

ipsogen[®] IDH1 RGQ PCR Kit

Instructions for Use

IVD

For In Vitro Diagnostic Use

For use with Rotor-Gene Q[®] MDx 5plex HRM instrumentFor use with QIAamp[®] DSP DNA Blood Mini Kit

This Instructions for Use is applicable for:

**REF****Version***ipsogen* IDH1 RGQ PCR Kit

24 samples

675223

Version 1



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1. Intended Use

The *ipsogen* IDH1 RGQ PCR Kit is a qualitative real-time PCR assay for the detection of single nucleotide variants (SNVs) coding five IDH1 mutations (R132C, R132H, R132G, R132S, and R132L), using genomic DNA extracted from fresh or frozen human whole blood or bone marrow aspirate specimens collected in K2-EDTA tubes from patients with acute myeloid leukemia (AML).

The test is intended to be used as a companion diagnostic device to aid clinicians in identifying AML patients who may be eligible for treatment with TIBSOVO® (ivosidenib) based on the detection of an IDH1 mutation. TIBSOVO may be administered in combination with azacitidine, in accordance with its approved indications. The test is not intended to guide treatment decisions for azacitidine.

Fresh or frozen human whole blood or bone marrow aspirate specimens, collected in K2-EDTA tubes, are processed manually using the QIAamp DSP DNA Blood Mini Kit.

The QIAGEN® Rotor-Gene Q MDx 5plex HRM instrument is used for automated amplification and detection.

The *ipsogen* IDH1 RGQ PCR Kit is not an automated device; however, the analysis is assisted by a dedicated software.

The *ipsogen* IDH1 RGQ PCR Kit is to be used by trained personnel in a professional laboratory environment.

The *ipsogen* IDH1 RGQ PCR Kit is intended for in vitro diagnostic use.

2. Intended User

For use with the QIAamp DSP DNA Blood Mini Kit (cat. no. 61104) using the manual isolation and purification of genomic DNA by microcentrifuge procedure.

The product is to be used only by trained personnel in a professional laboratory environment.

3. Description and Principle

3.1. Summary and explanation

Isocitrate dehydrogenase 1 is an enzyme that is encoded by the IDH1 gene on chromosome 2 in humans. Wild type isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. IDH1 hotspot mutations at R132 in IDH1 lead to gain-of-function mutations that result in the production of D-2-hydroxyglutarate (2HG) (1). 2HG is an oncometabolite involved in DNA and histone hypermethylation leading to gene transcription deregulation and cell differentiation blockage (2, 3). IDH1 mutations are present in hematological malignancies, mainly in AML (approximately 15–25%) (4), but also in myelodysplastic syndromes (MDS, approximately 2–7%) (5, 6).

The *ipsogen*® IDH1 RGQ PCR Kit is an assay for the detection and identification of five specific mutations within codon 132 of the IDH1 gene (Table 1).

Table 1. List of mutations and COSMIC identities

Mutation	Base change	COSMIC ID*
R132H	395G>A	COSV61615239
R132C	394C>T	COSV61615256
R132S	394C>A	COSV61615649
R132G	394C>G	COSV61615456
R132L	395G>T	COSV61615420

* COSMIC IDs are taken from the *Catalogue of Somatic Mutations in Cancer* (7) (<https://cancer.sanger.ac.uk/cosmic>).

3.2. Principle of the procedure

The *ipsogen* IDH1 RGQ PCR Kit is based on the detection by selective amplification of five IDH1 mutations in genomic DNA (gDNA) extracted from whole blood or bone marrow aspirate (BMA) specimens. Allele-specific technology allows accurate and highly reproducible detection of target IDH1 mutations, based on the use of specific forward and reverse primer and probe sets; mutation-specific primers bind selectively to the target DNA, which promotes extension and amplification in the PCR reaction.

Result reporting is fully automated. If both the run controls and the sample results are valid, and the assay target amplification is below the predetermined cutoff threshold, the report will display the IDH1 mutation(s) detected in each sample.

3.2.1. DNA purification

The *ipsogen* IDH1 RGQ PCR Kit has been validated for use with DNA extracted from whole blood and BMA samples using the QIAamp® DSP DNA Blood Mini Kit, following the procedure for manual isolation and purification of genomic DNA by microcentrifuge. Do not use any other DNA extraction products, and do not use either the QIAcube® or vacuum systems for extracting DNA with the QIAamp DSP DNA Blood Mini Kit.

The QIAamp DSP DNA Blood Mini Kit manual microcentrifuge procedure comprises 4 steps. Samples are first lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN® Protease (QP) and Lysis Buffer (AL). Each lysate is then applied to a QIAamp Mini spin column, and genomic DNA is adsorbed onto the silica membrane as the lysate is drawn through by centrifugal force. While the genomic DNA remains bound to the QIAamp Mini spin column membrane, contaminants are efficiently removed using wash buffers. Finally, genomic DNA is eluted from the QIAamp Mini spin column membrane using 200 µL Elution Buffer (AE). The eluted DNA is ready for use with the *ipsogen* IDH1 RGQ PCR Kit.

3.2.2. 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. Three types of oligonucleotides are used to detect each target base sequence: a pair of conventional PCR primers that are complementary to sequences up and downstream of the target sequence and form a PCR amplicon, and a probe molecule that is complementary to the precise target sequence and incorporates both a fluorescent reporter dye and a fluorescence quencher in close proximity.

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 1).

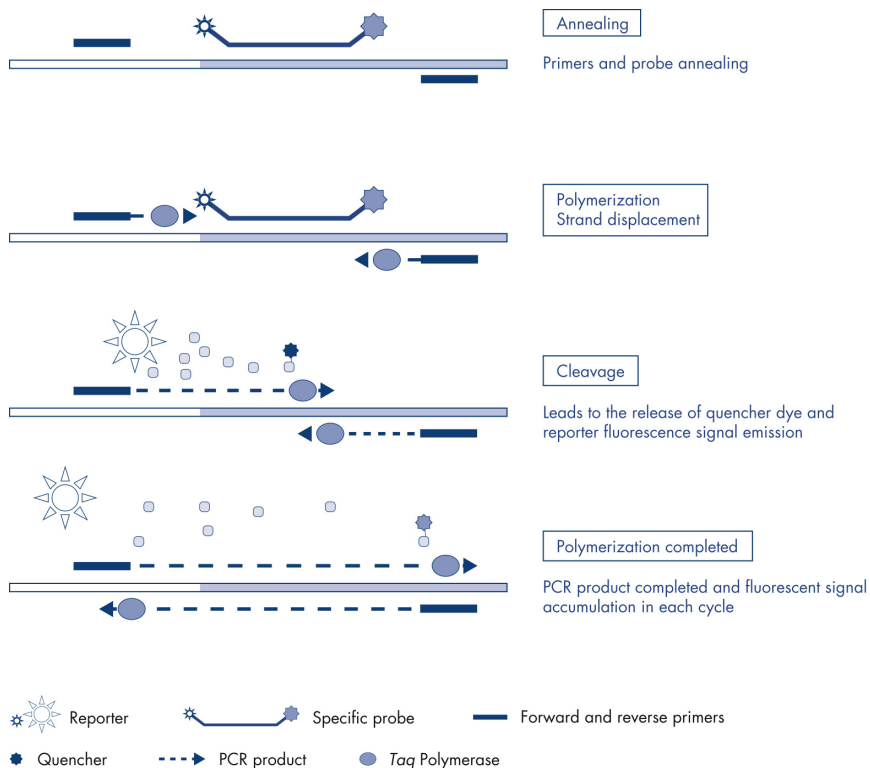


Figure 1. The TaqMan real-time reaction principle.

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.

Each IDH1 mutation assay contains an allele-specific primer that binds selectively to DNA sequences containing the mutation. A mismatch at the 3' end of the allele-specific primer (e.g., in the presence of a wild type sequence or different mutation) reduces the efficiency of primer extension, thus increasing specificity for the designated mutation.

Specificity for IDH1 R132 mutations is further increased by the use of PCR blockers. Oligonucleotides that are complementary to the wild type DNA sequence are included in PCR reaction mixtures. In the presence of wild type DNA, the oligonucleotide blockers hybridize selectively to the DNA, and inhibit the recognition of wild type sequences by mutation allele-specific primers.

The *ipsogen* IDH1 RGQ PCR Kit contains 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.

3.3. Platform and software

The *ipsogen* IDH1 RGQ PCR Kit is specifically designed for use with:

- Rotor-Gene® Q MDx 5plex HRM Platform* (cat. no. 9002032 [platform] or 9002033 [system]), operating with a personal computer installed with:
 - Rotor-Gene AssayManager® v2.1 (version 2.1.1 or later) software (cat. no. 9024203)

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

- Gamma Plug-in version 1.0 (or later) (cat. no. 9025511)
- *ipsogen* IDH1 RGQ PCR Kit Assay Profile ("AP_ipsogen_IDH1_Blood_BMA_V1_0_x.iap"
where $x \geq 0$)

Note: The *ipsogen* IDH1 RGQ PCR Kit Assay Profile is only compatible with Rotor-Gene AssayManager v2.1 or later.

Refer to the relevant individual user manuals for information regarding the Rotor-Gene Q MDx 5plex HRM instrument, Rotor-Gene AssayManager v2.1 Core Application software, or the Rotor-Gene AssayManager v2.1 Gamma Plug-in software.

The Rotor-Gene Q MDx 5plex HRM instrument must be maintained according to the requirements in the user manual.

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

4. Materials Provided

4.1. Kit contents

<i>ipsogen</i> IDH1 RGQ PCR Kit			
Catalog number			675223
Number of tests			24
Reagent	Cap color	Tube ID	Volume
IDH1 Reaction Mix 1	Green	IDH1 Mix 1	950 µL
IDH1 Reaction Mix 2	Purple	IDH1 Mix 2	950 µL
IDH1 Positive Control	Red	PC	120 µL
Taq DNA Polymerase	Mint	Taq	85 µL
Water for NTC	White	NTC	1.9 mL
Water for sample dilution	White	Dil.	1.9 mL

There are no accessories provided with the *ipsogen* IDH1 RGQ PCR Kit.

4.2. Components of the kit

Reagent	Critical / active / reactive ingredient(s)	Concentration range
IDH1 Reaction Mix 1 and IDH1 Reaction Mix 2	Target-specific oligonucleotides: primers	0.1–1 µM
	Target-specific oligonucleotides: fluorophore-labeled detection probes	0.1–1 µM
	IDH1 oligonucleotide PCR blocker	0.1–2 µM
	Deoxynucleotide triphosphates (dNTPs)	<1 mM
IDH1 Positive Control	DNA plasmids containing target-specific sequences	<100,000 copies/µL

Reagent	Critical / active / reactive ingredient(s)	Concentration range
Taq DNA Polymerase	Taq DNA Polymerase	1–10 units

Note: The device contains additional components that may influence the measurements.

4.2.1. Reaction mixes

IDH1 Reaction Mix 1 and IDH1 Reaction Mix 2 contain reagents to perform PCR reactions that detect the IDH1 mutations reported in Table 1.

Each reaction mix also amplifies a control reaction, as described below.

4.2.2. Control reaction

The control reaction uses a forward and reverse primer and labeled probe to detect the amplification of a conserved, non-IDH1 sequence. The control reaction is used to determine if an appropriate level of amplifiable DNA is present in the sample and is a factor in the analytical calculations that determine mutation status.

The control target is used to define a sample both as IDH1 mutation detected or no mutation detected, and as a DNA loading control. All samples will give a signal in the control reaction in a predefined Ct range if sufficient DNA has been added to the reaction tube.

