July 2020

therascreen® IDH1/2 RGQ PCR Kit Handbook

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Version 1

For detection of 12 *IDH1* and *IDH2* mutations in glioma

IVD

For in vitro diagnostic use

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

CE

REF 873011

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Sample to Insight

QIAGEN

Contents

Intended Use

The *therascreen* IDH1/2 RGQ PCR Kit is an in vitro diagnostic test based on PCR technology intended for the qualitative detection of 7 mutations of the *IDH1* gene and 5 mutations of the *IDH2* gene, and for direct identification of 3 major mutations, in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) human brain tissue.

The *therascreen* IDH1/2 RGQ PCR Kit is intended to be used as an aid for the classification of gliomas.

Summary and Explanation

Mutations in the isocitrate dehydrogenase (IDH) genes, *IDH1* and *IDH2*, are frequent in adult World Health Organization (WHO) grade II and III gliomas and WHO grade IV secondary glioblastomas (GBM). In addition to their diagnostic value, the presence of *IDH1/2* mutations is associated with positive prognosis of glioma patients (1–13).

The *therascreen* IDH1/2 RGQ PCR Kit is an assay for detection of 12 specific *IDH1/2* mutations: 6 within codon 132 of the *IDH1* gene, 5 within the homologous codon 172 of *IDH2* and, 1 within codon 100 of *IDH1* (Table 1). The kit also directly identifies the major *IDH1* and *IDH2* mutations leading to *IDH1* R132H, *IDH1* R132C, and *IDH2* R172K substitutions.

Table 1. IDH1 and IDH2 mutations detected using the *therascreen* IDH1/2 RGQ PCR Kit

Gene	Mutation	Base change	COSMIC ID*
IDH1	Arg132His (R132H)	395G>A	COSM28746
	Arg132Cys (R132C)	394C>T	COSM28747
	Arg132Ser (R132S)	394C>A	COSM28748
	Arg132Gly (R132G)	394C>G	COSM28749
	Arg132Leu (R132L)	395G>T	COSM28750
	Arg132Val (R132V)	394_395CG>GT	COSM28751
	Arg100Gln (R100Q)	299G>A	COSM88208
IDH2	Arg172Lys (R172K)	515G>A	COSM33733
	Arg172Met (R172M)	515G>T	COSM33732
	Arg172Trp (R172W)	514A>T	COSM34039
	Arg172Ser (R172S)	516G>T	COSM34090
	Arg172Gly (R172G)	514A>G	COSM33731

* COSMIC IDs are taken from the Catalog of Somatic Mutations in Cancer (**www.sanger.ac.uk/genetics/CGP/cosmic**).

Principle of the Procedure

The *therascreen* IDH1/2 RGQ PCR Kit provides reagents to perform 9 separate amplification reactions for detection of 12 mutations (Table 1):

- 3 total amplification reactions of codons 132 and 100 of the *IDH1* gene and of codon 172 of the *IDH2* gene
- 3 mutation amplification reactions of codons 132 and 100 of the *IDH1* gene and of codon 172 of the *IDH2* gene
- 3 mutation-specific amplification reactions of *IDH1* R132H, *IDH1* R132C, and *IDH2* R172K mutations

Total reaction mixes

The Total Primers and Probe Mixes (PPM-Total) use primers and probes to amplify both mutated and wild-type target sequences (Figure 1).

Mutation detection reaction mixes

The mutation detection primers and probe mixes combine primers and probes, to amplify both mutated and wild-type target sequences, plus an oligonucleotide, 3′ blocked with the addition of a phosphate group to prevent elongation (PCR clamping), which is specific to the wild-type target sequence.

When the PCR template contains the wild-type sequence, the 3′-phosphate oligonucleotide will dominate over PCR primer binding due to higher affinity. There is no or low extension by the DNA polymerase and no or low amplification is observed.

When a mutated sequence is present, PCR primer binding will dominate over the 3′-phosphate oligonucleotide binding and amplification will proceed (Figure 1).

Mutation identification reaction mixes

Allele-specific amplification is achieved by ARMS (Amplification Refractory Mutation System), which exploits the ability of the DNA polymerase to distinguish between a match and a mismatch at the 3′ end of a PCR primer.

When the PCR primer is fully matched, the amplification proceeds with full efficiency. When the 3′ base is mismatched, only low-level background amplification occurs (Figure 1).

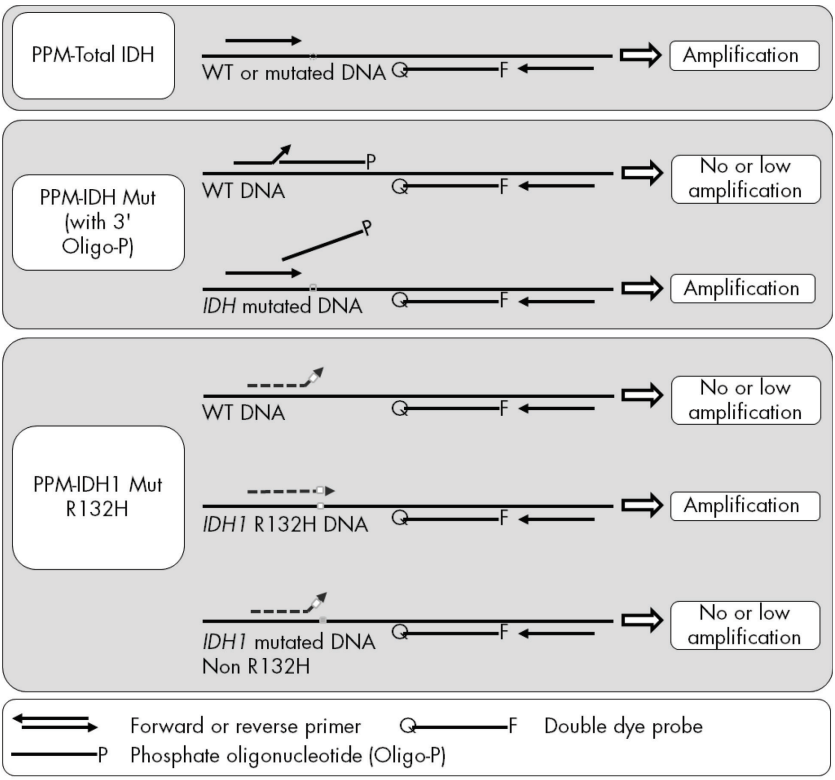

Figure 1. Results obtained with the primers and probe mixes in the *therascreen* IDH1/2 RGQ PCR Kit. The same principle shown to detect *IDH1* R132H applies for *IDH1* R132C and *IDH2* R172K.

