 44 Fus-2	Tube 52 Fus-2	Tube 60 Fus-2	Tube 68 Fus-2
E	Tube 5 Mut-1	Tube 13 Mut-1	Tube 21 Mut-1	Tube 29 Mut-1	Tube 37 Mut-1	Tube 45 Mut-1	Tube 53 Mut-1	Tube 61 Mut-1	Tube 69 Mut-1
F	Tube 6 Mut-2	Tube 14 Mut-2	Tube 22 Mut-2	Tube 30 Mut-2	Tube 38 Mut-2	Tube 46 Mut-2	Tube 54 Mut-2	Tube 62 Mut-2	Tube 70 Mut-2
G	Tube 7 Fus-1	Tube 15 Fus-1	Tube 23 Fus-1	Tube 31 Fus-1	Tube 39 Fus-1	Tube 47 Fus-1	Tube 55 Fus-1	Tube 63 Fus-1	Tube 71 Fus-1
H	Tube 8 Fus-2	Tube 16 Fus-2	Tube 24 Fus-2	Tube 32 Fus-2	Tube 40 Fus-2	Tube 48 Fus-2	Tube 56 Fus-2	Tube 64 Fus-2	Tube 72 Fus-2

Figure 4. Pipetting scheme of PCR reaction mix. Rows A and E (orange): Mutation-1 Reaction Mix. Rows B and F (purple): Mutations-2 Reaction Mix, Rows C and G (yellow): Fusion-1 Reaction Mix. Rows D and H (green): Fusions-2 Reaction Mix. Colors correspond with the PCR tube caps in the kit.

- 7. Vortex the cDNA samples for 3 seconds, then centrifuge briefly to collect the drops from the lids and sides of the tubes.
- 8. Add 5 µL of NTC, test sample, or PC from the Reverse Transcription reaction to the 0.1 mL PCR strip tubes, according to the pipetting scheme in Figure 5. Set the pipette to 5 µL and pipet each sample up and down 5–10 times to mix; immediately cap the tubes.

Note: Where possible, the addition of PC cDNA template to the tubes should take place in a different area or laboratory, away from the area or laboratory used to add the NTC and sample cDNA. This is to minimize the potential for sample contamination.

72 Well Plate									
	1	2	3	4	5	6	7	8	9
A	Tube 1 PC	Tube 9 Sample 1	Tube 17 Sample 3	Tube 25 Sample 5	Tube 33 Sample 7	Tube 41 Sample 9	Tube 49 Sample 11	Tube 57 Sample 13	Tube 65 Sample 15
B	Tube 2 PC	Tube 10 Sample 1	Tube 18 Sample 3	Tube 26 Sample 5	Tube 34 Sample 7	Tube 42 Sample 9	Tube 50 Sample 11	Tube 58 Sample 13	Tube 66 Sample 15
C	Tube 3 PC	Tube 11 Sample 1	Tube 19 Sample 3	Tube 27 Sample 5	Tube 35 Sample 7	Tube 43 Sample 9	Tube 51 Sample 11	Tube 59 Sample 13	Tube 67 Sample 15
D	Tube 4 PC	Tube 12 Sample 1	Tube 20 Sample 3	Tube 28 Sample 5	Tube 36 Sample 7	Tube 44 Sample 9	Tube 52 Sample 11	Tube 60 Sample 13	Tube 68 Sample 15
E	Tube 5 NTC	Tube 13 Sample 2	Tube 21 Sample 4	Tube 29 Sample 6	Tube 37 Sample 8	Tube 45 Sample 10	Tube 53 Sample 12	Tube 61 Sample 14	Tube 69 Sample 16
F	Tube 6 NTC	Tube 14 Sample 2	Tube 22 Sample 4	Tube 30 Sample 6	Tube 38 Sample 8	Tube 46 Sample 10	Tube 54 Sample 12	Tube 62 Sample 14	Tube 70 Sample 16
G	Tube 7 NTC	Tube 15 Sample 2	Tube 23 Sample 4	Tube 31 Sample 6	Tube 39 Sample 8	Tube 47 Sample 10	Tube 55 Sample 12	Tube 63 Sample 14	Tube 71 Sample 16
H	Tube 8 NTC	Tube 16 Sample 2	Tube 24 Sample 4	Tube 32 Sample 6	Tube 40 Sample 8	Tube 48 Sample 10	Tube 56 Sample 12	Tube 64 Sample 14	Tube 72 Sample 16

Figure 5. Pipetting scheme of samples tested with the *therascreen* FGFR RGQ RT-PCR Kit. Colors correspond with the PCR tube caps in the kit.

9. Ensure the PCR reaction mix is collected at the base of the 0.1 mL PCR strip tubes.
10. Open the *therascreen* FGFR FFPE JP Assay Profile version 1.0.4 in Rotor- Gene AssayManager version 2.1 software. See Table 5 for cycling conditions.

Table 5. Cycling conditions

Step	Time	Temperature	Additional comments
PCR initial activation step	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
2-step cycling*:			
Denaturation	60 sec	94°C	Combined annealing/extension step with fluorescence data collection.
Annealing/extension	90 sec	60°C	
Number of cycles	45		

* Optimal performance is only assured using these cycling conditions.

11. Place all 4 strip PCR tubes into the 72-well rotor. Take extra care to ensure that tubes are transferred to the correct positions in 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 5plex HRM instrument is maintained.

12. Place the 72-well rotor into the Rotor-Gene Q MDx 5plex HRM instrument. Ensure that the locking ring supplied with the Rotor-Gene Q MDx 5plex HRM instrument is placed on top of the rotor to secure the tubes during the run.
13. To start the real-time PCR run, follow the instructions "Using Rotor-Gene AssayManager version 2.1 software" below.

Note: The Rotor-Gene Q MDx 5plex HRM instrument cycling conditions for use with the *therascreen* FGFR RGQ RT-PCR Kit are detailed in Table 5.

9.5. Using Rotor-Gene AssayManager version 2.1 software

9.5.1. Procedure

1. Double-click the Rotor-Gene AssayManager version 2.1 software icon  on the desktop of the laptop connected to the Rotor-Gene Q MDx 5plex HRM instrument. The "Setup" environment appears by default.
2. Click **New manual work list** to create a new work list (Figure 6)

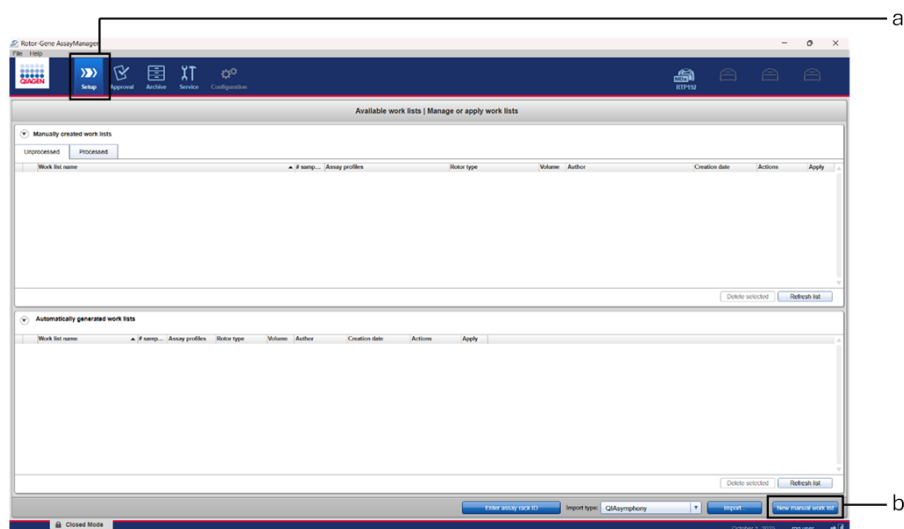


Figure 6. Setting up new manual work list. a = Select Setup environment, b = New manual work list.

3. Select the **Assays** tab on the left-hand side of the main window. Select **FGFR FFPE JP** from the list of available assay profiles and click the blue arrow to select the assay profile. If the assay profile name is truncated, move the mouse pointer over the assay profile to see the full name (Figure 7).