4.2.3. IDH1 Positive Control

The *ipsogen* IDH1 RGQ PCR Kit also contains the IDH1 Positive Control. The positive control is a mixture of DNA plasmids representing each of the mutations detected by the kit. Detection of the positive control confirms the proper functioning of each of the reaction mixes in the kit.

4.2.4. Negative Control

The *ipsogen* IDH1 RGQ PCR Kit contains nuclease-free water for no template control (tube NTC) to be used for the “no template control” reaction. The no template control serves as a negative control and assesses potential contamination during assay setup.

4.2.5. Taq DNA Polymerase

Taq DNA polymerase is the enzyme for the polymerase chain reaction used by the *ipsogen* IDH1 RGQ PCR Kit.

4.2.6. Water for sample dilution

An additional tube containing nuclease-free water is used, if required, to dilute gDNA that is too concentrated for the *ipsogen* IDH1 RGQ PCR Kit.

5. Materials Required but Not Provided

5.1. Additional reagents

- QIAamp DSP DNA Blood Mini Kit (cat. no. 61104, see “DNA purification” section)
- DNAZap™ PCR-degrading solutions or equivalent cleaning solutions
- Distel High Level Laboratory Disinfectant and isopropyl alcohol (IPA) wash or equivalent cleaning solution

5.2. Consumables

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

5.3. Equipment

- Platform and software as described on page 10
- Permanent marker
- Dedicated pipettes* (adjustable) for sample preparation
- Dedicated pipettes* (adjustable) for PCR master mix preparation
- Dedicated pipettes* (adjustable) for dispensing of template DNA
- Benchtop centrifuge* with rotor for 1.5 mL tubes

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

- Thermomixer*, heated orbital incubator*, heating block*, or water bath* capable of incubation at 37°C and 56°C
- Loading Block 72 × 0.1 mL Tubes, aluminum block for manual reaction set up (cat. no. 9018901)
- 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
- Locking Ring 72-Well Rotor, for locking Strip Tubes and Caps 0.1 mL in the 72-Well Rotor (cat. no. 9018904)
- Spectrophotometric quantification instrument*
- Benchtop vortexer

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

6. 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 its authorized representative and the regulatory authority in which the user and/or the patient is established.

6.1. Warnings

For in vitro diagnostic use.

For use with the Rotor-Gene Q MDx 5plex HRM Platform.

For use with the QIAamp DSP DNA Blood Mini Kit using the manual isolation and purification of genomic DNA by microcentrifuge procedure.

6.2. 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. 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 QIAamp DSP DNA Blood Mini Kit (cat. no. 61104), see the *QIAamp DSP DNA Blood Mini Kit Handbook*.

6.2.1. Emergency information

CHEMTREC

Outside USA & Canada +1 703-527-3887

6.3. Precautions

The user must always adhere to the following precautions when using the *ipsogen* IDH1 RGQ PCR Kit:

Taq DNA Polymerase



Contains: Ethoxylated nonylphenol. Toxic to aquatic life with long lasting effects. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. Collect spillage. Dispose of contents/ container to an approved facility in accordance with local, regional, national and international regulations.

- The test is for use with whole blood or bone marrow aspirate specimens collected in K2-EDTA tubes.
- 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 *ipsogen* IDH1 RGQ PCR Kit are diluted optimally. Do not dilute reagents further as this may result in a loss of performance. Do not use reaction volumes (reaction mix plus sample) of less than 25 μ L.
- All reagents in the *ipsogen* IDH1 RGQ PCR Kit are formulated specifically for use with the tests provided in the *ipsogen* IDH1 RGQ PCR Kit.

- All reagents supplied in the *ipsogen* IDH1 RGQ PCR Kit are intended to be used solely with the other reagents supplied in the same *ipsogen* IDH1 RGQ PCR Kit. Do not substitute the reagents in the *ipsogen* IDH1 RGQ PCR Kit or among the *ipsogen* IDH1 RGQ PCR Kits, as this may affect performance.
- Only use the Taq DNA polymerase (tube Taq) that is provided in the *ipsogen* IDH1 RGQ PCR Kit. Do not substitute with Taq DNA polymerase from other kits of the same or any other type, or with Taq DNA polymerase from another supplier.
- Refer to the *Rotor-Gene Q MDx 5plex HRM Platform User Manual* for additional warnings, precautions, and procedures.
- Install the Rotor-Gene Q MDx 5plex HRM instrument according to the installation procedures and site requirements.
- Do not use expired or incorrectly stored components.
- Use extreme caution to prevent contamination of the control and reaction mix reagents with the synthetic materials that are contained in the positive control reagent.
- Use extreme caution to prevent cross-contamination between samples. Cap the tubes promptly after addition of each sample.
- Thoroughly decontaminate the loading block before using it for preparation of assay master mixes. The use of DNAZap PCR-degrading solutions followed by Distel High Level Laboratory Disinfectant and IPA wash is recommended. The loading block must be dry before use.
- Use individual, dedicated pipettes for setting up reaction mixes and adding positive control reagents.
- Perform preparation and dispensing of reaction mixes in an area separate from the one used for the addition of the positive control.

- Fluorescently labelled molecules included in the reaction mix reagents are light sensitive. Protect control and reaction mix reagents from light to avoid photo bleaching.
- Do not open the Rotor-Gene Q MDx instrument until the run has finished.
- Do not open Rotor-Gene Q tubes after the run has finished. If an error has occurred during the run, the samples in the Rotor-Gene Q MDx 5plex HRM instrument must be disposed of and not retested.
- Caution must be exercised to ensure correct sample testing to avoid incorrect sample entry, loading errors and pipetting errors.
- To minimize flags for the controls and samples, strict compliance to the *ipsogen IDH1 RGQ PCR Kit Instructions for Use* should be followed, including but not limited to:
 - Correct reagent mixing is required and must be ensured at each mixing step during the assay setup.

7. Disposal

Discard sample and assay waste according to local safety procedures.

8. Storage and Handling

8.1. Reagent storage and handling

The *ipsogen* IDH1 RGQ PCR Kit is shipped on dry ice and must still be frozen on arrival. If the *ipsogen* IDH1 RGQ PCR Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note or reagents, please contact QIAGEN Technical Support or local distributors (visit www.qiagen.com).

The *ipsogen* IDH1 RGQ PCR Kit should be stored immediately upon receipt at -30°C to -15°C in a constant-temperature freezer. The *ipsogen* IDH1 RGQ PCR Kit must be protected from light to avoid photo-bleaching and loss of performance. When stored under the specified conditions, the *ipsogen* IDH1 RGQ PCR Kit is stable until the expiration date printed on the outer package.

Under these conditions, the components are stable for 11 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Repeated thawing and freezing should be avoided. Do not exceed a maximum of 3 freeze-thaw cycles within a maximum of 90 days. The reagents must be fully thawed at room temperature ($15\text{--}25^{\circ}\text{C}$) for a minimum of 30 minutes before use. Reagents must be returned to the freezer within 4 hours of being removed.

Reagents in the *ipsogen* IDH1 RGQ PCR Kit are diluted optimally, and no further purification or treatment is required prior to their use in analysis as directed by the *ipsogen* IDH1 RGQ PCR Kit Instructions for Use (this document).

Note: 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.

8.1.1. In-use stability

Do not use if stored outside the specifications, if the packaging has been damaged, or if other signs of deterioration or malfunction are visible.

Once opened, reagents can be stored in their original packaging at -30°C to -15°C for 90 days, or until the stated expiration date on the packaging, whichever is earliest.

8.2. Specimen storage and handling

The *ipsogen* IDH1 RGQ PCR Kit is for use with fresh or frozen human whole blood (WB) or bone marrow aspirate (BMA) specimens collected in K2-EDTA tubes.

All samples should be treated as potentially hazardous. Discard sample and assay waste according to local safety procedures.

8.2.1. Specimen collection and storage

Whole blood and bone marrow aspirate samples should be collected in K2-EDTA tubes according to manufacturer's recommendations. Specimens must be transported according to ISO 20658:2023 and must adhere to the storage conditions (including maximum freeze-thaw cycles) outlined below in this section to ensure specimen quality. DNA may be stored following extraction, prior to testing.

Recommended storage conditions for specimens are described below:

- If refrigerated, up to 7 days at 2–8°C.
- If frozen, up to 3 weeks for WB and up to 9 weeks for BMA specimens at –30°C to –15°C.
Do not exceed a maximum of two freeze-thaw cycles for either WB or BMA specimens.

Recommended storage conditions for gDNA are described below:

- If refrigerated, up to 2 weeks at 2–8°C.
- If frozen, up to 9 weeks at –30°C to –15°C.

8.2.2. Interfering substances in samples

Substances within whole blood or bone marrow aspirate samples could impact the performance of the *ipsogen* IDH1 RGQ PCR Kit.

The impact of potential interfering substances was assessed; refer to “Performance Characteristics” section for further details and for a list of substances tested. An interference was observed with the following agents: cytarabine, decitabine, etoposide, idarubicin hydrochloride, daunorubicin hydrochloride, azacitidine, and triglycerides.

9. Protocol: Sample Preparation

Important points before starting

- Read “Warnings and Precautions”.
- Ensure that all materials required but not provided are available (see page 15).
- Ensure that all reagents and consumables are stored correctly and are within expiry date.
- Ensure that all equipment has been checked and calibrated according to the manufacturer’s recommendations.

DNA purification

DNA purification must be carried out according to the instructions for the manual microcentrifuge extraction protocol in the *QIAamp DSP DNA Blood Mini Kit Handbook*.

Note: Use 200 µL of WB or BMA for extraction. Ensure that the samples are mixed well (e.g., by inversion) prior to extraction.

Note: DNA shall be eluted with 200 µL Elution Buffer (AE).

Note: The *ipsogen* IDH1 RGQ PCR Kit has been developed for use with the QIAamp DSP DNA Blood Mini Kit with manual microcentrifuge extraction protocol. To ensure optimal performance, other extraction products, protocols, and/or automated systems are not permitted.

DNA quantification and normalization

gDNA extracted from whole blood or bone marrow aspirate samples should be quantified (e.g., using a spectrophotometric quantification instrument) according to local procedures.

Note: Make sure the correct elution buffer is used to calibrate the quantification instrument.

- If measured concentration is both ≥ 1 and ≤ 6 ng/ μ L, sample normalization is not required.
- If measured concentration is less than 1 ng/ μ L, the sample must be reextracted.
- If measured concentration is greater than 6 ng/ μ L, the sample must be diluted to 5 ng/ μ L using the Water for Dilution (Dil.) provided in the *ipsogen* IDH1 RGQ PCR Kit, according to the formula:

$$C_i \times V_i = C_f \times V_f$$

Where

- C_i : Initial concentration of the extracted gDNA
- C_f : Final concentration to be targeted = 5 ng/ μ L
- V_f : Final volume needed to perform an *ipsogen* IDH1 RGQ PCR run (i.e., 10 μ L + extra volume for pipetting error)
- V_i : Initial volume of the extracted gDNA to be pipetted and diluted with the Water for Dilution (Dil.) provided in the *ipsogen* IDH1 RGQ PCR Kit

Note: Volume of water to add to extracted *gDNA* = $V_f - V_i$

Note: Use the provided Water for Dilution (Dil.) tube to dilute sample, if required.

Worked example

- For example, if gDNA concentration is measured at 20 ng/ μ L, then the volume to add to the gDNA can be determined as below:
 - C_i = 20 ng/ μ L [or as measured]
 - C_f = 5 ng/ μ L [fixed value]
 - V_f = 15 μ L [sufficient volume with overage for PCR reactions]
 - V_i is calculated as below:

$$20 \text{ ng}/\mu\text{L} \times V_i = 5 \text{ ng}/\mu\text{L} \times 15 \mu\text{L}$$

$$V_i = \frac{5 \times 15}{20} = 3.75 \mu\text{L}$$

- Volume of water to add is calculated as below:

$$\text{Volume of water to add} = V_f - V_i = 15 - 3.75 = 11.25 \mu\text{L}$$

Dispense 11.25 μL of Water for Dilution into an appropriate tube. Add 3.75 μL of gDNA sample, then mix thoroughly.