Materials Provided

Kit contents

***therascreen* IDH1/2 RGQ PCR Kit**		**(20)**
Catalog number		**873011**
Number of reactions		**20**
Primers and Probe Mix for the detection of total *IDH1*/R132 (Wild Type and Mutated)	PPM-Total IDH1/R132 25x	40 µl
Primers and Probe Mix for the detection of Total *IDH2*/R172 (Wild Type and Mutated)	PPM-Total IDH2/R172 25x	40 µl
Primers and Probe mix for the detection of Total *IDH1*/R100 (Wild Type and Mutated)	PPM-Total IDH1/R100 25x	40 µl
Primers and Probe Mix (including Oligo-P) for the detection of Mutated *IDH1*/R132	PPM-IDH1/R132 Mut 25x	40 µl
Primers and Probe Mix (including Oligo-P) for the detection of Mutated *IDH2*/R172	PPM-IDH2/R172 Mut 25x	40 µl
Primers and Probe mix (including Oligo-P) for the detection of Mutated *IDH1*/R100	PPM-IDH1/R100 Mut 25x	40 µl
Primers and Probe Mix for the identification of *IDH1* Mut R132H	PPM-IDH1 Mut R132H 25x	40 µl

Table continued on next page

Kit contents (continued)

***therascreen* IDH1/2 RGQ PCR Kit**		**(20)**
Catalog number		**873011**
Number of reactions		**20**
Primers and Probe Mix for the identification of *IDH1* Mut R132C	PPM-IDH1 Mut R132C 25x	40 µl
Primers and Probe Mix for the identification of *IDH2* Mut R172K	PPM-IDH2 Mut R172K 25x	40 µl
IDH1/IDH2 Wild Type Genomic DNA	IDH1/IDH2 WT Control	270 µl
IDH1/IDH2 Mutated Positive Control	IDH1/IDH2 Positive Control	270 µl
Mix of *Taq* DNA Polymerase, dNTPs, MgCl2, and buffer for qPCR	qPCR Master Mix 2x	5 x 900 µl
Nuclease-Free Water	Nuclease-Free Water	5 x 525 µl
therascreen *IDH1/2 RGQ PCR Kit Handbook* (English)		1

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.

Important: Make sure that instruments used in this procedure have been checked and calibrated according to the manufacturer's recommendations.

Reagents (manual DNA extraction)

- DNA extraction kit: QIAamp® DNA FFPE Tissue Kit (cat. no. 56404)
- RNase A (17,500 U) (cat. no. 19101)
- Xylene or Histolemon™ (Carlo Erba, cat. no. 454911, **www.carloerbareagents.com**)
- Ethanol (96–100%)
- 1x TE buffer, pH 8.0

Reagents (automated DNA extraction)

- DNA extraction kit: QIAsymphony® DSP DNA Mini Kit (cat. no. 937236)
- Buffer ATL (cat. nos. 19076 or 939016)
- RNase A (cat. no. 19101)
- Xylene or Histolemon (Carlo Erba, cat. no. 454911, **www.carloerbareagents.com**)
- Ethanol (96–100%)
- 1x TE buffer, pH 8.0

Consumables

- Scalpels
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters
- 2.0 ml or 1.5 ml nuclease-free tubes

- Strip Tubes and Caps, 0.1 ml, for the Rotor-Gene Q (cat. nos. 981103 or 981106)
- Ice

Additional consumables for automated DNA extraction

- Sample Prep Cartridges, 8-well (cat.no.997002)
- 8-Rod Covers (cat.no.997004)
- Filter-Tips, 200 µl, Qsym SP (cat. no. 990332) and Filter-Tips, 1500 µl, Qsym SP (cat. no. 997024)
- Elution Microtubes CL (cat.no. 19588)
- Micro tubes 2.0 ml Type H (Sarstedt®, cat. no. 72.693, **www.sarstedt.com**)

Equipment

- Slide rack and 2 compatible slide baths for xylene/Histolemon and ethanol
- Microliter pipets dedicated for PCR (1–10 µl; 10–100 µl; 100–1000 µl)
- Benchtop centrifuge with rotor for 0.5 ml/1.5 ml reaction tubes and microplates (capable of attaining 13,000–14,000 rpm)
- Benchtop vortexer
- Real-time PCR instrument: Rotor-Gene Q MDx 5plex HRM and associated specific material
- Rotor-Gene Q MDx software version 2.1.0 or higher
- Biophotometer
- Thermomixer, heated orbital incubator, heating block or water bath capable of incubation at 56°C and 90°C

Additional equipment for automated purification

- QIAsymphony SP instrument
- QIAsymphony SP software version 4.0 or higher

Warnings and Precautions

For in vitro diagnostic use

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 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 purification kit used, see the relevant kit handbook. For safety information regarding instruments, see the relevant instrument user manual.

General precautions

- The test is for use with buffered formalin-fixed, paraffin-embedded (FFPE) surgical resection tissue specimens.
- All chemicals and biological materials are potentially hazardous. Specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard sample and assay waste according to your local safety procedures.
- Reagents for the *therascreen* IDH1/2 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 µl.
- All reagents supplied in the *therascreen* IDH1/2 RGQ PCR Kit are intended to be used solely with the other reagents supplied in the same kit. Do not substitute any reagent between *therascreen* IDH1/2 RGQ PCR Kits, as this may affect performance.
- Refer to the Rotor-Gene Q MDx 5plex HRM instrument user manual for additional warnings, precautions, and procedures.

- Alteration of incubation and temperatures may result in erroneous or discordant data.
- Do not use expired or incorrectly stored components.
- Primers and probe mixes may be altered if exposed to light.
- Use extreme caution to prevent contamination of the mixes with the synthetic materials that are contained in the positive control reagent.
- Use extreme caution to prevent contamination by DNase, which might cause degradation of the template DNA.
- Use individual, dedicated pipets for setting up reaction mixes and adding templates.
- Perform preparation and dispensing of reaction mixes in an area separate from the one used for the addition of the templates.
- Do not open the Rotor-Gene Q MDx 5plex HRM instrument until the run is finished.
- Do not open Rotor-Gene Q MDx 5plex HRM tubes after the run is finished.
- Caution must be observed to ensure correct sample testing with emphasis to wrong sample entry, loading error, and pipetting error.

Reagent Storage and Handling

Shipping conditions

The *therascreen* IDH1/2 RGQ PCR Kit is shipped on dry ice. If any component of the *therascreen* IDH1/2 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, handbook, or the reagents, please contact one of the QIAGEN Technical Services or local distributors (see **www.qiagen.com**).

Storage

The *therascreen* IDH1/2 RGQ PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light.

Stability

When stored under the specified storage conditions, the *therascreen* IDH1/2 RGQ PCR Kit is stable until the stated expiration date.

Once opened, reagents can be stored in their original packaging at –30 to –15°C until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided. Do not exceed a maximum of 5 freeze–thaw cycles.

Specimen Handling and Storage

The *therascreen* IDH1/2 RGQ PCR Kit is for use with DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue from surgical resections collected from brain cancer subjects. All tissue samples should be treated as potentially hazardous.

