July 2020

IDH1/2 RGQ PCR Kit Handbook

20

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

For research use only. Not for use in diagnostic procedures

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

873001

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Sample to Insight

QIAGEN

Contents

Intended Use

The IDH1/2 RGQ PCR Kit is for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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

Principle of the Procedure

The 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

Table 1. IDH1 and IDH2 mutations detected using the 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**).

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).

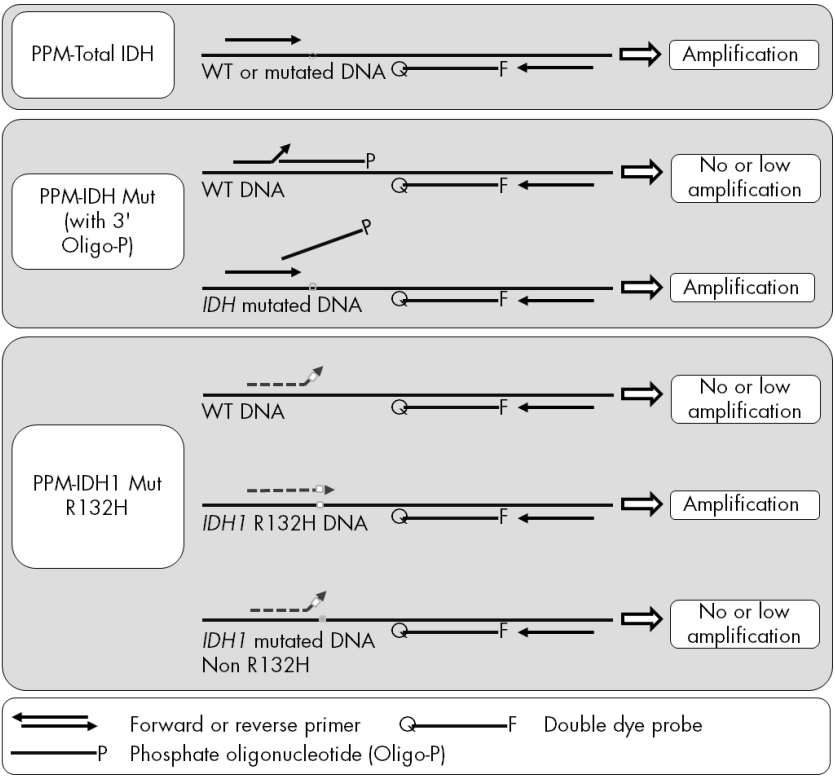

Figure 1. Results obtained with the primers and probe mixes in the IDH1/2 RGQ PCR Kit. The same principle shown to detect *IDH1* R132H applies for *IDH1* R132C and *IDH2* R172K.

Materials Provided

Kit contents

IDH1/2 RGQ PCR Kit		**(20)**
Catalog number		**873001**
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)

IDH1/2 RGQ PCR Kit		**(20)**
Catalog number		**873001**
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

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

- Sterile 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 5plex HRM and associated specific material
- Rotor-Gene Q 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

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 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 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 IDH1/2 RGQ PCR Kits, as this may affect performance.
- Refer to the Rotor-Gene Q 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 5plex HRM instrument until the run is finished.
- Do not open Rotor-Gene Q 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 IDH1/2 RGQ PCR Kit is shipped on dry ice. If any component of the 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 or the reagents, please contact one of the QIAGEN Technical Services or local distributors (see **www.qiagen.com**).

Storage

The 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 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 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) must 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 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 if long-term storage is required.

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 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", 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 nuclease-free 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 nuclease-free 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 14).

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", 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 nuclease-free 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 nuclease-free 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, and then for 8 weeks at –25 to –15°C if long-term storage is required.

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 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 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 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 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.