10. Protocol: IDH1 Mutation Detection

Important points before starting

- Read “Warnings and Precautions”.
- Ensure that all materials required but not provided are available (see page 15).
- Ensure that all reagents and consumables are stored correctly and are within expiry date.
- Before starting the protocol, familiarize yourself with the Rotor-Gene Q MDx 5plex HRM instrument. See the user manuals for the instrument, the Rotor-Gene AssayManager v2.1, and the Gamma plug-in for details.
- Ensure that all equipment has been checked and calibrated according to the manufacturer’s recommendations.
- Do not vortex Taq DNA polymerase (Taq) as this may inactivate the enzyme.
- Pipette Taq DNA polymerase (Taq) by placing the pipette tip just under the liquid surface to avoid the tip being coated in excess enzyme.
- Before starting, the following settings must be configured from the configuration tab (Figure 2 and Figure 3).

Finish run

☒ Run has to be released before starting approval
☐ Release of run has to be signed

Figure 2. Ensure that “Run has to be released before starting approval” and “Release of run has to be signed” check boxes are not checked.

Work list

Format of generated work list names
 WL_20110513_0430

User-definable section
 WL

☒ Date
☒ Time
☐ Operator

☒ Enable processing of unclear samples
☒ Enable checksum for LIMS import

Closed mode	UDT mode
☐ Material number required	☐ Material number required
☒ Valid expiry date required	☐ Valid expiry date required
☒ Lot number required	☐ Lot number required

Figure 3. Ensure that “Material number required” is not checked.

10.1. Things to do before starting

- Before each use, thaw all reagents, except for Taq DNA polymerase (Taq) and samples, at room temperature (15–25 °C) for at least 30 minutes. Vortex for 5–12 seconds to mix, then centrifuge briefly to collect the contents at the bottom of the tube. Return IDH1 reaction mixes, PC and NTC to the freezer (–30°C to –15°C) within 4 hours of initiating the thawing process.

Note: Only take out the Taq DNA polymerase (Taq) when the reagents have thawed.

- Ensure samples to be tested are completely thawed and mixed well.
- Download and install all required software. For further information on software installation, refer to the Installation of Gamma plug-in and importing the assay profile, next page.
 - Rotor-Gene AssayManager v2.1 (version 2.1.1 or later) software (cat. no. 9024203)

- Rotor-Gene AssayManager Gamma Plug-in v1.0 (or later) (cat. no. 9025511)
- *ipsogen* IDH1 RGQ PCR Kit Assay Profile

10.1.1. Installation of Gamma plug-in and importing the assay profile

For details about the installation of the Rotor-Gene AssayManager v2.1 core software, please refer to the *Rotor-Gene AssayManager v2.1 MDx Core Application User Manual*.

The installation and importing of the Gamma plug-in and the assay profile are detailed in the *Rotor-Gene AssayManager v2.1 MDx Core Application User Manual*.

1. Download the Gamma plug-in from the QIAGEN website (www.qiagen.com).
2. Start the installation process by double-clicking the `RGAM_V2_1_Gamma__Plug-in.Installation.v1_0_x.msi` file (where x is ≥ 0) and following the installation instructions. For a detailed description of this process, please refer to the “Installing Plug-ins” section in the *Rotor-Gene AssayManager v2.1 MDx Core Application User Manual*.
3. After successful installation of the plug-in, a person with administrator rights for the Rotor-Gene AssayManager v2.1 software will need to import the latest assay profile version, as follows.
 - a. Open the Rotor-Gene AssayManager v2.1 software by clicking the icon below.



The following window opens:

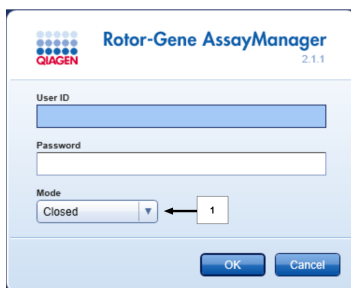
The image shows a login window for Rotor-Gene AssayManager V2.1.1. The window has a light blue background. At the top left is the Qiagen logo, consisting of a grid of blue dots above the word "QIAGEN" in red. To the right of the logo is the text "Rotor-Gene AssayManager" in blue, with "2.1.1" in smaller blue text below it. Below the header, there are three input fields: "User ID" (a blue rectangular box), "Password" (a white rectangular box), and "Mode" (a dropdown menu showing "Closed" with a downward arrow). To the right of the "Mode" dropdown is a small white box containing the number "1" with an arrow pointing left towards the dropdown. At the bottom right of the window are two buttons: "OK" and "Cancel", both in blue.

Figure 4. Rotor-Gene AssayManager V2.1.

- b. Log-in to Rotor-Gene AssayManager v2.1 software with your user ID and password. Do not change the "Closed" mode. Click **OK**. The following window opens:

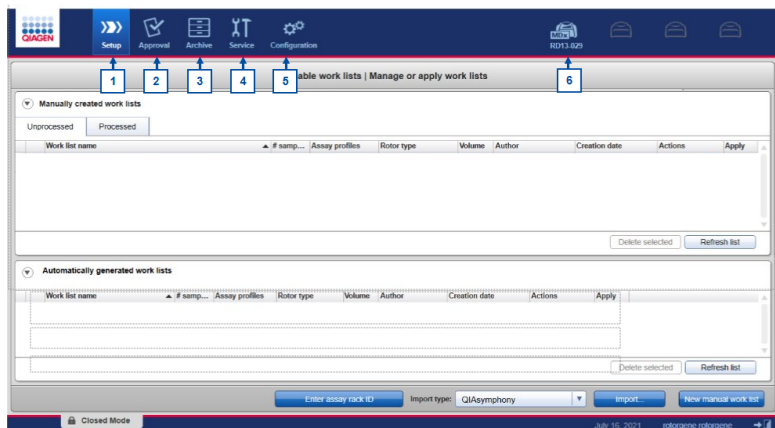


Figure 5. Rotor-Gene AssayManager v2.1.1. **1: Setup tab.** This tab allows you to manage or apply work. **2: Approval tab.** This tab enables you to find previous experiments. **3: Archive tab.** This tab allows you to find old experiments already approved. **4: Service tab.** In this tab, an audit trail of each file generated by the software is reported. **5: Configuration tab.** This tab allows configuration of all software parameters. **6: Rotor-Gene Q (RGQ) icon.**



- c. Select the configuration environment.
- d. Select the **Assay Profiles** tab.
- e. Click **Import**.
- f. Select the assay profile to be imported in the dialog then click **Open**.
- g. Once the assay profile has been successfully imported, it can be used in the "Setup" environment.

Note: The same version of an assay profile cannot be imported twice.

10.1.2. Procedure

1. Thaw all reagents except for Taq DNA polymerase (Taq) at room temperature (15–25°C) for a minimum of 30 minutes and a maximum of 2 hours before use.

Note: Only take out the Taq DNA polymerase (Taq) when the reagents have thawed.

Note: Return IDH1 reaction mixes, PC and NTC to the freezer (–30°C to –15°C) within 4 hours of initiating the thawing process.

Note: It is recommended to return IDH1 reaction mixes and Taq to the freezer (–30°C to –15°C) immediately after preparing the master mixes.

2. Mix all reagents, except for Taq, by vortexing for 5–12 seconds to avoid localized concentrations of salts and then centrifuge briefly to collect the contents at the bottom of the tube.

Note: The Taq DNA polymerase (Taq) should not be vortexed.

Note: The total time from start of PCR setup to the start of the run should not exceed 2 hours.

3. Prepare sufficient master mixes (IDH1 reaction mixes plus Taq DNA polymerase [Taq]) for the DNA samples, one Positive Control reaction, and one No Template Control reaction according to the volumes given in Table 3 and Table 4.

Note: Include reagents for $n + 2$ extra samples to allow sufficient overage for the PCR setup.

Note: The maximum number of runs using the optimal number of samples for use with *ipsogen* IDH1 RGQ PCR Kit is three runs of eight samples per run.

Note: The master mix contains all of the components needed for PCR except the sample and controls.

- Return the IDH1 reaction mixes and Taq to the freezer (–30°C to –15°C) immediately after preparing the master mixes.

Table 2. Number of reactions for Rotor-Gene Q MDx 5plex HRM instrument with 72-well rotor

Samples	Reaction
With the IDH1 Master Mix 1	
24 gDNA samples	24 reactions
Positive Control (PC)	1 reaction
No Template Control (NTC)	1 reaction
With the IDH1 Master Mix 2	
24 gDNA samples	24 reactions
Positive Control (PC)	1 reaction
No Template Control (NTC)	1 reaction

Table 3. Preparation of IDH1 Master Mix 1 (MM1)*

Component	Volume
IDH1 Reaction Mix 1	$19.6 \mu\text{L} \times (n + 2)^*$
Taq DNA Polymerase (Taq)	$0.4 \mu\text{L} \times (n + 2)^*$
Total volume	20.0 μL/reaction

* n = number of reactions (samples plus controls). When preparing the master mix, prepare enough for two extra samples ($n + 2$) to allow for sufficient coverage for the PCR setup.

Table 4. Preparation of IDH1 Master Mix 2 (MM2)*

Component	Volume
IDH1 Reaction Mix 2	$19.6\ \mu\text{L} \times (n + 2)^*$
Taq DNA Polymerase (Taq)	$0.4\ \mu\text{L} \times (n + 2)^*$
Total volume	20.0 μL/reaction

* n = number of reactions (samples plus controls). When preparing the master mix, prepare enough for two extra samples ($n + 2$) to allow for sufficient overage for the PCR setup.

- Thoroughly mix the master mix by vortexing for 5–12 seconds and then centrifuge briefly to collect the contents at the bottom of the tube.
- Place the appropriate number of 0.1 mL PCR strip tubes in the loading block according to the layout in Table 5.

Note: Keep the PCR tubes in the loading block until PCR reaction set up is complete. This reduces the likelihood of errors during PCR setup.

- Immediately add 20 μL master mix to each 0.1 mL PCR strip tube (not provided).

Note: Add MM1 and MM2 to alternate rows.

Note: Do not cap the tubes. Caps will remain in the plastic container until required.

- Immediately add 5 μL of Water for No Template Control (NTC) to the No Template Control tubes (PCR tube numbers 3 and 4).

Note: Ensure that the Water for No Template Control tube is used for the NTC. The Water for Sample Dilution tube should not be used as an NTC.

- Cap the tube containing the NTC (PCR tube numbers 3 and 4).