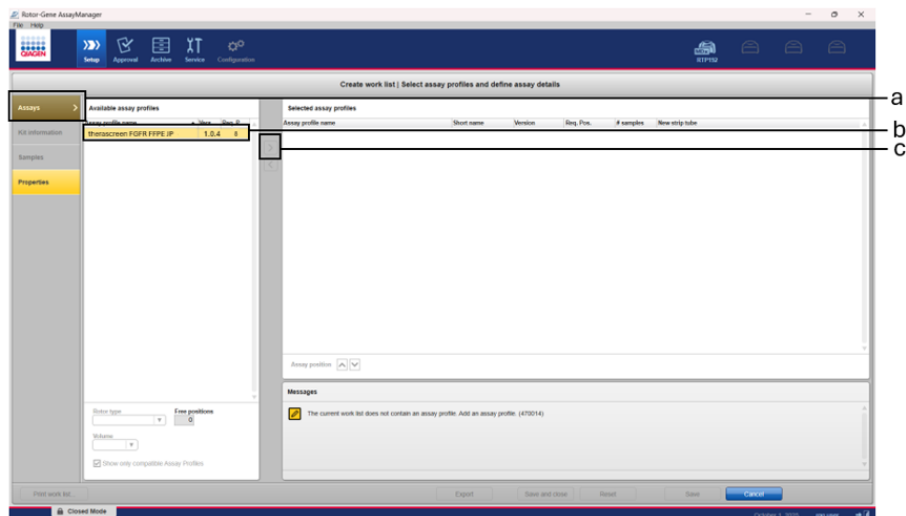


Figure 7. Setting up new manual work list; choosing assay profile name. a = Assays tab, b = Select therascreen_FGFR_FFPE_JP from the available assay profiles, c = Click the arrow.

4. In the "Selected assay profiles" window, enter the number of test samples to be tested excluding the number of run controls (Figure 8).

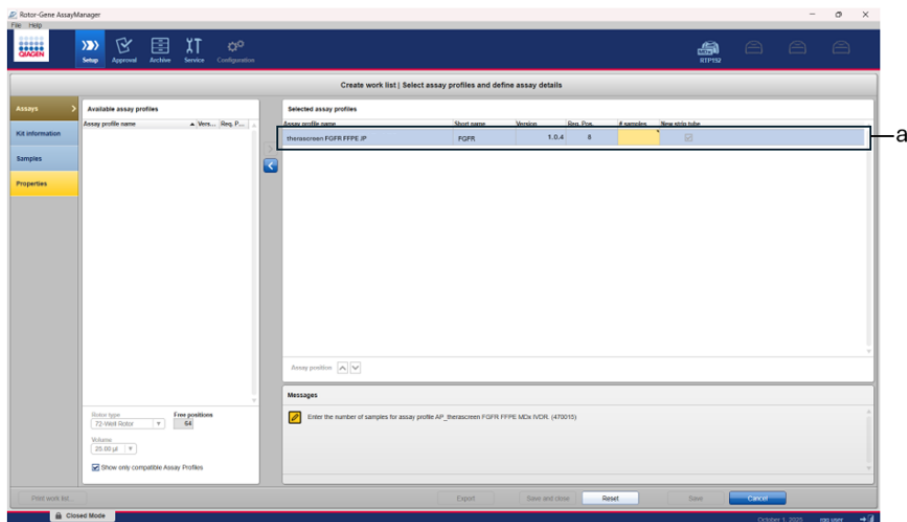


Figure 8. Create work list main window. a = Add the number of samples in the Assay profile name.

5. Click the **Kit information** tab. Select **Enter kit information manually** and enter the kit information (Figure 9).
 - a. In the **Material number** field, enter **0874755**.
 - b. Enter the lot number of the kit in the **Lot number** field.

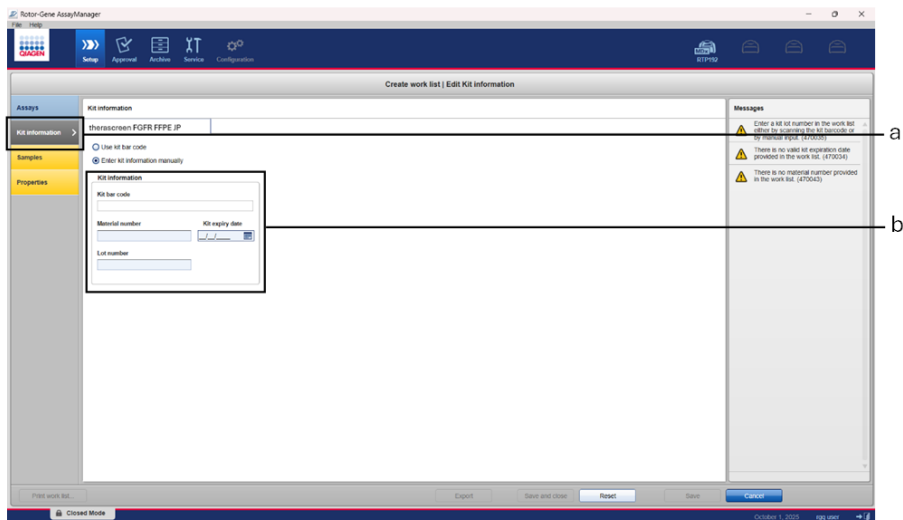


Figure 9. Create work list main window. a = Kit information tab, b = Enter the kit information.

6. Click the **Samples** tab to enter sample information. Enter the sample names manually (Figure 10).

Important: Ensure correct sample names are entered if using a work list generated for a previous Rotor-Gene AssayManager run.

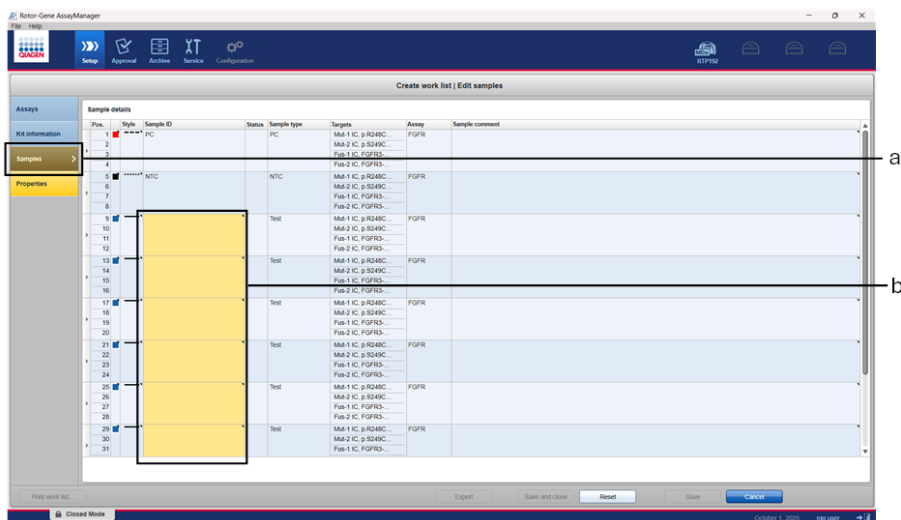


Figure 10. Create work list main window. a = Samples tab, b = Enter sample names.

- Click the **Properties** tab and enter the name of the work list. After the work list name is entered, ensure that the **is editable** and **work list is complete** boxes are checked. Then, click **Apply** in the bottom right-hand corner to apply the work list (Figure 11).
- Enter the experiment name in the **Experiment name** field. Select a cyclers from the list of available cyclers and ensure that the **Ring attached** box is checked. After all steps have been performed, click **Start run**. A progress indicator will appear below the icon at the top right of the screen to indicate that the run has started (Figure 12).

Note: The "Cycler" icon changes its appearance depending on the progress and the result of the run (Figure 13). Full descriptions of these cycler icons can be found in the *Rotor-Gene AssayManager version 2.1 Core Application User Manual*.