- Tissue specimen must be fixed in 4–10% neutral buffered formalin (NBF).
- 10 µm serial sections must be cut from the paraffin block and mounted on glass slides.
- A trained individual (such as a pathologist) should assess tumor content and area on an adjacent Hematoxilyn and Eosin (H&E) stained section. Use serial sections for DNA extraction.
- Only sections with ≥40% tumor content are eligible for the test.
- For sections containing <50 mm^2 tissue area, we recommend to process a sufficient number of sections to increase the total tissue area to at least 50 mm^2 (100 mm^2 for automated extraction on the QIAsymphony SP).
- Label, handle, and store tumor specimens, blocks, slides, and samples ready for extraction in a controlled fashion according to local procedures.
- Store FFPE blocks and slides at room temperature. Slides may be stored at ambient temperature for up to 4 weeks prior to DNA extraction for use with the *therascreen* IDH1/2 RGQ PCR Kit.
- Following extraction, genomic DNA may be stored for up to 1 week at 2–8°C or 8 weeks at –25 to –15°C.

Procedure

DNA extraction and preparation

Use the QIAamp DNA FFPE Tissue Kit (cat. no. 56404) or the QIAsymphony DSP DNA Mini Kit (cat. no. 937236) for purifying genomic DNA from samples prepared from FFPE brain cancer specimens.

Note: The *therascreen* IDH1/2 RGQ PCR Kit has only been validated in combination with the QIAamp DNA FFPE Tissue Kit or the QIAsymphony DSP DNA Mini Kit. Do not use any other DNA extraction product.

Using the QIAamp DNA FFPE Tissue Kit

CAUTION 	Please read carefully the following modifications that need to be applied to the QIAamp protocol.

- See "Starting material" in the *QIAamp DNA FFPE Tissue Handbook* and Specimen Handling and Storage, page 17 in this handbook, for preparation of samples prior to deparaffinization and DNA extraction.
- The QIAamp DNA FFPE Tissue Kit must be used manually only.
- The RNase step described in the *QIAamp DNA FFPE Tissue Handbook* must be performed.
- Do not use the QIAGEN Deparaffinization Solution. Use only the xylene/ethanol method for deparaffinization as described in "Slide deparaffinization procedure when using the QIAamp DNA FFPE Tissue Kit", below. Xylene can be replaced by Histolemon (xylene substitute).
- Proteinase K digestion must be performed for 1 hour.

- The samples must be eluted twice into 30 µl of elution buffer (Buffer ATE) from the QIAamp DNA FFPE Tissue Kit.

Slide deparaffinization procedure when using the QIAamp DNA FFPE Tissue Kit

1. Place the slides in a specific slide rack.
2. Put the slide rack into a slide bath containing xylene or Histolemon for 2 minutes. Shake by 2 or 3 movements backward and forward.
3. Place the rack into a second slide bath containing ethanol (96–100%) for 2 minutes. Shake by 2 or 3 movements backward and forward.
4. Dry the slides at 15–37°C. This will take a few minutes.
5. Label 1.5 ml microcentrifuge tubes for each sample, and add 180 µl Buffer ATL (from the QIAamp DNA FFPE Tissue Kit) to each tube.
6. Place a few drops of Buffer ATL onto the tissue sections on the slides (enough to cover the tissue surface).
7. Scrape the tissue area with a sterile scalpel, and add the scraped tissue to the corresponding labeled microcentrifuge tube.
8. Add 20 µl proteinase K (from the QIAamp DNA FFPE Tissue Kit) to each tube, and mix by vortexing.
9. Incubate at 56°C for 1 hour.

Continue with the 90°C incubation step in the QIAamp DNA FFPE Tissue Kit protocol (step 12 in the *QIAamp DNA FFPE Tissue Handbook*, June 2012, page 13).

Using the QIAsymphony DSP DNA Mini Kit

CAUTION 	Please read carefully the following modifications that need to be applied to the QIAsymphony SP Protocol Sheet: Tissue_LC_200_V7_DSP.

- See "Specimen Handling and Storage", page 17, for preparation of samples prior to deparaffinization and DNA extraction.
- The RNase step described in the QIAsymphony SP Protocol Sheet must be performed.
- Do not use the QIAGEN Deparaffinization Solution. Use only the xylene/ethanol method for deparaffinization as described in Slide deparaffinization procedure when using the QIAsymphony DSP DNA Mini Kit below. Xylene can be replaced by Histolemon (xylene substitute).
- Proteinase K digestion must be performed for 1 hour.
- The 50 µl elution volume must be selected in the touchscreen.

Slide deparaffinization procedure when using the QIAsymphony DSP DNA Mini Kit

Carry out deparaffinization according to the following steps, which differ from the protocol in the QIAsymphony SP Protocol Sheet: Tissue_LC_200_V7_DSP.

1. Place the slides in a specific slide rack.
2. Put the slide rack into a slide bath containing xylene or Histolemon for 2 minutes. Shake by 2 or 3 movements backward and forward.
3. Place the rack into a second slide bath containing ethanol (96–100%) for 2 minutes. Shake by 2 or 3 movements backward and forward.
4. Dry the slides at 15–37°C. This will take a few minutes.
5. Label 1.5 ml microcentrifuge tubes for each sample, and add 220 µl Buffer ATL to each tube.

6. Place a few drops of Buffer ATL onto the tissue sections on the slides (enough to cover the tissue surface).
7. Scrape the tissue area with a sterile scalpel, and add the scraped tissue to the corresponding labeled microcentrifuge tube.
8. Add 20 µl proteinase K (from the QIAamp DNA FFPE Tissue Kit) to each tube, and mix by vortexing.

Continue with the 56°C incubation step in the QIAsymphony SP Protocol Sheet: Tissue_LC_200_V7_DSP protocol (step 12 in the "Deparaffinization using xylene" protocol, April 2012). Incubate at 56°C for 1 hour.

Genomic DNA

Store genomic DNA at 2–8°C for up to 1 week post extraction or for 8 weeks at –25 to –15°C.

DNA quantity should be determined by measuring the optical density (OD) of the sample at 260 nm.

Dilute DNA to a concentration of 5 ng/µl in 1x TE buffer at pH 8.0.

The PCR reaction is optimized for samples containing 25 ng purified genomic DNA.

Protocol: Detection of *IDH1/2* mutations

Important points before starting

- To use the *therascreen* IDH1/2 RGQ PCR Kit optimally, samples must be grouped into batches of 4. Smaller batch sizes will mean that fewer samples can be tested with the *therascreen* IDH1/2 RGQ PCR Kit.
- We recommend testing all samples once per PCR run, as indicated in Table 2 and with a loading block layout and rotor setup as indicated in Table 3 and Figure 2.