Table 5. Layout of sample tests in the loading block (numbers denote positions in the loading block and indicate final rotor position)

Mix								
PC MM1 (1)	Sample 3 MM1 (9)	Sample 7 MM1 (17)	Sample 11 MM1 (25)	Sample 15 MM1 (33)	Sample 19 MM1 (41)	Sample 23 MM1 (49)	–	–
PC MM2 (2)	Sample 3 MM2 (10)	Sample 7 MM2 (18)	Sample 11 MM2 (26)	Sample 15 MM2 (34)	Sample 19 MM2 (42)	Sample 23 MM2 (50)	–	–
NTC MM1 (3)	Sample 4 MM1 (11)	Sample 8 MM1 (19)	Sample 12 MM1 (27)	Sample 16 MM1 (35)	Sample 20 MM1 (43)	Sample 24 MM1 (51)	–	–
NTC MM2 (4)	Sample 4 MM2 (12)	Sample 8 MM2 (20)	Sample 12 MM2 (28)	Sample 16 MM2 (36)	Sample 20 MM2 (44)	Sample 24 MM2 (52)	–	–
Sample 1 MM1 (5)	Sample 5 MM1 (13)	Sample 9 MM1 (21)	Sample 13 MM1 (29)	Sample 17 MM1 (37)	Sample 21 MM1 (45)	–	–	–
Sample 1 MM2 (6)	Sample 5 MM2 (14)	Sample 9 MM2 (22)	Sample 13 MM2 (30)	Sample 17 MM2 (38)	Sample 21 MM2 (46)	–	–	–
Sample 2 MM1 (7)	Sample 6 MM1 (15)	Sample 10 MM1 (23)	Sample 14 MM1 (31)	Sample 18 MM1 (39)	Sample 22 MM1 (47)	–	–	–
Sample 2 MM2 (8)	Sample 6 MM2 (16)	Sample 10 MM2 (24)	Sample 14 MM2 (32)	Sample 18 MM2 (40)	Sample 22 MM2 (48)	–	–	–

10. Add 5 µL of each sample to the sample tubes (e.g., PCR tube numbers 5–52).
11. Cap the tubes containing samples.
12. Add 5 µL IDH1 Positive Control (PC) to the positive control tubes (PCR tube numbers 1 and 2).
13. Cap the tubes containing PC.
14. Using a permanent marker, mark the lids of the tubes to show the direction to load the tubes into the Rotor-Gene Q MDx 5plex HRM instrument.

15. After all PCR tubes are capped, perform a visual check on sample tube fill levels to ensure sample has been added to all tubes.
16. Place the PCR strip tubes into the appropriate positions in the 72-well rotor (Table 5). If the rotor is not fully occupied, all empty positions on the rotor must be filled with a capped, empty tube.
17. Immediately place the 72-well rotor into the Rotor-Gene Q MDx 5plex HRM instrument. Make sure that the locking ring (accessory of the Rotor-Gene Q MDx 5plex HRM instrument) is placed on top of the rotor to secure the tubes during the run.

Performing an IDH1 mutation analysis run

1. The *ipsogen* IDH1 RGQ PCR Kit must be run on the Rotor-Gene Q MDx 5plex HRM instrument. Rotor-Gene AssayManager software v2.1 and Gamma plug-in must be installed before use.
2. The Rotor-Gene AssayManager v2.1 core software and Gamma plug-in each have a separate user manual. For installation instructions, see the “Installing Rotor-Gene AssayManager v2.1” and “Installing plug-ins” sections in the *Rotor-Gene AssayManager v2.1 MDx Core Application User Manual*.
3. The *ipsogen* IDH1 RGQ PCR Kit Assay Profile must be imported into Rotor-Gene AssayManager before use. For instructions, see “Installation of Gamma plug-in and importing the assay profile” section on page 29. The assay profile contains all the necessary parameters to execute a PCR run and perform automatic analysis and result interpretation.
4. A PDF viewer must be installed to view run reports produced by Rotor-Gene AssayManager.

The following instructions detail the setup of an IDH1 mutation analysis run using Rotor-Gene AssayManager v2.1 software. For advanced instructions, refer to the respective user manuals.

5. Open the Rotor-Gene AssayManager v2.1 software by double-clicking on the desktop icon (Figure 6).



Figure 6. Rotor-Gene AssayManager desktop icon.

6. At the Rotor-Gene AssayManager login screen (Figure 7), enter the user ID and password, select **Closed** mode, and click **OK**. The main Rotor-Gene AssayManager window will then open.

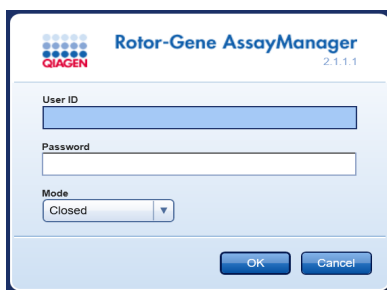


Figure 7. Rotor-Gene AssayManager login screen.

7. Select **New manual work list** from the main window to create a new work list (Figure 8, indicated by box 1).

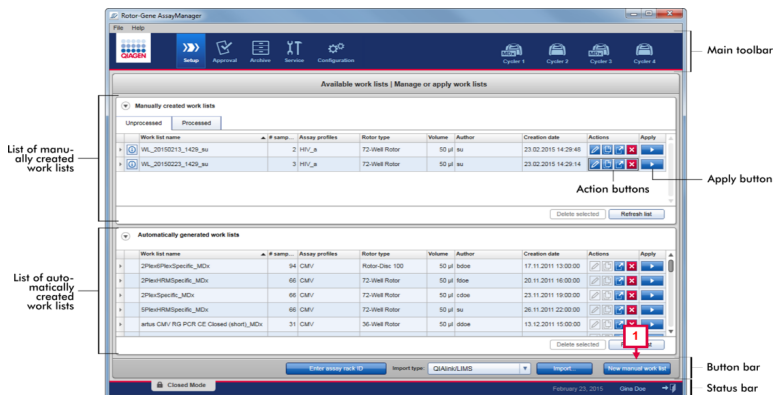


Figure 8. Rotor-Gene AssayManager main window. Box 1 indicates New manual work list button.

8. To perform an IDH1 run, ensure that the **Assays** tab is selected. Click the **ipsogen_IDH1_Blood_BMA** assay profile in the “Available assay profiles” window (Figure 9, indicated by box 1) and click the **blue arrow** to select the assay profile (Figure 9, indicated by box 2). Move the mouse pointer over the assay profile to see the full name if required. The assay profile should now appear in the “Selected assay profiles” window (Figure 9).

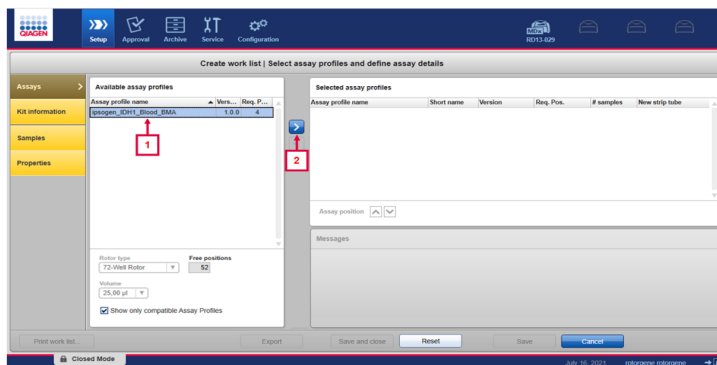


Figure 9. “Create work list” window. Box 1 indicates Assay profile to select. Box 2 indicates how to select chosen assay profile.

9. In the “Selected assay profiles” window (Figure 10, indicated by box 1), enter the number of samples to be tested (excluding controls). Next, click the **Samples** tab to enter “sample information” (Figure 10, indicated by box 2).

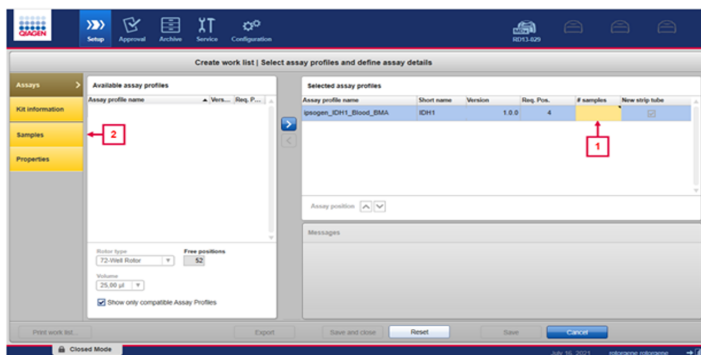


Figure 10. “Create work list” window on the “Assays” tab. Box 1 indicates where to add number of samples. **Box 2** indicates Samples tab.

10. Enter the sample names into the “Sample ID” column (Figure 11, indicated by box 1). Enter any comments into the “Comments” column. Next, click the **Properties** tab (Figure 11, indicated by box 2).

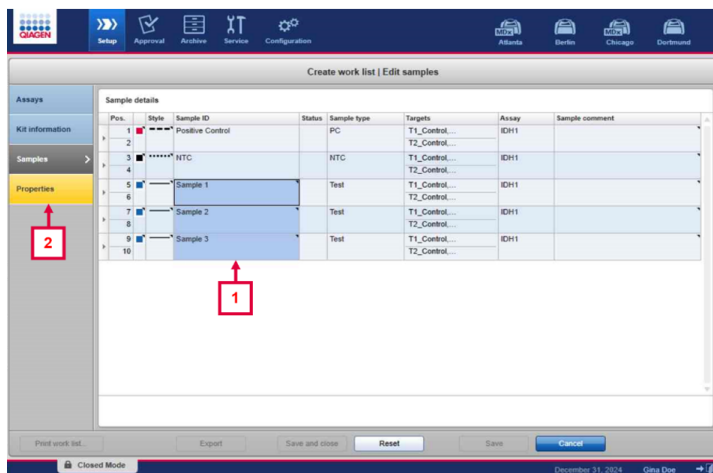


Figure 11. “Create work list” window on the “Samples” tab. Box 1 indicates Samples ID column. **Box 2** indicates Properties tab.

11. Enter a work list name into the “Work list name” text box and check the “work list is complete” (can be applied) box (Figure 12, indicated by box 1 and box 2). Click **Apply** to apply the work list (Figure 12, indicated by box 3).

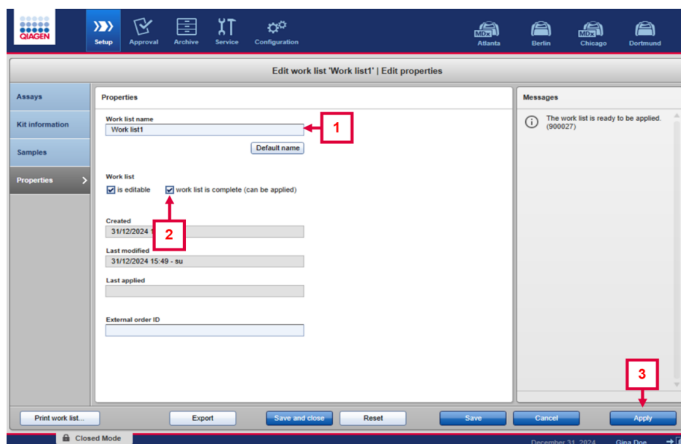


Figure 12. “Create work list” window on the “Properties” tab. Box 1 indicates where to write work list name. **Box 2** indicates “worklist complete” checkbox. **Box 3** indicates **Apply** button.

12. Enter an experiment name into the “Experiment name” text box (Figure 13, indicated by box 1). Ensure that the correct cyclers has been selected and the “Ring attached” check box has been checked (Figure 13, indicated by boxes 2 and 3). Click **Start run** to proceed (Figure 13, indicated by box 4).

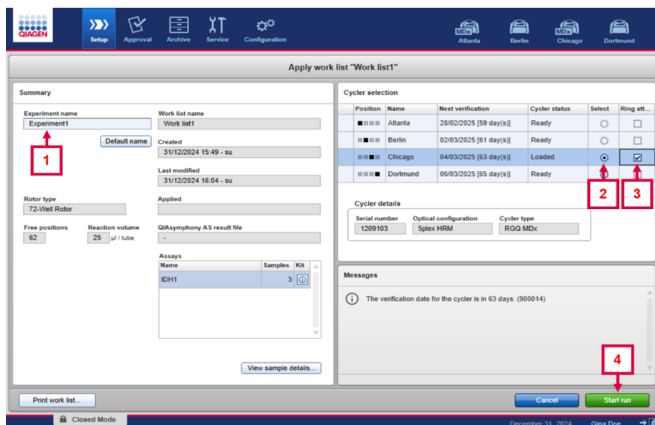


Figure 13. “Apply work list” window. Box 1 indicates where to add the experiment name. Boxes 2 and 3 indicate where to select the correct cycler and confirm the ring has been attached. Box 4 indicates the **Start run** button.