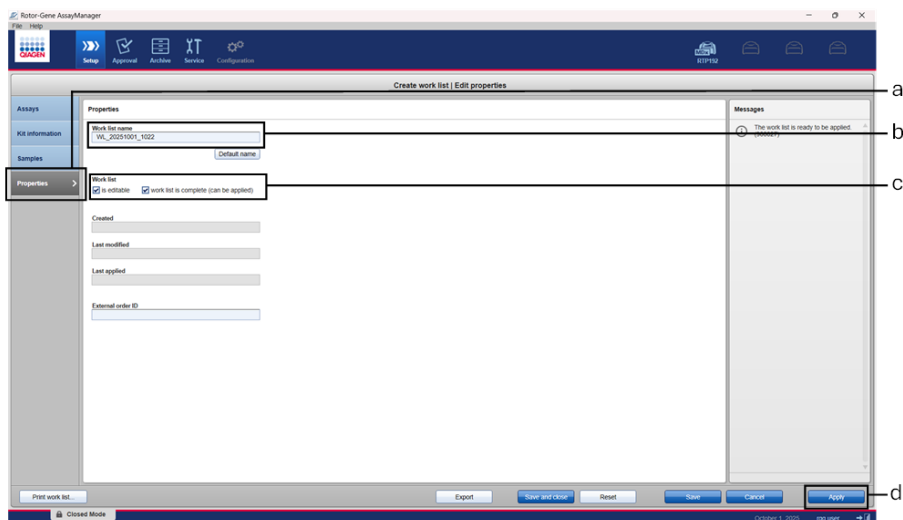


Figure 11. Create work list main window. a = Properties tab, b = Enter work list name, c = Select **is editable** and the work list is **complete**, d = **Apply**.

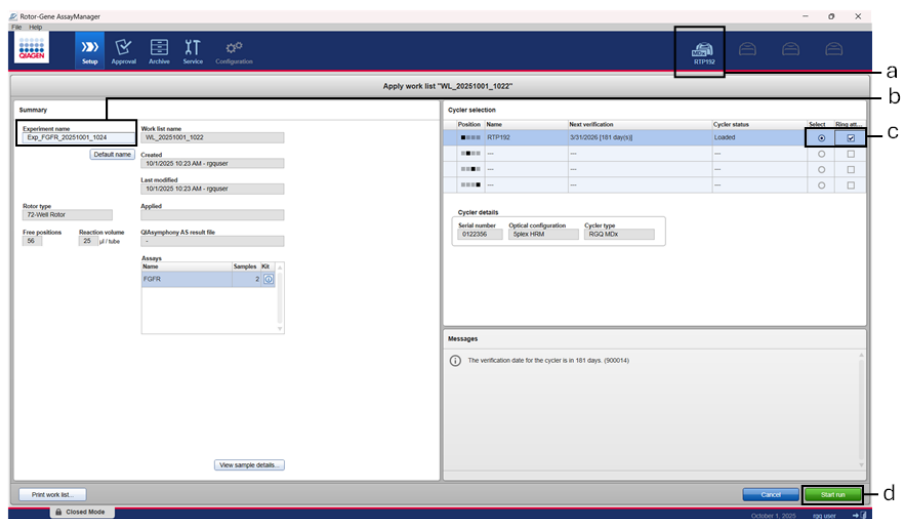


Figure 12. Applying work list and run start. a = Enter experiment name, b = Select the instrument, c = Ensure that **Ring attached** is selected, d = **Start run**.

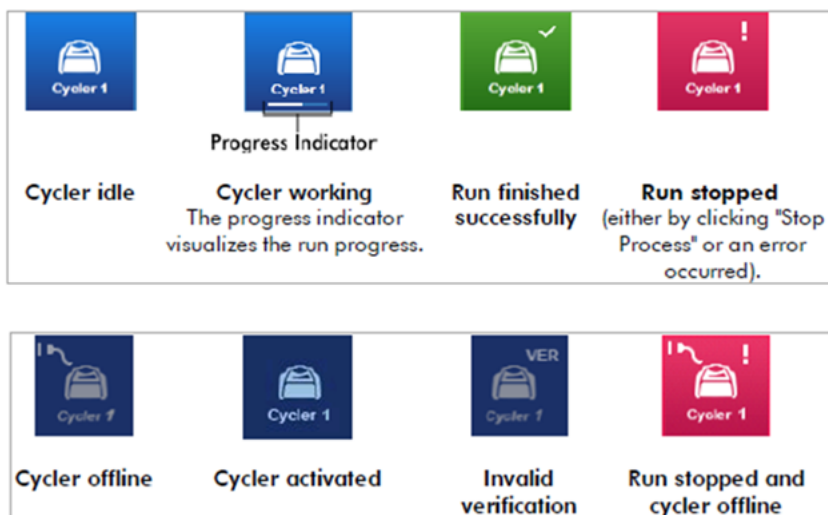


Figure 13. Rotor-Gene Q MDx 5plex HRM cycler icons that may be displayed.

9. After the run is complete, click **Finish run** and the Finish run dialog box will open (Figure 14).

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 dialog box when the run is in progress as data loss may occur.

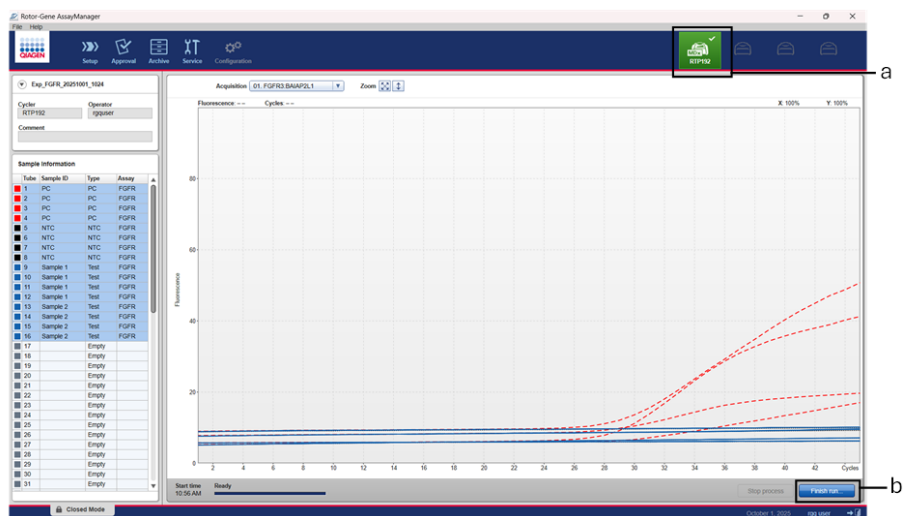


Figure 14. Finishing a run. a = Icon appearing when run successfully finished, b = Finish run.

- Click **Release and go to approval** to enter the Approval environment and release the Rotor-Gene Q MDx 5plex HRM instrument. The RGQ icon at the upper right of the screen will change from green to blue indicating that the RGQ instrument is ready to perform another run. Regardless of whether a run is successful or not, the RGQ instrument must be released (Figure 15).

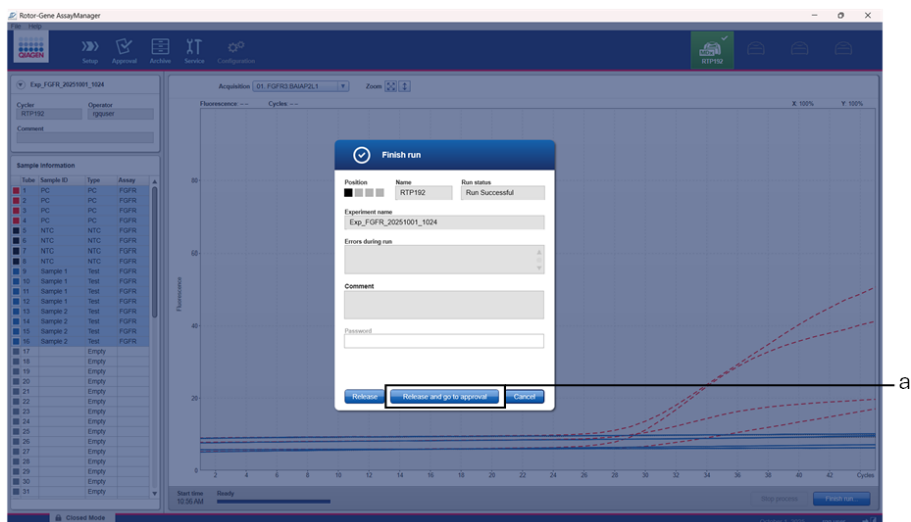


Figure 15. Finish Run pop up window. a = Select **Release and go to approval**.