Table 2. Number of reactions for Rotor-Gene Q MDx instruments with 72-tube rotor

Samples	Reactions
n DNA samples	n x 1 reaction
2 DNA controls	2 reactions: Positive and WT controls, each one tested once per PCR run
Water control	1 reaction

Table 3. Suggested loading block for an experiment with the *therascreen* IDH1/2 RGQ PCR Kit

Sample	Total *IDH1*/ R132	*IDH1*/ R132 Mut	*IDH1* Mut R132H	*IDH1* Mut R132C	Total *IDH2*/ R172	*IDH2*/ R172 Mut	*IDH2* Mut R172K	Total *IDH1*/ R100	*IDH1*/ R100 Mut
Mut PC*	1	9	17	25	33	41	49	57	65
WTC†	2	10	18	26	34	42	50	58	66
S1	3	11	19	27	35	43	51	59	67
S2	4	12	20	28	36	44	52	60	68
S3	5	13	21	29	37	45	53	61	69
S4	6	14	22	30	38	46	54	62	70
H_2O	7	15	23	31	39	47	55	63	71
Empty tube	8	16	24	32	40	48	56	64	72

* PC: Positive control.
† WTC: Wild-type control.

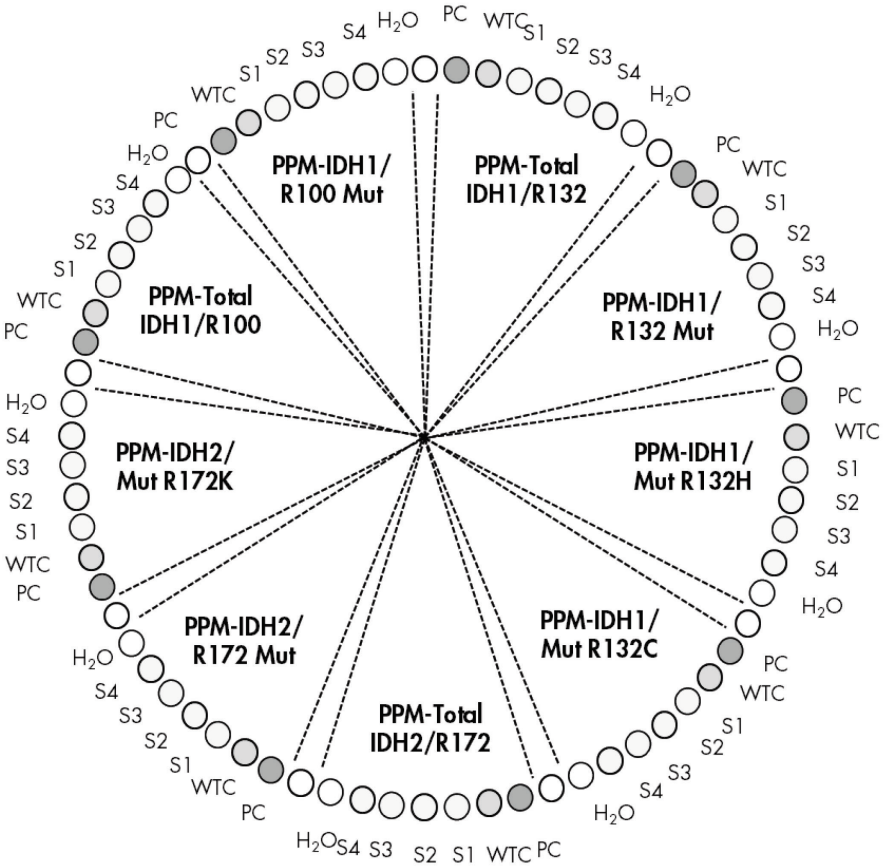

Figure 2. Suggested rotor setup for an experiment with the *therascreen* IDH1/2 RGQ PCR Kit.

Important: Make sure to always place a sample in position 1 of the rotor. Otherwise the instrument will not perform calibration, and incorrect fluorescence data will be acquired.

Procedure

1. Thaw all necessary components and place them on ice.
2. Prepare the following PCR mixes according to the number of samples being processed.

   Note: All concentrations are for the final volume of the reaction.

   Table 4 describes the pipetting scheme for the preparation of one reagent mix, calculated to achieve a final reaction volume of 25 µl. A pre-mix can be prepared for each PPM, according to the number of reactions. Extra volumes are included to compensate for pipetting errors.

Table 4. Preparation of PCR mixes

Component	1 reaction (µl)	Pre-mix: 7 + 1 reactions (µl)	Final concentration
qPCR Master Mix, 2x	12.5	100	1x
PPM,* 25x	1	8	1x
Nuclease-Free Water	6.5	52	–
Sample or control† (to be added at step 4)	5	5 each	–
Total volume	**25**	**25 each**	**–**

* Prepare 9 pre-mixes, one with each of the PPMs provided in the kit.
† Positive control, negative control or water control.

3. Dispense 20 µl of the pre-mix solution per Rotor-Gene tube (Table 3).
4. Add 5 µl of the material to be quantified (25 ng sample genomic DNA or control) in the corresponding tube (total volume 25 µl; Table 3).
5. Mix gently by pipetting up and down.
6. Place the tubes in the adapter provided with the instrument (Figure 2).

   Note: Unused positions need to be filled with empty tubes.
7. Load the full adapter into the Rotor-Gene Q MDx instrument.

8. Program the Rotor-Gene Q MDx instrument with the thermal cycling program as indicated in Table 5.

Table 5. Temperature profile

Hold	Temperature: 95°C
	Time: 10 min
Cycling	40 times
	95°C for 15 sec
	60°C for 60 sec with acquisition of FAM™ fluorescence in channel Green: Single

9. Click **Gain Optimisation** in the New Run Wizard dialog box to open the Auto-Gain Optimisation Setup dialog box. Set the range for Green channel from **2Fl** for **Min Reading** to **10Fl** for **Max Reading**.
10. Check the **Perform Optimisation Before 1st Acquisition** box, and close the Auto-Gain Optimisation Setup dialog box.
11. Start the thermal cycling program.
12. Once the thermal cycling has ended, perform the following.
    - 12a. Select **Options** > **Crop Start Cycles**. Remove data before cycle **10** so as to discard any artifacts.
    - 12b. Select **Analysis** > **Cycling A. Green from 10**, indicated on the report as "left threshold = 10.00".
    - 12c. Select **Dynamic Tube** as a normalization method and **Slope Correct** to correct the noise slope.
    - 12d. Set the **Outlier Removal** to **0%** (corresponding to the NTC threshold).
    - 12e. Set the **Reaction Efficiency Threshold** to be disabled.
    - 12f. Define the threshold at **0.03**.
    - 12g. Set the graph to linear scale.
    - 12h. Select **Digital Filter: Light**.

Interpretation of Results

Water controls

Water controls (no template controls) should give zero C_T values for all primers and probe mixes.