13. Upon completion, click **Finish run** to release and approve the run (Figure 14, indicated by box 1).

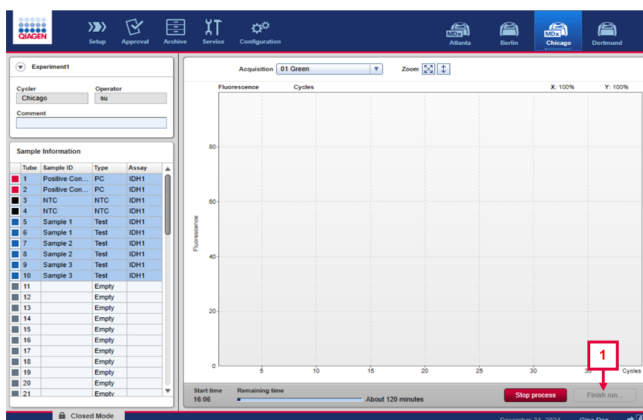


Figure 14. An example of “Cycler Environment” window. Box 1 shows the **Finish run** button.

14. To view the run information and IDH1 assay results, click **Release and go to approval** (Figure 15). This also releases the Rotor-Gene Q MDx 5plex HRM instrument ready for the next run.

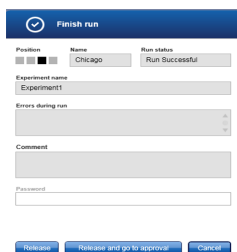


Figure 15. “Finish Run” dialog.

15. The `ipsogen_IDH1_Blood_BMA` assay profile contains all of the rules for automatic assay and sample analysis and result interpretation. The software will therefore assess the validity or invalidity of samples and controls automatically.

Note: For more information on interpretation of assay results, see “Interpretation of Results” section.

16. Processed data, experiment information, assay, and audit trail information can be found in the “Plots and information” window. Assay results can be found in the “Assay Results” window. If the Positive Control and No Template Control results are within an acceptable range, the “Overall sample result” column will report “valid” (Figure 16); otherwise, an “INVALID” result will appear (Figure 17).

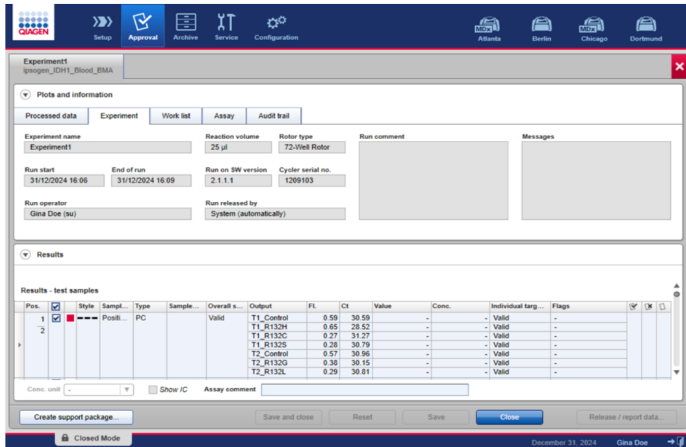


Figure 16. Valid "Assay Results" window.

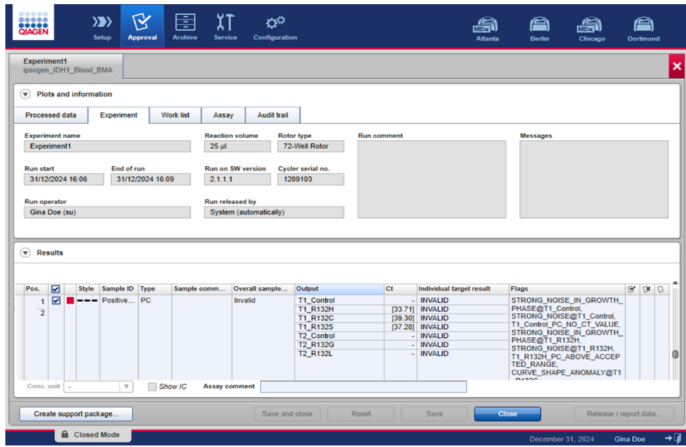


Figure 17. Invalid "Assay Results" window.

17. If the Sample Control results are within an acceptable range, the “Overall sample result” column will report either “No Mutation Detected” or “IDH1 Mutation Detected” (Figure 18). This indicates that the sample DNA concentration is suitable for mutation analysis.

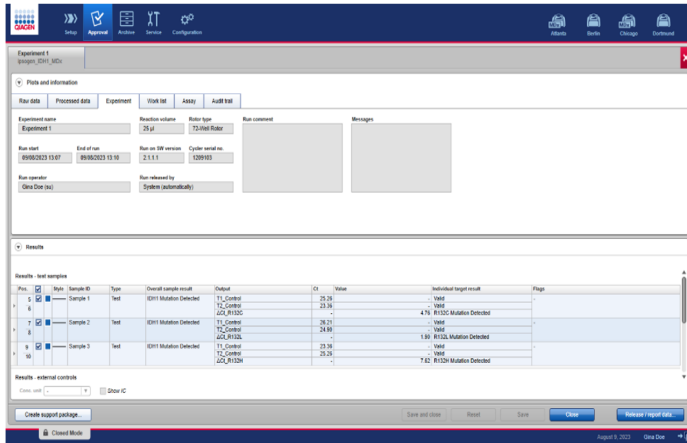


Figure 18. Sample results window.

18. Click **OK** to save the experiment to the archive and create a LIMS output and run report (Figure 19). Run reports and LIMS exports will be saved in the default report directory. The default directory can be found in the data export directories in the “Configuration” tab.

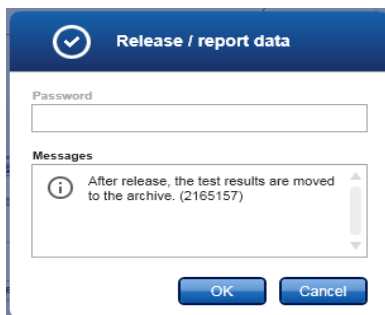


Figure 19. “Release/report data” dialog.

19. The “Status of release” dialog box will appear. Click **OK** to accept (Figure 20).

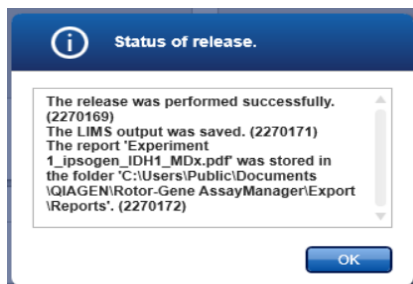


Figure 20. “Status of release” dialog.

20. Released experiments can now be retrieved from the “Archive” environment. To find an experiment, click the **Archive** tab and enter the necessary search criteria.

11. Interpretation of Results

The analysis and mutation calls are performed automatically by the Rotor-Gene AssayManager v2.1 software using the *ipsogen* IDH1 RGQ PCR Kit Assay Profile once a run is completed, and require no manual interpretation.

The following information explains how the analysis and mutation calls are made.

Analysis and mutation calls

The PCR cycle at which the fluorescence from a particular reaction crosses the predefined threshold value given by the *ipsogen_IDH1_Blood_BMA* Assay Profile is defined as the Ct value. Ct values indicate the quantity of specific input DNA. Low Ct values indicate higher input DNA levels and high Ct values indicate lower input DNA levels. For each IDH1 mutation assay, a calculation is performed to determine the ΔCt value using the equation:

$$\Delta Ct = [\text{mutation assay Ct value}] - [\text{control assay Ct value}]$$

Based on predetermined analytical Ct and ΔCt values, the *ipsogen_IDH1_Blood_BMA* Assay Profile qualitatively determines the mutation status of the DNA samples and reports if a sample contains one or more of the specified IDH1 mutations.

The controls (PC, NTC, Sample Control) are assessed to ensure that acceptable Ct values are met, and the reactions have been performed successfully.

If the Ct values are outside of the acceptable range, then an "INVALID" result is obtained, and one or more flags reported. For further information, see "ipsogen IDH1 RGQ PCR Kit Assay Profile Flags", next page. For example, if the sample control Ct is below the acceptable range this means that DNA input is too high and the sample needs to be diluted.

Note: IDH1 mutation status is not reported in the event of an invalid sample or invalid run.

The Rotor-Gene AssayManager v2.1 software determines the result for each target by combining all relevant analysis results according to core analysis algorithms such as normalization, sample, and assay rules defined in the corresponding assay profile.

The following results may be assigned to an individual sample:

- IDH1 Mutation Detected
- No Mutation Detected
- INVALID: If one or more flags are assigned to the sample during analysis by the Rotor-Gene AssayManager v2.1 software that are defined to set the target result to “INVALID”.

Note: If an error has occurred during the run, the samples in the Rotor-Gene Q MDx 5plex HRM instrument must be disposed of and not retested.

ipsogen IDH1 RGQ PCR Kit Assay Profile Flags

Flags corresponding to the Rotor-Gene AssayManager v2.1 Gamma Plug-in that are not specific to this *ipsogen*_IDH1_Blood_BMA Assay Profile only are listed in Table 6.

Table 6. Rotor-Gene AssayManager v2.1 Gamma Plug-in related flags

Flag	Description	Action
ANALYSIS_FAILED	Assay is set to invalid because the analysis has failed.	Contact QIAGEN Technical Support.
ASSAY_INVALID	Sample is invalid because at least one external control is invalid.	Repeat run. See Table 7.
CONSECUTIVE_FAULT	A target that was used for calculation of this target is invalid.	No additional actions required

Flag	Description	Action
CURVE_SHAPE_ANOMALY	The raw data amplification curve shows a shape that deviates from the established behavior for this assay. There is a high likelihood of incorrect results or a result misinterpretation.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
FLAT_BUMP	The amplification curve shows a shape like a flat bump deviating from the established behavior for this assay. There is a high likelihood of incorrect results or result misinterpretation (e.g., wrong Ct value determination).	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
MULTIPLE_THRESHOLD_CROSSING	The amplification curve crosses the threshold more than once. An unambiguous Ct cannot be determined.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
NO_BASELINE	No initial baseline has been found. The subsequent analysis cannot be performed.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
OTHER_TARGET_INVALID	Another target for the same sample is invalid.	No additional actions required
RUN_FAILED	Invalid Assay is set to invalid due to a problem with the cyclor or the cyclor connection.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
RUN_STOPPED	Invalid Assay is set to invalid because the run has been stopped manually.	Repeat run.
SATURATION	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
SPIKE	A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the Ct is determined.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.

Flag	Description	Action
SPIKE_CLOSE_TO_CT	A spike is detected in the amplification curve close to the Ct.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
STEEP_BASELINE	A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
STRONG_BASELINE_DIP	A strong drop in the baseline for the raw data fluorescence is detected in the amplification curve.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
STRONG_NOISE	Strong noise outside the growth phase of the amplification curve detected.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
STRONG_NOISE_IN_GROWTH_PHASE	Strong noise is detected in the growth (exponential) phase of the amplification curve.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.
WAVY_BASE_FLUORESCENCE	Wavy baseline for the raw data fluorescence detected in the amplification curve.	Retest. Contact QIAGEN Technical Support if this flag occurs during the retest.

Table 7 and Table 8 list the possible flags that may be generated by the *ipsogen_IDH1_Blood_BMA* Assay Profile along with their meanings and actions to be taken.