11. Select the experiment in the Assay selection part of the Approval environment.
 "Raw data", "Processed data", "Experiment", "Assay" and "Audit trail" information can be found in the Plots and information section. Assay results can be found in the Results section (Figure 16).

Note: The FGFR FFPE JP assay profile file associated with the *therascreen* FGFR RGQ RT-PCR Kit contains all the software instructions required for automatic sample analysis and result interpretation. Following PCR cycling, the Rotor-Gene AssayManager software assesses the validity of the run controls and samples automatically. If the Positive Control and No Template Control data are within the preset acceptable range for the test, the run controls will report "Valid". If both run controls are valid, the samples will be analyzed on an individual basis; if the internal control data are within the pre-set acceptable range for the test, the sample will report "Valid", if outside the pre-set acceptable range for the test the sample will report "Invalid". If the external controls are outside of the pre-set acceptable range for the test, an "Invalid" overall sample result will be reported. If either of the run controls fail, the entire run will be invalidated. All samples will then be flagged as "ASSAY_INVALID". If this occurs, see "Interpretation of Results" on page 42 for instructions regarding how to proceed.

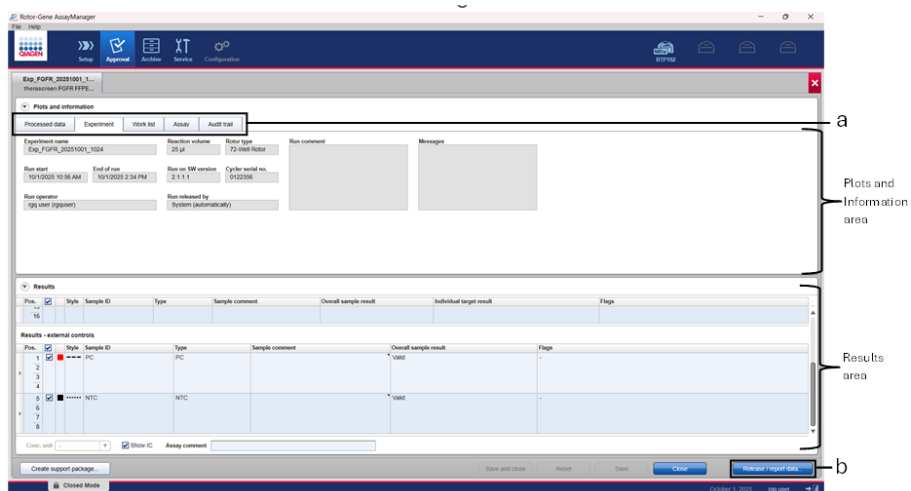


Figure 16. Example of assay results main windows. a = Experiment tab in Plots and information. b = Release/report data. Plots and information area with results area present.

The results of all test samples must be approved (accepted or rejected) in the Results area of the Approval environment and released.

12. Click **Release/report data**. The Release/report data dialog box will appear, as shown in Figure 17.

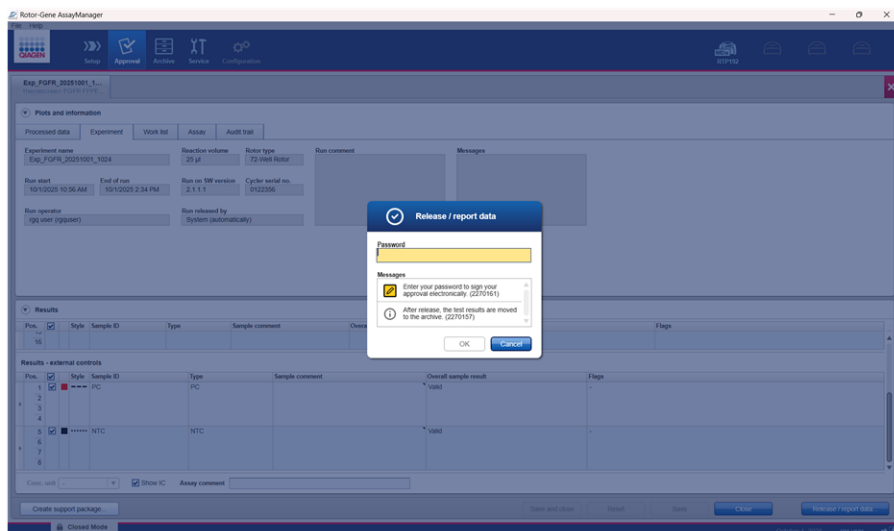


Figure 17. The Release/report data dialog box.

13. Click **OK** to save the experiment to the archive and create a LIMS output and run report. 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" section of the software environment.
14. To view an experiment stored in the experiment archive, click the **Archive** software environment and search for the experiment using the search criteria in the Filter Options section. Click **Apply filter** to search. To select an experiment, check the box next to the experiment you would like to view and click **Show assays**. (Figure 18).
15. If an experiment fails and an error code is displayed, a list of potential failures and error codes that can be presented by Rotor-Gene AssayManager is contained within the *Rotor-Gene AssayManager version 2.1 User Manual* and/or *Rotor-Gene AssayManager version 2.1 Gamma MDx Plug-in User Manual*.

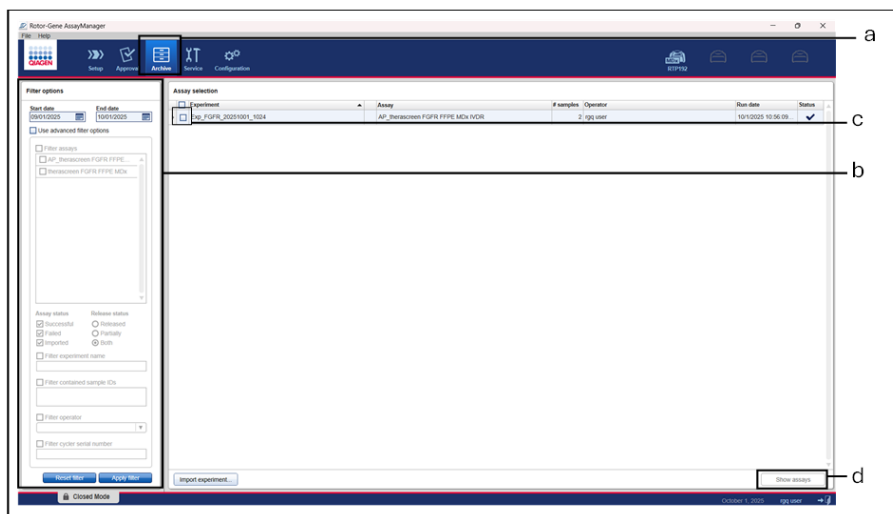


Figure 18. The “Experiment Archive” main window. a = “Archive” environment selection, b = Search/Filter options, c = Experiment name selection, d = “Show assays” control.

10. Interpretation of Results

All data analysis steps are performed automatically, with no requirement for manual interpretation. Rotor-Gene AssayManager version 2.1 automatically checks run validity and sample validity criteria and will not report mutation status results in the event of an invalid run or sample. The analytical result is determined by combining all relevant PCR data according to core analysis algorithms defined in the FGFR FFPE JP assay profile.