If a positive C_T value is obtained with a water control, this results from a cross-contamination. See "Troubleshooting guide", page 36, to find a solution.

Quality control using C_T values of controls

The *IDH1/2* wild-type control (WTC) and the mutated *IDH1/2* positive control (Mut-PC) allow the validation of the experiment.

- If there is no Ct value, the control is classified as mutation-negative for the respective detection assay.
- If Ct values are detected, calculate the ΔC_T as follows for each control

  $\Delta C_{T\ IDH1/R132\ Mut} = C_{T\ IDH1/R132\ Mut} - C_{T\ Total\ IDH1/R132}$

  $\Delta C_{T\ IDH2/R172\ Mut} = C_{T\ IDH2/R172\ Mut} - C_{T\ Total\ IDH2/R172}$

  $\Delta C_{T\ IDH1/R100\ Mut} = C_{T\ IDH1/R100\ Mut} - C_{T\ Total\ IDH1/R100}$

  $\Delta C_{T\ IDH1\ Mut\ R132H} = C_{T\ IDH1\ Mut\ R132H} - C_{T\ Total\ IDH1/R132}$

  $\Delta C_{T\ IDH1\ Mut\ R132C} = C_{T\ IDH1\ Mut\ R132C} - C_{T\ Total\ IDH1/R132}$

  $\Delta C_{T\ IDH2\ Mut\ R172K} = C_{T\ IDH2\ Mut\ R172K} - C_{T\ Total\ IDH2/R172}$

Controls are classified as mutation-positive if the ΔC_T values are less than or equal to the respective ΔC_T cutoff values, listed in Table 6. If the ΔC_T value is higher than the cut-off, the control is classified as mutation-negative for the considered mutation assay.

Table 6. Cutoff values for each mutation assay

Mutation assay	Cutoff (ΔC_T)
IDH1/R132 Mut	5.34
IDH2/R172 Mut	6.42
IDH1/R100 Mut	4.65
IDH1 Mut R132H	6.87
IDH1 Mut R132C	7.14
IDH2 Mut R172K	8.49

- The *IDH1/2* wild-type control must be detected as mutation-negative for each mutation assay (Table 7).
- The mutated *IDH1/2* positive control must be detected as mutation-positive for each mutation assay (Table 7).

The entire experiment is rejected if one or both conditions are not met.

Table 7. Example of run validation on controls

Value	Water (NTC)	IDH1/IDH2 WT Control	IDH1/IDH2 Positive Control
$C_{T\ Total\ IDH1/R132}$	Undetected	25.45	23.95
$C_{T\ IDH1/R132\ Mut}$	Undetected	34.32	25.76
$\Delta C_{T\ IDH1/R132\ Mut}$	Undetected	8.87	1.81
$C_{T\ Total\ IDH2/R172}$	Undetected	25.42	24.93
$C_{T\ IDH2/R172\ Mut}$	Undetected	34.36	26.36
$\Delta C_{T\ IDH2/R172\ Mut}$	Undetected	8.94	1.43
$C_{T\ Total\ IDH1/R100}$	Undetected	26.30	24.69
$C_{T\ IDH1/R100\ Mut}$	Undetected	33.04	26.39
$\Delta C_{T\ IDH1/R100\ Mut}$	Undetected	6.74	1.70
$C_{T\ IDH1\ Mut\ R132H}$	Undetected	35.20	26.48
$\Delta C_{T\ IDH1\ Mut\ R132H}$	Undetected	9.75	2.53
$C_{T\ IDH1\ Mut\ R132C}$	Undetected	37.16	27.07
$\Delta C_{T\ IDH1\ Mut\ R132C}$	Undetected	11.71	3.12
$C_{T\ IDH2\ Mut\ R172K}$	Undetected	Not detected	27.97
$\Delta C_{T\ IDH2\ Mut\ R172K}$	Undetected	N/A	3.04

Sample input validation

A sample input must be validated before interpretation.

The C_T value obtained for a sample with each PPM-Total ($C_{T\ Total\ IDH1/R132}$, $C_{T\ Total\ IDH2/R172}$ and $C_{T\ Total\ IDH1/R100}$) must be lower than 32.00. $C_{T\ Total}$ values ≥32.00 are due to poor quality of the DNA. The sample must be tested again. If the quantity of DNA is still insufficient, extract more tumor tissue, if available (see "Troubleshooting guide", page 36).

Sample results

IDH1/2 mutation detection

For each sample, calculate the ΔC_T values obtained with each detection mutation assay (PPM-IDH1/R132 Mut, PPM-IDH2/R172 Mut, PPM-IDH1/R100 Mut) as follows.

$$\Delta C_{T\ IDH1/R132\ Mut} = C_{T\ IDH1/R132\ Mut} - C_{T\ Total\ IDH1/R132}$$

$$\Delta C_{T\ IDH2/R172\ Mut} = C_{T\ IDH2/R172\ Mut} - C_{T\ Total\ IDH2/R172}$$

$$\Delta C_{T\ IDH1/R100\ Mut} = C_{T\ IDH1/R100\ Mut} - C_{T\ Total\ IDH1/R100}$$

If there is no Ct value for a mutation detection assay, the sample must be classified as mutation-negative for the considered mutation.

Samples are classified as mutation-positive if the ΔC_T value is less than or equal to the ΔC_T cutoff value of the respective mutation detection assay, listed in Table 8.

Table 8. Cutoff values for each mutation detection assay

Mutation assay	Cutoff (ΔC_T)
IDH1/R132 Mut	5.34
IDH2/R172 Mut	6.42
IDH1/R100 Mut	4.65

IDH1/2 mutation identification

For each sample, calculate the ΔC_T values obtained with each identification mutation assay (PPM-IDH1 Mut R132H, PPM-IDH1 Mut R132C, PPM-IDH2 Mut R172K) as follows.

$\Delta C_{T\ IDH1\ Mut\ R132H} = C_{T\ IDH1\ Mut\ R132H} - C_{T\ Total\ IDH1/R132}$

$\Delta C_{T\ IDH1\ Mut\ R132C} = C_{T\ IDH1\ Mut\ R132C} - C_{T\ Total\ IDH1/R132}$

$\Delta C_{T\ IDH2\ Mut\ R172K} = C_{T\ IDH2\ Mut\ R172K} - C_{T\ Total\ IDH2/R172}$

If there is no Ct value for a mutation identification assay, the sample must be classified as mutation-negative.

The sample mutation is identified if the ΔC_T value is less than or equal to the ΔC_T cutoff value of the respective mutation identification assay, listed in Table 9. Examples of ΔC_T interpretation are shown in Table 10 and Table 11.