The flag names are constructed to provide information on the affected component of the kit, the sample or control affected and the failure mode.

For example:

T1_Control1_PC_NO_CT_VALUE = The Positive Control (PC), Control Assay for Tube 1 has no Ct value

See also “Troubleshooting Guide” section.

Table 7. Software flags used by the *ipsogen* IDH1 RGQ PCR Kit Assay Profile for the PC and NTC

Flag	Meaning	Action
T1_<target>_PC_NO_CT_VALUEor T2_<target>_PC_NO_CT_VALUE	Invalid run. No Ct value for PC.	Repeat run.
T1_<target>_PC_ABOVE_ ACCEPTED_RANGEor T2_<target>_ PC_ABOVE_ACCEPTED_RANGE	Invalid run. PC above the specification range.	Repeat run.
T1_<target>_PC_BELOW_ ACCEPTED_RANGEor T2_<target>_ PC_BELOW_ACCEPTED_RANGE	Invalid run. PC below the specification range.	Repeat run.
T1_<target>_ NTC_UNEXPECTED_ CT_VALUE or T2_<target>_NTC_ UNEXPECTED_CT_VALUE	Invalid run. Ct value has been detected in NTC.	Repeat run.

Table 8. Software flags used by the *ipsogen* IDH1 RGQ PCR Kit Assay Profile for samples

Flag	Meaning	Action
T1_Control1_DNA_ INPUT_TOO_ HIGH or T2_Control1_DNA_ INPUT_TOO_ HIGH	Invalid sample. Sample Control Ct value below the control working range.	Repeat the DNA quantification and nor- malization. After retest, if the control Ct value is still invalid, reextract the sample. If the control Ct value is invalid after reextraction and two further rounds of testing, then the sample should be reported as indeterminate mutation status.
T1_Control1_ABOVE_ ACCEPTED_ RANGE or T2_Control1_ABOVE_ ACCEPTED_ RANGE	Invalid sample. Sample control Ct value above the control working range.	Repeat the DNA quantification and nor- malization. After retest, if the control Ct value is still invalid, reextract the sample. If the control Ct value is invalid after reextraction and two further rounds of testing, then the sample should be reported as indeterminate mutation status.

Flag	Meaning	Action
T1_Control1_NO_CT_ VALUE or T2_Control1_NO_CT_ VALUE	Invalid sample. No Ct value in sample.	Repeat the DNA quantification and normalization. After retest, if the control Ct value is still invalid, reextract the sample. If the control Ct value is invalid after reextraction and two further rounds of testing, then the sample should be reported as indeterminate mutation status.

Note: If a retested sample is invalid for a different reason upon repeat, this is still classed as a second repeat and a reextraction of the sample should be performed. See Figure 21 for retest and reextraction procedures.

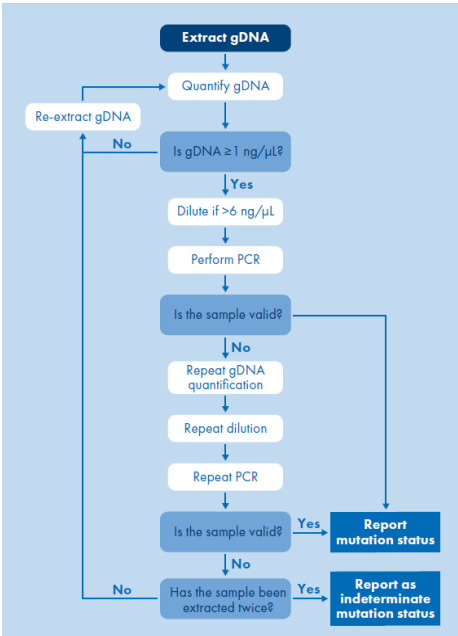


Figure 21. Flowchart for sample retest procedure.

12. Limitations

The test is not intended to be used as a companion diagnostic device to aid clinicians in identifying patients with locally advanced or metastatic cholangiocarcinoma who may be eligible for treatment with TIBSOVO based on the detection of an IDH1 mutation.

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

The test is designed to detect five mutations within codon 132 of the IDH1 gene. Samples with results reported as No Mutation Detected may contain IDH1 mutations not detected by the assay.

The *ipsogen* IDH1 RGQ PCR Kit uses a polymerase chain reaction (PCR) procedure. As with all PCR procedures, samples may be contaminated by external sources of DNA in the test environment and the DNA in the positive control. Use caution to avoid contamination of samples and reaction mix reagents.

The *ipsogen* IDH1 RGQ PCR Kit is only for use with whole blood and bone marrow aspirate samples collected in K2-EDTA tubes.

The *ipsogen* IDH1 RGQ PCR Kit is only for use with the QIAamp DSP DNA Blood Mini Kit.

Strict compliance with the *ipsogen IDH1 RGQ PCR Kit Handbook* 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 *ipsogen* IDH1 RGQ PCR Kit may only be used when all reaction components from the same kit lot are used together.

The product is intended only for use on the Rotor-Gene Q MDx 5plex HRM instrument.

The *ipsogen* IDH1 RGQ PCR Kit is a qualitative test. The test is not for quantitative measurements of percent mutation.

Performance of the *ipsogen* IDH1 RGQ PCR Kit is unknown if microbial contamination is introduced during assay procedures.

Performance of the *ipsogen* IDH1 RGQ PCR Kit could be affected in the presence of cytarabine, decitabine, triglycerides, etoposide, idarubicin hydrochloride, daunorubicin hydrochloride, or azacitidine in the test samples.

13. Performance Characteristics

13.1. Analytical performance

13.1.1. Limit of Blank

The Limit of Blank (LoB) is defined in CLSI guideline EP17-A2 as “the highest measurement result that is likely to be observed (with a stated probability) for a blank sample”. For the *ipsogen* IDH1 RGQ PCR Kit, this is the data point that corresponds to the upper 95th percentile in blank (wild type, i.e., R132 mutation negative) samples.

The LoB has been determined by measuring the levels of breakthrough for each of the five IDH1 mutation assays of the *ipsogen* IDH1 RGQ PCR Kit, where breakthrough is defined as the non-specific, low level amplification of a DNA template. For the *ipsogen* IDH1 RGQ PCR Kit, this refers to non-specific binding of a mutant probe to the wild type DNA.

Table 9. Summary of LoB results

IDH1 assay	LoB (Ct value)
R132H	No amplification
R132C	No amplification
R132S	No amplification
R132G	No amplification
R132L	No amplification

Based on this analysis, the LoB for the assay has been determined as the absence of a Ct value.

13.1.2. Limit of Detection

The Limit of Detection (LoD) is defined as the lowest allele frequency of IDH1 R132 mutation (in a background of wild type DNA) that is possible to detect 95% of the time. Genomic DNA was extracted from whole blood (WB) and BMA specimens spiked with R132 mutant cell line DNA, and was diluted into normalized DNA extracted from wild type clinical WB and BMA specimens. The genomic DNA was serially diluted to % MAF levels above, at, and below the estimated LoD for each assay.

At least 60 replicates of each % mutant allele frequency (MAF) dilution in the series at a low DNA input level were tested using three *ipsogen* IDH1 RGQ PCR Kit lots. The LoD for each assay was determined using a Probit model (Table 10).

Table 10. Summary of LoD results

IDH1 assay	LoD (% MAF)
R132H	1.25
R132C	2.70
R132S	4.70
R132G	3.20
R132L	2.90

The LoD was verified across the validity range of *ipsogen* IDH1 RGQ PCR Kit lots.

13.1.3. Assay cutoff

The assay cutoff is a specific ΔC_t value used to determine whether a sample is classed as positive or negative for an IDH1 R132 mutation. Samples which generate ΔC_t values at or below the cutoff are classified as R132 mutation-positive (i.e., R132 mutation detected) and ΔC_t values generated above the cutoff are classified as R132 mutation negative (i.e., no mutation detected). The false-negative and false-positive rates for each IDH1 mutation-specific

assay were used to determine a cutoff value for each mutation assay, such that a result equal to or less than the cutoff will result in an IDH1 Mutation Detected classification. The cutoff for each assay within the *ipsogen* IDH1 RGQ PCR Kit is shown in Table 11.

Table 11. Summary of assay cutoff values

IDH1 assay	Assay cutoff (ΔC_t value)
R132H	14.75
R132C	14.75
R132S	14.75
R132G	14.75
R132L	14.75

13.1.4. Genomic DNA input range

The DNA input level is defined as the total quantity of amplifiable DNA in a sample as determined by the C_t value from the control reaction. To demonstrate that the performance of *ipsogen* IDH1 RGQ PCR Kit is consistent across the control reaction working range (CWR) (21.54–25.25 C_t), a 7-level serial dilution of varying DNA input levels was tested (with the highest and lowest levels being outside of the CWR). This was conducted for wild type and for each of the five R132 mutations (at 1.5 $\times$ LoD), and for both WB and BMA.

The evaluation was performed using three *ipsogen* IDH1 RGQ PCR Kit lots with six replicates tested per DNA input level (two replicates per kit lot). The data was analyzed using regression analysis to determine the linear range. For the assay to be determined as linear across the DNA input range, there should be no change across the range in ΔC_t for mutation positive samples (i.e., no statistically significant linear, quadratic, or cubic effect). For wild type sample, for the assay to be determined as linear across the DNA input range, there should be a statistically significant linear effect in control C_t with respect to dilution level and no statistically significant quadratic, or cubic effect.

Parameter estimates for the tested dilutions demonstrated linearity for all samples. A linear ΔC_t and control C_t range was observed across the DNA input range tested for the assay.

13.1.5. Accuracy of measurement

Precision

The repeatability of the *ipsogen* IDH1 RGQ PCR Kit was assessed by testing contrived samples at two mutation levels (1× LoD and 2× LoD) at the standard DNA input of 5 ng/μL, and wild type samples at the standard (5 ng/μL) and low DNA input (3.5 ng/μL) levels were tested at one site (located in the UK). The samples were tested in triplicate with four runs per sample across multiple days, Rotor-Gene Q MDx 5plex HRM instruments, and operators and using three lots of *ipsogen* IDH1 RGQ PCR Kit to generate a total of a minimum of 106 replicates per sample (Table 12 and Table 13).

Table 12. Assay repeatability for bone marrow aspirate samples*

Mutation	Level	Fractional proportion of valid results	Correct calls (%)	Lower two-sided 95% CI
Wild type	3.5 ng/μL	108 / 108	100.00	96.64
	5 ng/μL	106 / 106	100.00	96.58
R132H	1× LoD	106 / 108	98.15	93.47
	2× LoD	107 / 107	100.00	96.61
R132C	1× LoD	105 / 106	99.06	94.86
	2× LoD	108 / 108	100.00	96.64
R132S	1× LoD	99 / 106	93.40	86.87
	2× LoD	108 / 108	100.00	96.64
R132G	1× LoD	107 / 108	99.07	94.95
	2× LoD	108 / 108	100.00	96.64

Table 12. Assay repeatability for bone marrow aspirate samples* (continued)

Mutation	Level	Fractional proportion of valid results	Correct calls (%)	Lower two-sided 95% CI
R132L	1× LoD	107 / 108	99.07	94.95
	2× LoD	108 / 108	100.00	96.64

* Number of correct calls and lower two-sided 95% confidence limits for each IDH1 mutation at 1× and 2× LoD, and wild type samples tested across one site.