First, run control analyses are performed:

- The PC reaction is checked for the presence of amplification within the positive control assays and the IC assays. This run control is valid if the Ct value of each PC is within the relevant PC specifications.
- The NTC reaction is checked for the absence of specific amplification within the no template control assays and the IC assays. This run control is valid if no Ct value is observed or if the Ct value is above the NTC specification.
- **Note:** PC and NTC may be invalid due to performance of the FGFR3 fusion FGFR-BAIAP2L1 and FGFR2 fusions FGFR2-BICC1 and FGFR2-CASP7 targets. The *therascreen* FGFR RGQ RT-PCR Kit is also designed to detect these alterations, but results are not reported for samples due to the absence of clinical validation data.

If any of these run controls do not conform to specifications, the run will be invalidated, and the analysis will need to be performed again from the reverse transcription step.

If all run control analyses conform to specifications, analysis of the sample reactions is performed. Based on predetermined Ct values, the *FGFR* gene alteration status of each cDNA sample is qualitatively determined and reported.

The following results may be assigned to an individual sample:

- FGFR Alteration Detected ("Valid")
- No Alteration Detected ("Valid")
- INVALID: If one or more flags are assigned to the sample during analysis by Rotor-Gene AssayManager version 2.1 that are defined to set the FGFR alteration result to "INVALID".

Note: A tumor may contain more than one *FGFR* alteration. In such instances, more than one *FGFR* alteration will be reported.

Note: The report generated at the end of the run displays the results obtained from the experimental samples and run controls, with invalidating flags displayed in front of any invalid results. If an error occurs during the RGQ run, the samples in the instrument must be discarded and repeat testing should be performed from the extracted RNA sample.

All possible flags corresponding to Rotor-Gene AssayManager version 2.1 Gamma MDx Plug-in are listed in the *Rotor-Gene AssayManager version 2.1 Gamma MDx Plug-in User Manual*. Additional flags which are specific to *therascreen* FGFR FFPE JP are shown in Table 6.

Table 6. Example software flags which may be displayed

Software flags	When this flag is shown	Action to be taken
PC_ABOVE_ACCEPTED_RANGE	PC above the specification range	Invalid run. Test to be repeated from the extracted RNA if available, or re extracted if it is not.
PC_BELOW_ACCEPTED_RANGE	PC below the specification range	Invalid run. Test to be repeated from the extracted RNA.
PC_NO_CT_VALUE	No Ct value for PC in PC tubes	Invalid run. Test to be repeated from the extracted RNA.
NTC_UNEXPECTED_CT_VALUE	Ct value detected in NTC	Invalid run. Test to be repeated from the extracted RNA.
(Alteration) IC_ABOVE_ACCEPTED_RANGE	Sample above specification range	Invalid sample. Sample to be re-extracted.
(Alteration) IC_BELOW_ACCEPTED_RANGE	Sample below specification range	Invalid sample. Sample to be re-extracted.
(Alteration) IC_NO_CT_VALUE	No Ct value for sample	Invalid sample. Test can be repeated from the extracted RNA or sample to be re-extracted.
(Alteration) UNEXPECTED_EARLY_CT	Amplification curve crossed threshold early in cycling	Anomalous sample result. Repeat test from the extracted RNA.
STRONG_BASELINE_DIP (Alteration)	Dip detected in baseline	No user action needed; assay profile auto-adjusts for the dip

Note: If repeated invalid runs are obtained, contact QIAGEN Technical Service. PC and NTC may be invalid due to performance of the FGFR3 fusion FGFR-BAIAP2L1 and FGFR2 fusions FGFR2-BICC1 and FGFR2-CASP7 targets. The *therascreen* FGFR RGQ RT-PCR Kit is also

designed to detect these alterations, but results are not reported for samples due to the absence of clinical validation data.

11. Limitations

- Results from the product must be interpreted within the context of all relevant clinical and laboratory findings and are not to be used alone for diagnosis.
- The *therascreen* FGFR RGQ RT-PCR Kit is only to be used to test RNA derived from specimens of FFPE UC tumors.
- The *therascreen* FGFR RGQ RT-PCR Kit is only to be used to test RNA prepared using the RNeasy DSP FFPE Kit (cat. no. 73604).
- The *therascreen* FGFR RGQ RT-PCR Kit is also designed to identify FGFR3 fusion FGFR3-BAIAP2L1 and FGFR2 fusions FGFR2-BICC1 and FGFR2-CASP7 because these *FGFR* fusion patients were eligible for the 42756493-BLC2001 clinical trial of BALVERSA (erdafitinib). However, the test is not clinically validated to detect these 3 fusions due to a lack of required clinical specimens. Drug safety and efficacy has not been established for cases of UC harboring these fusions and therefore results for these alterations are not reported for samples. However, these targets are relevant for the purposes of PC and NTC validity.
- Samples with results reported as "No FGFR Alteration Detected" may harbor *FGFR* alterations that are not detected by the *therascreen* FGFR RGQ RT-PCR Kit.
- Detection of *FGFR* alterations is dependent on sample integrity and the amount of amplifiable cDNA which can be derived from the sample.
- If the internal control (IC) assay for a particular sample has no Ct value or is outside the specified range, the testing procedure should be repeated for this sample.
- The product is to be used only by personnel specially instructed and trained in in vitro diagnostics procedures and operation of the Rotor-Gene Q MDx 5plex HRM instrument.
- The product is intended for use only on a Rotor-Gene Q MDx 5plex HRM real-time PCR cycle.
- Strict compliance with the *therascreen FGFR RGQ RT-PCR Kit Instructions for Use* is required for optimal results. Dilution of the reagents, other than as described in this handbook, is not recommended and will result in a loss of performance.
- The instructions provided in this handbook are to be used with Rotor-Gene AssayManager version 2.1 with automated *FGFR* alteration status calling, together with the Gamma MDx Plug-in version 1.0.0 and *therascreen* FGFR FFPE JP Assay Profile version 1.0.4 Assay Profile.
- The *therascreen* FGFR RGQ RT-PCR Kit does not demonstrate any detectable cross reactivity (resulting in a report of "Alteration Detected") between the *FGFR* gene alteration

assays it includes.

- The *therascreen* FGFR RGQ RT-PCR Kit provides a qualitative test result, generating either a positive or negative result call for each *FGFR* alteration.
- The *therascreen* FGFR RGQ RT-PCR Kit uses a 2-step RT-PCR procedure. As with all similar procedures, samples may become contaminated by external sources within the test environment, or potentially by the positive control. Test operators must exercise due caution to avoid contamination of samples and kit reagents.
- The effect of microbial contamination upon the performance of the *therascreen* FGFR RGQ RT-PCR Kit is unknown; operators must exercise due caution to avoid introduction of microbial contaminants during testing procedures and should not use kit components if evidence of microbial growth is observed.
- 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.

12. Performance Characteristics

12.1. Limit of blank

The limit of blank (LoB) is defined in CLSI guideline EP17-A2 as “the highest result that can reasonably be expected from a blank sample (i.e., a sample with concentration at or near zero) for a given error probability α ”. For the *therascreen* FGFR RGQ RT-PCR Kit, this is the data point that corresponds to the upper 95th percentile in *FGFR* alteration-negative samples. The LoB was determined by measuring the levels of breakthrough for each of the 9 *FGFR* alteration assays of the *therascreen* FGFR RGQ RT-PCR Kit, where breakthrough is defined as the nonspecific, low-level amplification of an *FGFR* alteration-negative RNA sample. The LoB was determined by the analysis of 60 clinical wild-type samples, using 180 data points per assay, across 3 *therascreen* FGFR RGQ RT-PCR Kit and 3 instruments. The LoB for each assay within the *therascreen* FGFR RGQ RT-PCR Kit is shown in Table 7.

Table 7. Summary of LoB results

FGFR assay	LoB (Ct value)
p.R248C (c.742C>T)	39.64
p.S249C (c.746C>G)	No amplification
p.G370C (c.1108G>T)	No amplification
p.Y373C (c.1118A>G)	No amplification
FGFR3-TACC3v1	No amplification
FGFR3-TACC3v3	No amplification

12.2. Assay cutoff and cross-reactivity

12.2.1. Assay cutoff

The assay cutoff value is a specific Ct value used to determine whether a sample is classed as positive or negative for an *FGFR* alteration. Samples which generate Ct values at or below the cutoff are classified as *FGFR* alteration-positive (i.e., *FGFR* alteration detected) and Ct values generated above the cutoff are classified as *FGFR* alteration-negative (i.e., no alteration detected). The false-negative and false-positive rates for each assay were used to determine a cutoff value for each *FGFR* alteration-specific assay, such that a result equal to or less than the

cutoff will result in an *FGFR* alteration detected classification. The cutoff for each assay within the *therascreen* *FGFR* RGQ RT-PCR Kit is shown in Table 8.