Table 9. Cutoff values for each mutation identification assay

Mutation assay	Cutoff (ΔC_T)
IDH1 Mut R132H	6.87
IDH1 Mut R132C	7.14
IDH2 Mut R172K	8.49

Table 10. Example of *IDH1/2* mutation detection

Value	Sample 1	Sample 2
$C_{T\ Total\ IDH1/R132}$	26.39	26.32
$C_{T\ IDH1/R132\ Mut}$	33.86	28.29
$\Delta C_{T\ IDH1/R132\ Mut}$	7.47	1.97
$C_{T\ Total\ IDH2/R172}$	26.79	25.79
$C_{T\ IDH2/R172\ Mut}$	35.13	35.21
$\Delta C_{T\ IDH2/R172\ Mut}$	8.34	9.42
$C_{T\ Total\ IDH1/R100}$	27.20	27.37
$C_{T\ IDH1/R100\ Mut}$	33.83	33.76
$\Delta C_{T\ IDH1/R100\ Mut}$	6.63	6.39
Mutation detection	No mutation detected	R132 mutation detected

Table 11. Example of *IDH1/2* mutation identification

Value	Sample 1	Sample 2
$C_{T\ Total\ IDH1/R132}$	26.39	26.32
$C_{T\ IDH1\ Mut\ R132H}$	33.82	28.27
$\Delta C_{T\ IDH1\ Mut\ R132H}$	7.43	1.95
$C_{T\ Total\ IDH1/R132}$	26.39	26.32
$C_{T\ IDH1\ Mut\ R132C}$	37.94	Not detected
$\Delta C_{T\ IDH1\ Mut\ R132C}$	11.55	N/A
$C_{T\ Total\ IDH2/R172}$	26.79	25.79
$C_{T\ IDH2\ Mut\ R172K}$	Not detected	Not detected
$\Delta C_{T\ IDH2\ Mut\ R172K}$	N/A	N/A
Mutation identification	No mutation detected	Mutation detected for R132H

Interpretation of *IDH1/2* mutations

The procedure used to assign the *IDH1/2* mutation type to samples positive for an *IDH1/2* mutation is shown in Table 12. An example of interpretation is shown in Table 13.

Table 12. Interpretation guide

		Mutation identification			
		***IDH1* Mut R132H detected**	***IDH1* Mut R132C detected**	***IDH2* Mut R172K detected**	**No mutation detected**
Mutation detection	**R132 mutation detected**	R132H mutation detected	R132C mutation detected	–	R132 mutation but neither R132H nor R132C
	R172 mutation detected	–	–	R172K mutation detected	R172 mutation but not R172K
	R100 mutation detected	–	–	–	R100
	No mutation detected	Low content of mutation R132H detected (between 1% and 2%)*	Low content of mutation R132C detected (between 1% and 4%)*	Low content of mutation R172K detected (approximately 1%)*	No mutation detected

* These cases might occur rarely, and all samples and technical acceptance criteria should be checked, especially tumor cell content. If all criteria are met, the sample should be retested.

Table 13. Example of *IDH1/2* mutation reporting and interpretation

	Sample 1	Sample 2
Mutation detection	No mutation detected	R132 mutation detected
Mutation identification	No mutation detected	Mutation detected for R132H
Result interpretation	No mutation detected nor identified	R132H mutated

Note: If a sample has 2 or more ΔC_T values less than or equal to the ΔC_T cutoff values, then the mutant status is assigned to the mutation with the greatest difference between the cutoff and the obtained ΔC_T. See example in Table 14.

Table 14. Example of interpretation in case of multiple positive results

	Sample 3	Sample 4
ΔC_T IDH1/R132 Mut	1.24	5.24
ΔC_T cutoff IDH1/R132 Mut	5.34	5.34
(ΔC_T cutoff – ΔC_T) IDH1/R132 Mut	4.10	0.10
ΔC_T IDH2/R172 Mut	5.32	5.95
ΔC_T cutoff IDH2/R172 Mut	6.42	6.42
(ΔC_T cutoff – ΔC_T) IDH2/R172 Mut	1.10	0.47
Result interpretation	R132 mutated	R172 mutated

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, visit **www.qiagen.com**.

		Comments and suggestions
Clogged column during DNA extraction		
	Incomplete lysis	Repeat centrifugation. The remaining lysate can be transferred to a new column. Repeat the extraction run with less FFPE tissue.
Insufficient DNA in the extraction eluate		
	Insufficient FFPE tissue area	Repeat the extraction run with more FFPE tissue section(s).
IDH1/2 WT control not detected		
a)	Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and setup of the reaction. Repeat the PCR run.
b)	Inappropriate storage of kit components	Store the *therascreen* IDH1/2 RGQ PCR Kit at –30 to–15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 16. Do not exceed a maximum of 5 freeze–thaw cycles.
c)	The *therascreen* IDH1/2 RGQ PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and, if necessary, use a new *therascreen* IDH1/2 RGQ PCR Kit.
***IDH1/2* positive control not detected**		
a)	Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and setup of the reaction.

		Comments and suggestions
		Repeat the PCR run.
b)	Inappropriate storage of kit components	Store the *therascreen* IDH1/2 RGQ PCR Kit at –30 to–15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 16. Do not exceed a maximum of 5 freeze–thaw cycles.
c)	The *therascreen* IDH1/2 RGQ PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and, if necessary, use a new *therascreen* IDH1/2 RGQ PCR Kit.
No signal, including no signal for controls		
a)	No reaction tube in position 1 of the Rotor-Gene Q MDx instrument	Make sure to always place a sample in position 1 of the rotor. Otherwise the instrument will not perform calibration, and incorrect fluorescence data will be acquired.
b)	Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and setup of the reaction. Repeat the PCR run.
c)	Inappropriate storage of kit components	Store the *therascreen* IDH1/2 RGQ PCR Kit at –30 to–15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 16. Do not exceed a maximum of 5 freeze–thaw cycles.
d)	The *therascreen* IDH1/2 RGQ PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and, if necessary, use a new *therascreen* IDH1/2 RGQ PCR Kit.
e)	Incorrect detection channel chosen	Set the detection channel to Cycling Green or 530 nm/640 nm.
f)	No data acquisition program	Check the cycling program. See Table 5, page 26.

		Comments and suggestions
		Select acquisition mode **Single** at the end of each annealing segment of the PCR program.
Fluorescence intensity varies		
	Pipetting errors or omitted reagents; tube or well inversions	Check pipetting scheme and setup of the reaction. Repeat the PCR run.
Fluorescence intensity too low		
a)	Inappropriate storage of kit components	Store the *therascreen* IDH1/2 RGQ PCR Kit at –30 to–15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling", page 16. Do not exceed a maximum of 5 freeze–thaw cycles.
b)	The *therascreen* IDH1/2 RGQ PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and, if necessary, use a new *therascreen* IDH1/2 RGQ PCR Kit.
c)	Very low amount of target DNA	Always check the DNA concentration before starting. See "DNA extraction and preparation", page 18.