Table 13. Assay repeatability for whole blood samples*

Mutation	Level	Fractional proportion of valid results	Correct calls (%)	Lower two-sided 95% CI
Wild type	3.5 ng/μL	107 / 107	100.00	96.61
	5 ng/μL	108 / 108	100.00	96.64
R132H	1× LoD	108 / 108	100.00	96.64
	2× LoD	107 / 107	100.00	96.61
R132C	1× LoD	107 / 108	99.07	94.95
	2× LoD	107 / 107	100.00	96.61
R132S	1× LoD	97 / 108	89.81	82.51
	2× LoD	107 / 107	100.00	96.61
R132G	1× LoD	106 / 107	99.07	94.90
	2× LoD	108 / 108	100.00	96.64
R132L	1× LoD	105 / 106	99.06	94.86
	2× LoD	106 / 106	100.00	96.58

* Number of correct calls and lower two-sided 95% confidence limits for each IDH1 mutation at 1× and 2× LoD, and wild type samples tested across one site.

13.1.6. Intermediate precision

The precision of the *ipsogen* IDH1 RGQ PCR Kit within laboratory (repeatability) was assessed. The precision of ΔC_t values (the difference in C_t values between a mutation reaction and the control reaction) for positive test samples and control C_t values for negative test samples are reported in Table 14.

Table 14. Intermediate precision of ΔC_t and control C_t^*

Sample type	Analysis variable	Target mutation	Assay	N	Mean	Intermediate precision (SD)
BMA	ΔC_t	R132C	RM1	108	6.17	0.8177
		R132G	RM2	108	7.22	0.6411
		R132H	RM1	107	5.92	0.6161
		R132L	RM2	108	6.54	0.3887
		R132S	RM1	108	7.68	0.5616
	Control C_t	WT	RM1	106	23.67	0.0903
			RM2	106	23.72	0.1277
WB	ΔC_t	R132C	RM1	107	6.77	1.4127
		R132G	RM2	108	6.40	1.1413
		R132H	RM1	107	6.15	0.8026
		R132L	RM2	106	7.03	0.4312
		R132S	RM1	107	7.00	0.6764
	Control C_t	WT	RM1	108	23.67	0.0890
			RM2	108	23.76	0.1123

* Mean and standard deviation (intermediate precision) for the ΔC_t of each mutation assay for 2x LoD mutation-positive samples, and for the control C_t for WT samples at 5 ng/ μ L DNA input, including including both BMA and WB sample types .

13.1.7. Reproducibility

The reproducibility of the *ipsogen* IDH1 RGQ PCR Kit was assessed by testing contrived samples at two mutation levels (1× LoD and 2× LoD) at the standard DNA input of 5 ng/μL, and wild type samples at the standard (5 ng/μL) and low DNA input (3.5 ng/μL) levels were tested at three sites (one located in UK and two located in USA). The samples were tested in duplicate with six runs per sample across multiple days, Rotor-Gene Q MDx 5plex HRM instruments, and operators and using three lots of *ipsogen* IDH1 RGQ PCR Kit to generate a total of a minimum of 178 replicates per sample (Table 15 and Table 16).

Table 15. Assay reproducibility for bone marrow aspirate samples*

Mutation	Level	Fractional proportion of valid results	Correct calls (%)	Lower two-sided 95% CI
Wild type	3.5 ng/μL	179 / 179	100	97.96
	5 ng/μL	178 / 178	100	97.95
R132H	1× LoD	178 / 180	98.89	96.04
	2× LoD	179 / 179	100	97.96
R132C	1× LoD	177 / 178	99.44	96.91
	2× LoD	179 / 180	99.44	96.94
R132S	1× LoD	167 / 178	93.82	89.21
	2× LoD	179 / 180	99.44	96.94
R132G	1× LoD	179 / 180	99.44	96.94
	2× LoD	180 / 180	100	97.97
R132L	1× LoD	179 / 180	99.44	96.94
	2× LoD	180 / 180	100	97.97

* Number of correct calls and lower two-sided 95% confidence limits for each IDH1 mutation at 1× and 2× LoD, and wild type samples tested across three sites.

Table 16. Assay reproducibility for whole blood samples*

Mutation	Level	Fractional proportion of valid results	Correct calls (%)	Lower two-sided 95% CI
Wild type	3.5 ng/μL	179 / 179	100	97.96
	5 ng/μL	180 / 180	100	97.97
R132H	1× LoD	180 / 180	100	97.97
	2× LoD	179 / 179	100	97.96
R132C	1× LoD	178 / 180	98.89	96.04
	2× LoD	179 / 179	100	97.96
R132S	1× LoD	159 / 180	88.33	82.72
	2× LoD	179 / 179	100	97.96
R132G	1× LoD	178 / 179	99.44	96.93
	2× LoD	180 / 180	100	97.97
R132L	1× LoD	177 / 178	99.44	96.91
	2× LoD	178 / 178	100	97.95

* Number of correct calls and lower two-sided 95% confidence limits for each IDH1 mutation at 1× and 2× LoD, and wild type samples tested across three sites.

13.1.8. Lot interchangeability

The object of this study was to demonstrate that regardless of which lots of the QIAamp DSP DNA Blood Mini Kit and *ipsogen* IDH1 RGQ PCR Kit (IDH1 Kit) are used, that the mutation call status for all samples, in both peripheral blood (PB) and bone marrow aspirate (BMA), is consistent for all targets.

In order to assess the lot interchangeability of the kits, this study used five mutation samples (one for each mutation target detected by the IDH1 Kit) and one WT sample, for each sample type (12 samples in total). Four extraction replicates for each sample were extracted using three independent extraction kit lots. DNA concentration for each replicate was quantified and subsequently normalized to 5 ng/μL (if not already within a range of 1–6 ng/μL). Each

replicate was then assessed by PCR, one replicate per reaction mix using three independent IDH1 Kit lots.

In order to verify Lot Interchangeability, it was required that across all combinations of the QIAamp DSP DNA Blood Mini and *ipsogen* IDH1 RGQ PCR Kit lots, that >90% correct call rate must be observed for each target in each sample type (PB and BMA).

It was observed that all targets had a >90% correct call rate. Except for R132H in the PB sample type, a correct call rate of 100% (36/36) was obtained. The R132H PB sample type returned a correct call rate of 91.67% (33/36). This met the requirement for lot interchangeability of the QIAamp DSP DNA Blood Mini Kit and *ipsogen* IDH1 RGQ PCR Kit.

13.1.9. Cross-contamination / analytical carryover

The purpose of this study was to evaluate the *ipsogen* IDH1 RGQ PCR Kit for carryover when IDH1 mutation positive samples are tested adjacent to IDH1 mutation negative (wild type) samples. This study examined the entire IDH1 System from extraction to PCR amplification and investigated if carryover occurred between samples, extractions, and within or between runs.

This study was performed with mutation positive (either R132C or R132L) and wild type WB or BMA specimens. Two independent sets of samples were extracted following a predefined extraction matrix designed to introduce risk of sample cross-contamination.

Extraction cross-contamination was tested across six extractions, and PCR cross-contamination was tested across six PCR runs, following a run matrix designed to introduce the risk of sample cross-contamination. Extraction and PCR runs were set up consecutively by the same operator (one operator per independent set of samples) using the same equipment, with no other runs set up using this instrument between these runs and separate instruments used for each sample set. One lot of QIAamp DSP DNA Blood Mini Kit and one lot of *ipsogen* IDH1 RGQ PCR Kit were used throughout this study.

A total of 144 wild type replicates were tested, with no wild type replicate generating either a R132C or R132L mutation detected call. Therefore, demonstrating the absence of cross-contamination of the wild type samples by mutation positive samples sharing the same DNA extraction and run set up procedure.

13.1.10. Specimen handling

This study assessed sample handling variability during the DNA extraction process using the QIAamp DSP DNA Blood Mini Kit. Whole blood (WB) and bone marrow aspirate (BMA) samples were tested across three independent laboratories. Each of the three sites processed four extractions per sample (12 samples – five IDH1 mutation samples and one wild type sample, for both sample types), resulting in a total of 144 individual gDNA extracts.

All samples were blinded before extraction. Each sample was extracted, quantified, and normalized at the respective sites, and then tested using the *ipsogen* IDH1 RGQ PCR Kit at QIAGEN Manchester.

Across all mutation and WT samples, the proportion of correct calls was 100%, supporting the reproducibility and repeatability of the *ipsogen* IDH1 RGQ PCR Kit at the pre-analytical step of DNA extraction.

13.1.11. Interfering substances (analytical specificity)

The study aimed to evaluate the impact of exogenous and endogenous substances on the performance of the *ipsogen* IDH1 RGQ PCR Kit. The study included at least nine replicates for each sample type (whole blood and bone marrow aspirate) and mutation status (wild type, R132H, and R132G), in combination with each interferent.

Exogenous substances present in the DNA extraction process were tested by spiking into the gDNA eluate after DNA extraction and normalization. Samples tested with potential

exogenous interferents were spiked at concentrations representing the highest feasible level of the interfering substance carryover into a sample. The exogenous substances tested included:

- K2-EDTA
- Lysis buffer
- QIAGEN Protease
- Ethanol
- Buffer AW1
- Buffer AW2

Endogenous substances, including pharmaceuticals and naturally occurring biological substances, were spiked into whole blood or bone marrow aspirate samples before DNA extraction. Test concentrations were determined based on C_{max} values (3× concentration of a substance expected in patient sample) or CLSI EP07-ED3 recommendations. The endogenous substances tested included:

- Cytarabine
- Decitabine
- Etoposide
- Idarubicin hydrochloride
- Daunorubicin hydrochloride
- Azacitidine
- Fludarabine phosphate
- Hemoglobin
- Unconjugated bilirubin
- Cholesterol

- Albumin
- Triglycerides

Up to four exogenous or four endogenous substances were pooled using a single solvent, and spiked samples were compared to a solvent-only control sample. If required, individual substances from a pool that demonstrated an interfering effect were then tested in isolation.

Cytarabine, decitabine, etoposide, idarubicin hydrochloride, daunorubicin hydrochloride, azacitidine, and triglycerides were noted to potentially reduce sample validity rates in low DNA input samples but did not lead to incorrect results.

Fludarabine phosphate, hemoglobin, unconjugated bilirubin, cholesterol, albumin, and all exogenous substances tested met the acceptance criteria, demonstrating no significant impact on the performance of the *ipsogen* IDH1 RGQ PCR Kit.

13.2. Clinical Performance

13.2.1. Diagnostic sensitivity and specificity

The *ipsogen* IDH1 RGQ PCR Kit (IDH1 Kit) was compared with a next-generation sequencing (NGS) reference test, the Archer VariantPlex Core Myeloid panel (validated by ICON) in newly diagnosed AML patients (using both BMA and PB samples, Table 17). The overall diagnostic sensitivity was 98.48% (Clopper–Pearson (Exact) Binomial 95% Confidence Interval (CI): (91.84%, 99.96%)) and diagnostic specificity was 100% (95% CI: (96.11%, 100%)) (Table 18).

Table 17. *ipsogen* IDH1 RGQ PCR Kit vs reference test (ICON NGS) for newly diagnosed AML – Analysis for all mutations

Frequency		Reference test		
		IDH1 mutation detected	IDH1 mutation not detected	Total
<i>ipsogen</i> IDH1 RGQ PCR Kit	IDH1 mutation detected	65	0	65
	IDH1 mutation not detected	1	93	94
	Total	66	93	159

Table 18. Diagnostic sensitivity and specificity for newly diagnosed AML – Analysis for all mutations

	Frequencies	Clinical performance	Lower two-sided 95% CI	Upper two-sided 95% CI
Diagnostic sensitivity	65 / 66	98.48	91.84	99.96
Diagnostic specificity	93 / 93	100.00	96.11	100.00

The analysis of clinical performance was also performed on a per-mutation basis as presented in Table 19 and Table 20.