Table 8. Summary of assay cutoff results

<i>FGFR</i> assay	Assay cutoff (Ct value)
p.R248C (c.742C>T)	36.00
p.S249C (c.746C>G)	39.09
p.G370C (c.1108G>T)	41.00
p.Y373C (c.1118A>G)	43.00
FGFR3-TACC3v1	43.00
FGFR3-TACC3v3	43.00

12.3. Assay cross-reactivity and analytical specificity

12.3.1. Assay cross-reactivity

Assay cross-reactivity is defined as nonspecific amplification of an *FGFR* alteration by the *therascreen* *FGFR* RGQ RT-PCR Kit reagents, other than the intended target of an assay, which gives a Ct value below the selected cutoff for that assay. High *FGFR* alteration level samples were tested with the *therascreen* *FGFR* RGQ RT-PCR Kit and no off-target amplification below the cutoff was observed in any of the assays. Therefore, no cross-reactivity was observed between the assays for *FGFR* alterations that comprise the *therascreen* *FGFR* RGQ RT-PCR Kit.

12.3.2. Analytical specificity

The level of potential cross-reactivity between primers, probes, and blockers used within the *therascreen* *FGFR* RGQ RT-PCR Kit and non-*FGFR* 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 blockers used with the *therascreen* *FGFR* RGQ RT-PCR Kit assays bind non-specifically within any genome, including the human genome. An additional *in silico* analysis was performed to determine whether the oligonucleotides used within each multiplex assay bind non-specifically 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 either wild-type *FGFR*

alleles or any *FGFR* alterations not detected by the *therascreen* FGFR RGQ RT-PCR Kit and therefore will not generate a false signal.

12.4. Limit of detection

The LoD is defined as the lowest number of *FGFR* alteration RNA copies/ μ L for each target that it is possible to detect 95% of the time. For the *FGFR* alteration-specific assays of the *therascreen* FGFR RGQ RT-PCR Kit, the LoD is reported as *FGFR* alteration RNA copies/ μ L. Individual *FGFR* alteration in vitro transcripts, one for each alteration, were spiked into a pool of normalized RNA extracted from wild-type clinical specimens and serially diluted at levels above, at, and below the estimated LoD for each assay.

Sixty replicates of each dilution point in the series were tested using 3 *therascreen* FGFR RGQ RT-PCR Kit lots. The LoD for each assay was determined using a Probit model and reported as the highest RNA copy number/ μ L (i.e., worst case) LoD detected across the 3 *therascreen* FGFR RGQ RT-PCR Kit lots tested (Table 9).

Table 9. Summary of assay cutoff results

<i>FGFR</i> assay	LoD (copies/ μ L)
p.R248C (c.742C>T)	75.80
p.S249C (c.746C>G)	289.82
p.G370C (c.1108G>T)	141.57
p.Y373C (c.1118A>G)	274.71
FGFR3-TACC3v1	25.26
FGFR3-TACC3v3	45.75

The LoDs of the p.R248C (c.742C>T), p.S249C (c.746C>G), p.G370C (c.1108G>T), p.Y373C (c.1118A>G), FGFR3-TACC3v1, and FGFR3-TACC3v3 assays were verified using *FGFR* alteration-positive clinical UC samples.

12.5. Repeatability and reproducibility

The repeatability (within laboratory) of the *therascreen* FGFR RGQ RT-PCR Kit was assessed by testing contrived samples at 3x LoD, representing the 9 alterations detected by the *therascreen* FGFR RGQ RT-PCR Kit and an *FGFR* alteration-negative sample.

Repeatability was assessed by testing these samples at one site across multiple days, Rotor-Gene Q instruments, and operators to generate a total of 60 replicates per sample (Table 10).

Table 10. Assay repeatability: Number of correct calls and two-sided 95% confidence limits for each *FGFR* alteration at 3x LoD and wild-type samples tested across one site

Template	No. of correct calls	Percentage of correct calls (%)	Lower two-sided 95% confidence limit (%)	Upper two-sided 95% confidence limit (%)
p.R248C (c.742C>T)	60/60	100	94.04	100.00
p.S249C (c.746C>G)	60/60	100	94.04	100.00
p.G370C (c.1108G>T)	60/60	100	94.04	100.00
p.Y373C (c.1118A>G)	60/60	100	94.04	100.00
FGFR3-TACC3v1 *	59/60	98.3	91.06	99.96
FGFR3-TACC3v3	59/60	98.3	91.06	99.96
WT (Mut-1 Reaction Mix)	60/60	100	94.04	100.00
WT (Mut-2 Reaction Mix)	60/60	100	94.04	100.00
WT (Fus-1 Reaction Mix)	60/60	100	94.04	100.00
WT (Fus-2 Reaction Mix)	59/60	98.3	91.06	99.96

* From 1xLoD samples.

Reproducibility was measured by testing contrived samples at 3x LoD level, clinical samples close to LoD and wild-type samples across the 3 different sites (1 internal QIAGEN site in the United Kingdom and 2 additional external sites in the USA). The contrived samples for all *FGFR* alterations at 3xLoD and wild-type samples were tested by 3 operators (per site) over 5 days using 3 Rotor-Gene Q MDx instruments at each external site. In addition, RNA extracted from formalin-fixed paraffin embedded (FFPE) UC clinical samples was used to test the device reproducibility. Testing was performed at the LoD level for each target (p.R248C (c.742C>T), p.S249C (c.746C>G), p.G370C (c.1108G>T), p.Y373C (c.1118A>G), FGFR3-TACC3v1, and FGFR3-TACC3v3) utilizing clinical samples. The analysis also combined lot-to-lot variability within the study design. All 1xLoD clinical samples were tested at each of the 3 sites using the *therascreen* FGFR RGQ RT-PCR Kit (2 biological replicates x 2 *therascreen* FGFR RGQ RT-PCR Kit lots x 2 operators x 3 days = 24 replicates at each site). These total replicates were tested across 3 Rotor-Gene Q MDx instruments at each site and 2 of the 3 *therascreen* FGFR RGQ RT-PCR Kit lots were used alternatively in each site (Table 11).

Table 11. Assay reproducibility: Number of correct calls and two-sided 95% confidence limits for each *FGFR* alteration at LoD, 3x LoD and wild-type samples tested across all sites

Mutation	Target level	Specimen type	No. correct calls results	Percent correct calls (%)	Lower two-sided 95% confidence limit (%)	Upper two-sided 95% confidence limit (%)
p.R248C (c.742C>T)	3x LoD	Contrived	120/120	100.00	96.97	100.00
	1x LoD	Clinical	72/72	100.00	95.01	100.00
p.G370C (c.1108G>T)	3x LoD	Contrived	120/120	100.00	96.97	100.00
	1x LoD	Clinical	71/72	98.61	92.50	99.96
p.S249C (c.746C>G)	3x LoD	Contrived	120/120	100.00	96.97	100.00
	1x LoD	Clinical	72/72	100.00	95.01	100.00
p.Y373C (c.1118A>G)	3x LoD	Contrived	120/120	100.00	96.97	100.00
	1x LoD	Clinical	71/72	98.61	92.50	99.96
FGFR3-TACC3v3	3x LoD	Contrived	119/120	99.17	95.44	99.98
	1x LoD	Clinical	71/72	98.61	92.50	99.96
FGFR3-TACC3v1	1x LoD	Contrived	119/120	99.17	95.44	99.98
	½x LoD	Clinical	63/72	87.50	77.59	94.12
WT (Mut-1)	NA	Clinical	120/120	100.00	96.97	100.00
WT (Mut-2)	NA	Clinical	120/120	100.00	96.97	100.00
WT (Fus-1)	NA	Clinical	120/120	100.00	96.97	100.00
WT (Fus-2)	NA	Clinical	116/120	96.67	91.69	99.08

12.6. Specimen handling

This study assessed sample handling variability, specifically during the RNA extraction step. FFPE UC clinical specimens were divided into 3 independent sets to be extracted with the RNeasy DSP FFPE Kit at 3 independent laboratories. Each set contained a predefined number of *FGFR* wild-type and *FGFR* positive (i.e., *FGFR* alteration detected) specimens. All specimens were blinded before extraction. Each set was extracted 3 times by 2 operators at 3 sites. All RNA samples were then tested using the *therascreen FGFR RGQ RT-PCR Kit* at one site.