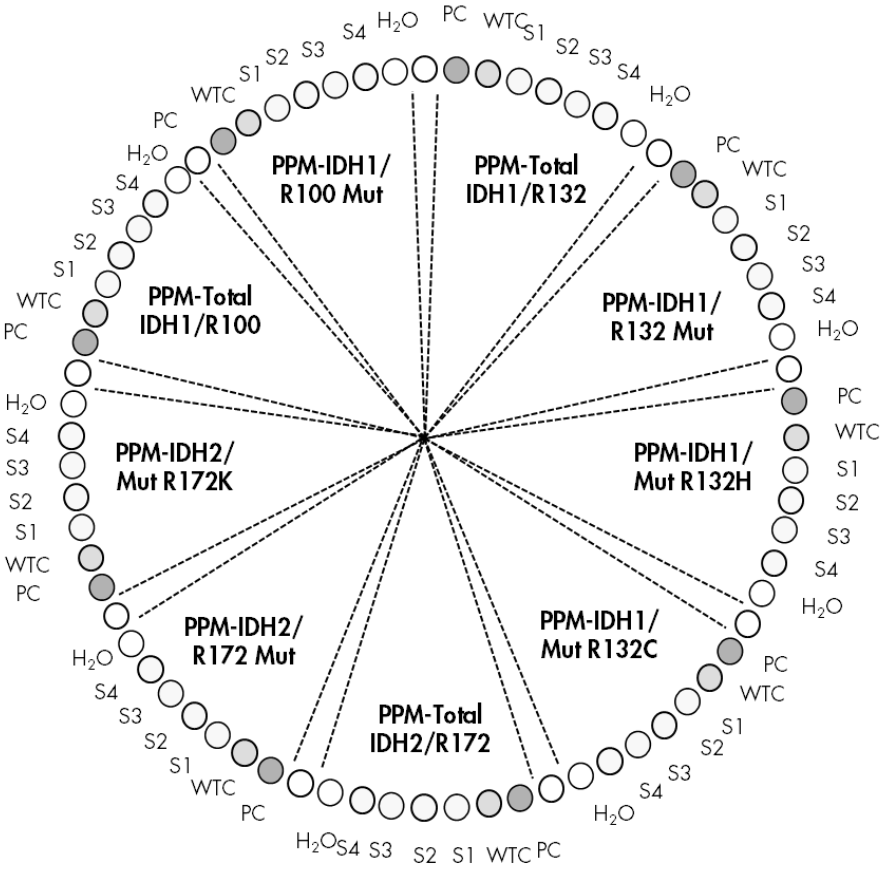

Figure 2. Suggested rotor setup for an experiment with the IDH1/2 RGQ PCR Kit.

Note: 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).

   Important: Unused positions need to be filled with empty tubes.

7. Load the full adapter into the Rotor-Gene Q instrument.

8. Program the Rotor-Gene Q 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, carry out the following.
    12a. Select **Options** and **Crop Start Cycles**. Remove data before cycle **10** to discard any artifacts.
    12b. Select **Analysis** and **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. Specify the threshold as **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", 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 an experiment to be qualified.

For each control, calculate the ΔC_T values as follows.

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

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

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

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

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

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

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

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.

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 either condition is 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").

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 mutation identification 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 detection assay, the sample must be classified as mutation-negative for the considered mutation.

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 IDH1/2 RGQ PCR Kit at –30 to –15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling". Do not exceed a maximum of 5 freeze–thaw cycles.
c)	The 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 IDH1/2 RGQ PCR Kit.
***IDH1/2* positive control not detected**		
a)	Pipetting errors or omitted	Check pipetting scheme and setup of the reaction.

		Comments and suggestions
	reagents; tube or well inversions	Repeat the PCR run.
b)	Inappropriate storage of kit components	Store the IDH1/2 RGQ PCR Kit at –30 to –15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling". Do not exceed a maximum of 5 freeze–thaw cycles.
c)	The 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 IDH1/2 RGQ PCR Kit.
No signal, including no signal for controls		
a)	No reaction tube in position 1 of the Rotor-Gene Q 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 IDH1/2 RGQ PCR Kit at –30 to –15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling". Do not exceed a maximum of 5 freeze–thaw cycles.
d)	The 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 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.

		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 IDH1/2 RGQ PCR Kit at –30 to –15°C and keep primers and probe mixes protected from light. See "Reagent Storage and Handling". Do not exceed a maximum of 5 freeze–thaw cycles.
b)	The 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 IDH1/2 RGQ PCR Kit.
c)	Very low amount of target DNA	Always check the DNA concentration before starting. See "DNA extraction and preparation".

	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 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".

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of 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**.

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
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.
IDH1/2 RGQ PCR Kit (20)	For 20 reactions: 9 Primer and Probe Mixes, WT Control, Positive Control, Master Mix, Nuclease-Free Water	873001
Rotor-Gene Q and other accessories		
Rotor-Gene Q 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650
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

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Document Revision History

Date	Changes
July 2020	Revised information in Sample input validation section about classification of sample when there is no Ct value available in mutation detection assay Revised IDH1/IDH2 WT Control column in Table 7 for C_T IDH Mut R172K and $\Delta C_{T\ IDH2\ Mut\ R172K}$ Revised Sample 1 and Sample 2 columns in Table 11 for $C_{T\ IDH1\ Mut\ R132C}$, $\Delta C_{T\ IDH1\ Mut\ R132C}$, $C_{T\ IDH2\ Mut\ R172K}$, and $\Delta C_{T\ IDH2\ Mut\ R172K}$

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