Table 19. *ipsogen* IDH1 RGQ PCR Kit vs Reference test (ICON NGS) for newly diagnosed AML – Analysis per mutation

Frequency		Reference test							Total
		Mutation not detected	R132C	R132G	R132H	R132H and R132C	R132L	R132S	
<i>ipsogen</i> IDH1 RGQ PCR Kit	Mutation not detected	93	1	0	0	0	0	0	94
	R132C	0	39	0	0	0	0	0	39
	R132G	0	0	5	0	0	0	0	5
	R132H	0	0	0	16	0	0	0	16
	R132H and R132C	0	0	0	0	1	0	0	1
	R132L	0	0	0	0	0	1	0	1
	R132S	0	0	0	0	0	0	3	3
	Total	93	40	5	16	1	1	3	159

Table 20. Diagnostic sensitivity and specificity for newly diagnosed AML – Analysis per mutation

Mutation	Diagnostic sensitivity	Two-sided 95% CI	Diagnostic specificity	Two-sided 95% CI
R132C	97.6% (40 / 41)	87.1%, 99.9%	100.00% (118 / 118)	96.92%, 100.00%
R132G	100.0% (5 / 5)	47.8%, 100.0%	100.00% (154 / 154)	97.63%, 100.00%
R132H	100.0% (17 / 17)	80.5%, 100.0%	100.00% (142 / 142)	97.44%, 100.00%
R132L	100.0% (1 / 1)	2.5%, 100.0%	100.00% (158 / 158)	97.69%, 100.00%
R132S	100.0% (3 / 3)	29.2%, 100.0%	100.00% (156 / 156)	97.66%, 100.00%

13.3. Clinical utility/efficacy of the *ipsogen* IDH1 RGQ PCR Kit with TIBSOVO treatment in combination with azacitidine in newly diagnosed AML

Subjects screened in the AG120-C-009 clinical study who had available Abbott RealTime IDH1 CTA results were selected to evaluate concordance between the Abbott assay (CTA) and the *ipsogen* IDH1 RGQ PCR Kit (CDx). The maximum number of leftover samples positive for IDH1 mutations based on the CTA assay and meeting the study inclusion/exclusion criteria were included in the bridging analysis.

As of the March 18, 2021 data cutoff, 146 IDH1-mutation-positive subjects (with the CTA) had been identified and randomized to receive either ivosidenib (TIBSOVO) + azacitidine (N = 72) or placebo + azacitidine (N = 74).

From this Full Analysis Set (FAS), subjects were excluded for the following reasons:

- Withdrawal from trial: 12 subjects, and lack of consent for future testing: 8 subjects
- Samples collected in China: 12 subjects, excluded due to export restrictions
- No leftover samples available: 2 subjects

After these exclusions, 112 samples remained available for the Bridging Study.

Of these 112 samples, 111 were IDH1-mutation-positive with the CTA and formed the positive Bridging set. One sample had been randomized based on a local test and was CTA-negative, and therefore served as the negative Bridging sample. Note that two samples were non-evaluable and a further two samples were tested outside of the workflow, resulting in a total of 107 evaluable samples.

In addition, a separate subset of CTA-negative subjects was used to generate the negative sample set. A total of 148 CTA-negative samples were available for testing with the *ipsogen* IDH1 RGQ PCR Kit to identify patients eligible for treatment with ivosidenib (TIBSOVO) plus azacitidine. Additionally, to determine the clinical efficacy using the IDH1 Kit, as shown by improved event-free survival (EFS) and other clinical outcomes consistent with the AG120-C-009 clinical study.

The Bridging Study was a non-interventional, retrospective analysis using leftover BMA and PB specimens from newly diagnosed AML patients. The primary objectives of the Bridging Study were to assess concordance between the *ipsogen*IDH1 RGQ PCR Kit and the Abbott CTA and to evaluate the ability of the IDH1 Kit to identify patients eligible for treatment with ivosidenib (TIBSOVO) plus azacitidine. Additionally to determine the clinical efficacy using the *ipsogen*IDH1 RGQ PCR Kit, as shown by improved even-free survival (EFS) and other clinical outcomes consistent with the AG120-C-009 clinical study.

The study population was representative with respect to demographics, disease characteristics, and sample type. No significant imbalances were observed between CDx-evaluable and non-evaluable subjects.

13.4. Clinical concordance

The Clinical Bridging Study demonstrated that Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) met the predefined concordance threshold of $\geq 95\%$, establishing concordance between the *ipsogen* IDH1 RGQ PCR Kit and the Abbott CTA.

Concordance results were as follows:

- PPA: 95.33% (102/107; 95% CI: 89.43–98.47)
- NPA: 99.31% (144/145; 95% CI: 96.22–99.98)
- Overall Percent Agreement (OPA): 97.62% (246/252; 95% CI: 94.89–99.12)

Table 21. Concordance between the IDH1 Kit (CDx) and the Abbott CTA with CTA as the reference method

CDx mutation status		CTA mutation status	
Frequency	Mutation detected	No mutation detected	Total
Mutation detected	102	1	103
No mutation detected	5	144	149
Total	107	145	252

13.5. Clinical efficacy

Clinical efficacy of the *ipsogen* IDH1 RGQ PCR Kit was assessed by evaluating treatment outcomes in newly diagnosed AML patients identified as IDH1-mutation-positive by the CDx. These outcomes, including event-free survival (EFS), were compared with the clinical efficacy observed in the AG120-C-009 study, in which patient selection was performed using the Abbott RealTime IDH1 CTA.

Table 22 shows the EFS for the two treatment groups of AG120-C-009 based on the clinical efficacy, and Table 23 for the two treatment groups of AG120-C-009 based on the CDx positive results.

Table 22. Clinical trial efficacy event-free survival results (AG120-C-009)

	Tibsovo + azacitidine (N = 72)	Placebo + azacitidine (N = 74)
Subjects (%) with events	46 (63.9%)	62 (83.8%)
EFS Rate (%) (95% CI)		
1 day	41.7% (30.2%, 52.7%)	20.3% (12.0%, 30.0%)
3 months	41.7% (30.2%, 52.7%)	20.3% (12.0%, 30.0%)
6 months	39.9% (28.6%, 51.0%)	20.3% (12.0%, 30.0%)
9 months	39.9% (28.6%, 51.0%)	20.3% (12.0%, 30.0%)
12 months	37.4% (25.9%, 48.9%)	12.2% (4.3%, 24.4%)
18 months	33.3% (20.9%, 46.2%)	6.1% (0.7%, 20.9%)
24 months	22.2% (6.6%, 43.4%)	0.0% (-, -) NE*
36 months	0.0% (-, -) NE	0.0% (-, -) NE*
Hazard ratio (relative to placebo + azacitidine) (Stratified)	0.33	
95% CI	(0.16, 0.69)	
P-value for HR=1 (one-sided)	0.0011	

* NE = Non-evaluable.

Table 23. Summary of overall event-free survival based on CDx results for Population 1

	Tibsovo + azacitidine (N = 50)	Placebo + azacitidine (N = 52)
Subjects (%) with events	32 (64%)	46 (88.5%)
Subjects (%) without events (censored)	18 (36.0%)	6 (11.5%)
EFS Rate (%) (95% CI)		
1 day	40.0% (26.5%, 53.1%)	13.5% (5.9%, 24.1%)
3 months	40.0% (26.5%, 53.1%)	13.5% (5.9%, 24.1%)
6 months	37.8% (24.5%, 51.0%)	13.5% (5.9%, 24.1%)
9 months	37.8% (24.5%, 51.0%)	13.5% (5.9%, 24.1%)
12 months	37.8% (24.5%, 51.0%)	9.0% (2.4%, 21.1%)
18 months	37.8% (24.5%, 51.0%)	9.0% (2.4%, 21.1%)
24 months	25.2% (7.4%, 48.2%)	0.0% (-, -) NE*
36 months	0.0% (-, -) NE	0.0% (-, -) NE*
Hazard Ratio (relative to Placebo+azacitidine) (Stratified)	0.438	
95% CI	(0.254, 0.757)	
P-value for HR=1 (one-sided)	0.0016	

* NE = Non-evaluable.

When compared against the clinical trial efficacy population, an even split between the treatment groups was observed in the CDx + subjects and the percentage of events between the groups was similar when compared against the clinical trial efficacy. The hazard ratio (HR) for the clinical trial was 0.33 and statistically significant (0.0011) (Table 22), the HR for the efficacy for the CDx + Population was 0.438 and statistically significant (0.0016) (Table 23). Although the CDx HR is higher than the clinical trial efficacy, there is still an improvement

from placebo as both the CTA and CDx HRs are <1 which means that the treatment is working and both of them are statistically significant.

- EFS and secondary endpoints in CDx-positive patients were consistent with the main clinical trial results.

The *ipsogen* IDH1 RGQ PCR Kit demonstrated high concordance with the clinical trial assay and effectively identified ND AML patients eligible for targeted therapy.

13.6. Summary of safety and performance

The summary of safety and performance can be found on the EUDAMED website.

14. References

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15. Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Support 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	
"NO_CT_VALUE" flag in Positive Control (PC)	
a. Incorrect configuration of the PCR	Check your pipetting scheme and repeat the PCR.
b. The storage conditions for one or more kit components did not comply with the instructions given in "Reagent storage and handling" section.	Check the storage conditions (see the kit label) of the reagents and use a new kit, if necessary.
"UNEXPECTED_CT_VALUE" flag in NTC	
a. Contamination occurred during preparation of the PCR	Ensure the area has been decontaminated. Repeat the PCR with new reagents. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure that workspace and instruments are decontaminated at regular intervals.
"ABOVE_ACCEPTABLE_RANGE" or "BELOW_ACCEPTABLE_RANGE" flag in PC	
a. Error during preparation of the PCR	Repeat the PCR ensuring accurate pipetting.
"DNA_INPUT_TOO_HIGH" flag in the sample tube	
a. Sample is too concentrated	Repeat DNA quantification and normalization. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]).

"ABOVE_ACCEPTED_RANGE" and "NO_CT_VALUE" flags in sample tube

- a. Insufficient starting DNA template present in sample

Repeat DNA quantification and normalization. Samples should be diluted using the water provided in the kit (Water for Dilution [Dil.]). After retest, if the control Ct value is still invalid, reextract the sample. If the control Ct value is invalid after reextraction and two further rounds of testing, then the sample should be reported as indeterminate mutation status.

16. Symbols

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

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalogue number
	Lot number
	Material number (i.e., component labelling)
	Components
	Contains
	Number
	Global Trade Item Number
	Unique Device Identifier
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



Warning/caution



Health hazard



Environment

17. Contact Information

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

18. Ordering Information

Product	Contents	Cat. no.
<i>ipsogen</i> IDH1 RGQ PCR Kit	For 24 reactions: IDH1 Reaction Mix 1, IDH1 Reaction Mix 2, IDH1 Positive Control, Taq Polymerase, Water for NTC, Water for sample dilution	675223
QIAamp DSP DNA Blood Mini Kit — for purification of genomic DNA from whole blood or bone marrow aspirate		
QIAamp DSP DNA Blood Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase, Buffers, Collection Tubes (2 mL)	61104
Rotor-Gene Q MDx 5plex HRM and accessories		
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
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
Loading Block 72 × 0.1 mL Tubes	Aluminum block for manual reaction setup with a single-channel pipette in 72 × 0.1 mL tubes	9018901
72-Well Rotor	For holding Strip Tubes and Caps, 0.1 mL, with reaction volumes of 10–50 µL; requires Locking Ring 72 Well Rotor	9018903
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 mL in the 72-Well Rotor	9018904
Strip Tubes and Caps, 0.1 mL (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 mL (2500)	10 × 250 strips of 4 tubes and caps for 10,000 reactions of 10–50 µL	981106

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 Support or your local distributor.

19. Document Revision History

Revision	Description
R1, June 2026	Initial release

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