Across all *FGFR* alterations, the proportion of correct calls was 96.22%, supporting the reproducibility and repeatability for the *therascreen* *FGFR* RGQ RT-PCR System at the preanalytical step of RNA isolation.

12.7. Lot-to-lot interchangeability

This study addressed the potential for lot-to-lot variability to impact *FGFR* alteration detection by the *therascreen* *FGFR* RGQ RT-PCR Kit. The *FGFR* System utilizes 2 separate kits: RNeasy DSP FFPE Kit for isolation of RNA from clinical UC FFPE specimens and *therascreen* *FGFR* RGQ RT-PCR Kit for the amplification and detection of *FGFR* alterations.

Lot-to-Lot interchangeability was demonstrated using 3 lots of the RNeasy DSP FFPE Kit and 3 lots of the *therascreen* *FGFR* RGQ RT-PCR Kit. A combination of clinical and contrived samples was used for this study. Four clinical FFPE specimens harboring the p.R248C (c.742C>T), p.S249C (c.746C>G), *FGFR3*-TACC3v1 alterations and a clinical wild-type FFPE specimen were extracted in duplicate using 3 RNeasy DSP FFPE Kit lots and tested with 3 different *therascreen* *FGFR* RGQ RT-PCR Kit. In addition, contrived samples for the p.G370C (c.1108G>T), p.Y373C (c.1118A>G), and *FGFR3*-TACC3v3, alteration were produced at the 3x LoD level and tested using the 3 *therascreen* *FGFR* RGQ RT-PCR Kit lots. All samples were normalized and diluted to the 3x LoD level for each assay. A total of 36 replicates for each sample were generated. The overall percentage of correct calls for all samples across all *therascreen* *FGFR* RGQ RT-PCR Kit and RNeasy DSP FFPE Kit lots was 99.65% (286/287 samples).

12.8. Cross-contamination/analytical carryover

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

Clinical UC FFPE samples were divided into 2 independent sets. Both sets comprised 18 wild-type samples and 12 *FGFR* alteration-positive samples. The RNA extraction, RT, and PCR reaction setup followed a matrix designed to introduce the risk of sample cross-contamination. Each set was tested by a different operator using the same *therascreen* *FGFR* RGQ RT-PCR Kit lot. A total of 128 wild-type replicates were tested and the percentage of false-positive calls for the wild-type samples was 3.13% (4/128 samples).

12.9. Interfering substances

This study investigated the impact of potential endogenous and exogenous interferents on *FGFR* alteration status detection across the RNeasy DSP FFPE Kit and the *therascreen FGFR* RGQ RT-PCR Kit. Samples were spiked with one of 4 interferents (hemoglobin, Buffer RPE, Deparaffinization Solution, or paraffin wax), either during the extraction step or the RNA sample normalization step. A total of 60 replicates per interferent across each of the 9 *FGFR* alteration-positive samples and wild-type samples were tested. The 4 interferents showed no statistically significant difference in results between the control samples and test samples, with the presence of interferents not affecting assay performance or target call.

12.10. Correlation to reference method

To demonstrate the accuracy of the *therascreen FGFR* RGQ RT-PCR Kit (relative to RT droplet digital PCR (RT-ddPCR)), an accuracy study was conducted with specimens from the 42756493-BLC2001 clinical trial, supplemented with specimens obtained from the same intended use (intent to diagnose) population. The *therascreen FGFR* RGQ RT-PCR Kit and ddPCR testing for *FGFR* alterations was performed on the same samples extracted from 307 specimens (271 clinical trial specimens and 36 procured specimens).

Samples with valid results for both *therascreen FGFR* RGQ RT-PCR Kit and ddPCR (n = 306) were analyzed to assess the Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and Overall Percent Agreement (OPA), based on agreement between the 2 methods for overall *FGFR* alteration status (*FGFR* Alteration Detected or No Alteration Detected). These percentages, together with the corresponding two-sided 95% confidence intervals (CI) are summarized in Table 12.

Table 12. *therascreen FGFR* RGQ RT-PCR Kit versus ddPCR (with ddPCR as orthogonal method)

Measure of agreement	Percent agreement % (N)	Two-sided 95% CI
Positive percent agreement (PPA)	99.04 (103/104)	94.76, 99.98
Negative percent agreement (NPA)	97.52 (197/202)	94.32, 99.19
Overall percent agreement (OPA)	98.04 (300/306)	95.78, 99.28

For the 6 overall *FGFR* alteration status discordant results, one sample gave no alteration detected result by the *therascreen FGFR* RGQ RT-PCR Kit and gave alteration detected results by ddPCR while 5 samples gave *FGFR* alteration detected results by the *therascreen FGFR*

RGQ RT-PCR Kit and gave no alteration detected results by ddPCR. Table 13 shows the PPA of the target alteration with ddPCR as the reference method.

Table 13. PPA with ddPCR as orthogonal method, along with the two-sided 95% confidence intervals by alterations

Alteration	Percentage agreement % (N correct/N Total)	Two-sided 95% CI
p.R248C (c.742C>T)	93.33 (14/15)	68.05, 99.83
p.S249C (c.746C>G)	100.00 (56/56)	93.62, 100.00
p.G370C (c.1108G>T)	100.00 (2/2)	15.81, 100.00
p.Y373C (c.1118A>G)	100.00 (18/18)	81.47, 100.00
FGFR3-TACC3v1	100.00 (16/16)	79.41, 100.00
FGFR3-TACC3v3	100.00 (5/5)	47.82, 100.00
FGFR3-BAIAP2L1	100.00 (1/1)	2.50, 100.00

12.1.1. Clinical outcome data

The 42756493-BLC3001 trial was a Phase 3 study to demonstrate superiority of single agent erdafitinib over established (chemotherapy) and emerging (anti PDL-1/PD-L1 monoclonal antibodies) standard of care options in relapsed/refractory subjects with selected *FGFR* alterations.

During the Phase 3 study, erdafitinib was compared to vinflunine or docetaxel (cohort 1) or pembrolizumab (cohort 2) in subjects with advanced urothelial cancer and selected *FGFR* gene mutations. The primary endpoint of overall survival (OS) was evaluated in 2 cohorts:

- Cohort 1: erdafitinib versus chemotherapy (docetaxel or vinflunine) in subjects who have received prior anti-PD-(L)1 agent
- Cohort 2: erdafitinib versus pembrolizumab in subjects who have not received prior anti-PD-(L)1 agent

This study was conducted using the *therascreen* FGFR RGQ RT-PCR Kit as a CDx to screen patients for an *FGFR* mutation. Only cohort 1 was used for registration purposes.

For all observed *FGFR* mutation-positive (*FGFR+*) subjects in cohort 1, the primary efficacy endpoint of OS showed a statistically significant improvement in OS for subjects treated with

erdafitinib vs chemotherapy (HR=0.628; 95% CI: 0.438, 0.900; p-value: 0.0106). This represents a 37.2% reduction in the risk of death for subjects in the erdafitinib treatment group vs. the chemotherapy treatment group. In the erdafitinib group, the median OS was 10.94 months (95% CI: 10.12, 16.76). In the chemotherapy group, the median OS was 7.72 months (95% CI: 6.54, 11.30) (Table 14, Figure 19). These results demonstrated comparable efficacy with the drug intention to treat (ITT) population (HR=0.64; 95% CI: 0.47, 0.88; refer to drug labeling).