	Comments and suggestions
Negative control (H_2O) gives a positive result	
Cross-contamination, reagent contamination, instrument error, well or capillary inversion, or probe degradation	Replace all critical reagents, or use a new *therascreen* IDH1/2 RGQ PCR Kit. Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carryover contamination. Keep primers and probe mixes protected from light. Check for false positives on fluorescence curves. Check the setup of the reaction. See "Protocol: Detection of *IDH1/2* mutations", page 22.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *therascreen* IDH1/2 RGQ PCR Kit is tested against predetermined specifications to ensure consistent product quality. Certificates of analysis are available on request at www.qiagen.com/support/.

Limitations

The kit is intended for professional use.

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

This kit should be used following the instructions given in this manual, in combination with a validated instrument mentioned in "Materials Required but Not Provided", page 12.

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

The *therascreen* IDH1/2 RGQ PCR Kit is validated only for buffered formalin-fixed paraffin-embedded brain tissue.

The *therascreen* IDH1/2 RGQ PCR Kit is validated only for use with the QIAamp DNA FFPE Tissue Kit or with the QIAsymphony DSP DNA Mini Kit.

Only the Rotor-Gene Q MDx 5plex HRM (for PCR) and the QIAsymphony SP (for sample preparation) have been validated.

Any off-label use of this product and/or modification of the components will void QIAGEN's liability

It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance studies.

The test is designed to detect 7 mutations in codons 132 and 100 of the *IDH1* gene and 5 mutations in codon 172 of the *IDH2* gene. Samples with results reported as "no mutation detected" may harbor *IDH1* or *IDH2* mutations not detected by the assay.

Detection of mutations is dependent on sample integrity, the tumor content, and amplifiable DNA present in the specimen.

Any diagnostic results generated with the product must be interpreted within the context of all relevant clinical or laboratory findings.

Performance Characteristics

Limit of blank (LOB)

Limit of blank (LOB) was determined (following CLSI/NCCLS EP17-A guideline; 14) on negative samples (FFPE normal brain, 8 samples, 64 measurements/lot, 2 lots).

LOB results are presented in Table 15.

Table 15. Limit of blank (LOB)

Assay	LOB	Final LOB
R132 Mut	Validation lot 1: 6.57 Validation lot 2: 6.32	6.32
R132H Mut	Validation lot 1: 7.91 Validation lot 2: 8.22	7.91
R132C Mut	Validation lot 1: 8.04 Validation lot 2: 8.20	8.04
R172 Mut	Validation lot 1: 7.74 Validation lot 2: 7.59	7.59
R172K Mut	Validation lot 1: 9.93 Validation lot 2: 10.58	9.93
R100 Mut	Validation lot 1: 6.52 Validation lot 2: 5.19	5.17

Limit of detection (LOD)

Limit of detection (LOD or analytical sensitivity) was determined based on the "precision profile approach" described in the CLSI/NCCLS EP17-A guideline (14). Five low positive samples (plasmid DNA spiked into glioma wild-type DNA) were used per mutation (30 to 110 measurements per mutation type and mutation percentage).

LOD results are presented in Table 16.

Table 16. Limit of detection (LOD)

Assay	Mutations	LOD	Assay cutoff	Sensitivity (%)
R132H Mut	R132H	6.87	6.87	0.78
R132C Mut	R132C	7.14	7.14	1.19
R172K Mut	R172K	8.49	8.49	0.61
R132 Mut	R132H	5.50	5.34	2.32
	R132C	5.34		4.35
	R132L	5.42		2.30
	R132G	5.61		2.23
	R132S	5.42		2.75
	R132V	5.56		2.24
R172 Mut	R172K	6.42	6.42	1.06
	R172G	6.58		3.00
	R172M	6.66		3.31
	R172S	6.42		14.93
	R172W	6.68		2.36
R100 Mut	R100Q	4.65	4.65	3.45

A mutation is detected if ΔC_T is less than or equal to the LOD.

Effect of DNA input

DNA was extracted from 4 different glioma tumor samples: 2 with wild-type *IDH1/2* and 2 carrying the *IDH1* R132H (395G>A) mutation.

Three different DNA amounts (including the recommended one for the protocol) were tested to evaluate the impact of DNA input on qualitative results. Results showed that DNA input had no impact on qualitative results. However, more technical failures ($C_{T\ Total}$ QC failures) were observed for DNA input lower than the recommended input (<25 ng DNA). Consequently, an input of 25 ng DNA in a volume of 5 µl is recommended to run the test.

Repeatability and reproducibility

The precision study was performed on 4 different samples (plasmid DNA spiked into glioma wild-type DNA representative of wild-type (WT), mutant and cutoff sample) tested 40 times in duplicate (n = 80 measurements).

Standard deviations (SD) and coefficients of variation (CV) are presented in Table 17.

Table 17. Precision results

Assay	Sample	Mean ΔC_T	SD_R*	SD_{Run}†	SD_{Total}‡	CV_{Total} (%)‡	Correct calls rate
R132C Mut	WT	11.58	1.08	0.00	1.11	10	100% (78/78)
	5%	5.19	0.26	0.23	0.46	9	100% (76/76)
	10%	4.37	0.27	0.14	0.48	11	100% (78/78)
	30%	2.62	0.20	0.21	0.46	18	100% (78/78)
R132H Mut	WT	10.87	1.48	0.00	1.48	14	100% (78/78)
	5%	4.46	0.27	0.05	0.31	7	100% (78/78)
	10%	3.57	0.28	0.14	0.31	9	100% (76/76)
	30%	1.86	0.21	0.20	0.30	16	100% (72/72)
R172K Mut	WT	12.20	0.31	0.17	0.39	3	100% (66/66)
	5%	6.19	0.50	0.00	0.63	10	100% (76/76)
	10%	5.23	0.32	0.20	0.48	9	100% (76/76)
	30%	3.68	0.18	0.11	0.36	10	100% (76/76)

* R: Repeatability.
† Run: Between-run reproducibility.
‡ Total: Total precision (including inter-instrument, inter-operator and inter-lot).

Table continued on next page

Table 17. Precision results (continued)

Assay	Sample	Mean ΔC_T	SD_R*	SD_{Run}†	SD_{Total}‡	CV_{Total} (%)‡	Correct calls rate
R100 Mut	WT	7.21	0.41	0.27	0.52	7	100% (70/70)
	5%	3.68	0.27	0.16	0.33	9	100% (76/76)
	10%	2.93	0.24	0.15	0.32	11	100% (76/76)
	30%	1.56	0.25	0.07	0.26	17	100% (76/76)
R132 Mut	WT	8.01	0.76	0.00	0.78	10	100% (152/152)
	R132H 5%	4.29	0.30	0.15	0.48	11	99% (151/152)
	R132C 5%	4.44	0.30	0.00	0.56	13	
	R132H 10%	3.49	0.27	0.22	0.46	13	99% (151/152)
	R132C 10%	3.69	0.27	0.23	0.53	14	
	R132H 30%	1.87	0.21	0.02	0.33	18	100% (152%152)
	R132C 30%	2.00	0.26	0.28	0.59	29	
R172 Mut	WT	9.47	0.91	0.87	1.45	15	100% (66/66)
	5%	4.45	0.35	0.12	0.56	13	100% (76/76)
	10%	3.55	0.29	0.02	0.53	15	100% (76/76)
	30%	2.05	0.18	0.15	0.47	23	100% (76/76)

* R: Repeatability.
† Run: Between-run reproducibility.
‡ Total: Total precision (including inter-instrument, inter-operator and inter-lot).