Table 14 and Figure 19 show these efficacy results for observed QIAGEN *FGFR*+ population.

Table 14. Summary of overall survival: All *FGFR*+ within cohort 1 analysis set (CDx efficacy analysis)

	Chemotherapy	Erdafitinib
Analysis set: All <i>FGFR</i> + within cohort 1	89	105
Event	57 (64.0%)	64 (61.0%)
Censored	32 (36.0%)	41 (39.0%)
Time to event (months)		
25th percentile (95% CI)	3.06 (2.20, 6.31)	8.02 (5.65, 9.49)
Median (95% CI)	7.72 (6.54, 11.30)	10.94 (10.12, 16.76)
75th percentile (95% CI)	19.61 (11.60, 25.89)	24.48 (17.94, NE)
Range	(0 [‡] , 34 [‡])	(0.2 [‡] , 38 [‡])
6-months event free rate (95% CI)	0.67 (0.56, 0.76)	0.81 (0.72, 0.88)
12-months event free rate (95% CI)	0.36 (0.24, 0.47)	0.49 (0.38, 0.59)
18-months event free rate (95% CI)	0.27 (0.16, 0.39)	0.35 (0.24, 0.45)
24-months event free rate (95% CI)	0.15 (0.06, 0.29)	0.25 (0.16, 0.36)
30-months event free rate (95% CI)	0.04 (0.00, 0.16)	0.16 (0.07, 0.29)
p-value*		0.0106
Hazard ratio (95% CI)* [†]		0.628 (0.438, 0.900)

* A p-value is from a log-rank test.

[†] Hazard ratio is from unstratified proportional hazards model. Hazard ratio <1 favors erdafitinib

Note: [‡] = censored observation, NE = not estimable

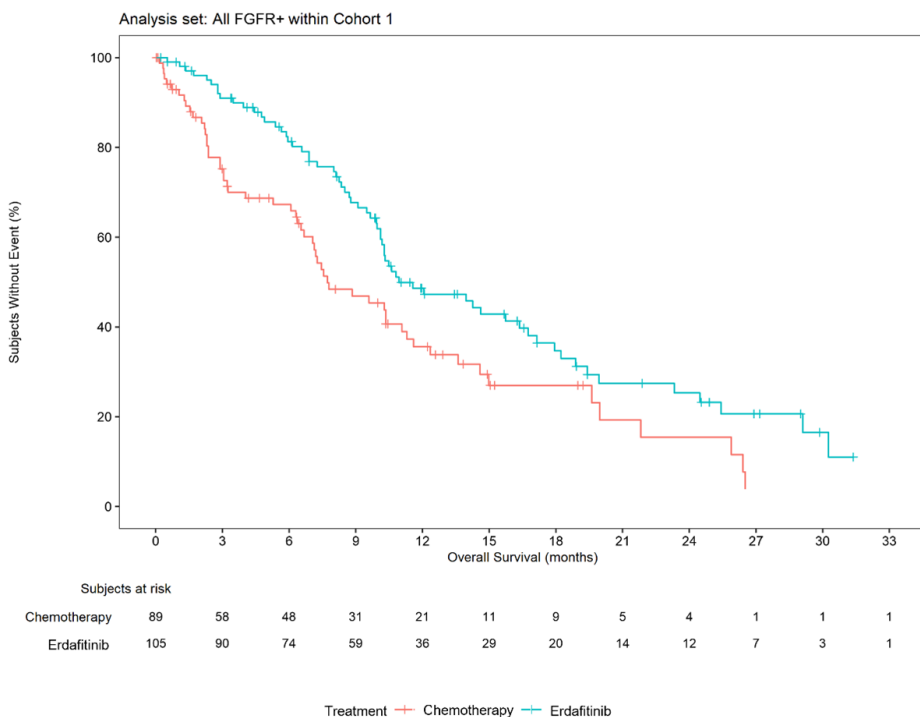


Figure 19. Overall survival within cohort 1 analysis set.

13. References

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2. Knowles, M. A., & Hurst, C. D. (2015). Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nature reviews cancer, 15(1), 25-41.
3. Rodriguez-Vida, A., Saggese, M., Hughes, S., Rudman, S., Chowdhury, S., Smith, N. R., ... & Arkenau, H. T. (2015). Complexity of FGFR signalling in metastatic urothelial cancer. Journal of hematology & oncology, 8(1), 119.
4. Holland, P. M., Abramson, R. D., Watson, R., & Gelfand, D. H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5'→3'exonuclease activity of *Thermus aquaticus* DNA polymerase. Proceedings of the National Academy of Sciences, 88(16), 7276-7280.
5. BALVERSA (Erdafitinib) Prescribing Information.

14. 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 (for contact information, visit www.qiagen.com). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit www.qiagen.com).

Comments and suggestions	
NTC sample shows invalid result	
a. Detection of an assay target has occurred in the absence of template material	Repeat the test from the RT step using sample RNA if available. If sample RNA is not available, repeat the test from the RNA extraction step. 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.
PC sample shows invalid result	
a. Incorrect configuration of the RT and PCR	Check your work steps by means of the pipetting scheme and repeat from the RT step, if necessary.
b. The storage conditions for one or more kit components did not comply with the instructions given in "Storage and Handling" on page 19	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
c. The <i>therascreen</i> FGFR RGQ RT-PCR Kit has expired.	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
IC shows invalid result	
a. IC is outside preset acceptable range; RNA sample quality is unsuitable for testing with the <i>therascreen</i> FGFR RGQ RT-PCR Kit	Repeat RNA extraction if sufficient FFPE tissue is available.

15. 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)
	Global Trade Item Number
	Unique Device Identifier
	Contains
	Component
	Number
Rn	R is for revision of the Instructions for Use and n is the revision number

Symbol

Symbol definition



Temperature limitation



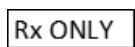
Manufacturer



Consult instructions for use



Keep away from sunlight



Prescription use only



Warning/Caution

16. Contact Information

For technical assistance and more information, please see our Technical Support Center at support.qiagen.com.

17. Ordering Information

Product	Contents	Cat. no.
<i>therascreen</i> FGFR RGQ RT-PCR Kit (24)	For 24 reactions: RT Buffer 1, RT Buffer 2, RT Primer Mix, Reverse Transcriptase, Positive Control, PC Diluent, Mutations-1 Reaction Mix, Mutations-2 Reaction Mix, Fusions-1 Reaction Mix, Fusions-2 Reaction Mix, Water for Sample Dil., Water for NTC	874755
RNeasy DSP FFPE Tissue Kit		
RNeasy DSP FFPE Kit	For 50 RNA preps: RNeasy MinElute® Spin Columns, Collection Tubes, Elution Tubes, Deparaffinization Solution, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers and RNase-Free Water.	73604
Rotor-Gene Q MDx 5plex HRM and accessories		
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cyclers and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002033
PCR Tubes, 0.2 mL (1000)	1000 thin-walled tubes for 1000 reactions of 20–50 µL	981005
Strip Tubes and Caps, 0.1 mL (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
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
Loading Block 96 x 0.2 mL PCR Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 96 x 0.2 mL PCR tubes	9018905
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

Product	Contents	Cat. no.
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 ml in the 72-Well Rotor	9018904

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use. QIAGEN kit instructions for use are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

18. Document Revision History

Revision	Description
R1, June 2025	Initial release for Japan
R2, January 2026	Updated freeze-thaw cycles for FGFR kit from 3 to 5 Updated freeze-thaw cycles for RNA from 3 to 5 Updated the Materials Required but not Provided section to add calibrated spectrophotometer Adaption of Rotor-Gene AssayManager throughout the document • Updated screenshots to RGAM UI • Updated procedural steps for RGAM use • Revised Interpretation of Results section to add information regarding RGAM flags
Rev. 03, August 2026	FGFR FFPE JP Assay Profile version update from 1.0.3 to 1.0.4 Updated Warnings and Precautions section to remove PCR artefact mitigation/false-negative rate statement

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