Methods comparison

Comparison to immunohistochemistry (IHC) for detection of *IDH1*/R132H.

A study was conducted to demonstrate the concordance between mutation status as assessed with the *therascreen* IDH1/2 RGQ PCR Kit and by IHC (Anti-human IDH1R132H antibody clone H09, DIANOVA).

A total of 103 clinical glioma samples were selected. The oldest block was 10 years old.

All samples passed quality controls for both the *therascreen* IDH1/2 RGQ PCR Kit and IHC.

The results demonstrated a positive percentage agreement (PPA) of 100%, a negative percent agreement (NPA) of 98%, and an overall agreement (OA) of 99% (Table 18).

Table 18. Analysis of agreement between therascreen RGQ PCR Kit and IHC

Measure of agreement	Frequency (%)	95% Confidence interval
PPA	45/45 (100%)	[92;100]
NPA	57/58 (98%)	[91;100]
OA	102/103 (99%)	[96;100]

Comparison to bidirectional sequencing

A study was conducted to demonstrate the concordance between mutation status as assessed with the *therascreen* IDH1/2 RGQ PCR Kit and by bidirectional sequencing.

A total of 103 clinical tumor samples from glioma patients were selected. The oldest block was 10 years old.

All 103 samples passed quality controls for the *therascreen* IDH1/2 RGQ PCR Kit, and 101 samples returned results for the bidirectional sequencing.

The results demonstrated a positive percentage agreement (PPA) of 100%, a negative percent agreement (NPA) of 92%, and an overall agreement (OA) of 96% (Tables 19 and 20).

Table 19. *therascreen* IDH1/2 RGQ PCR Kit vs. bidirectional sequencing

therascreen IDH1/2 RGQ PCR Kit	Sanger bidirectional sequencing				
	R132*	**R132C**	**R132H**	**R172†**	**WT**
R132*	6	0	0	0	0
R132C	0	2	0	0	0
R132H	0	0	42	0	3
R172†	0	0	0	0	1
WT	0	0	0	0	47

* R132 means that the sample was found mutated for a R132 mutation but neither R132H nor R132C.
† R172 means that the sample was found mutated for a R172 mutation but not R172K.

Table 20. Analysis of agreement with bidirectional sequencing

Measure of agreement	Frequency (%)	95% Confidence interval
PPA	50/50 (100%)	[93;100]
NPA	47/51 (92%)	[81;97]
OA	97/101 (96%)	[90;98]

References

1. Louis, D.N. et al. (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. **114**, 97.

2. Parsons, D.W. et al. (2008) An integrated genomic analysis of human glioblastoma multiforme. Science **321**, 1807.

3. Yan, H. et al. (2009) IDH1 and IDH2 mutations in gliomas. N. Engl. J. Med. **360**, 765.

4. Hartmann, C. et al. (2009) Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol. **118**, 469.

5. Ichimura, K. et al. (2009) IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro-oncology **11**, 341.

6. Von Deimling, A., Korshunov, A., and Hartmann, C. (2011) The next generation of glioma biomarkers: MGMT methylation, BRAF fusions and IDH1 mutations. Brain Pathol. **21**, 74.

7. Hartmann, C. et al. (2010) Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol. **120**, 707.

8. Sanson, M. et al. (2009) Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J. Clin. Oncol. **27**, 4150.

9. Houillier, C. et al. (2010) IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. Neurology **75**, 1560.

10. Watanabe, T., Nobusawa, S., Kleihues, P., and Ohgaki, H. (2009) IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am. J. Pathol. **174**, 1149.

11. Nobusawa, S., Watanabe, T., Kleihues, P., and Ohgaki, H. (2009) IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin. Cancer Res. **15**, 6002.

12. Weller, M. et al. (2009) Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. J. Clin. Oncol. **27**, 5743.

13. Riemenschneider, M.J., Jeuken, J.W.M., Wesseling, P., and Reifenberger, G. (2010) Molecular diagnostics of gliomas: state of the art. Acta Neuropathol. **120**, 567.

14. Clinical and Laboratory Standards Institute (CLSI) (2004). *Protocols for determination of limits of detection and limits of quantitation: Approved Guideline,* 2nd ed. CLSI Document EP17-A. Wayne, PA: Clinical and Laboratory Standards Institute (formerly NCCLS).

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

 <N>	Contains reagents sufficient for <N> reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number (i.e., component labeling)
COMP	Components (i.e., a list of what is included)
CONT	Contains (contents)
NUM	Number (i.e., vials, bottles)

Rn	R is for revision of the Handbook and n is the revision number
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Caution

Ordering Information

Product	Contents	Cat. no.
therascreen IDH1/2 RGQ PCR Kit (20)	For 20 reactions: 9 Primer and Probe Mixes, WT Control, Positive Control, Master Mix, Nuclease-Free Water	873011
Rotor-Gene Q MDx and accessories		
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002033
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032
Loading Block 72 x 0.1ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Strip Tubes and Caps, 0.1ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
QIAamp DNA FFPE Tissue Kit — for purification of genomic DNA from paraffin-embedded tissues		
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

Product	Contents	Cat. no.
QIAsymphony DSP DNA Mini Kit — for automated purification of DNA from 1–96 samples		
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each: includes 2 reagent cartridges and enzyme racks and accessories	937236
QIAsymphony SP and accessories		
QIAsymphony SP System	QIAsymphony sample prep module: includes installation and training, 1 year warranty on parts and labor	9001751
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µl, Qsym SP (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332
Filter-Tips, 1500 µl, Qsym SP (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS instruments	997024
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588

Product	Contents	Cat. no.
Reagents		
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
R5, July 2020	Revised the Interpretation of Results section to add information regarding classification of controls and samples depending on Ct value detection Revised IDH1/IDH2 WT Control column in Table 7 for C_T IDH Mut R172K and ΔC_T IDH2 Mut R172K Revised Sample 1 and Sample 2 columns in Table 11 for C_T IDH1 Mut R132C, ΔC_T IDH1 Mut R132C, C_T IDH2 Mut R172K, and ΔC_T IDH2 Mut R172K

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Limited License Agreement for *therascreen* IDH1/2 RGQ PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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